Preface

This issue of the Toxicologist is devoted to the abstracts of the presentations for the platform and poster sessions of the 24th Annual Meeting of the Society of Toxicology, held at the Town & Country Hotel, San Diego, CA, March 18-22, 1985.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), appears on pages 329-338.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 257. The Keyword Index was prepared by Elton R. Homan, Union Carbide Corporation, and Edward Miedel, Conquest Computer Company.

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2 GENTAMICIN NEPHROTOXICITY IN RATS WITH PRIOR RENAL INSUFFICIENCY. M.P. Carver and J.E. Riviere, North Carolina State University, Raleigh, NC.

Gentamicin (G) nephrotoxicity has not been thoroughly investigated in animals with experimental renal disease. The present study in 241 Sprague-Dawley rats examines two models, subtotal (2/3) surgical nephrectomy (NX) and polyvinyl alcohol (PVA) glomerulonephritis, using total daily G doses from 0-120 mg, administered for 6 or 12 days. Renal function was estimated by creatinine (Cr) clearance, fractional Na and K excretion, serum Cr and urea nitrogen, and urinary N-acetyl-β-D-glucosaminidase excretion, as well as light microscopic evaluation. Comparison of dose-response curves in NX rats with controls (no pretreatment) demonstrated a resistance to G nephrotoxicity in NX rats after 6 days, which was partially reversed by 12 days despite less severe morphological lesions. PVA pretreatment, resulting in an otherwise benign glomerulopathy, potentiated G toxicity at 6 days, but caused no differences after 12 days. Renal G concentrations at 6 days were not consistently correlated with toxicity. These findings demonstrate altered G toxicokinetics in two renal pathophysiological states in rats.

4 STUDIES ON THE SEX DIFFERENCE IN HEXACHLORO-1:3-BUTADIENE-INDUCED NEPHROTOXICITY. E.A. Lock, and J. Ishmael, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Hexachloro-1:3-butadiene (HCB) shows a marked sex difference in nephrotoxicity, females being four times more sensitive than males. We have examined in male and female rats the nephrotoxicity of the glutathione (HCB-GSH) and cysteine (HCB-CYS) conjugates, and the liver and kidney concentration of total and bound radioactivity from [14C]-HCB. Adult rats of both sex were dosed with HCB-GSH or HCB-CYS at doses ranging from 12-145 μmol/kg ip and killed 24 h later. Renal damage was assessed by plasma urea and histopathology. Both metabolites produced a marked sex difference in nephrotoxicity similar to that seen with the parent compound. The tissue distribution of HCB was determined at an equimolar dose, which is nephrotoxic to females but not males. Rats of both sex killed 4h after [14C]-HCB (192 μmol/kg, ip) showed no difference in total renal protein or plasma radiolabel. Despite the similar total renal content, covalently bound radiolabel was four times higher in female kidney versus male kidney. These findings suggest a sex difference in the renal activation of HCB conjugates.
5 THE EFFECT OF PROBENCID ON HEXACHLORO-1,3-BUTADIENE-INDUCED NEPHROTOXICITY. E.A. Lock, and J. Ishmael, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

Hexachloro-1,3-butadiene (HCB) and its glutathione (HCB-GSH), cysteine (HCB-CYS) and mercapruate (HCB-NAC) conjugates are potent nephrotoxins, causing necrosis to the pars recta of the proximal tubule. We have examined the effect of probenecid, a competitive inhibitor of renal organic anion transport on the nephrotoxicity produced by these compounds. Female rats were given probenecid (5000μmol/kg, ip) 0.5h before and 7.5h after HCB (192μmol/kg); HCB-GSH (47μmol/kg); HCB-CYS (36μmol/kg) or HCB-NAC (64μmol/kg). Animals were killed 24h after dosing and plasma urea and renal morphology assessed. Treatment with probenecid completely prevented the nephrotoxicity produced by these compounds. The concentration of radioactivity in the renal cortex and plasma of rats receiving 64μmol/kg [14C]-HCB-NAC in the presence and absence of probenecid was determined. Probenecid treatment reduced the renal cortex to plasma ratio of radioactivity from 4.2 in animals given HCB-NAC alone to <1.0 in animals given HCB-NAC plus probenecid. These findings indicate that the conjugates of HCB are transported into renal tubular cells via a probenecid sensitive transport system, and that accumulation is related to nephrotoxicity.

6 PATHOLOGY OF ACUTE HYDROCARBON NEPHROTOXICITY IN MALE RATS. B.G. Short, V.L. Burnett, J.T. Martin, and J.A. Sweeney, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

Acute hydrocarbon-induced nephrotoxicity is associated with the isoparaffinic fraction of gasoline. In previous work, whole body autoradiography performed 72 hours after oral administration of 2.0g/kg (14C)-2,2,4 trimethylpentane (TMP) revealed localization of radioactivity in the renal cortex in a radial pattern, suggesting deposition of the compound in the proximal tubules. After administration of 0.5 or 2.0g/kg TMP the earliest exacerbation of hyaline droplet accumulation was observed histologically 24hrs after treatment in the P2 segment of the proximal tubule. This accumulation decreased in severity 72 hours following administration. Ultrastructure studies revealed large, crystalloid, polyhedral lysosomes in P2 corresponding to the hyaline droplets as early as 8 hours after dosing. This alteration persisted until the eighth day following treatment. No difference between dose groups was observed by light or electron microscopy. These observations suggest that the hyaline droplet accumulation in the kidney may be attributable directly to TBP or a metabolite. These data will be compared with the effects found in multiple dose studies.

7 EFFECTS OF 1,3-DICHLOROPROPENE (DCP) ON RENAL TRANSPORT AND GLUTATHIONE (GSH) IN THE RAT. H.M. Jeffrey, J.M. Baggett, M.A. Berendt. Dept. of Pharmacology, College of Medicine, Univ. of Nebraska Medical Center, Omaha, NE 68105.

DCP is a pesticide used for the control of nematodes. The purpose of this study was to determine the renal effects of DCP. Male Sprague-Dawley rats were given single doses of 40 to 200 mg/kg intraperitoneally (ip) or 100 to 325 mg/kg orally. Intraperitoneal and oral 1250's were found to be approximately 175 mg/kg and 325 mg/kg respectively. Renal transport was assessed using 14C-paeminophipharosine (PAH) and 14C-tetraethylammonium (TEA) 24 hours following oral or ip administration of DCP. PAH transport was depressed significantly following oral administration of 175, 250 and 325 mg/kg DCP with no significant effect on TEA transport. PAH transport also decreased significantly following ip administration of 75, 150, 175 and 200 mg/kg DCP with no significant effect on TEA transport noted. Renal GSH content was measured 30 minutes, 2 hours and 24 hours after ip administration of DCP. Hepatic GSH content 30 minutes after dosing ranged from 56% of control at 40 mg/kg to 12% of control at 200 mg/kg. Renal GSH content 30 minutes after dosing ranged from 75% of control at 40 mg/kg to 34% of control at 200 mg/kg. Hepatic and renal GSH 2 hours after injection ranged from 63% to 18% of control and 85% to 25% respectively. By 24 hours after administration of DCP hepatic GSH levels were either back to or above control values (123% of control at 200 mg/kg). Renal GSH levels were also back to or above control values 24 hours after injection (146% of control at 150 mg/kg). These data suggest that DCP metabolism may be important in the disruption of renal transport. (Supported by ES 03712).

8 PRECHRONIC (14-DAY) AND SUBCHRONIC (13-WEEK) TOXICITY OF ORALLY ADMINISTERED 4-VINYLCYCLOHEXENE TO FISCHER 344 RATS AND B6C3F1 MICE. J.J. Collins; M. Powers; A. Manus*; NICHS, NTP, RTP, NC; *LBI, Kensington, MD. Sponsor: R. Yang

4-Vinylcyclohexene (VCH), a dimer of 1,3-butadiene present in significant quantities in the gases discharged during tire curing, was examined for its toxic effects in F344 rats and B6C3F1 mice by 14-day prechronic and 13-week subchronic testing. In the 14-day studies, VCH was administered orally in corn oil (gavage) at 0, 300, 600, 1250, 2500 or 5000 mg/kg body weight to both sexes and species while the doses for the 13-week studies were 0, 50, 100, 200, 400, or 800 mg/kg/day for rats and 0, 75, 150, 300, 600 or 1200 mg/kg/day for mice (5 days/week). Extensive mortality was seen in rats and mice in the 14-day studies at VCH levels >1250 mg/kg, although no chemically-related gross or histopathologic effects were observed. Effects on final body weights (>5%) were seen in the 13-week studies in male and female rats and female mice receiving 1600, 800, or 600 mg/kg, respectively, while extensive mortality was observed in mice dosed at 1200 mg/kg. VCH-induced histologic effects were manifested as hyaline droplet degeneration of the proximal convoluted kidney tubules in 13-week dosed male rats with the severity of the lesions being dose-related. No chemically-related gross or histopathologic effects were evident in dosed mice or female rats in the 13-week studies and no lesions of any kind were identified in the stomach, the presumed target organ, in rats or mice in either the 14-day or 13-week studies.
9 CHRONIC TOXICITY AND ONCOCENICITY STUDIES ON ETHYLENE GLYCOL (EG) IN RATS AND MICE. L.R. DePass, M.D. Woodside, R.H. Carman and C.E. Weil, Bushy Run Research Center, Export, PA. These studies were performed to assess the chronic toxicity and oncogenicity of EG in the diet. Groups of 130 F344 rats and 80 CD-I mice per sex were fed 1.0, 0.2, or 0.04 g/kg/day EG. Two control groups for each species received no EG. Mortality was increased in high-dose male rats all of which died by 475 days. The following effects were also observed in high-dose male rats: reduced body weight gain, increased water intake, increased blood urea nitrogen and creatinine, reduced red cell count, hematocrit and hemoglobin, increased neutrophil count, increased urine volume, and reduced urine specific gravity and pH. Urinary calcium oxalate crystals increased kidney weight were seen in all high-dose rats. Uric acid crystals, reduced urine volume and pH and increased specific gravity were seen in high-dose female rats. Histopathologic changes in high-dose male rats included tubular cell hyperplasia, tubular dilation, peritubular nephritis, parathyroid hyperplasia, and soft tissue mineralization. Fatty change of the liver was seen in high-dose female rats. No clinical signs or gross or microscopic evidence of toxicity were seen in mice at the dosages used. Water intake and clinical pathology parameters were not measured in the mouse study. The data from both studies provided no substantial evidence for an oncogenic effect of EC.

10 SENSITIVITY OF DEVELOPING RAT PUPS TO NEPHROTOXINS. R.J. Kavlock, Developmental Biology Division, USEPA, RTP, NC. Sponsor: Neil Chernoff.

The kidneys of newborn rats, which are both morphologically and physiologically immature, have been shown to be relatively insensitive to the nephrotoxic effects of several chemicals. To examine the specificity of these age-related differences, pups received 20 mg Amphotericin B (AB)/kg or 250 mg folic acid (FA)/kg on postnatal days 1, 8 or 15. GFR (estimated by the clearance of creatinine) and fractional excretions (FE) of water, urea, and electrolytes (Na, K, Cl) were determined during a three hour clearance period at 1, 2 or 6 days after treatment. Kidney weights (KW) and histopathology (HP) were also obtained for these animals. In another group, maximal urinary concentrating ability (UMAX) was determined following a six hour period of fluid deprivation. We observed no differences in degree of renal toxicity with age, but repair of renal damage tended to proceed slower in the youngest animals. AB treatment resulted in a slight increase in KW without accompanying HP. Clinical signs included uremia, increased FE of water and Na, decreased FE of urea and a greatly diminished UMAX, but no change in GFR. FA treatment resulted in increased KW with marked HP. Clinical signs included uremia, decreased GFR and UMAX, and increased FE of water. Thus, the relative morphological and physiological immaturity of the kidneys of neonatal rats does not assure protection from all nephrotoxic agents.

11 THE EFFECTS OF UNILATERAL NEPHRECTOMY (UNIX) ON THE TOXICITY AND RENAL HANDLING OF MERCURIC CHLORIDE IN THE RAT. M.T. Houser and W.O. Berndt. University of Nebraska Medical Center, Omaha, NE

The remaining kidney of an animal subjected to UNIX undergoes profound structural and physiologic alterations. Although these changes are well characterized, little attention has been paid to the effects of UNIX on the toxicity and/or handling of nephrotoxins. Because of this, we studied the effect of mercuric chloride (2mg/kg) on animals 2 days following UNIX or sham surgery (SS). One day following injection, the kidney content of mercury was significantly (p<0.05) higher in the UNIX group (20.7 μg/100 mg dry wt vs 18.8) although liver content was similar (1.1 μg/100 mg dry wt vs 0.9). Urinary excretion of mercury during the first day was also significantly (p<0.01) higher in the UNIX group (34.9 μg/gm kidney wt/day) as compared to SS control (20.2).

Following in vitro incubation with mercuric chloride (10-5 M for 2 hours), mercury uptake in tissues from animals 2 days post UNIX was significantly (p<0.05) higher than SS control (13.3 μg/100 mg dry wt vs 13.4). Glomerular filtration rate (GFR) decreased dramatically in both groups and was significantly (p<0.02) lower in the UNIX group on Days 1-4 and 10 following injection. Furthermore, the percent reduction in GFR in the UNIX group was significantly (p<0.05) higher as compared to saline injected controls on all the same days. Our data suggests that following UNIX, mercuric chloride induces a more severe renal injury as assessed by GFR and reveals significant differences in the in vivo and in vitro handling of mercury.

12 MORTALITY AND Hg2O3 AND Cr51 IN THE KIDNEY AND LIVER FOLLOWING GLUTATHIONE DEPLETION WITH DIETHYLMALATE PLUS BUTHIONINE SULFOXIMINE. J. McC. Baggett and W.O. Berndt, Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE, 68105.

Renal concentrations of mercury have been directly associated with glutathione (GSH) levels by several investigators. It was found that rat mortality increased when HgCl2 (4 mg/kg) was given subsequent to GSH depletion with the combination of DEM and BSO (DEM-BSO), whereas 100% survival was seen when HgCl2 followed either of the depleting agents alone. All animals receiving HgCl2 (4 mg/kg) after GSH depletion with DEM-BSO died between 8 and 24 hours. Sixty-four percent died within 24 hours of 2 mg/kg HgCl2 following DEM-BSO. GSH was reduced more in both organs by DEM-BSO than by DEM alone. Renal uptake of Hg2O3-HgCl2 was 28% of control after DEM-BSO and 45% of control after DEM alone. Hepatic uptake of Hg2O3-HgCl2 was not different from control. Renal and hepatic levels of Cr51-potassium dichromate (10 mg/kg) given simultaneously with mercuric chloride (4 mg/kg) were not different from control.

Ethylenediaminetetraacetic acid (EDTA) is currently the chelant of choice in the treatment of lead (Pb) toxicosis but its ability to mobilize lead from soft tissues is limited. This study compared the excretion and disposition of Pb following the administration of five chelating agents [EDTA, ethylenediaminetetraacetic acid (EDTA), dimercapto succinic acid (DMSA), dimercaptopropanesulphonate (DMPS), and penicillamine (PA)] each administered at doses of 50, 100, and 150 mg/kg. Male Swiss mice (20g) were administered lead acetate at 5 mg Pb/Kg body weight ip daily for 5 days followed by administration of a chelant ip for 3 days. EDTA and DMSA were equally efficacious in promoting urinary excretion of lead (250% increase over prechelation values) and were significantly better (P<0.01) than either DMPS or PA (146 and 130%, respectively). Lead concentrations in bone differed significantly (P<0.01) from control only following chelation therapy with either EDTA or DMSA. However, EDTA was slightly more effective than DMSA in mobilizing lead from bone. PA and DMSA were the most effective drugs in removing lead from liver, whereas DMPS proved most effective in the kidney. The data indicate that the overall effect of DMSA as a chelating agent is equal to that of EDTA. Although EDTA is better at mobilizing lead from bone, a major reservoir of lead for the body, DMSA is superior in removing lead from critical soft tissue depots.

Thymic atrophy and humoral immunosuppression by PCBs is associated with the aromatic hydrocarbon (Ah) receptor. We examined the relation between these Ah receptor-mediated toxic effects. 3,3',4,4'-Tetrachlorobiphenyl (TCB), which causes immunosuppression and thymic atrophy, and 2,3,3',4,4'-pentachlorobiphenyl (PCB), which causes immunosuppression without thymic atrophy, were administered ip to C57BL/6 mice at 0.5, 35, and 350 umol/kg body wt 2 days before iv immunization with 10 ug E. coli lipopolysaccharide (LPS), a thymic independent antigen. Both congeners caused suppression of anti-IgG plaque forming cells (FFC)/spileen and serum antibody titers. TCB was also administered 2 days before either 10 or 100 ip immunization with sheep erythrocytes (SRBC). TCB before iv immunization had no effects on the II response whereas treatment before only the III response inhibited. PCF responses were suppressed but was most severe, and accompanied by thymic atrophy, only when exposure occurred 2 days before immunization. These results demonstrate that thymic atrophy is not a sensitive measure of Ah receptor mediated immunosuppression, that this effect is not necessarily a consequence of thymic toxicity, and suggest that terminal differentiation of the B lymphocyte is impaired during activation of the Ah gene complex. (NIH grant ES02897)

TOXIC MANIFESTATION OF IN VIVO CISPLATIN ON ISOLATED PERFUSED KIDNEY FUNCTION. K. Murag, D.A. Pasino, R.S. Goldberg and J.B. Hook. Dept. Investigative Toxicology, Smith Kline and French Laboratories, Philadelphia, PA

The antineoplastic drug, cisplatin, is nephrotoxic in experimental animals and in humans. Cisplatin also causes glucosuria associated with hyperglycemia in rats. The present experiments were conducted to examine the effects of cisplatin on tubular function using the isolated perfused kidney (IPK). Kidneys of male Sprague-Dawley rats (250-350 g) were perfused with modified Krebs-Ringer bicarbonate buffer with 7% bovine serum albumin. Addition of cisplatin (44 µmol/L) into the perfusate did not affect renal perfusion flow, GFR or fractional reabsorption of water, Na, K, and glucose. However, 2 days after a single injection of cisplatin (10 mg/kg), pronounced functional changes in renal function were observed. BUN and serum glucose were reduced from 13.9 ± 0.1 to 5.38 ± 8.8 mg% and from 146 ± 8 to 314 ± 64 mg%, respectively. GFR in the IPK was reduced >50%; reabsorption of water, Na and glucose was reduced >10%; K reabsorption was converted to secretion. Thus, the IPK appears to offer a functional model of cisplatin nephrotoxicity and the preliminary data suggest that glucosuria is attributable not only to hyperglycemia but also impaired tubular reabsorption of glucose.


In vivo exposure to DMBA suppresses cellular and humoral immunity (TAP 22, 299, 1984; Dean et al., Cell. Immunol., in press). An in vitro model of DMBA-induced immunomodulation was developed to study the mechanism of this suppression. Spleen cells from B6C3F1 mice were cultured in medium containing DMBA (final concentration: 40, 20 or 4 µM in 0.1% DMSO). Splenocyte subpopulations were quantitated by cytofluorometry, while function was evaluated in oneway mixed leukocyte cultures (MLC), Mishell Dutton (MD) and cytotoxic T-lymphocyte (CTL) assays. In vitro exposure to DMBA resulted in a concentration-dependent suppression of CTL-mediated tumor cytotoxicity (92%), the MD response (73%), and numbers of sheep erythrocyte antibody plaque forming cells in the MD assay (97%). These results were similar to those obtained following in vivo DMBA exposure. Addition of the T-cell lymphokine interleukin (IL)-2 restored CTL and MLC responses while macrophage-derived IL-1 failed to abrogate MLC and CTL suppression. Relative percentages of helper (LY+) and cytotoxic/suppressor (LY2,3+) T-cells were not significantly altered following in vitro exposure to DMBA and/or IL-2. These results suggest a DMBA-induced functional deficit in T-helper cells which is reversible by addition of exogenous IL-2.
17 DELTA-9-TETRATHYDROCARBONOL DECREASES HOST RESISTANCE TO HERPESVIRUS INFECTION IN THE MOUSE. E.M. Minkin, M.P. Holsapple, and G.A. Cahral. Departments of Pharmacology and Toxicology and of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA. Sponsor: A.E. Munsen.

This study was undertaken to determine whether Delta-9-tetrahydrocannabinol (Delta-9-THC) decreases host resistance to herpes simplex virus type 2 (HSV2) infection in the female B6C3F1 mouse. Animals were administered Delta-9-THC (15mg/kg to 200 mg/kg) or vehicle intraorally on days 0-3. HSV2 was introduced intravenously (i.v.) or intravaginal (i.vag.) at 2300 PPU or 10a.5 PPU, respectively, on day 1. All mice receiving HSV2 were assessed for decreased resistance by comparing frequencies of mortalities to those of vehicle controls. Frequencies of lesion expression and virus shedding were also used to assess resistance in animals receiving HSV2 i.vag. Mice inoculated i.v. with HSV2 and treated with Delta-9-THC exhibited significantly higher mortalities when compared to vehicle controls. Mice receiving HSV2 i.vag. showed dose-related increases in the frequency of mortalities, titers of vaginal virus shed, and expression of lesions. These results indicate that Delta-9-THC decreases host resistance to HSV2 infection in the mouse. This decrease may be elicited at an early stage in the host response since drug administration diminished the primary humoral response to T-dependent antigens.


The respiratory burst associated with cell membrane perturbation of monocytes and macrophages results in an increased production and concomitant release of reactive oxygen metabolites. Superoxide anion, HzO2 and products of their interaction, e.g., the hydroxyl radical and singlet oxygen, have all been implicated as important mediator molecules in the oxygen-dependent immune response of monocytes and macrophages to various intracellular parasites and tumor cells. Activation of oxygen-derived metabolites has been shown to be a mechanism of activated versus unstimulated macrophages showing an enhanced capacity to kill intracellular pathogens. This respiratory burst is accompanied by an emission of light termed chemiluminescence (CL) which can be enhanced by luminol and readily measured with a liquid scintillation counter.

Hyperchlorination of drinking water has been reported to have adverse effects on various macrophage functions, including reduction of tumoricidal activity. The purpose of this research was to investigate the effects of several chloro compound on the ability of the rat peritoneal cells to produce reactive oxygen metabolites. Male weaning Sprague-Dawley rats were exposed to the following chloro compounds in the drinking water for a 10-week period: sodium hypochlorite (0, 5, 15 or 30 ppm), or chlorine dioxide (0, 15, 15, or 50 ppm). Sodium hypochlorite at 15 or 30 ppm resulted in suppressed CL at 1 min, post-PMA addition and the CL response from animals receiving 30 ppm remained lower than controls at 11 min. Post-PMA addition at 12.5 or 25, 50 ppm chlorine dioxide, showed lower CL at 1 min, post-PMA. This chlorine-induced alteration of CL responses by rat macrophages may have important implications in host defense against microorganisms and neoplasms. In a parallel study, rats injected subcutaneously with 2 mg of dexamethasone/kg body weight had suppressed CL for greater than 60 min, post-PMA. The application of CL for macrophage function and relevance to immunotoxicology will be discussed.


Exposure of rats to dietary TBTO levels of 20 and 80 mg/kg for 6 weeks suppressed parameters of thymus-dependent immunity and of nonspecific resistance (Vos et al., Toxicol. Appl. Pharmacol. 75 (1984) 387). Therefore, it was investigated whether these effects are also observed after long-term exposure. Weanling male Wistar rats were fed diets containing 0, 0.5, 5 or 50 mg TBTO/kg. Function tests were performed after 4-6 and 15-17 months exposure in expts. I and II, respectively. The resistance to Trichinella spiralis infection was dose-related suppressed at the 0 and 50 mg/kg level in both expts., as shown by increased counts of muscle larvae and depressed serum IgG titers. In contrast to results after short-term exposure, delayed-type hypersensitivity was not suppressed after long-term treatment. Regarding the nonspecific resistance, TBTO reduced macrophage function at 50 mg/kg in both expts., as shown by impaired splenic clearance of Listeria monocytogenes, whereas enhanced clearance was found at 0.5 mg/kg in expt. I, and at 0.5 and 5 mg/kg in expt. II. TBTO treatment also impaired natural cell-mediated cytotoxicity against tumor target cells as indicated by reduced activity of natural killer cells in spleen at 50 mg/kg in expt. I, and all treatment groups in expt. II. It is concluded that long-term TBTO exposure alters both acquired and natural host resistance.


Prolonged exposure to vanadium-containing dusts in industry has been correlated with increased occurrences of respiratory disease. In vitro studies have indicated that vanadium can interfere with functional activities of cells involved in the immune response. Female B.C. mice were injected IP with NH4VO, for 3, 6, 9 of 3 weeks and then assayed for both humoral and cell mediated immune responses. Resistance to E. coli endotoxin lethality, Listeria monocytogenes lethality, EAC rosette formation and peritoneal macrophage phagocytic activity were assayed. Mice were dosed with vanadium at 2.5, 5.0 or 10.0 mg kg body weight for the exposure periods. Resistance to endotoxin lethality was increased in a time and dose dependent manner, with the highest dose (10 V) providing 100% protection after 6 weeks of vanadium exposure. Conversely, Listeria lethality was increased in a time and dose dependent manner. At the lowest exposure, 100% mortality was observed after 3 weeks. Resistance was decreased by 200 fold from exposures, by comparing the LD50 values. Macrophage phagocytic indices were decreased in a dose dependent manner. EAC rosette formation was stimulated through 6 weeks, but decreased after 9 weeks. The results indicate that vanadium can significantly alter CMI responses in vivo after short periods of exposure.

Our purpose was to compare effects of NiCl₂ and CdCl₂ on susceptibility to murine cytomegalovirus with effects on virus-augmented and spontaneous natural killer cell activity (NK). NK was determined using a 4 hr chromium release assay with splenic cell effector targets (50:1). Susceptibility was determined by incidence of mortality following a normally sublethal virus dose. NiCl₂ (20 mg/kg) given intramuscularly (i.m.) 2 days post infection resulted in increased NK 3, 4 and 5 days post infection. Depressed NK was also seen in 4 days post infection after 10 mg NiCl₂/kg but not after 5 mg/kg. Significant mortality following viral infection was seen only after 20 mg NiCl₂/kg. Similarly, while mortality was only marginally affected by 6.25 mg CdCl₂/kg given i.m. 2 days post infection, NK activity was significantly depressed. Neither were affected by 3.13 mg CdCl₂/kg. No mortality was observed in mice treated by inhalation with 1 mg/m³ CdCl₂, 2 hours/day, for 5 days or 2.5 mg/m³ 4 days post infection. Virus augmented NK 4 days post infection was slightly enhanced by this treatment while spontaneous NK was slightly depressed. We conclude that effects of these metals on NK activity reflect effects on susceptibility to virus infection. Also inhalation and i.m. exposures did not produce the same results. This abstract does not necessarily reflect EPA policy.

22 IMMUNOTOXIC EFFECTS OF AFLATOXIN B₁ IN BALB/c MICE. R.V. Reddy, M. Ahara and R.P. Sharma. Utah State University, Logan, UT

We have previously reported on the immunotoxic effects of aflatoxin B₁ (AFB₁), a secondary fungal metabolite of Aspergillus flavus in outbred CD-1 mice. Literature on AFB₁ suggests considerable interspecies and strain difference in susceptibility to hepatotoxic and hepatocarcinogenic effects. Male Balb/c mice received 0, 0.03, 0.145 and 0.70 mg/kg body weight of AFB₁ orally every other day for 4 weeks. Splenic lymphocytes from these mice were cultured without or with optimum concentrations of mitogens, lipopoly saccharide (LPS) pokeweed mitogen (PWM), phytohemagglutinin (PHA) and concanavalin A (Con A). AFB₁ caused a dose-related decrease in DNA synthesis in lymphocyte cultures with or without the mitogens. No effect on protein synthesis and a mild suppression of ribonucleic acid synthesis in high dose level were observed. There was a dose-dependent decrease in peripheral leucocyte counts and natural killer cell function. Splenic lymphocytes from normal Balb/c mice cultured with 10⁻⁶ to 10⁻⁴ M AFB₁ resulted in a decreased DNA synthesis at >2.5 x 10⁻⁶ M, a decreased protein synthesis at >10⁻⁴ M, and a decreased ribonucleic acid synthesis at >2.5 x 10⁻⁵ M. The Con A induced suppressor factor was decreased by AFB₁ at >5 x 10⁻⁵ M. The results suggest that Balb/c mice are susceptible to immunotoxic effects of AFB₁ at the dose levels employed.

23 PATHOLOGICAL AND BIOCHEMICAL EFFECTS OF VOMITOXIN IN RODENTS. F. Iverson, E. Lok and E.A. Mera. Food Directorate, Health Protection Branch, Tunney's Pasture, Ottawa, Canada. R1A 0L2.

Vomitoxin (DON, deoxynivalenol) is a tricothecene mycotoxin that has been detected in wheat and corn in North America. Several studies are underway at FBH Ottawa to delineate toxicological effects of vomitoxin. The following short term studies were designed to look for sex and species differences in rodents based on pathology and alterations in protein synthesis (14 C-leucine incorporation) and DNA synthesis (3H-thymidine incorporation) in vivo.

Male rats given 7.5 mg/kg vomitoxin po daily for 15 days exhibited decreased thymus and spleen weights. Thymidine incorporation was increased in the esophagus and decreased in the jejunum and spleen. Male mice receiving 7.5 mg/kg vomitoxin po died within 3 days. Female mice were unaffected. At 3.75 mg/kg po vomitoxin daily for 15 days, male mice showed decreased thymus and spleen weights. Thymidine incorporation was increased in the esophagus and decreased in the jejunum. Vomitoxin is more toxic to mice than rats, and more toxic towards males than females. The early changes in protein and DNA synthesis also correlate well with pathological changes observed in animals given lower levels of vomitoxin for extended time periods.

24 EXPERIMENTAL INDUCTION OF DERMAL AND RESPIRATORY HYPERSENSITIVITY TO TOLEUME DIISOCYANATE BY INHALATION EXPOSURE. Karol, M.H. and Stadler, J.C., Dept. Ind. Env. Hlth. Sci., Univ. of Pittsburgh, Pittsburgh, PA 15261

Both respiratory and dermal sensitivity have been associated with industrial exposure to toluene diisocyanate (TDI). Using an animal model, experiments were undertaken to determine the coexistence of both types of sensitivity, as well as the production of antibodies to TDI in animals sensitized by inhalation exposure. Guinea pigs were sensitized by inhalation of 0.92 ppm TDI for 3 hours/day on 5 consecutive days. Animals were evaluated for dermal sensitivity (days 8, 28), respiratory sensitivity (days 10, 21, 23, 29, 30), and for antibody to TDI (day 21). Of eight guinea pigs, 4 developed dermal sensitivity by day 8. Inhalation challenge on days 10, 21, 23 with 50 ppb TDI was negative for all animals. However, challenge using TDI-GSA on days 29/30 produced immediate-onset responses in 4 of 8 animals. Antibody titers to TDI were noted in all animals. These results indicated that: (1) dermal sensitivity, respiratory sensitivity and antibody production are optimally detected at different times following exposure to TDI (2) these sensitivities may exist independently of one other in the animal model. Supported by NIHES ES01332.
THE IMMUNE RESPONSE OF RATS TO INHALED TRIMELLITIC ANHYDRIDE (TMA): I. IMMUNE-MEDIATED PATHOLOGY OF THE LUNG. N.S. Hatoum, C.L. Leach, J.C. Roger, L.J. Garvin, and V.S. Bac, IIT Research Institute and Standard Oil Company (Ind), Chicago, IL 60616

Trimellitic anhydride (TMA) is a widely used chemical intermediate. Previous studies have shown that respiratory syndromes in TMA workers can be immune-mediated. Sprague-Dawley rats were exposed via inhalation to TMA at concentrations of 10, 30, 100, and 300 ug/m^3 for 2 weeks. Some rats were allowed 12 days of recovery and half of these were challenged with a single TMA exposure. Other rats were allowed to recover 12 weeks with no challenge. Rats whose lungs were increased in the 100 and 300 ug/m^3 groups after 2 weeks of exposure. Rested/challenged rats had increased lung weights over rested/non-challenged rats. After 2 weeks of exposure there was a dose-related increase in the number of hemorrhagic lung foci. Rested/challenged rats also showed a dose-related increase in lung foci, whereas the foci in nonchallenged rats had mostly disappeared. Histologically, there was a dose-related increase in macrophage accumulation, alveolar hemorrhage, and pneumonitis following the exposure. Rested/challenged rats also showed an increase in these parameters whereas the nonchallenged rats had nearly recovered. Complete recovery from all pathological findings was apparent in all rats 12 weeks after the final exposure.

THE IMMUNE RESPONSE OF RATS TO INHALED TRIMELLITIC ANHYDRIDE (TMA): II. EFFECTS ON SERUM ANTIBODY, LUNG IgG, AND LUNG COMPLEMENT. C.L. Leach, N.S. Hatoum, H.V. Ratajczak, C.R. Zeiss, J.C. Roger, and L.J. Garvin, IIT Research Institute and Standard Oil Company (Ind), Chicago, IL 60616

Inhalation of trimellitic anhydride (TMA) by workers induces a variety of immunological syndromes. In order to reproduce manifestations of these syndromes, rats were exposed to 10, 30, 100, and 300 ug/m^3 for 1 and 2 weeks, allowed 12 days to recover and either challenged or not challenged, or allowed 12 weeks of recovery. Total serum antibody (Ab) specific against TMA, and nonspecific IgG and complement (C3) in the lungs were determined. No serum Ab was detected after 1 week, however a dose-related increase in serum Ab was detected after 2 weeks of exposure, with a maximum of 40 ng bound/ml of serum. The rested/challenged rats showed a greater immune-response, with nearly twice the Ab in every dose group. Rested/nonchallenged rats exhibited Ab levels higher than those after 2 weeks of exposure, but not as high as the rested/challenged group. Serum Ab remained high through the 12-week recovery. C3 and IgG levels in exposed rats were different from controls in macrophages, large vessels, and alveoli. These effects were most pronounced after 2 weeks of exposure and in the rested/challenged rats; rested/nonchallenged and 12-week recovery rats returned to control levels.


Alveolar macrophages were infected with influenza virus to develop a model of pulmonary antiviral immunity that assessed (a) the direct antiviral effect of alveolar macrophages, and (b) the production of interferon as a result of this virus-cell interaction. Alveolar macrophages were obtained by bronchoalveolar lavage from male Fisher 344 rats that were exposed to O3, NO2, or air. Alveolar macrophages were then infected with influenza virus for 1 hour, and virus not adsorbed was removed by washing. Alveolar macrophages were assayed for and virus infectivity by testing the NaDodSO4 lysis assay using the Madin-Darby canine kidney cell line to determine the number of cells infected with influenza virus. Infectious center assays were carried out at various time points up to 48 hours post infection. The kinetics of alveolar macrophage inactivation of influenza virus was compared for animals exposed and not exposed to the various pollutants. In addition, the interaction of influenza virus with alveolar macrophages resulted in the production of interferon. The kinetics of interferon production by alveolar macrophages infected with influenza virus was determined for animals exposed and not exposed to pollutants. This study does not necessarily reflect EPA policy.


Benzene is a potent bone marrow toxicant. While all blood cell types are targets for benzene poisoning, lymphocytes have been shown to be particularly sensitive. The immunotoxic consequences of benzene or its metabolites have been demonstrated in a number of in vitro studies. Apparently no data exist regarding the effects of benzene on host resistance to infectious agents. The objective of this investigation was to systematically examine the effects of benzene on marine resistance to an infectious agent, Listeria monocytogenes. Four concentrations of benzene were employed, 10, 30, 100, and 300 ppm, in an effort to determine how benzene might alter the immune response when administered prior to infection (5 days, pre-exposure) or prior to and during infection (12 days, continuous exposure). Appropriate air controls were maintained. Bacterial counts were performed from spleen cell homogenates of 5 mice sacrificed at days 1, 4, and 7 of infection. An alternate group of mice given the same benzene and bacterial exposure regimens were used to determine spleen cell populations at the same sacrifice periods. The results demonstrate that pre-exposure to benzene results in increased bacterial numbers at day 4 of the infection only at the highest benzene concentration (300 ppm ~ 750 % of control). Continuous exposure resulted in increased bacterial numbers at day 4 in all but the lowest benzene-treated group (300 ppm ~ 950% of control; 100 ppm ~ 750% of control; 300 ppm ~ 720% of control). No significant increase in bacteria were observed in any benzene treated group at day 1 or day 7 of infection. The increased bacterial numbers at day 4 suggests an effect on cell-mediated immune responses. Determination of spleen cell populations at identical sacrifice periods show both T and B lymphocytes to be particularly sensitive to benzene with reductions at all concentrations 30 ppm for both exposure regimens. These cellular effects were dose dependent. Esterase positive cells were relatively resistant to benzene effects. The results point to a benzene induced delay in the immune response to L. monocytogenes.
The short-term toxicology profile of the novel antihypertensive agent 3-((4-(3-ethoxyphenyl)-1-piperazinyl)butyl)amino)-5,5-dimethyl-2-cyclohexen-1-one was evaluated after oral dosing in mice, rats and dogs. Following single oral doses, LD50 values were 469 and 444 mg/kg in male and female mice, and 367 and 338 mg/kg for male and female rats, respectively. In subacute studies, dose-dependent clinical signs of toxicity included ptosis of eyelids, depression, muscle stiffness, epiphora and hypothermia. Plasma creatinine and liver weights increased in females at dose levels of 40 mg/kg and greater; while plasma creatinine and chloride levels increased at doses greater than 20 mg/kg in males. Hypertrophy of the adrenal cortex was observed at 160 mg/kg with ataxia, miosis, diarrhea and depression occurring at higher dose levels. With repeated daily administration, LD10 levels were elevated and heart rates were increased after dosing in females. No significant pathologic lesions were observed in Beagle dogs. These data provided adequate safety margins for clinical trials.

Methylphenidate hydrochloride (M) is an adrenergic stimulant used to treat minimal brain dysfunction in prepubescent children, wherein it is suspected to cause growth retardation. In order to evaluate its toxic potential, M was supplied in the diet to male and female Fischer 344 rats and B6C3F1 mice for 13 weeks at 0, 125, 250, 500, 1000 or 2000 ppm. Body weight and length were monitored weekly, and selected organs were weighed and examined histologically at termination. Bone (femur) length and density were also determined. No M-related deaths occurred, and body weight gain was reduced slightly at the higher doses. Liver weight was increased in both species at 1000 and 2000 ppm, while kidney weight was increased slightly in rats at these doses. Microscopic changes were restricted to mild hepatocellular hypertrophy in mice at 500-2000 ppm and in rats at 1000 and 2000 ppm. Testicular weight, sperm density, and spermat morphology were all normal in the males, while the estrus cycle may have been lengthened in the females. Bone length and density and the rate of body lengthening were unaffected by M in rats, and serum growth hormone concentrations, although highly variable, were not consistently reduced. These data indicate that prolonged exposure to M produces hepatocellular hypertrophy and hepatomegaly in rodents.
A 90-DAY SUBCHRONIC TOXICITY STUDY WITH DOXYLAMINE ADMINISTERED TO FISCHER 344 RATS AND B6C3F1 MICE. Carlton D. Jackson and Boon-Nam Blackwell, National Center for Toxicological Research, Jefferson, AR. Sponsor: W.T. Allaben

Doxyamine hydrochloride was administered in the feed to male and female Fischer 344 rats at dose levels of 0, 162, 405, 1012, 2530, and 6325 ppm and to male and female B6C3F1 mice at dose levels of 0, 80, 162, 325, 750, and 1500 ppm. There were no treatment related deaths during the study in either species. In rats, doxyamine produced a reduction in final body weight of 13% at 6325 ppm (males) and 5%, 10%, and 14% at 1012 ppm, 2530 ppm, and 6325 ppm, respectively (females). In mice, the final body weights were reduced 8% and 10% in males and females, respectively, in the highest dose group compared to controls. In rats, doxyamine produced changes in the liver which were observed in the two highest dose groups. Parotid salivary glands exhibited cystomegaly and vacuolated basophilic cytoplasm. In mice, microscopic lesions were confined to cell cytoplasm or karyomegaly of the liver which varied from mild at low doses to severe at the highest dose levels. Serum levels of hepatic enzymes were consistent with the hepatotoxicity observed.


Techniques commonly described for inducing lesions of the rat gastric mucosa by administration of irritants result in variable degrees of damage. A technique is described below using a flexible endoscope for precise lesion induction and subsequent monitoring over long time periods in non-surgically prepared rats. Male Wistar rats weighing 250-350g are anaesthetised with intravenous alphaxalone/ alphadolone and a tracheal cannula inserted. The animal is artificially ventilated and an Olympus Bronchoscope Type BF-3C4 passed down the oesophagus into the stomach which is then inflated with 4-6ml/100g bodyweight of air. Upto 70% of the stomach can then be inspected and photographed. The stomach must be empty before examination and the animals are maintained on a restricted feeding regime of 6-8 hours/day access to food. Lesions are induced by passing a fine nylon cannula through an air-tight seal down the endoscope channel. Irritants are deposited directly onto a pre-selected region of the mucosa under visual control. The technique has been used to induce and monitor acute lesions. Chronic lesions taking 6-12 months to develop have also been observed generating data on incidence and progression without the need to sacrifice large numbers of animals.

TOXIC ALPHA, BETA-UNSATURATED ALDEHYDES INHIBIT SUPEROXIDE ANION RADICAL PRODUCTION IN VITRO IN STIMULATED PHAGOCYTIC CELLS. G Witz, N J Lawrie, H A Amoroso and B D Goldstein. UMDS--Rutgers Medical School/Rutgers University Joint Graduate Program in Toxicology, Piscataway, NJ.

Alpha,beta-unsaturated aldehydes are a class of ubiquitous toxic compounds. They occur as environmental air pollutants, e.g. crotonaldehyde (CRO) and acrolein (ACR); they may be generated in vivo as lipid peroxidation products, e.g. trans-4-hydroxyxenon (4-OH-NON), or as metabolites of xenobiotics, e.g. mucosaldehyde (MUC), a postulated benzene metabolite. Previous studies in our laboratory have shown that these alpha, beta-unsaturated aldehydes inhibit superoxide anion radical (O2·−) production in stimulated human polymorphonuclear leukocytes (PMN). The present studies show that these compounds also inhibit O2·− production in vitro in phorbol myristate acetate-stimulated pulmonary macrophages (PAM) from rats. The concentrations at which 50% inhibition occurred (IC50) in PAM were similar to those observed in human PMN. They are: ACR, 2.4x10−6M; MUC, 5.1x10−6M; 4-OH-NON, 7.7x10−6M; CRO, 2.4x10−7M. These studies suggest that reactive aldehydes may affect antibiotic defense mechanisms by inhibiting the membrane oxidase of phagocytic cells which metabolizes oxygen to bactericidal reactive oxygen species.

These studies were supported by ES02530.

IN-VIVO MODEL FOR GASTROINTESTINAL IRRITATION. R.S. Eydeloth, Merck Sharp & Dohme Research Laboratories, West Point, PA.

Gastrointestinal mucosal irritation is an important side effect of several classes of orally administered therapeutic agents. A canine cannulated intestinal, fistula model was developed to test the local irritation potential of various sustained release drug delivery formulations. Transabdominal cannulated duodenal fistulae are surgically prepared in beagle dogs. These canulae provide ready access to the duodenal lumen for placement of test devices, endoscopic evaluation, pH determinations, mucosal biopsies, etc. Test formulations are suspended from the canula plug by a radiopaque apparatus allowing for radiographic documentation of its position after placement in the duodenal lumen. Mucosal alterations are evaluated endoscopically and documented with video and/or still photography. If necessary, mucosal changes can be evaluated further at necropsy and histomorphologically.

This model depicts prolonged, fixed source local exposure of the intestinal mucosa to a drug delivery system and accurately defines the local irritation potential for that formulation.
TCDD modulates a variety of responses in epidermal cells in vivo and in culture, some of which are associated with altered cell proliferation, differentiation or both. The growth of epidermal cells in culture is regulated by several biochemical mediators including EGF. We examined the actions of TCDD on EGF binding in a basal cell population from a human keratinocyte cell line. TCDD reduced [125I]EGF binding by 40% within 96h and the loss could not be attributed to changes in the state of differentiation as assessed by cell morphology and cornified envelope competence. Modulation of EGF binding by TCDD was dose dependent (ED50 ~ 10 nm) and stereospecific, suggesting that the response is mediated by the TCDD receptor. Scatchard analysis of EGF binding data indicated a single class of binding sites in both control and TCDD treated cultures with equivalent Ks values; however, the total number of EGF binding sites was diminished by 40% after exposure to TCDD. This loss of EGF binding sites was concomitant with the loss of EGF-stimulated DNA synthesis. These data support the hypothesis that the basal cell population of epidermal cells in culture is a target for the actions of TCDD. The regulation of EGF binding and subsequent EGF-mediated responses in basal cells may be one mechanism by which TCDD alters epidermal cell differentiation.

The skin penetration of residual acrylamide monomer (AAM) following contact with 1% aqueous solutions of polycrpolamides was examined using Fischer 344 rat skin. In vivo measurements with [14C]-AAM monomer over 24 hr demonstrated that about 26% of the applied monomer penetrated skin and was systematically distributed. Another 35% was recovered from skin at the dosing site (not removed by washing) and was considered available for further absorption. In vitro cumulative penetration of rat skin by [14C]-AAM monomer in 24 hr (54% in effluents, 11% in washed skin) showed good overall agreement with in vivo results and chemical analysis confirmed the presence of only AAM in effluents (not metabolized in skin).

When three polycrpolamides were applied as 1% aqueous solutions to rat skin in vitro, residual monomer was well absorbed (67.9% mean penetration) over 24 hr. Also, in an in vitro examination of dilute monomer alone suggested a similar absorption profile when compared to residual monomer in several polycrpolamides. Overall, this work suggests that residual monomer in polymer flake materials may be significantly absorbed when in contact with human skin for extended time periods.

Presently there are no convenient methods for predicting blood and tissue levels from vapor penetration across the skin. In this study, we investigated the dermal vapor penetration of dibromomethane (DBM) and bromochloromethane (BCM). Rates were closely clipped to remove fur and exposed to DBM and BCM vapes in an exposure chamber which afforded respiratory protection with a face mask and allowed whole body skin exposure. Transdermal flux and permeability constants were calculated from blood levels of parent chemicals and their metabolites. Measured permeability constants were used to provide the input function for a physiological model of the dinalomethanes. The skin was described as a homogeneous compartment connected with a blood skin compartment and the skin surface. The rate constants for skin penetration were based on permeability x area products (PA) and skin partition coefficients (.546 for DBM and .267 for BCM). 2A5 for a 300 gram rat were 374 ml/hr for DBM and 262 ml/hr for BCM. These values indicate that in an inhalation exposure approximately 5% of the absorption of these chemicals would be through the skin route. The model successfully predicted parent and metabolite kinetics for dermal vapor exposures to other concentrations of DBM and BCM.
41 SUBCHRONIC DERMAL TOXICITY OF VINYLCOLOXENE DIOXIDE IN RATS AND MICE. R.S. Chhabra, C. Montgomery,* A. Peters, and D. Donofrio.† National Toxicology Program, NIEHS, Research Triangle Park, NC* and Battelle Columbus Laboratories, Columbus, OH.†

Vinylcyclohexene dioxide (VCĐ) is primarily used as a reactive diluent for epoxy resins. Groups of ten rats and mice of each sex were administered VCĐ by dermal application for 13 weeks. Rats received 300 μL of VCĐ in acetone at concentrations of 200, 100, 50, 25, 12.5 and 0 mg/mL. Mice received 100 μL of VCĐ in acetone at concentrations of 100, 50, 25, 12.5, 6.25 and 0 mg/mL. There were no chemical related effects on survival or body weight, except for high dose group in rats which showed substantial decrease in body weight gains. The organ weights were not affected by VCĐ administration. The skin lesions were observed in the top two dose levels of both species and sexes. The lesions consisted of sebaceous gland hyperplasia and/or hypertrophy, acanthosis, parakeratosis and hyperkeratosis. In mice, at 100 and 50 mg/mL dose levels diffuse ovarian follicular hypoplasia was observed which was characterized by decreased numbers of primary and secondary Graafian follicles. The results from this study showed that skin was target tissue for VCĐ toxicity in both rats and mice. Ovaries were identified as target organs for VCĐ toxicity only in mice.

43 DIETHYLNDITROASINE (DEN) AND PHENOBARBITAL (PB) INDUCED ALTERATIONS IN RAT HEPATIC POLYPOLYDODI. C. Tinchalk, A.K. Charles and R. Abraham. Dept. of Pharm. & Tox., Albany Medical College, N.Y.

Previous studies have shown that alterations in hepatic polyplody can be induced by hepatocarcinogens. In this study newborn SD rats were given ip single (S) (day 1) or multiple (M) (9 doses, 3/wk, for 3 wk) doses of DEN (15 mg/kg). Rats were weaned at 3 wk and given 0.05% PB in the diet for 76 days. Controls received basal or PB diet. Rats were killed 1 hr after [3H]-TdR injection (1 μCi/g). Hepatic nuclei were isolated and separated on a sucrose gradient into ploidy classes (2n, 4n, 8n). Changes in TdR incorporation, nuclear ploidy, liver protein and DNA were determined. Compared to controls, PB alone produced a decline in 4n nuclei with a corresponding increase in 2n population in both sexes; the effect on females being more marked. DEN(S)+PB produced a similar but significant reduction in 4n and induction of 2n. The effect of DEN(M)+PB showed the largest reduction of 4n and induction of 2n. No apparent changes were noted in 8n hepatocytes. DEN(S)+PB produced an enhanced incorporation of TdR into total liver DNA, for both sexes, while DEN(M)+PB females had a significant increase in total DNA. Alterations in ploidy may play a role in early neoplastic changes leading to hepatic tumor formation in rodents. 1/30 hepatic carcinomas were seen in these investigations. (Supported by Shell Internationale Res. Maatschappij B.V.)


The toxicologic potential of enoxacin, a quinolone carbonylic acid antibacterial agent, was evaluated in rodents and Beagle dogs following acute and repeated intravenous doses. Intravenous LD50 values in rodents were approximately 300 mg/kg in B6C3F1 mice and Wistar rats of both sexes. Enoxacin, administered by daily IV injection to rats for 4 weeks was well tolerated at 20 mg/kg but caused concentration-dependent necrosis at the injection site at doses levels greater than 40 mg/kg and dose-related suppression of body weight gain at 60 and 80 mg/kg. In Beagle dogs intravenous infusions were tolerated at dose levels up to 30 mg/kg. At 40 mg/kg intolerance was manifested as depression, ataxia and collapse. There was no evidence of local irritation at clinical concentrations, although at higher concentrations local irritation at the injection site was concentration-dependent and occurred in one of six animals administered daily doses of 30 mg/kg for 4 weeks. Systemic clinical signs, evident during drug infusion, included transient erythema and edema of the dermis which occurred at 15 to 30 mg/kg and diminished following repeated administration. These data provided adequate safety margins for clinical trials in man.


Cytosolic proteins were examined in rat livers at various stages of tumor development induced by two hepatocarcinogens, MX and DEN. Neonatal SD rats were given multiple doses of DEN (ip 15mg/kg; 3 doses/week) and MX (oral gavage 1mg/kg; 5 doses/week) for 21 days. After weanling, the rats were killed before and after giving 0.05% phenobarbital (PB) in the diet for 76 days. Cytosolic fractions were prepared by centrifugation for 2 h at 105,000 x g and analyzed by SDS-PAGE electrophoresis. At day 21, DEN induced two proteins of Mr 46K and 68K, while MX induced three different proteins of Mr 21K, 25K and 30K. Addition of PB elevated the levels of the latter 3 proteins in the MX group with no such changes in DEN and PB group. The most striking finding was the appearance of a 21K Mr protein in MX animals, but not in DEN rats at 21 days. The mobility of the 21K protein was similar to that found by Eriksson et al. (B.B.R.C. 117, 740, 1983). These results demonstrate the presence of a possible oncogene product (2) in the preneoplastic liver cytosol and also suggest that an epigenetic carcinogen like MX induces an oncogene product-like protein; an effect similar to the one exhibited by genotoxic chemicals. The detection of this protein in preneoplastic liver may be an important diagnostic marker in preneoplasia. (Supported by Shell Internationale & Amer. Cancer Soc. grant #5A)
Continuous exposure of four-week old male F-344 rats to 40 ppm of diethylnitrosamine (DEN) in drinking water resulted in the accumulation of O'-ethyl-deoxothymidine (O'-EtTdhd) in hepatocyte DNA and in the induction of γ-glutamyltranspeptidase positive (GGT+) hepatocellular foci (PNAS 81:1692-1695, 1984). Kinetic analysis of these processes was undertaken to facilitate assessment of carcinogenic risk associated with exposure to DEN. Dose rates were estimated from water consumption and body weight. Rates of O'-EtTdhd accumulation were modelled as the difference between rates of dose-dependent adduct formation and first-order DNA repair:

$$\frac{da}{dt} = k_a \cdot \text{dose} - k_r \cdot a$$

where A = O'-EtTdhd, and k_a and k_r are rate constants for formation and loss of adducts, respectively. In four-week old rats, the accumulation of O'-EtTdhd adducts paralleled formation of GGT+ foci, an observation consistent with a single-stage model of foci induction via alkylation of hepatocyte DNA.

Chlorencid acid (CA, CAS # 115-28-6) was selected for testing because it is a high production chemical used as a chemical intermediate in preparation of polyester resins (fire retardant) and plasticizers. In 14-d and 90-d prechronic feeding studies high concentrations of CA resulted in mortality, decreased weight gain, and liver lesions (centrilobular cytomegaly and mitotic alterations) in both rats and mice. Based on these data, doses selected for a 2-yr carcinogenesis study were 0, 625 and 1250 ppm CA fed ad libitum to groups of 50 rodents of each species and sex. In both species, survival and food consumption was similar to that of concurrent controls, but decreased body weight occurred in low and high dose female rats and high dose female mice. In 2-yr studies, primary tumor incidence was: (1) hepatocellular tumors (neoplastic nodule and carcinoma combined) in male (con, 4/50; low dose, 2/50; high dose, 27/50) and female (1/50, 5/49, 15/50) rats and male (13/50, 23/49, 27/50) and female (3/50, 7/49, 7/50) for mice, and (2) pancreatic acinar cell hyperplasia and adenoma (combined) in male rats (0/49, 8/50, 9/50). The relationship between these results and the carcinogenic potential of chlorencid acid are currently undergoing evaluation. These results are not considered final until completion of the NTP peer review process.
Phenobarbital (PB) administered to adult mice has been previously shown to be a tumor promoter however when its administration started at weaning, it inhibited tumorigenesis. We will report that PB, 500 ppm in the drinking water (DW), promoted liver cancer in B6C3F1 mice initiated as adults with diethylnitrosamine (DENA). PB (500 ppm in DW) at weaning also promoted tumorigenesis in the following mice initiated on day 15 of age: Balb/c with DENA, and Swiss with ethynitrosourea. PB induced gamma-glutamyltranspeptidase (GGT) activity only in tumors of Swiss mice and not in tumors of Balb/c and B6C3F1 mice. PB administered either acutely or chronically did not induce GGT activity in normal liver or in preneoplastic lesions of the 3 strains of mice, contrary to Fischer-344 rats. In conclusion, 1) PB promoted hepatocarcinogenesis when started either at weaning or at maturity which is contrary to some published results and 2) PB induced GGT activity only in tumors of Swiss mice and not in preneoplastic lesions of any strain. Therefore, PB promotion in mouse liver, does not result in the appearance of early preneoplastic lesions containing GGT that could be used as a marker in short-term assays. (This abstract does not necessarily reflect EPA policy).

**Dietary vitamin A effects on hepatic tumor promotion by polybrominated biphenyls in rats. N.S. Rezabek, S.D. Sleight, R.K. Jensen, S.D. Austin, D. Dixon, Deps. of Path. and Biochem., Michigan State University, East Lansing, MI 48824.**

Vitamin A inhibits development of some chemically-induced tumors. Polybrominated biphenyls (PBB) are tumor promoters which affect vitamin A homeostasis. We hypothesized that dietary vitamin A levels influence tumor promotion by PBB. Partially hepatocarcinized Sprague-Dawley rats were initiated with diethylnitrosamine. Beginning on day 7, rats were fed diets containing 2000 IU (low A) or 200,000 IU (high A) retinyl acetate/kg feed. From day 30, diets contained no PBB, 10 ppm Firemaster BP-6 (FM), 10 ppm 2',4',5',3',4',5'-hexabromobiphenyl (245-HBB) or 1 ppm 3,4,5,3',4',5'-hexabromobiphenyl (345-HBB). Rats were killed on day 180. Average numbers of y-glutamyltranspeptidase positive foci/cm² of liver were: low A/no PBB, 12 ± 17; high A/no PBB, 5 ± 7; low A/FM, 809 ± 725; high A/FM, 478 ± 337; low A/245-HBB, 92 ± 29; high A/245-HBB, 49 ± 42; low A/345-HBB, 458 ± 214; high A/345-HBB, 350 ± 160. Although numbers of foci were low in all groups, differences were significant only in rats given 245-HBB. This congener causes minimal changes in vitamin A homeostasis, whereas FM and 345-HBB have marked effects. Thus while dietary vitamin A levels may influence promotion by certain PBB congeners, the mechanisms appear to be independent of PBB effects on vitamin A homeostasis. Supported by NIEHS Grant ES-02781.

**Failure of various pure and complex mixtures of PCBs to initiate carcinogenesis during liver growth and regeneration. N.A. Hayes, S.H. Safe, D. Armstrong and R.G. Cameron, Department of Pathology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.**

The abilities of various PCBs to initiate y-glutamyltranspeptidase (y-GT)-positive resistant hepatocyte nodules were evaluated in F344 rats in which hepatocytes were proliferating. The PCBs examined were 2,2',4',4',5',5'-hexachlorobiphenyl, 2,2',4,4',4'-tetrachlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, Aroclor 1254, and a mixture of pure PCBs (BHM-PCB) that resembles the PCBs found in human breast milk. PCBs were administered once weekly for 3 weeks (equivalent of 400 µmol/kg/week) to suckling male and female rats which were later subjected to selection with 2-acetylaminofluorene (2-AAF) (3x20mg/kg/day) and partial hepatectomy (PH). None of these PCBs generated y-GT positive nodules 2 or 4 weeks after selection, whereas diethylnitrosamine, 2-AAF, benzo(a)pyrene (B(a)P), and 3-methylcholanthrene (3-MC) were all positive. Aroclor 1254 and BHM-PCB were also negative when given in conjunction with PH in rats selected with 2-AAF and necrotizing CCl₄ (0.5 ml/kg), whereas 3-MC and B(a)P were positive. These observations suggest that brief exposure to PCBs does not initiate resistant hepatocytes in proliferating liver in an assay which detects both hepatic and non-hepatic initiating carcinogens. Supported by NSERC Canada (#90901).

**Alternatives to dietary administration of 2-acetylaminofluorene in resistant hepatocyte assays for carcinogenicity. M.A. Hayes, E. Roberts and E. Farber. Department of Pathology, University of Toronto, Toronto, Ontario, Canada M5S 1A8. Sponsor: I.C Munro**

The resistant hepatocyte model is used for sequential analysis of cancer development, and in short-term bioassays of carcinogens with initiating activity. Resistant (initiated) hepatocytes have been routinely stimulated to grow by 2-acetylaminofluorene (2-AAF) in the diet (0.02%) for 2 weeks, coupled with partial hepatectomy (PH). We have evaluated the efficacy of 2-AAF by gavage in selecting resistant (γ-glutamyltranspeptidase positive) liver nodules in F344 rats initiated with diethylnitrosamine (DEN; 200 mg/kg). Three or four consecutive daily doses of 2-AAF (20 mg/kg/day) in 12 aqueous carboxymethyl cellulose followed by PH on day 4 were equivalent to dietary 2-AAF. The fourth dose of 2-AAF before PH could be replaced by a reduced dose (5 mg/kg) given 3 days after PH. 2-AAF by gavage resulted in numerous resistant nodules at 1,2 and 4 weeks after PH, persistent nodules at 4 and 6 months, and hepatocellular carcinomas at 12 months. Nodules were rarely selected in control rats not given DEN. These alternative selection regimens can be varied in intensity and can replace dietary feeding of 2-AAF in sequential studies or bioassays. Supported by MRC Canada, NCI Canada and NCI-NIH.
53 EFFECTS OF 1,1 DICHLOROETHANE (1,1 DCE), 1,2 DICHLOROETHANE (1,2 DCE), AND CHLOROFORM (CHCl₃) IN DRINKING WATER ON LUNG AND LIVER TUMOR INCIDENCE IN MICE. J.E. Klaugia, R.J. Ruch, and M.A. Pereira. Dept. of Pathology, Medical College of Ohio, Toledo, OH and NHERL, U.S. EPA, Cincinnati, OH.

CHCl₃, 1,1 DCE and 1,2, DCE have been shown to induce liver tumors in B6C3F₁ mice when administered by gavage in corn oil. We examined the effects of continuous treatment in drinking water (d.w.) of CHCl₃, 1,1 DCE, 1,2 DCE and phenobarbital (PB) on liver and lung tumorigenesis in B6C3F₁ male mice. Mice (35) received one of two concentrations of CHCl₃, 1,1 DCE, or 1,2 DCE or PB (500 ppm) in the d.w. starting 4 weeks post-weaning. An additional 35 mice from each group were initiated with diethylnitrosamine (10 ppm in the d.w. for 4 weeks) at weaning and prior to treatment with one of the 4 compounds. None of the compounds increased the number or incidence of lung and liver tumors by themselves. PB promoted liver tumor formation (but not lung tumors) in initiated mice. 1,1 DCE and 1,2 DCE did not affect the incidence of liver and lung tumors in initiated animals. CHCl₃ inhibited liver and lung tumorogenesis in the initiated mice. Mice were sampled and the tumor incidence quantified 6 months (10 mice) and 12 months (25 mice) after treatment was begun. These results opposed those found when these chemicals were administered in corn oil. (This abstract does not necessarily reflect EPA policy.)

55 1,1,1-TRICHLOROETHANE FORMULATION: A CHRONIC INHALATION TOXICITY AND ONCOGENICITY STUDY IN RATS AND MICE - PART I. RESULTS OF FINDINGS IN MICE. J.F. Quast, L.L. Calhoun, and M.J. McKenna, Dow Chemical U.S.A., Mammalian Environmental Toxicology Research Laboratory, Midland, MI.

Groups of male and female B6C3F₁ mice (80 sex/group) were exposed to vapor concentrations of 0, 150, 500, or 1500 ppm of 1,1,1-trichloroethane formulation for 6 hours/day, 5 days/week for 2 years. Ten mice/sex from each group were pre-designated for interim sacrifices after 6, 12, and 18 months of exposure. Fifty mice/sex from each group were assigned to the study to be terminated after 24 months. The exposure levels used in the 2-year study were based upon findings in a 90-day subchronic study in which degenerative and inflammatory changes were observed in the nasal olfactory epithelium at 2000 ppm. In addition, minimal hepatic effects characterized by decreased glycogen and centrilobular swelling were observed in some mice in this group. Parameters measured during the 2-year study included mortality, in-life clinical signs of toxicity, hematology, clinical chemistry, body weight, organ weights (liver, kidneys, brain, heart, testes), gross pathology, and histopathology. Inhalation exposure of male and female B6C3F₁ mice to 1500 ppm or less of vapors of 1,1,1-trichloroethane formulation for 2 years did not result in any toxic or oncogenic effect considered due to the test chemical.


Four chlorinated hydrocarbon (CH) solvents—tetrachloroethylene (TTC), 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane (STTE), and hexachloroethane (HCE)—were tested for carcinogenic promotion and initiation activity in a rat liver foci assay using γ-glutamyl transpeptidase (GGT) as a putative preneoplastic marker. Groups of 10 young adult male Osborne-Mendel rats each were given partial hepatectomies and treated with CH in protocols previously described (The Toxicologist 4:729). TTC, STTE, and HCE produced 3.38 ± 0.61, 4.36 ± 0.85, and 4.38 ± 1.04 foci/cm², respectively, significantly elevated compared with controls given diethylnitrosamine (DEN) alone (1.77 ± 0.49 foci/cm²). In the promotion protocol, in the absence of DEN initiation, TTC and STTE produced smaller increases 1.64 ± 0.49 and 1.16 ± 0.36 foci/cm², respectively, that were significant relative to controls given corn oil alone (0.15 ± 0.15). None of these solvents induced GGT+ foci in the initiation protocol. In the course of this and previous investigations, GGT+ foci promoted by the chlorinated aliphatics were found to differ in appearance from the classic phenobarbital-promoted foci. The basis for this difference is not known but may relate to some degree of remodeling in the CH-induced lesion. (Supported by EPA Contract 68-02-3703).


Rainbow trout have been shown to be a useful animal model in studying the effects of modulators of chemical carcinogenesis. Several exposure protocols were used to differentiate classes of chemical modulators. When trout embryos were initiated via water bath exposure to a carcinogen ( aflatoxin, AFB; N-methyl-N'-Nitro-N-nitrosoguanidine, MNG), then, after hatching, fed one of several modulators (polychlorinated biphenyls, PCB; p-naphthoflavone, PNF; indole-3-carbinol, ICI: 17'-estradiol; phenobarbital; soy protein isolate; DDT; freeze-dried garlic and onion), the resulting alteration in tumor incidence can be classified as either promotion (increase vs control) or post-initiation inhibition (decrease vs control). Tumor incidences altered when a modulator is fed prior to or simultaneously with the initiator (AFB, MNG, diethylstilbestrol, methylazoxymethanol acetate) can be classified as either anti-initiation (decrease vs control) or enhancement (increase vs control). Using these protocols, we have observed that some modulators can have mixed action (i.e. anti-initiation or promotion for PNF and ICI and phenobarbital), depending on the time of exposure relative to carcinogen exposure. Similarly a single modulator (i.e. PCB) can act differently with two different carcinogens. (Supported by ES-000092, ES-000040, ES-00210, and CA-34732)

Indole-3-carbinol (I3C), butylated hydroxyanisole (BHA) and Aroclor 1254 (PCB) are inducers of the hepatic mixed function oxidase system in rainbow trout. These alterations can influence the metabolism of xenobiotics and their toxicological manifestations. In vivo and in vitro metabolism and DNA binding was investigated in rainbow trout prefed these compounds at levels of 100, 3000 and 2000 ppm for PCB, BHA and I3C respectively. PCB and I3C resulted in 35X and 70% less DNA binding of injected AFB1 in vivo than controls while BHA had no effect. AFB1 metabolism in freshly isolated hepatocytes from fish prefed these compounds showed 20% less DNA binding in I3C hepatocytes with a two-fold increase in the more polar metabolite aflatoxin M1 (AFM1). Per unit of AFB1 metabolized, PCB fish hepatocytes had DNA binding 5X% more than control and 3X fold more AFM1 and 50% less aflatoxicol. BHA hepatocytes had similar AFB1 DNA binding and metabolite profiles as controls. These findings suggest that PCB and I3C inhibit AFB1 hepatocarcinogenesis in trout by an anti-initiation mechanism. BHA inhibition has not been tested in trout but would appear, from these results, to be ineffective as an anti-initiator. (Supported by ES-00092, ES-00040, ES-00210, and CA-34722)


A recent epidemiologic study (MacMahon et al. NEJM 304:630, 1981) implicating coffee as an etiologic factor for cancer of the pancreas has caused considerable concern and controversy. We utilized a well-defined, short-term rat model of pancreatic cancer to investigate the role of coffee in initiating or enhancing pancreatic tumors. Male, Lewis rats were weaned to one of 4 groups of 30 rats each. Controls were fed AIN-76A diet. Two other groups were fed instant caffeinated or decaffeinated coffee. The fourth group drank freshly brewed coffee as their sole source of liquid. One half of each group received the potent pancreatic carcinogen azaserine (5 mg/kg) weekly for a total of 15 weeks. All rats were autopsied at 24 weeks of study. Pancreases were examined by quantitative light microscopic methods for atypical acinar cell foci. In the saline-injected rats, there was no difference between the number (<= per pancreas) or size (0.2 mm^2) of the foci observed in the 4 groups. The coffee preparations enhanced neither the number nor size of the azaserine-induced foci. There were approximately 300 foci per cu cm of pancreas and their average diameter was 340 m. In this model coffee neither induced pancreatic tumors nor enhanced the growth of carcinogen-induced tumors. Supported by NIH-NCI grant CA32379.

58 BENZO(A)PYRENE METABOLISM AND DNA BINDING IN TELEROST SKIN AND LIVER CULTURE. D.M. Lucas, J.E. Klaunig, R.J. Ruch, and R. Teel, Department of Pathology, Medical Center of Ohio, Toledo, OH, and Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, CA. Sponsor: M.A. Pereira

Epidermal and hepatic neoplasms have been detected in bottom-dwelling fish of polyaromatic hydrocarbon-contaminated sediments of rivers and lakes. Cultures of liver and skin from normal channel catfish (Ictalurus punctatus) and brown bullhead (Ictalurus nebulosus) were established to examine DNA binding and metabolism of benzo(a)pyrene (BP) in these tissues. Skin was cultured in rocking organ cultures. Liver was dissociated with collagenase and grown in short-term primary culture. Cultures maintained in 15 medium at 20°C were treated with 3H BP for 24 and 48 hours. BP binding was greater in bullhead skin than catfish skin. Liver cultures displayed similar binding in both species. Bullhead skin metabolized approximately 15% and catfish skin metabolized approximately 5% of the BP after 24 hours of incubation. Both bullhead and catfish liver metabolized approximately 36% of the BP after 24 hours and 50% after 48 hours. The major metabolites detected in the liver cultures included 9,10 and 7,8 dihydrodiols, phenols and quinones.

60 ADDUCT LEVELS IN METHYLBENZYLNITROSAMINE ESOPHAGEAL CARCINOGENESIS: T.F. Scharger, J.M. Newberne, S.A. Broitman. Boston University Medical School; 2Massachusetts Institute of Technology, Boston, MA.

Zinc deficiency significantly enhanced methylbenzyl nitrosamine (MBN) esophageal carcinogenesis in rats. The effect of this diet on the formation and persistency of 7-methylguanine (7-MG) and 6-methyl guanine (6-MG) was examined to determine a possible role of adduct levels to subsequent tumor formation. Levels were compared in the esophagus and the liver (a non-target organ) after one (initiation) dose and after six (carcinogenic) doses. Four hours after a single dose zinc deficiency significantly enhanced levels of both adducts. Levels were reduced in a control diet group paired to the reduced food intake of the zinc deficient group (this group also has decreased tumor incidence). 6-MG:7-MG ratios were lowest in the pair fed group and highest in the zinc deficient group. In the liver 7-MG levels were 3-4 fold less than in the esophagus and 6-MG levels were 5-10 fold less; there were no significant differences between the dietary groups in adduct levels. After 6 doses adduct levels increased in all diet groups for both organs. 7-MG levels increased proportionately more than 6-MG levels so the ratio of the two declined. Adduct levels decreased more rapidly in the control diet groups than in the deficient group over a 48 hour period; 6-MG levels fell more rapidly than 7-MG levels. Zinc deficient enhancement of MBN esophageal carcinogenesis may be mediated by increased levels of 6-MG and a slower rate of its removal.
Deltamethrin is a pesticide used worldwide in agriculture and in vector control programs; however, no published data are available on its long-term effects. Recent mutagenicity studies on several synthetic pyrethroids provided no evidence for the mutagenicity of Deltamethrin. (M. Pluijmen et al., Mutat. Res., 137 (1984) 7-15). The purpose of the present study was to verify whether Deltamethrin induces tumors in mice. Deltamethrin was administered by gavage, in corn oil, to male and female C57BL/6 mice, 6 weeks old, at four dose levels (0.1, 1, 4 and 8 mg/kg bw) on 5 days a week for 104 weeks. After completion of the treatment, the mice were observed until 120 weeks of age, when all survivors were killed. The treatment had a slight effect on body growth and survival rates, especially in the group treated with the highest dose. Various types of tumors were observed in all experimental groups. An increased incidence of lymphoma was observed in mice receiving 1 and 4 mg/kg bw, but not in the group treated with 8 mg/kg bw Deltamethrin. No significant difference in the incidence of lung adenomas, liver-cell tumors or other tumors was observed in treated groups when compared with controls.

Currently, a 2-Year Toxicity-Oncogenicity Study in Fischer 344 Rats. S. J. Gorzinski, K. A. Johnson, K. M. Bodner, R. A. Campbell, R. C. Kraeka, R. W. Nast and C. H. Wolf. Mammalian and Environmental Toxicology Laboratory, Dow Chemical USA, Midland, MI 48640.

Male and female F-344 rats were given 0, 0.01, 0.1, 0.3 or 2 mg/kg/day of acrylamide in their drinking water. Subgroups of animals were sacrificed at 6, 12 and 18 months to assess chronic toxicity and peripheral nerve ultrastructure. Beginning at 12 months, focal axonal degeneration of the tibial nerve was apparent in rats of all groups, including controls, but was exacerbated by acrylamide in the 2 mg/kg group. Light microscopic and ultrastructure examination were comparable in delineating axonal degeneration. Increased mortality occurred at 21-24 months in females given 2 mg/kg. At 24 months, tumor incidence at several sites was increased in rats given 2 mg/kg: females - mammary gland, central nervous system, thyroid gland, mouth, uterus and clitoral gland; males - scrotum and thyroid gland. The incidence of scrotal mesothelioma was slightly increased above control at 0.1 mg/kg and statistically increased at 0.5 and 2 mg/kg. There were no statistically significant alterations in tumor incidence in either sex given 0.01 mg/kg. This study was sponsored by: the American Cyanamid Company, Dow Chemical USA, Nalco Chemical Company, and The Standard Oil Company (Ohio).
A series of collaborative studies were undertaken to evaluate the potential role of electron transport reactions as mediators of asbestos toxicity. For this purpose, samples of asbestos were heated to 400°C, with and without subsequent X-irradiation. Heat treatment reduces the population of "trapped" electrons while X-irradiation restores this population. Characterization of the asbestos after heating indicated that no changes in physical or chemical composition had taken place. Heating, however, significantly reduced biological activity in a variety of in vitro assays employing the pulmonary alveolar macrophage, human fibroblasts and tracheal organ culture systems. Furthermore, macromolecular adsorption and binding to asbestos were reduced with heat treatment. In contrast, the biological activity of the heated samples was restored with X-irradiation. These data support the hypothesis that asbestos toxicity is mediated through uni-electron transport reactions.

The pulmonary tumor response of strain A mice has been reported to be a rapid and efficient predictor of carcinogenic potential for a variety of chemicals. The route of exposure has usually been by intraperitoneal injection of solubilized materials. We compared intratracheal (i.t.) instillation as a more representative route typical of human exposures, with intraperitoneal (i.p.) injection of nickel subsulfide, a potent animal carcinogen. Animals were sacrificed either 20 weeks after the first dosing which is the standard protocol for the tumorigenicity assay, or were held until 45 weeks after the first dosing. Urethane, a positive control, produced a significant increase in pulmonary tumor response after i.t. instillation as well as the usual i.p. injection. For nickel subsulfide treated animals there was no evidence of a dose-related increase in tumor incidence in any i.p. or i.t. treatment group when compared with age matched controls. In fact, fewer tumors than controls were seen after i.t. exposure. Our findings of a lack of tumorigenicity of nickel subsulfide, a most potent carcinogen, and nickel acetate (to be presented) a weak carcinogen, cast further doubt on the utility of the strain A mouse model for tumorigenicity evaluation. Supported by the Electric Power Research Institute.

Previous work has shown that chlorinated and brominated acetonitriles are capable of producing point mutations in Salmonella, inducing sister chromatid exchange in CHO cells and initiating skin tumors in mice. In the present study chloroacetonitrile (CAN), dichloroacetonitrile (DCAN), trichloroacetonitrile (TCAN), bromochloroacetonitrile (BCAN) and dibromoacetonitrile (DBAN) were administered at doses of 20 and 40 mg/kg 3X weekly for 24 weeks to female strain A/J mice in 2% emulphor to determine if they were capable of enhancing lung adenoma development. Each group included 40 animals. Treatments began at 10 weeks of age and animals were sacrificed at 54 months of age. All treatments increased the percent of animals that developed lung adenomas over the control incidence (12%). Although responses were not always dose-related for purpose of comparison, the tumor incidences for the 40 mg/kg treatments were: CAN, 28%; DCAN, 19%; TCAN, 20%; BCAN, 29%; and DBAN, 21%. These data indicate that haloacetonitriles do have weak activity for inducing lung adenomas in strain A/J mice and suggest that they may present some hazard to those consuming chlorinated drinking water. (This is an abstract of a proposed presentation and does not necessarily represent EPA policy.)
Female SENCAR mice were exposed to aqueous solutions of HOCl, NaOCl, ClO₂, and NH₂Cl. Exposure to disinfectant solutions involved immersion (except head) for a 10 minute period for 4 days. Animals were sacrificed the day following the last treatment and skin thickness measured. Concentrations of disinfectants were 1, 100, and 1000 mg/L. The minor and detectable in control animals measured 0.0144 + 0.0015 mm. At concentrations of 1000 mg/L HOCl and ClO₂ increased the skin thickness to 0.0390 ± 0.0067 and 0.0404 ± 0.0019 while NaOCl increased thickness to 0.0752 ± 0.0061 mm after 4 days treatment. NH₂Cl reduced skin thickness to 0.0097 ± 0.0015 mm. The response to 1000 mg/L HOCl appeared to be maximal after 4 days treatment and tended to decrease with further treatment, although sustaining a thickness of approximately 2X that over control. The response to HOCl was found to be dose-related, with the minimally effective dose being 10 mg/L. Examination of sections of treated skin indicate an increase in cell numbers. HOCl/OCl and ClO₂ are therefore capable of inducing hyperplastic responses in the mouse skin. The basis for the decrease in skin thickness with NH₂Cl treatment remains to be established.

(This is an abstract of a proposed presentation and does not necessarily represent EPA policy.)

Virgin (straight run) petroleum distillates which nominally boil in the range of 350-650°F have previously been considered to have zero or low carcinogenic activity. However, many of the mid-distillate fractions which have a boiling range predominantly between 350-700°F, such as heating oil and diesel fuels, today contain cracked petroleum fractions from refinery conversion processes such as Catalytic Cracking and Coking. Distillate boiling range materials from these processes tend to be of higher aromatic content, though containing low aromatics concentrations of the 4-ring aromatic type. Lifetime mouse skin cancer bioassays were conducted on commercial grade heating oils from varied refining operations and with varied crude sources. Both straight-run and heating oils with cracked stocks were tested. Two additional virgin mid-distillate materials without cracked stocks were also examined. Fifty male C57H/HaJ mice were treated 3 times per week with 25 mg/treatment for life or until tumors developed. Survival, tumor yield and latency were determined for all materials and the median latencies as estimated by the Weibull distribution were calculated. The results of these studies will be discussed in the context of the addition of cracked stocks to virgin distillates and with respect to process/compositional parameters of mid-distillate as a class.

Renewed interest in the development of synthetic fuels has stimulated investigations of the carcinogenic potential of liquids derived from non-petroleum sources. In the present study, the carcinogenic potential of Athabasca tar sands and six experimental liquids derived from crude bitumen was evaluated utilizing the mouse epidermal carcinogenesis model. Tar sands, crude bitumen and raw naphtha produced few, if any, tumors; minimal skin cancer hazards should be associated with the materials. Three thermally and catalytically cracked liquids, light (nominally 149-515°C) and heavy (nominally >315°C) gas oils and coker oil, produced a significant number of neoplasms. Cracking enriches the PNA content and contributes significantly to the carcinogenic activity exhibited by these liquids and by petroleum-derived materials of similar boiling range and process history. A synthetic crude oil, prepared by blending naphtha and light and heavy gas oils, was moderately carcinogenic; however, the activity of this sample fell within the range of values obtained in studies of crude petroleum samples. Since these streams do not differ significantly in carcinogenic potency from similar petroleum-derived materials, comparable industrial hygiene practices should be adequate to control the carcinogenic hazards.


A solvent-extracted lubricating oil base (B), a furnace oil distillate (P) and a dewaxed lube oil distillate (D) induced skin tumors in 0/50, 9/50 and 26/50 mice, respectively, during lifetime skin-painting bioassays. An abbreviated initiation/promotion (I/P) bioassay was conducted to assess the I/P potential of B, P and D. During the 28-week I phase, groups of 30 CD-1 mice were treated dermally once a day for 5 days with 25 or 50 μl of B, P or D, or 50 μl of acetone (A), rested for 2 weeks, and then treated twice per week for 25 weeks with 50 μl (0.1 mg/ml) of Phorbol-12-Myristate-13-Acetate (PMA). Only the D-treated groups had a significantly higher incidence of papillomas relative to the A control. During the 28-week P phase, groups of 30 CD-1 mice were treated once with either 50 μl of DMBA (1.0 mg/ml) or A, rested for two weeks, and then treated twice per week with B, P or D for the remaining 25 weeks. Only P and D showed a significantly higher incidence of papillomas in DMBA-initiated mice relative to their A-initiated controls. These data suggest that D is a complete carcinogen with initiating and promoting activity, F is a promoter only, and B is non-carcinogenic.

TUMOR INITIATION AND PROMOTION EFFECTS OF PETROLEUM FRACTIONS IN MOUSE SKIN. J.M. Gerhart, C.A. Halder, N.S. Hatoum, T.M. Warne, and P.J. Garbin, IIT Research Institute, Standard Oil Co. (Ind.), Chicago, IL, and Amoco Oil Company, Naperville, IL.
Newborn rat cutaneous epidermal keratinocytes, 6-7 days old, were pre-exposed (PE) to 0.05 MNNG for 1 hr at 8 AM, 2 and 8 PM for 2 consecutive days. Control (CPE) cultures received vehicle only. At 8 AM on the third day PE and CPE cells received a dose of 30 μM MNNG. Single-strand breaks and alkali-labile lesions were measured as decreases in % double-stranded DNA (DSDNA) using alkaline unwinding/hydroxylapatite chromatography. Typically, 1/2 hr exposure to 30 μM MNNG decreased DSDNA from 88% to 36% in both PE and CPE cultures. DNA repair was inferred from time-dependent return to control DSDNA after challenge. One hr post-challenge, DSDNA increased 22% in PE but only 10% in CPE culture. Two hr after challenge, PE cells had a further increase of 6% in DSDNA, while in CPE DSDNA was increased 16%. These differences in rates of repair between PE and CPE cells at 1.0 and 2.0 hr post-challenge disappeared at 6 hr when both PE and CPE had 70% DSDNA. No further increases in % DSDNA were observed 6 hr post-MNNG challenge. In summary, we report for the first time that non-DNA-damaging pre-exposure to MNNG can enhance the initial rate of repair of DNA damage incurred from MNNG challenge in rat epidermal keratinocyte culture. (USAMRC Contract No. DOC-C-DAMD 17-82-C-198)

**EVALUATION OF E. COLI HEAT STABLE TOXIN (ST) AS A SKIN TUMOR PROMOTER IN SENCAR MICE. M.R. Moore, Litton Bionetics, Inc., Kensington, MD 20895**

Produced and released by pathogenic strains of E. coli, STs are low molecular weight peptides differing in size, amino acid composition, methanol solubility, and host susceptibility. STs appear to induce cellular cyclic GMP by directly stimulating guanylate cyclase activation. The relative strength of skin tumor promoters may be due in part to their ability to stimulate cyclic GMP formation. The potential activity of ST as a tumor promoter in skin was evaluated in a 23-week study using 13 groups of female Sencar mice (30 mice/group). Twice weekly applications of ST 2 μg or 25 μg per application were ineffective for tumor promotion. Twice weekly applications of ST 2 μg for 20 wks was not effective as a second-stage tumor promotion stimulus. As a first-stage tumor promotion stimulus, twice weekly applications of ST 25 μg for 2 wks resulted in a tumor incidence (33%) and multiplicity (1.17) at 18 wks versus 13% and 0.33 in the appropriate control. At 23 wks the incidence and multiplicity was 50% and 1.53 in the ST group versus 33% and 0.76 in the control. In a second study, ST 25 μg 2x/wk for 2 wks resulted in an incidence and multiplicity of 31% and 0.72 versus 14% and 0.21 in the control at 19 wks. ST may have weak activity as a first-stage tumor promoter in skin. (Supported by HHS/FDA Contract No. 223-80-2295).

**TRANSPLENTAL CARCINOGENESIS AND MULTITH THEORY OF TUMOR DEVELOPMENT. D.G. Brauntetter, The Upjohn Co., Kalamazoo, MI, P. Conran, F.J. Goldblatt, Medical College of Ohio, Toledo, OH. Sponsor: T.J. Raczkik**

A widely accepted concept in the area of carcinogenesis is that multiple insults or "hits" must be received by an initially normal cell in order for it to be transformed over time into a clinically detectable and malignant neoplasm. Epidemiological studies of cancer incidence in man during aging and experimental studies in animals have also prompted numerous mathematical models of carcinogenesis which indicate that multiple discrete events must occur in the course of malignant tumor development. In transplantal carcinogenesis studies performed in the mouse, we have observed that liver and lung tumors induced early in organogenesis are much larger than those induced late in gestation or in adult mice. The proportional decrease in tumor size observed when they are induced at later stages of development roughly parallels the decrease in fetal growth rate during this same period and the large tumors induced early in gestation are composed of aggregations of smaller "adult" sized tumors. This suggests that many descendants of a single transformed fetal cell retain the capacity to become tumors in later life. If this is true, either the probability of additional "hits" occurring after the initial carcinogen related hit is fairly high or only a single hit is required for mouse liver and lung tumor development.
77 METHYLATION OF THE SERUM ALBUMIN GENE (SAG) AS COMPARED TO THE KRISTEN-RAS ONCOGENE (Ki-ras) IN HEPATOCYTES (H) AND NON-PARENCHYMAL CELLS (NPC) OF RAT LIVER.  R.L. Vorce and J.J. Goodman, Dept. of Pharmacology and Toxicology, Ctr for Environmental Toxicol, Michigan State University, E. Lansing, MI 48824, USA.

There is a negative correlation between the extent of methylation of a gene, i.e., percent of cytosine present as 5-methylcytosine (5-MeC), and its activity. DNA methylation was estimated by employing the restriction endonucleases Msp I and Hpa II. They recognize the sequence CCGG. Msp I, but not Hpa II, will cleave DNA if there is a 5-MeC adjacent to the guanine. The difference in the cleavage patterns provides a measure of the extent of methylation. H and NPC were isolated, DNA was purified and digested with Hpa II or Msp I and hybridized with a 32P-labelled cDNA probe, either SAG or Ki-ras. The results of this study indicate that the SAG is hypomethylated in H and hypermethylated in NPC. This is consistent with expression of the gene in the former cell type and nonexpression in the latter. In contrast, Ki-ras is hypermethylated in both H and NPC, suggesting that it is, at most, minimally expressed in normal liver. In view of the fact that cell proliferation is involved in the promotion stage of carcinogenesis and oncogenes appear to be involved in the regulation of cell proliferation, methylation of Ki-ras was assessed during liver regeneration following partial heptectomy. The extent of methylation was not altered during normal cell proliferation. These investigations provide a basis for testing the hypothesis that carcinogens can cause hypomethylation of oncogenes, leading to their increased expression. Supported by USPHS Grant CA-30635.

78 MNNG AND BCES-INDUCED DNA DAMAGE IN BASAL CELLS AND DIFFERENTIATED KERATINOCYTES. L.T. Mulholland and R.B. Conolly, Program in Toxicology, The University of Michigan, Ann Arbor, MI 48109-2029. Sponsor: I.A. Bernstein

Newborn rat cutaneous epidermal keratinocytes were grown under conditions favoring proliferation of basal cells with or without differentiation. Cultures were exposed to N-methyl-N-nitro-N-nitrosoguanidine (MNNG) or to di-benz(chlortetrahydrofluoride (BCES). DNA damage (alkali-labile lesions, single strand breaks, and cross-links) was measured using alkaline unwinding/hydroxylapatite chromatography. Repair of DNA damage was assessed by following its disappearance over time. With control DNA normalized as 100% double stranded (DDSDNA), 10 uM MNNG challenge reduced % DSDNA to 69.2 ± 1.4% and 41.0 ± 1.5% in basal and differentiated cells, respectively. Twelve hours after MNNG challenge, % DSDNA was 90.1 ± 1.2% and 52.7 ± 0.5% in basal and differentiated cells, respectively. BCES caused dose-dependent DNA crosslinks at concentrations from 50 uM to 200 uM in both basal cells and differentiated keratinocytes. In a typical experiment, with control % DSDNA 100%, 50 uM BCES resulted in 11.5% DSDNA in differentiated cells. Our data show that basal cells and differentiated keratinocytes differ significantly in susceptibility to MNNG-induced DNA damage and its repair but not with respect to BCES-induced DNA damage. (Supported by USAMRDC Contract No. DDC-C-DAMD 17-82-C-198)


Variations in carcinogenicity between species have been observed for a wide variety of chemicals and have often been related to differences in hepatic activation of procarcinogens. The hamster hepatocyte DNA repair assay has been shown to be useful for the detection of genotoxic agents and should also be useful for assessing species differences in metabolism of chemicals to genotoxic forms. Primary cultures of rat, mouse, hamster, and human hepatocytes were isolated by liver perfusion with a collagenase solution and incubated with known genotoxic agents and 3H-thymidine for 18-20 hr. UDS was measured by quantitative autoradiographic grain counting as net grains/nucleus (NG). Controls from all species showed <0 NG. 2-Acetethylaminofluorone yielded 21.0 NG in hamster (1 µg/ml), 8.3 NG in mouse (10 µg/ml), and 10.3 NG in human (10 µg/ml) hepatocytes. Dimethylthiourea yielded 57.8 NG in hamster (1 µM), 38.4 NG in rat (15 µM), 35.6 NG in mouse (20 µM), and 33.3 NG in human (20 µM) cells. Benzidine yielded 38.8 NG in hamster (10 µg/ml), 16.8 NG in rat (1 µg/ml), and 9.3 NG in human (1 µg/ml) hepatocytes. 4-Aminobiphenyl yielded 33.2 NG in rat and 34.1 NG in human (10 µg/ml) cells. These results indicate that both quantitative and qualitative differences may be observed in the genotoxicity of chemicals in hepatocytes from different species.


Methods have been developed to describe the formation of AFBl-DNA adducts within transfer RNA gene sequences isolated from the liver chromatin of male Fischer rats over a dose-range of 0.25 - 2.0 mg AFBl/kg. Nuclear chromatin was sheared via DNase II digestion, and DNase II sensitive chromatin DNA was treated under buffered alkaline conditions to stabilize AFBl-bound radioactivity. Alkali-treated DNA was hyridized with nuclear cDNA, and the cDNA hybrid were purified by two successive cesium salt density centrifugations. Transfer DNA regions were found to be preferential targets within nuclear DNA for AFBl modification; at two hours post dosing, transfer RNA gene sequences contained 5-6 times more bound AFBl moieties than unfractionated nuclear DNA over the 8-fold dose range observed. Comparison studies employing in vivo versus in vitro adducted nuclear DNA indicated that the observed preferential modification of cDNA regions is due to the relative accessibilities of these DNA sequences and to nucleotide content or sequence specificity. Supported by USDA grant AH 6658, TAMU CEIR grant 18845, and ERA grant VA 1401 from the Texas Agricultural Experiment Station.
Putative genotoxic lesions can be quantitated by DNA strand break analysis. In vivo studies of genotoxicity and genoprotection required the development of a rapid, sensitive method for measuring DNA strand breaks in soft tissues. DNA strand breaks were quantitated by measuring the rate of unwinding of double-stranded DNA at high pH. Optimal mouse liver homogenates were prepared by squashing whole livers in a tissue press followed by gentle homogenization in 0.9 % NaCl, 1.0 mM Na₂EDTA, pH 8.0, 0° C. Aliquots were gently mixed with equal volumes of 40 mM NaOH, 2.0 M NaCl, 0° C, final pH >10.5. At various time points samples were neutralized with NaH₂PO₄. Single-stranded DNA was sheared from double-stranded DNA by sonication and then removed by extraction with aqueous phenol.

Conventional colorimetric or fluorometric methods were used to measure DNA. Extraction of single-stranded DNA and recovery of double-stranded DNA approached 100%. Initially high DNA unwinding rates became negligible in about 30 minutes. At that time non-phenol-extractable, double-stranded DNA was proportional to the DNA strand breaks caused by treatment with diethylnitrosamine (G, 5, 10, 20 and 40 mg/kg, i.p.). This procedure provides a simple and convenient assay to quantitate DNA strand breakage in soft tissues.

(Supported by NIMH grant ES03373 and CA38277.)

Phthalate esters are widely used as plasticizing agents in synthetic rubbers to increase the resistance to stretching. They are released into the air during incineration of used tires or from automobile tires by friction against asphalt. Although phthalate esters have a low order of acute toxicity in rodents, their widespread distribution in the environment has led to concern regarding possible toxicological hazards. The dark brown solid material isolated from a steam distillate of synthetic rubber was found to contain di-(2-ethylhexyl)phthalate and two other components, and exhibited strong mutagenicity in Salmonella typhimurium strain TA 98 with a metabolic activation system. The sample showed only weak mutagenicity in TA 98 without S9 mix and in TA 100 with S9 mix. Since the standard samples di-(2-ethylhexyl)-, di-n-octyl-, and di-n-butyl-phthalate showed no mutagenicity in either strain in the presence or absence of S9 mix nor in plate incorporation or preincubation assay systems, the mutagenicity of the rubber sample is not directly due to di-(2-ethylhexyl)phthalate but may be due to an unstable component or to components acting as comutagens.
In a series of genetic toxicology studies addressing point mutations, clastogenesis, DNA damage/repair and chromosomal aberrations, carbosulfan (2,3-dihydro-2,2-dimethyl-7-benzofuranyl [(dibutylamino)thio]) methyl carbamate yielded negative results. Results obtained in the Ames test, DNA repair proficient and deficient tester strains of S. typhimurium and E. Coli, the L5178Y TK+/– mouse lymphoma assay, the micronucleus test, an in vivo cytogenetics test in rats and a dominant lethal test in mice indicated that carbosulfan did not cause point mutations, DNA damage or damage to mammalian chromosomes.

The effect of whole cigarette smoke exposure on bone marrow sister chromatid exchanges (SCEs) was studied in B6C3F1 mice. Animals were exposed nose-only to 10% (v/v) whole cigarette smoke 5 days per week for 2 weeks. Four dose levels of cigarette smoke (1, 4, 9, and 18 exposures/day) were studied using 2 cigarette types, Kentucky reference 3A1 (3A1) and American Blend (AB). A single exposure represented 1 cigarette, or 0.38mg Total Particulate Matter (TPM) for 3A1 and 18mg TPM for AB cigarettes. Carboxyhemoglobin levels, a monitor of smoke exposures, were significantly increased 10-30 fold in all smoke-exposed mice compared to sham controls. The sham-treated and untreated shelf controls demonstrated a normal distribution of SCEs/cell with ~5% of all cells containing >10 SCEs/cell. As the exposure levels increased, the distribution of SCEs/cell became non-parametric and the frequency of cells containing >10 SCEs increased, with ~30% of all cells scored at 18 exposures/day containing >10 SCEs/cell for both cigarette types. Maximal induction was observed at 18 exposures/day, with means of 11.63 SCEs/cell for 3A1 and 9.43 SCEs/cell for AB cigarette smoke, compared to 5.71 SCEs/cell for sham controls. A dose-dependent increase in SCEs was observed for both the 3A1 and AB cigarettes at dose levels which had no effect on bone marrow cell replication kinetics.

It was shown in 1977 (Yamasaki, E. & Ames, B.; Proc. Natl. Acad. Sci., 74-3555) that cigarette smokers have mutagenic urine. The purpose of the present study was to determine if the same was true for rats exposed to smoke so that factors influencing the mutagenicity (nicotine/tar content, medication, pollutants, etc.) could be evaluated in an animal model. Using XAD-2 urine concentrates of human smokers and of male rats exposed nose-only to cigarette smoke (20 min/day for 6 mo) and T98 S. typhimurium (+S9) as the test strain, it was observed that human urine was mutagenic but not rat urine. While the data suggested a species difference, further study demonstrated that this could not be concluded with certainty because of the potent bacteriocidal action of the rat urine. Results of in vitro and in vivo studies with 2AAF and other known mutagens demonstrated that extracts of only 5 ml of rat urine were sufficiently bacteriocidal to mask low levels of mutagenic activity, whereas extracts of 25 ml of human urine were non-toxic to the T98 strain. Ultimate use of the rat as a model for the study of smoking and urine mutagenicity will depend upon the successful separation of toxic components from mutagens, if any, before assay of urine extracts in the Ames systems. (Supported in part by THRI Grant No. 4E014)

We have measured the cytotoxicity and mutagenicity of complex mixtures of polycyclic aromatic hydrocarbons (PAH) from a coal conversion material with the CHO/HGPRT assay (Chinese hamster ovary cells) using rat liver S9 for metabolic activation. The mixture is highly cytotoxic at concentrations required for minimal detection of mutagenesis. To better understand the relative roles of cytotoxicity and mutagenicity, we separated the 2- to 6-ring PAH mixture into fractions defined by their number of aromatic rings. The 4- and 5-ring PAH were much more mutagenic than the parent PAH mixture, but were less cytotoxic. Conversely, the 2- and 3-ring PAH, which accounted for 75% of the mixture, were only slightly mutagenic and very cytotoxic. Furthermore, the cytotoxicity of the 2- and 3-ring PAH depended on metabolic activation. Results of experiments using cofactors and model compounds showed that the cytotoxicity of the 2-ring PAH fraction was due primarily to quinone and phenol metabolites of the non-mutagenic PAH. Our results suggest that the low response of mammalian cells to mutagens in complex PAH mixtures is related to competing effects of toxic and mutagenic components. (This work was supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38.)
The effect of several metal compounds in cultured human fibroblasts was studied. No DNA damage as measured by strand breaks on alkaline sucrose gradients was detected after treatment of cells with subcytotoxic concentrations of any of the metals tested. Using the more sensitive nick translation assay, DNA damage was observed following treatments with $5 \times 10^{-4}$M Cd$^{++}$ and 2mM Ni$^{++}$ whereas Hg$^{++}$, Mn$^{++}$, Co$^{++}$ produced no such damage. Data from this assay also indicated that Ni$^{++}$- and Cr$^{+++}$-induced DNA damage was repaired within 120 minutes. Cd-induced strand breaks were not repaired in this time. DNA damage induced by Cd$^{++}$ but not by Cr$^{+++}$ or Ni$^{++}$ was dependent on O$_2$ consistent with recent findings suggesting that generation of active O$_2$ species is necessary for Cd$^{++}$ induced DNA damage. Cd$^{++}$, Cr$^{+++}$, Ni$^{++}$ and Hg$^{++}$ were found to induce repair synthesis in unscheduled DNA synthesis or cesium chloride sedimentation studies.

Cynomolgus macaque monkeys were exposed to 0, 500, 1000, 2000, 3000 and 4500 ppm toluene for 50 min in a head-only exposure system and simultaneously treated for behavioral effects using a delayed matching-to-sample procedure. The behavioral indices of toxicity were short-term memory, response time, probability of responding and responding during the retention interval. Carbon dioxide production, the most sensitive index, increased in the second half of the session relative to the first half at all toluene concentrations. All other parameters showed concentration-related changes. Response time increased at 1000 ppm or more of toluene, and showed an acute, within-session tolerance at intermediate doses. Accuracy of matching at three retention intervals (0, 4, 16 sec), declined at concentrations of 2000 ppm or more, indicating an attentional deficit but not specific memory effects. Probability of responding decreased at 3000 ppm. Responding during the retention interval decreased at 4500 ppm. No behavioral measure exhibited either cumulative effects or tolerance to 4500 ppm during 3-day exposures. The results indicate that toluene produced dysfunction of cognitive and motor ability at concentrations below those causing ataxia and intention tremoring. (Support: ES-00260, ES-03461, ES-07065 all from NIH).

The ability of EDTA (long the agent of choice in treating increased lead (Pb) burden) to reverse performance deficits provoked by Pb exposure has not previously been studied. Long-Evans weaning rats were exposed chronically to 50 ppm sodium or Pb acetate in drinking water. At 55 days of age, behavioral evaluation on a fixed interval (FI) schedule of food reinforcement began. As in previous studies, response rates of Pb rats increased relative to controls over the first 35 sessions. We hypothesized that EDTA should lower rates of the Pb-treated group more rapidly than termination of Pb exposure alone. Thus, animals within each group were matched on the basis of FI rate, and Pb exposure terminated. Half the rats in each group received 5 consecutive daily i.p. injections of 75 mg/kg EDTA; the others, saline. Slight increases in control rate followed saline; EDTA increased rates even further. FI rates declined, as predicted, in the Pb-saline group (33 to 25 responses/minute). Pb+EDTA did not decrease response rates; in fact, they were even further increased (34 to 50 responses/minute). This occurred despite a marked elevation of urinary Pb (1031 μg/dl at 24 hr cf., 135 μg/dl in the Pb+saline group). Although chelation with EDTA accelerated Pb excretion, it had no impact, possibly even deleterious effects, on Pb-induced performance effects. NIEHS 03054, 03079, 01248.
93 INHALED TOluene PRODUCES PENTOBARBITAL-LIKE DISCRIMINATIVE StimULUS EFFECTS IN MICE. D.C. Rees, E. Coggeshall, R.L. Balster, Dept. of Pharm. and Tox., Medical College of Virginia, Richmond, VA 23298. Sponsor: J.P. Borzelleca.

Abuse of organic solvents remains a significant public health problem. Little is known regarding the type of intoxication these agents induce nor the determinants of their abuse potential. Available evidence indicates that solvents have similar pharmacological effects to those produced by central nervous system depressants such as alcohol, barbiturates or volatile anesthetics. Since the abuse potential of solvents may lie in their ability to produce an alcohol or barbiturate-like intoxication, a drug discrimination procedure was used to assess similarities in the discriminative stimulus properties between toluene, a typical abused solvent, and pentobarbital (PB), a classic CNS depressant. Ten mice were trained to discriminate i.p. PB (15 mg/kg) from saline in a two-lever operant task in which responding was under the control of a fixed-ratio 20 (FR20) schedule of food presentation. Stimulus generalization was examined following 20-min inhalation exposures to toluene (300-5400 ppm). Generally, drug-reversing responses increased in a concentration-dependent manner. Similarities in the discriminative stimulus properties of solvents and CNS depressants may be related to their abuse potential. (Supported by NIDA Grant DA-00490 and NIEHS Grant ES-07087).

95 EFFECTS OF TRIETHYL TIN-SO₄ (TET) IN MICE RESPONDING UNDER A MULTIPLE SCHEDULE OF FOOD PRESENTATION. G.R. Wengen and D.E. McMellan, University of Arkansas for Medical Sciences, Little Rock, AR.

The effects of TET on lethality and on responding under a multiple fixed-ratio 30, fixed-interval 600 sec (mult FR30 FI600) schedule of food presentation were determined in BALB/c mice. A single dose of either 10, 12.5 or 15 mg/kg TET was administered intraperitoneally, and the lethality was determined at 24-hr intervals for the next 2 weeks. During the first 24 hrs, no deaths were observed after 10 mg/kg, 4% (1/27) lethality was observed after 12.5 mg/kg, and 75% (21/28) lethality was observed after 15 mg/kg. The lethality 144 hrs after TET had risen to 20%, 95% and 100% for the 10, 12.5 and 15 mg/kg groups. No additional deaths were observed for the remainder of the 2-week period. At 5, 7.5 and 10 mg/kg TET, the rate of FR and FI responding was markedly depressed 3 hrs after administration. At 27 hrs after administration, no significant effects were observed at 5 and 7.5 mg/kg. The rate of FR and FI responding in the mice receiving 10 mg/kg showed a partial recovery at 27 hrs and was almost fully recovered by 51 hrs. In a fourth group, repeated administrations of 7.5 mg/kg TET at 2-week intervals produced no evidence of cumulative or diminished effects. Thus, the behavioral effects of TET in the mouse were of short duration with no cumulative effects. (This work supported by EPA Grant # RB0945201.)

94 DISCRIMINATIVE StimULUS PROPERTIES OF INJECTED TOluene IN MICE. D.C. Rees, R.L. Balster, Dept. of Pharm. and Tox., Medical College of Virginia, Richmond, VA 23298. Sponsor: J.P. Borzelleca.

Alcohol, barbiturates, and benzodiazepines share discriminative stimulus properties and all are subject to abuse. Other work in our laboratory demonstrated that animals trained to discriminate i.p. pentobarbital (PB) from saline would generalize to inhaled toluene. These experiments sought to further characterize the discriminative stimulus properties of toluene. Mice were trained to discriminate i.p. toluene (75 mg/kg) from vehicle in a two-lever operant task in which responding was under the control of a fixed-ratio 20 (FR20) schedule of food presentation. Acquisition of the discrimination required a minimum of 100 training days. Generalization tests following 20-min exposures to inhaled toluene (300-3600 ppm) resulted in concentration-dependent increases in responding on the toluene lever. Also, i.p. toluene generalized to i.p. PB. These effects could not be accounted for on the basis of response rate suppression nor stimuli associated with the route of administration. Toluene's discriminative stimulus properties are most likely mediated by the CNS. These results provide further evidence for an overlap in the stimulus properties of PB and both injected and inhaled toluene. (Supported by NIDA Grant DA-00490 and NIEHS Grant ES-07087).


Low, chronic doses of halothane, a commonly used surgical anesthetic, have been found to disrupt synaptogenesis during ontogeny (Uemura and Bowman, 1980; Exp Neurol. 69:135-142). This experiment was designed to determine the generality of this effect. Unilateral lesions of the entorhinal cortex were performed on albino rats. Three were exposed to 100 ppm of halothane in air for 8 hours/day on days 1-14 post lesion. Three other rats were controls. Synaptic density in CA3 of the hippocampus was 10,472±0.786 x 10⁸ synapses/mm² (Mean ± SD) while the halothane-exposed group averaged 7,883±0.813 x 10⁸ synapses/mm² (F(1,4)=15.71, p<.025). The rats were also tested for spatial alternation, a behavior sensitive to lesions of the entorhinal cortex. The halothane group showed slower behavioral recovery, but this effect was not significant. These results demonstrate that low, chronic exposure to halothane suppresses reactive synaptogenesis as well as synaptogenesis during development. Halothane may be useful as an experimental tool for manipulating the rate of synapse formation for studying critical events of synaptogenesis and for investigating relationships between synaptogenesis and behavior. (Supported by NIH Grant # 1T70.)
Eight male hooded rats were implanted with chronic bipolar electrodes in the medial forebrain bundle and trained to self-stimulate. Following training, the animals were placed in an experimental chamber equipped with six levers and cue lights above each lever. The task required an animal to depress the lever below an illuminated cue light within 1 sec in order to obtain a 250 ms 60 Hz brain stimulation pulse train as reinforcement. The lights were illuminated in a fixed sequence of 1–2–3–4–5–6–7–8–9–10–11–12–13 that was repeated 50 times each day. Incorrect responses or omissions were recorded but not punished. All eight animals completed training, performing with 83.7% correct responses after 25 weeks. Carbon monoxide (CO) exposures consisted of giving each animal an intraperitoneal injection of 100% CO 30 min prior to testing. The injections resulted in carboxyhemoglobin levels of 15, 25, 40, and 55% at 45 min post injection. The results indicated that performance deteriorated significantly at the highest CO levels only. Repeated (3 times) exposures to CO did not result in improved performance or behavioral tolerance. These results show that CO administered via the intraperitoneal route has very similar effects to inhaled CO on behavior, and also provides an easy method for administering CO to rats without the need for inhalation facilities.

Supported by ES 02277, 07094, and NH 08759


RDX (cycloextrimethylene nitromethane) is an explosive compound that produces spontaneous behavioral seizures in both animals and humans. In the present experiments, rats received p.o. the vehicle or RDX and were tested 2 or 8 hrs later for seizure susceptibility. At 8 hrs, audiogenic (1 min stimulation by ultrasound) seizures were elicited in RDX rats at the following frequencies: 60 mg/kg (9/11); 20 mg/kg (3/10); 10 mg/kg (1/10); 0 mg/kg (0/21). The duration of pentylentetrazol-induced (50 mg/kg i.p.) seizures also increased in rats given 60 mg/kg RDX (lower dosages not tested). In both experiments, a significant number of RDX animals died prior to (92%) or as a result of (75%) testing, an event never seen in controls. Preliminary observations suggest that dose-responses and time course functions of audiogenic and spontaneous seizures differ; audiogenic, but not spontaneous seizures, were evident at 10 mg/kg. At 50 mg/kg, spontaneous seizures were seen within 2 hrs after treatment, when audiogenic seizures could not be elicited.


Sulfolane is an industrial solvent that enhances seizure susceptibility following acute administration (Berdette and Dyer, 1984); little is known, however, about its other behavioral effects. The present experiments determined the effects of acute sulfolane administration on motor activity and flavor-aversine conditioning. Adult male Long-Evans hooded rats (N=6/group) were treated i.p. with nothing (NIC), the saline vehicle (V) or sulfolane (50-400 mg/kg), 75 min before a 25-min test in a photocell device that recorded horizontally (HA) and vertically (VA) directed motor activity. Sulfolane decreased motor activity at the largest dosage only; ED50 estimates (doses reducing activity to 50% of V) were 353 and 273 mg/kg for HA and VA, respectively. Additional rats (N=6/group) received 30-min access to saccharin solution (0.1%) and subsequently were treated i.p. with nothing (NIC), saline (V), or sulfolane (50-400 mg/kg). Three days later rats were given a choice between saccharin and tap water. Sulfolane produced a dosage-related reversal of the saccharin preference displayed by control rats (ED50 = 139 mg/kg). Sulfolane may produce prominent behavioral effects at doses lower than those that enhance seizure susceptibility.

*Supported by an NRC research associateship.
Endosulfan is a cyclodiene pesticide used widely on crops to which youths are likely to be exposed. Very little is known, however, about its toxicity at varying ages of exposure. The following experiments therefore compared the effects of acute endosulfan administration on lethality, motor activity and flavor-aversion conditioning in weanling (28d) and adult (67d) male Long-Evans hooded rats. The oral LD50 of endosulfan was smaller for weanlings (17.8 mg/kg) than adults (27.6 mg/kg). For motor activity assessment, rats (N=12/group) treated p.o. with endosulfan (2.5-20 mg/kg) or the corn-oil vehicle were tested 120-min later in a Motron chamber or 60-min later in a figure-eight maze. Endosulfan produced similar dosage-related decreases in motor activity at both ages. For flavor-aversion conditioning, rats (N=6/group) received 30-min access to saccharin solution (0.12) and were then treated p.o. with endosulfan (2.5-20 mg/kg), corn-oil, or nothing. Three days later, all rats were given a choice between saccharin and tap water. Endosulfan produced a dosage-related conditioned flavor aversion in adults but not in weanlings. Therefore, endosulfan produces multiple behavioral effects that are not simply related to age of exposure.

*Supported by an NRC research association.

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**ACUTE AND DELAYED TOXICITY OF ORGANOPHOSPHORUS ESTERS TO LAYING HENS IN PERCUTANEOUS AND ORAL DOSING REGIMENS.**


Thirteen organophosphorus esters were evaluated for their potential to cause organophosphorus ester induced delayed neurotoxicity (OPIDN) when administered topically and/or p.o. to white leghorn hens: tetrachlorvinphos, chlorpyrifos, DEF, dichlorvos, dimethoate, EPN, ethoprop, fenithion, isofenphos, lepto phos, rotenone, tetrachlorvinphos, terbufos, and trichlorfon. DEF induced ataxia if given topically or p.o. at over 21 mg/kg/day for up to 90 days. Hens treated with EPN developed irreversible ataxia after repeated doses of 1.3 mg/kg topically or 5 mg/kg/day p.o., while lepto phos was neurotoxic at doses of 6-7 mg/kg/day topically and 10 mg/kg/day p.o. Multiple treatments of chlorpyrifos, terbufos, dichlorvos and dimethoate caused death after varying periods of increasing debility; birds had difficulty walking, but did not display typical symptoms of OPIDN. Fenithion and isofenphos induced dramatic weight loss at low levels; isofenphos induced OPIDN, but hens did not survive. Dichlorvos given at 66 mg/kg/day p.o. or topically at 1 mg/kg/day produced cholinergeic symptoms; most hens died during treatment. At lower levels, dichlorvos induced no overt ataxia. No other compound in this series induced consistent ataxia whether administered p.o. or topically. Ethoprop (acute oral LD50 near 5 mg/kg, acute topical LD50 near 3 mg/kg) was too toxic to evaluate fully.

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**EFFECTS OF DIRECT AND INDIRECT-ACTING DopAMINERGIC AGONISTS ON LOCOMOTOR ACTIVITY OF RATS EXPOSED PERINATALLY TO POLYBROMINATED BIPHENYLS.**


Pregnant Sprague-Dawley rats ingested 0, 0.2 or 2 mg/kg polybrominated biphenyls (PBB) in a peanut butter vehicle daily from day 6 of gestation through day 24 postpartum. The offspring were assessed for stimulation of spontaneous locomotor activity by a direct and indirect-acting dopaminergic agonist following an accommodation period. L-DOPA (250 mg/kg) was the direct-acting agonist, preceded by 50 mg/kg of the peripheral l-amino acid decarboxylase inhibitor benzerazide. d-Amphetamine (d-A, 2 mg/kg) was the indirect-acting dopaminergic agonist. Control and treated offspring were comparable for the initial pattern of habituation. Following injection with l-DOPA, control and PBB-exposed offspring exhibited a comparable degree of stimulation, the peak occurring between 90 and 105 minutes following injection. Offspring from dams administered 2 mg/kg PBB were significantly less active than controls following d-A injection and the time-course of stimulation was shorter in PBB-exposed offspring of either sex. Female offspring exposed to 0.2 mg/kg PBB also showed a shorter duration of d-A stimulation, while males exposed to 0.2 mg/kg PBB showed a longer duration of d-A stimulation, each group related to their respective controls. Therefore, perinatal PBB exposure may produce deficits in dopaminergic systems which are presynaptic, rather than postsynaptic, in origin. (Supported by NIEHS grant ES 02763.)
107 INVESTIGATION OF THE BEHAVIORAL AND NEUROCHEMICAL PROPERTIES OF 11-HYDROXY-D9-TETRAHYDROCANNABINOL AND ITS FATTY ACID CONJUGATE IN THE RAT.


The compound 11-hydroxy-d9-tetrahydrocannabinol (11-OH-d9-THC) is a psychoactive metabolite of d9-tetrahydrocannabinol (THC), the major active constituent of cannabis. A long-retained cannabinoid metabolite has been detected in rat tissues after intravenous (I.V.) administration of THC and has been identified as a fatty acid conjugate of 11-OH-d9-THC, namely 11-palmitoyloxy-d9-tetrahydrocannabinol (11-palm-d9-THC). The objective of this study was to determine if 11-palm-d9-THC exhibited psychoactive properties in the rat. 11-Palm-d9-THC induced both catalepsy and analgesia following I.V. injection, responses similar to those produced by 11-OH-d9-THC, but less pronounced and more delayed. Neither compound produced changes in striatal dopamine (DA) uptake and hypothalamic norepinephrine (NE) uptake or altered levels of DA and NE in either region. Since it has been shown that 11-Palm-d9-THC can be hydrolyzed in vivo to 11-OH-d9-THC, it is possible that the effects of the fatty acid conjugate reflect metabolic conversion to 11-OH-d9-THC. (Supported by NIDA Grant No. DA00793-07.)


Currently there is no animal model for human bismuth (Bi) encephalopathy. Fifty-eight female Swiss-Webster mice received up to 3 IP injections of 2500 mg/kg (1st dose) or 1250 mg/kg (2nd and 3rd dose) of Bi Sublimate (BSN) as a 10% aqueous suspension on days 1, 38 and 52. Twelve mice received distilled H2O vehicle. Brain and whole blood were analyzed for Bi by anodic stripping voltammetry after wet ashing with acid. Sensitivity was approximately 25 ppb in blood and 150 ppb in brain, depending on the size of the sample.

Nine mice developed signs of neurotoxicity (myoclonus, ataxia, dementia, tremors and convulsions) which were similar to those observed in human cases. High blood Bi levels (1.1 ± 0.6 ppm) were measured in clinically affected mice. Brain Bi levels (8.4 ± 2.0 ppm) in these mice approximated those previously reported for autopsied humans. Blood (0.6 ± 0.2 ppm) and brain (4.3 ± 1.2 ppm) Bi levels were significantly lower (p < .05, sign test) in a paired group of clinically unaffected mice (n=9) receiving identical doses of BSN. Bi levels (control mice (n=9) were at or near detection limits. Both affected and clinically normal mice dosed with BSN exhibited moderate hydrocephalus. This is the first documented animal model of Bi encephalopathy.
**EFFECTS OF TRIMETHYLLIN ON DIURNAL RHYTHMS IN RATS AND MICE.** P.J. Bushnell and H.L. Evans. NYU Medical Center, New York, NY 10016.

The ability of trimethyllin (TMT) to disrupt diurnal patterns of feeding, drinking and spontaneous motor activity was examined in rats given 0, 3, 5, or 7 mg TMTCl/kg p.o. and observed in the home cage for two weeks thereafter. Mice were injected with TMTCl at 0, 0.3, 1, or 3 mg/kg p.o.; spontaneous motor activity and metabolic rate (as CO₂ production, VCO₂) were measured continuously for 3 days, and again for 48 hr periods at 10, 20, 30, and 60 days postinjection. TMT caused dose-related decreases in food consumption and body weight in both species. At the high dose, rats doubled their intake of water, and mice showed a persistent whole body tremor for 3 days. The nocturnal pattern of drinking and of rearing in rats changed at all doses of TMT from 3 to 9 days post dosing. Despite lowered VCO₂, mice became hyperactive for 48 hr after 3 mg/kg, then hypoactive for at least 60 days. The normal peak in motor activity at light offset was exaggerated in mice at low TMT doses and eliminated at the high dose. In most instances, the behavioral changes induced by TMT appeared as constraints on the normal amplitude of periodic fluctuations in spontaneous behavior. (Support: Center Grant ES-00260 and Contract ES-2-5017 from NIIEHS).


Children from low SES minority families are considered to be at risk for lead poisoning. Detergents in cognitive function as indexed by traditional IQ tests have been reported in several studies of children with low to moderate lead exposure, although these results are very controversial. An independent replication of a previous study (Schroeder et al. Environ. Res., in press) of the interaction of lead and social factors on Stanford-Binet IQ was performed on 80 low SES Black children screened by county health departments in North Carolina. Children's mean blood lead (PbB) level was 20.85 µg/dl (range, 6.3-47.4). Multivariate regression analyses showed no interactions between PbB and age, sex, maternal IQ, Caldwell home environment score or SES (Hollingshead Two-Factor Index). There was a highly significant negative relationship of both mean and maximum PbB levels on IQ (P<.002). That is, IQ decreased linearly as PbB increased. Tests for quadratic and cubic components were not significant. These results replicate our previous findings and are consistent with recent studies of PbB effects on cognitive function in children from low-income families.

**NEUROTOXICITY PRODUCED IN RATS BY CONCURRENT EXPOSURE TO TRIETHYL LEAD AND 2,5-HExAMEDINE.** Daniel M. Lapadula, Hugh A. Tilton, Gerald A. Campbell, and Mohamed B. Abou-Donia. Duke University Medical Center, Durham, NC, and Laboratory of Behavioral and Neurological Toxicology, NIEHS, Research Triangle Park, NC.

Triethyl lead chloride (TEL) and 2,5-hexamidine (2,5-HD) are known neurotoxins working through separate mechanisms. The effect of combined treatment of TEL and 2,5-HD, for 6 weeks on Fischer 344 rats was investigated. Ten rats were given 0.7 mg/kg TEL in a volume of 2 ml/kg by gavage while another group was given 0.5% 2,5-HD in the drinking water and vehicle by gavage (2 ml/kg). A third was given a combination of the two treatments. A fourth group served as controls and was given vehicle by gavage. 2,5-HD produced a reversible loss of body weight, decreased grip strength, and decreased horizontal motor activity. TEL alone increased hot plate latencies. 2,5-HD and TEL-treated animals recovered 4 weeks after cessation of treatment. Neither treatment alone produced fatalities. In combination (2,5-HD + TEL) decreases in body weight appeared additive and there was a 40% mortality by 6 weeks of dosing. Rats given TEL + 2,5-HD had significant loss of both grip strength and increased hot plate latencies. Neurobehavioral deficits and neuropathological changes were greater in the combined treatment with 2,5-HD and TEL than when either chemical was used alone. (Supported in part by NIOSH Grant OH00823).

**SUBCHRONIC INHALATION TOXICITY OF PINACOLYL ALCOHOL (3,3-DIMETHYL-2-BUTANOL) IN RATS.** J.T. James, R.D. Armstrong, G. Leach, W.C. Hall, R.J. Pellerin, Toxicology Division, Chemical Research and Development Center, Aberdeen Proving Ground, MD 21010. Sponsor E.J. Olajo.

The purpose of this study was to evaluate the subchronic inhalation toxicity of pinacolyl alcohol (PA). Rats were exposed to filtered air or PA vapor at concentrations of 0.20, 1.00 or 5.0 mg/l, 6 hours/day, 5 days/week, for 13 weeks. Some rats from each group were held for an additional 4 weeks after exposure. Lacrimation, ataxia and dental alopecia were observed exclusively in high-dose animals. There were no significant between-group differences in body weight during the study. Clinical chemistry, hematology and coagulation measurements revealed only increased cholesterol and bilirubin in high-dose males after 7 weeks and a slight increase in urea nitrogen in high and mid-dose males after 13 weeks. Spontaneous activity and passive avoidance tests at 7 and 13 weeks indicated no PA-induced abnormalities. Physiological testing after 13 weeks of exposure revealed no PA-induced abnormalities. Necropsy and histopathological studies at 7 and 13 weeks of exposure, and 4-weeks post-exposure, indicated minimal changes due to inhalation of PA. Based on the results of this investigation, the subchronic inhalation toxicity of PA is minimal.
113 ACUTE INHALATION TOXICITY OF PINACOLYL ALCOHOL (3,3-DIMETHYL-2-BUTANOL) IN SPARGUS-DARLEY RATS. J.T. James, B.P. Infesto and M.R. Landauer, Toxicology Division, Chemical Research and Development Center, Aberdeen Proving Ground, MD 21010. Sponsor: E.J. Olajos.

The purpose of this study was to evaluate the acute inhalation hazard associated with pinacolyl alcohol (PA). Rats were exposed in a 20L chamber to PA vapor generated by passing the intake air through a 5 cm column of the alcohol. The vapor concentration was 15 mg/L and exposures were for 15, 70 or 140 minutes. These exposures produced 0, 0 and 50% mortality, respectively. Survivors of the 70 and 140 minute exposures showed subnormal weight gain and increased red blood cell counts. Additional studies were conducted to measure the respiratory response and motor behavior of rats given a single 6-hour exposure to 5 mg/L. This exposure caused a 35% decrease in breathing rate and a 20% decrease in tidal volume. Immediately after exposure, control and experimental rats exhibited similar ambulatory activity levels; however, the experimental rats habituated more rapidly than controls. At the end of the 24-minute test, the experimental animals had only 1% of the control activity level. In contrast, rearing activity levels were less than 20% of control values throughout the test period. No mortality was observed in the rats exposed to 5 mg/L for 6 hours. While PA exhibits some acute toxicity, there is only minimal inhalation hazard from this alcohol.

114 INHALATION TOXICITY OF WELDING FUMES. R.A. Parent, Consultox, Ltd., Baton Rouge, LA; W.B. Coate, Hazleton Laboratories America, Inc., Vienna, VA

Groups of 40 rats were exposed for six hours to fumes from six welding materials which included those used in shielded metal, gas metal and flux core arc welding.

Urine was collected from one group of 10 rats which were subsequently necropsied and their lungs examined microscopically. Another group of rats was subjected to lung lavage at 20 hours post-exposure. Two additional groups were analyzed for cytogenetic effects (bone marrow) and wet/dry lung weight ratios.

Urine and marrow samples failed to show mutagenic activity (S. typh.) and cytogenetic effects. Lung weights taken at 14 days did show effects, but wet/dry lung weight ratios taken at 24 and 72 hours were unremarkable. Microscopic examination of lungs taken at 14 days showed pigmented macrophages for all fumes and occasional focal acute pneumatic and multifocal perivascular edema. Most lung lavage fluids were bloody or had reduced cell populations. Various parameters relating to macrophage viability were measured and effects were significant. The fume from the stainless steel electrode was determined to be the most toxic to rats. (Supported by the American Welding Society, Miami, Fl.)

115 SUBCHRONIC INHALATION TOXICITY OF GLUTARALDEHYDE. B.J. Greenspan, B. Ballantyne, E.H. Fowler, and W.M. Snellings, Bushy Run Research Center, Union Carbide Corporation, Export, PA

Glutaraldehyde (GA), a protein cross-linker and biocide, is a potent peripheral sensory irritant. As a basis for a subchronic toxicity study, two 9-day inhalation studies were conducted with male and female F-344 rats using vapor concentrations of 3.1, 1.4, 0.3 and 0 ppm, and 0.1, 0.63, 0.2 and 0 ppm; exposures were for 6-hr a day. The major findings were mortality at the high concentration, and depression in body weight gain, signs of sensory irritation, and inflammation of the nasal and olfactory mucosa at the high and intermediate concentrations. The subchronic study with male and female F-344 rats was conducted using GA vapor concentrations of 194, 49, 21 and 0 ppb, with exposures of 6-hr a day, 5 days a week for 14 weeks. The principal findings at 194 and 49 ppb were perinasal wetness and statistically significant decreases in body weight gain. There were increased activities of serum phosphokinese, lactic dehydrogenase and hydroxybutyric dehydrogenase; however, these were not dose-related and no histopathologic correlates were found. There was no histological evidence of inflammation in the nasal and olfactory mucosa. These findings show an absence of significant toxicity under the conditions of the subchronic study, but confirm the presence of sensory irritation at the concentrations used.

116 EFFECT OF ACROLEIN ON MACROPHAGE FUNCTIONS AND HOST DEFENSE IN MICE AND RATS. R.L. Sherwood, C.L. Leach, N.S. Hatcun, and G. Arany. IT Research Institute, Chicago, IL.

Acrolein, a toxic aldehyde component of cigarette smoke, has been shown to impair pulmonary defense mechanisms of experimental animals. Male Sprague-Dawley rats were exposed to 0.1, 1.0 or 3.0 ppm acrolein for 4 hr/day, 5 days/week for three weeks. Rats were tested one day following the last exposure and exhibited no change in pulmonary clearance of inhaled [35S]-Klebsiella pneumoniae at any acrolein concentration. Decreased numbers of peritoneal cells were obtained from exposed rats while the number of cells lavaged from the lungs was unchanged. In vitro phagocytosis of [51Cr]-chicken red blood cells by rat alveolar (AM) and peritoneal (PM) macrophages was significantly increased following inhalation of 0.1 and 1.0 but not 3.0 ppm of acrolein. AM from acrolein exposed rats had increased 5'nucleotidase and lyszyme activity. Female CD1 mice were exposed to 0.1 ppm acrolein or filtered air 3 hr/day for 1 or 3 days and tested within 1 hr post-exposure. No effects in pulmonary clearance of K. pneumoniae or mortality from infectious streptococcus aerosol challenge were observed after a single exposure. Exposure of five consecutive days produced a decrease in bacterial clearance with no increase in streptococcus mortality.

Supported in part by EPA Cooperative Agreements R806730 and R807034.
In vivo metabolic constants (Km and Vmax) were determined in Fischer rats for four chemicals: 1,1-dichloroethylene (1,1-DCE), diethyl ether (DE), bromochloromethane (BCM), and methy1 chloroform (MC). A series of uptake curves were obtained for each chemical at a variety of initial concentrations. The shapes of these curves depended on tissue solubilities and the rate of metabolism of the chemical. Tissue:air partition coefficients were determined for each chemical experimentally and incorporated into a physiological kinetic model which was used to simulate the uptake curves. An optimized fit of the family of uptake curves for each chemical was obtained by adjusting the metabolic constants. 1,1-DCE metabolism was represented as a single saturable process; BCM and DE exhibited a combination of both a saturable and a first order process; MC had only a first order pathway. Pyrazole, which blocks oxidative microsomal metabolism, competitively inhibited the saturable pathways of 1,1-DCE, BCM, and DE metabolism and abolished the first order pathway for MC. The simulation approach for analyzing gas uptake data distinguishes between single and multiple metabolic pathways and provides kinetic constants that can be used in predictive toxicokinetic models for constant concentration inhalation exposure or for exposure by other routes of administration.

118 COMPARISON OF SUBCHRONIC INHALATION TOXICITY OF FIVE ALIPHATIC NITRILES IN RATS. V. Roloff, R. Short, W. Ribelin and M. Dietrich. Monsanto Company, Environmental Health, St. Louis, MO.

Two mononitriles, two dinitriles and acetone cyanohydrin (ACY) were each evaluated for inhalation toxicity to male and female rats exposed for one- month durations at 6 hrs/day for 5 exposure days per week. Groups of animals were exposed to mean concentrations (mg/m³ in air) of either acetonitrile (ACN) at 0, 1038, 3104 and 10,485; or propionitrile (PN) at 0, 99, 260 and 743; or succinonitrile (SN) at 0, 14, 34 and 110; or adiponitrile (ADN) at 0, 64, 115 and 493; or ACY at 0, 32, 104 and 208. Irritation to the eyes and/or nose, decreases in body weight, death and various nervous system effects were observed in the mid- and/or high exposure animals of all those in the SN study. Respiratory difficulties were also noted but limited to those exposed to ACY and ACN. A mild anemia was apparent in animals exposed to ACN, PN and ADN. Hemoglobin pigmentation and hemopoiesis in the spleen were observed in mid- and high females of the ADN study. The "no-effect" levels for ACN, PN, SN, ADN and ACY were 1038, 260, >110, 64 and 32 mg/m³ in air, respectively. These data indicate that the mono- and dinitriles exhibit greater toxicity with increasing chain length. ACY was determined to be the most toxic of the nitriles tested. This is probably due to its rapid dissociation to cyanide.


As part of a study to assess background exposure to benzene in populations not occupationally exposed, we have measured ambient atmospheric benzene levels at various sites throughout the San Francisco Bay area and have compared levels found to breath levels of 15 urban smokers and 15 urban nonsmokers. Breath samples were collected in 5 liter sample bags, internal standard (benzene-d3) was added and benzene was trapped on silica gel. Atmospheric samples (also 5 lt) were usually trapped directly using an air sampling pump. Water was added to the silica gel and headspace samples were analyzed by selected ion recording (at m/z 78 and m/z 81) gas chromatography mass spectrometry. Atmospheric benzene levels within the city of San Francisco were generally found to be in the 1 to 5 ppb range. Breath levels in urban smokers (7.3±3.3 ppb) and nonsmokers (2.5±1.0 ppb) were both found to be higher than ambient atmospheric levels at the study site on their respective study days (3.4 ppb and 1.4 ppb). Thus, although atmospheric levels are a major contributor to background benzene exposure, there may be other significant sources of exposure even for nonsmokers.


Two commercial dyes that can become airborne during manufacturing processes have been tested for their inhalation toxicity. The dyes are 2-(2'quinonyl)-1,3-indandione or solvent yellow (SY), and a 30/70 (w/w) mixture of SY and 1,4-di- p-toluidinonapthquinone or solvent green (SG). Fischer-344 rats were exposed 6 hr/day, 5 days/week for 4 weeks to 10, 50, and 220 mg/m³ of SY or the SY/SG mixture. The aerosol mass median aerodynamic diameter was 3 to 5 μm, Cg ≥ 2.0. SY cleared from the lungs rapidly and produced no histopathology at any exposure level. Analysis of bronchoalveolar lavage fluid (BAL) also indicated no lung damage. However, animals exposed to the highest level failed to gain weight at control rates. The SG portion of the SY/SG did not clear the lungs as rapidly as SY. BAL analysis and histopathology indicated a mild inflammatory response in the lungs in the high level exposed animals, and to a much lesser degree in the medium level animals. This inflammation was reflected in pulmonary function changes consistent with some airflow obstruction. High level animals failed to gain weight at control rates. Toxicity was not observed with either dye at the lowest exposure concentration. (Research supported by USAMRDL under IAA No. AT(29-2)-2138 with the U.S. DOE Contract No. DE-AC04-76EVO1013.)
Talc is used in a variety of manufacturing processes. To gain information on pulmonary retention from occupational exposure to talc during its preparation and use, Fischer-344 rats and B6C3Fl mice were exposed to talc by inhalation for 6 hours per day, 5 days per week for 4 weeks. The mass median aerodynamic diameter was 3.3 μm and 2.7 μm in rats and mice. For the rats, the actual exposure concentrations were 0, 2.3, 4.3 and 17 mg/m³ with lung burdens of 78, 190 and 818 μg tcalc/g lung. For the mice, the exposure concentrations were 0, 2.2, 5.7 and 20 mg/m³ with lung burdens of 120, 330 and 1200 μg tcalc/g lung. Lung burdens were generally proportional to inhalation exposure concentrations in both rats and mice at all exposure levels. Based on deposition and retention models, rats exposed to 2, 6 and 18 mg/m³ of talc for two years would be expected to have lung burdens near 0.15, 0.5, and 2 mg tcalc/g of lung, if clearance was unimpaired. Mice would have <30% higher lung burdens (0.2, 0.7 and 2.6 mg/g). These data were used to select target exposure levels for a 2-year chronic exposure study. (Research performed for the National Toxicology Program under Interagency Agreement 22-Y-01-ES-20088 under DOE Contract No. DE-AC04-76EV10103.)

Talcubotrol is a β2 adrenergic agonist used in the treatment of chronic obstructive lung diseases. The purpose of this study was to determine the potential toxicity of inhaled talcubotrol in rats and dogs. Rats were whole-body exposed to aerosol gravimetric concentrations of 0, 0.01, 0.02 or 0.1 mg/m³ of tcalcubotrol-hydrochloride, 60 minutes/day for 28 days whereas dogs were exposed (via insufflation) to estimated daily doses of 0, 0.2, 1.0 or 6.0 mg/kg for an equal period. No dose-related changes in body weight, food consumption, hematological or serum chemistry parameters were observed in either species. Histopathologic lesions were noted in the anterior section of the nasal cavities of rats exposed to 0.02 mg/m³ (24% incidence) and 1.1 mg/l (82% incidence) tcalcubotrol. These lesions involved the nasal septum and/or turbinates and/or the dorsal-lateral wall of the nasal cavity and consisted of necrosis, supplicative rhinitis and exudate. Similar changes were not present in rats allowed to recover for 2 weeks. No drug-related lesions were detected in lungs of either species. These results indicate that repeated inhaled talcubotrol was without toxicity in dogs and that local tissue injury caused by tcalcubotrol in the nasal cavity of rats was reversible.

Studies examining the inhalation toxicity of combustion products involve measuring or controlling a number of variables simultaneously, including incident heat flux, temperature, and sample weight. Further, most combustion toxicity methods require monitoring of gas concentrations and biological endpoints. A computerized data acquisition and control system using an Apple II Plus and a Fluke 2400A measurement control link was developed for the radiant furnace test method to assist in conducting experiments, recording data and processing stored information. Precalibrated signals were sent every 10 seconds from data storage through the control link to the lamps to produce the desired experimental incident heat flux. The specimen mass loss was recorded every 10 seconds via signals sent from a universal transducing cell. Concentrations of O₂, CO and CO₂ in the exposure chamber and temperatures were recorded every 10 seconds using data acquisition. The system provided real-time display of O₂, CO and CO₂ concentrations, remaining sample weight, furnace temperature and chamber temperature. Biological endpoint information can also be entered. Report generation software provides data tabulation, graphic display, and statistical evaluation of parameters.

Minimal scientific criteria were established for screening combustion product test methods. Of 14 published methods, the National Bureau of Standards Protocol (NBS) and the Pittsburgh Test were considered acceptable for general use. For each test, the samples were highly reproducible as were interlaboratory data. Worst case NBS data were compared with Pittsburgh data. For 8 synthetic products (CPVC pipe, PTFE coated wire, ABS pipe, PVC conduit, polyurethane foam, PVC wall cover, nylon carpet and THHN coated wire) the values from the two tests were statistically indistinguishable. Cellulosic products (cotton fabric, wood ceiling tile, reprocessed paper, and Douglas Fir) differed by more than a factor of 4 in the two tests. Values from 9 products containing both natural and synthetic materials were more divergent than pure synthetic and less than cellulosic. It appeared that products which formed a char layer upon thermal decomposition were more favorably evaluated in the Pittsburgh test than the NBS test. Nonchar forming materials performed the same in both tests.


Investigations on the inhalation toxicity for rats of PTFE combustion-pyrolysis products have demonstrated that the particulate fraction (0.02 to 0.4 X 10^-6 m) produced from 430°C and above is the primary cause of toxicity. The inhaled particulate would be ingested by lung macrophages and possibly affect phagocytic competence. This was the objective of the present study. We also compared lethality from inhalation of PTFE fumes, filtered and non-filtered, in rabbits and in Fisher 344 and Sprague-Dawley rats. Rabbits and rats were exposed for 1 hr to fumes from PTFE heated at 430°C (0.1 mg/lit) to 450°C (2.0 mg/lit). Concentrations at 1.0 mg/lit and above proved lethal for all rats and half of the rabbits. Lung injury was edema and hemorrhage. Surviving animals showed labored respiration, lost body weight the first 24 hr and then regained at the control rate. Filtration of fumes generated at 450°C prevented mortality. Lung lavage fluid from surviving rabbits removed lung macrophages which were then incubated with 85strontium labelled microspheres (2.0-4.0 um) at post-exposure times of 15 min to 3 hr. Inhibition of phagocytes was evident at concentrations of 1.0 and 1.2 mg/liter up to 2 hr incubation. By 3 hr phagocytic competence was restored.


As contrasted with nonchar forming materials which appear to be equal in potency when measured by either of two combustion toxicity tests, values for combustion product potency measured by the Pittsburgh test (mice, dynamic exposure, ramped temperature 20°C/min) showed char forming materials less toxic than was indicated by the National Bureau of Standards (NBS) test (rat, static exposure and temperature). Wood, cotton, phenol formaldehyde and a char forming polyurethane foam were tested under a variety of temperature profiles. When the furnace of the Pittsburgh apparatus was held at a constant temperature, the LC50 value remained higher (less toxic) than the NBS worst case. When the NBS furnace was ramped at 20°C/ min, the LC50 value remained close to the NBS worst case LC50. In addition, when mice were used instead of rats in the NBS test, the LC50 for these test materials did not shift. Thus, neither species sensitivity nor furnace temperature profile explains the differences in measured potency for char forming material in these two tests.


Combustion toxicology studies have focused on obtaining LC50's and documenting acute respiratory and behavioral effects. Dose-response curves are generally very steep, and investigators are often concerned only in identifying potentially hazardous materials. Thus the radiant furnace test method was used to develop an approximate lethal exposure [AL(Ct)50] procedure. Groups of 3 animals were exposed for 30 minutes to smoke concentrations until 0%, and 33 or 67% mortality response groups were generated. A 100% lethal response group was recorded either by biological testing or by exceeding preset chemical criteria for CO (6000 ppm) or HCN (200 ppm). AL(Ct)50's were exposures producing 33 or 66% mortality response groups. The AL(Ct)50's obtained at 2.5 W/cm² heat flux in (mg/L) - minutes were 2356.5 for Douglas fir (D. fir), 1537.7 for D. fir plywood, 1906.8 for oriented strand board (OSB), 488.8 for rigid polyurethane foam (PU), and 1854.0 for acrylic rug (AR). The AL(Ct)50's obtained at 5.0 W/cm² in (mg/L) - minutes were 2903.0 for D. fir, 2698.7 for D. fir plywood, 2593.7 for OSB, 804.2 for PU, and 571.0 for AR. This screening procedure can be used as a basis for further investigations.

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Formaldehyde (HCHO) pretreatment of Fischer-344 (F-344) rats induces significant cross-tolerance to the sensory irritation properties of Cl₂. To determine if HCHO pretreatment would cause cross-tolerance to other aldehydes, whether they be saturated, cyclic, or unsaturated, male F-344 rats, weighing 190 - 210 g, were pretreated with 15 ppm HCHO, 6 hr/day for 9 days, and then challenged with a given aldehyde on the 10th day. To quantify the sensory irritation response in these animals, respiratory rate depression was measured in a head-only inhalation chamber using plethysmographic techniques. With the end-point response remaining the reflex decrease in respiratory rate, control animals, weighing 220 -270 g, were challenged identically without prior pretreatment. In naive animals, the concentration eliciting a 50% decrease in respiratory rate (RD₅₀) was 23 ppm or less for unsaturated aliphatic aldehydes. For cyclic and saturated aliphatic aldehydes, the RD₅₀ ranged from 600 - 1000 ppm and 3000 - 6800 ppm, respectively. Formaldehyde pretreatment resulted in cross-tolerance only with acetaldehyde (RD₅₀ increased 3.5-fold) and acrolein (RD₅₀ increased 5-fold). These studies indicate that the development of cross-tolerance following HCHO pretreatment is not a general phenomenon.

AUTORADIOGRAPHIC ASSESSMENT OF THE DISPOSITION OF DIFFERENT FORMS OF INTRATRACHEALLY ADMINISTERED CADMIUM IN RAT LUNG. K. Aihara, B.P. Sharma, and J.L. Shupe. Toxicology Program, Utah State University, Logan, UT

The half-life of the pulmonary clearance of soluble cadmium closely resembles that of insoluble form after intratracheal (IT) instillation. However, it has been suggested that IT instillation, especially in smaller volumes, results in uneven distribution, and in greater deposition in the lower respiratory tract as compared to inhalation. The present autoradiographic study was undertaken to compare the initial distribution patterns of soluble and less soluble forms of cadmium in the rat lung after IT instillation. Male Sprague-Dawley rats were divided into two groups, each group received either soluble or a less soluble cadmium (¹⁰⁹Cd) IT in 0.1 ml buffered saline. At 5, 30 and 90 min post-institution, rats were sacrificed and processed for autoradiography, and radioactivity of lung sections. ¹⁰⁹Cd was unevenly distributed in the lungs at 5 min for both forms of Cd. At 90 min post-instillation, ¹⁰⁹Cd was almost evenly distributed in the lungs instilled with soluble form, while in case of the less soluble form a spotty distribution of ¹⁰⁹Cd in bronchi was observed. ¹⁰⁹Cd was mainly translocated to liver and stomach, followed by kidney and intestine. Results indicated that the initial translocation of instilled ¹⁰⁹Cd from lung is relatively slow in the case of less soluble form as compared with the soluble form.

EVALUATION OF PRESSURE-VOLUME AND FLOW-VOLUME LOOPS TO DIFFERENTIATE BETWEEN OBSTRUCTION AND RESTRICTION, Schaper, M., Thompson, R.D. and Alarie, Y., Dept. Ind. Env. Hlth. Sci., Univ. of Pittsburgh, Pittsburgh, PA

A previously described method permits continuous measurement of airflow (V), inspired volume (VI), and plethysmographic pressure changes, (ΔP). To obtain such measurements, each animal was fitted with a head chamber and positioned in a whole-body plethysmograph. For the control, animals inhaled room-air, when placed in this system, and were then exposed to 10% CO₂ mixtures (18% O₂, 72% N₂) which induced significant and stable increases in VI and ΔP. When aerosols of histamine, carbarylcholine, serotonin, propanolol or sulfuric acid were added to CO₂ mixtures, declines in VI were evoked which were dependent on aerosol concentration. Utilizing this approach, the effects of carbarylcholine, serotonin, and propanolol on pressure-volume and flow-volume loops were examined. The aerosols consistently evoked reductions in VI, although two types of response patterns were identified. The first category, described as obstruction, was characterized by decreases in VI and respiratory frequency (f). Interruptions in expiratory flow occurred and resistance to airflow increased which resulted in greater work of breathing. The second category, termed reflex restriction, was also characterized by decreases in VI, but f increased significantly. Supported by NIHES Grant 1 R01-ES0247.


Three fractions of amosite fibers with different lengths and diameters - fine (F), medium (M), and coarse (C) - were prepared and intratracheally instilled into rats to study the inflammatory cell response and changes in epithelial permeability in the lung. Rats were instilled with 0, 100, or 500 μg of each fraction suspended in saline and sacrificed one day later. After dosing with 500 μg, numbers of peroxidase positive macrophages (PPMs), polymorphonuclear cells (PMNs), and lymphocytes increased and alveolar macrophages (AMS) decreased in the M- fraction group. The C- fraction group showed a decrease in AMS and a smaller increase in PMNs, while the F- fraction group showed no significant changes. Lung permeability was significantly increased only in the M- and C- groups. After dosing with 100 μg, PPMs and PMNs increased to a smaller degree in the M- and C- groups, while lung permeability increased significantly only in the C- group. A separate study evaluating the effects after 30 days with 500 μg showed a return of cell numbers to normal levels, but an increase in cellular uptake of ⁶⁷Ga-citrate, a marker for inflammation, was evident in the M- and C- groups. The results indicate a size-dependent toxicity of amosite fibers. A correlation was noted between numbers of lavable PMNs and increase in epithelial permeability and may indicate a causal relationship.
133 STEREOREALYSIS OF THE BHT-DIQUAT INTERAC-
TION IN MURINE PULMONARY ALVEOLUS. P.A. Coulombe,
P.R. Filion and M.G. Côté, Dépt. de pharmacologie,
Université de Montréal, Montréal, Canada.

In this study the reparative processes in the
mice lung alveoli are studied following an experi-
mental acute injury, using stereological methods.
Swiss-Webster mice were treated with Butylated
hydroxytoluene (400 mg/kg i.p.) and sacrificed at
times of 1, 3, 5, 7 and 14 days thereafter; 24 h
prior to each sacrifice, the herbicide diquat was
administered (4 mg/kg i.p.) as a challenge to the
ongoing reparative events in the pulmonary alveo-
li. A morphometric analysis was performed on
CM-A-embedded histological sections, with special
emphasis on the inflammatory and epithelial re-
generative components of the reaction. Following
BHT, type I epithelium damage and necrosis are
evidenced by its decreased volumic (Vv) and sur-
face (Sv) densities. Epithelial regeneration
ensues, through a biphasic pattern of prolifera-
tion and differentiation of the pneumocyte II;
proliferative peaks occur at days 3 and 7. The
inflammation is shown by the increased numerical
(Nv) and volumic densities of alveolar macro-
phages at days 3 through 14; PMN neutrophils also
increase, but at days 3 and 7 only. The pinpoint
administration of diquat at critical times of
alveolar regeneration induces some modifications
in the proliferative kinetic of these cell types.
These results further document the homostatic
mechanisms of regeneration in a situation of
toxic alveolar injury.

Supported by the MRC of Canada et l’IRSSS, Québec.

135 Effect of Methylcyclopentadienyl Manganese Tri-
carbonyl (MMT) on Systemic and Pulmonary Hemody-
namics. D. Cox, D. Penney, R. Gaddis, J. Orr, R.
Hanzlik and G. Traiger. Dept. of Pharmacol. & Tox-
icol. and Med. Chem., School of Pharmacy, Univ.
of Kansas, Lawrence, KS 66045.

Administration of MMT to laboratory animals
produces convulsions accompanied by hemorrhagic
pulmonary edema. Manifestations of toxicity appear
similar to that of convulsant agents which cause
neurogenic pulmonary edema (NPE). NPE is associ-
ated with a centrally-mediated sympathetic discharge
leading to elevated pressure in the pulmonary vas-
cular beds. Studies are directed to the
effect of MMT on the systemic and pulmonary blood
pressures and examine the role of the sympathetic
nervous system in MMT-induced pulmonary injury.

Hemodynamic changes were studied in conscious
dogs following IV dosing (2.5, 5, 15, 30 mg/kg). MMT
produced a dose related increase in systemic and
pulmonary arterial blood pressures. Pressures re-
mained elevated for 1 min after the lower doses,
higher doses resulted in marked increases for a
15 min period. Wedge pressure was significantly
increased after 15 and 30 mg/kg. Plasma epinephrine
and norepinephrine levels were markedly increased
1 min after the 15 and 30 mg/kg doses. MMT in-
creased systemic arterial blood pressure in con-
scious rats. Treatment of rats with phenoxybenz-
amine or dihydrothreter protected against the occu-
rence of MMT-induced pneumotoxicity. The data sug-
gest that MMT-induced pneumotoxicity is mediated
in part via the sympathetic nervous system and is
similar to that of NPE. (Supp. by Ethyl Corp.)

136 EARLY INDICATORS OF PULMONARY CHANGE
INDUCED BY COMBUSTION-GENERATED PAR-
TICULATES. S.J. Stoner, J.W. Clayton and S.E.
Wilson, University of Arizona, Tucson, AZ

Changes in lung lavage fluid parameters were
used to assess pulmonary toxicity of combustion-
generated particulates. Male Sprague-Dawley rats
were dosed by intratracheal instillation with saline
suspensions of diesel and polyurethane foam
combustion-generated particulates, gallium oxide
and silica (comparative controls), and saline (vehicle
control). Animals were terminated and lungs lavaged
with cold 0.9% saline at 1, 4, 7, 20 and 60 days post-
exposure. Lung lavage fluid was assayed for lactate
dehydrogenase, acid and alkaline phosphatase, and
total soluble protein. In addition, at 60 days lung
tissue was homogenized and analyzed for protein,
collagen and DNA content, wet and dry lung and
body weights were obtained, and sections taken for
histopathological evaluation. The combustion-
generated particulates induced little change in lung
lavage fluid parameters from the vehicle controls.
Silica produced the greatest elevation, followed by
gallium oxide. The 60-day lung tissue biochemical
analysis, lung and body weight ratios, and histo-
pathology confirmed the pulmonary changes
indicated by early elevations in lung lavage fluid
parameters. The model developed by these methods
is adequate to assess short- and long-term effects of
these types of particulates on the lung.

134 COLLAGEN METABOLISM IN NORMAL AND DAMAGED
MOUSE LUNG TISSUE FOLLOWING TREATMENT WITH
PREDNISOLONE. J.P. Ehrlich, Division of
Pharmacology & Toxicology, College of Pharmacy, The University of
Texas, Austin, TX 78712-1074.

Treatment of mice with high doses of prednisolone on Days 1-5 after
the induction of lung damage with butylated hydroxytoluene (BHT) enhances
the development of fibrosis within 3 weeks. The conversion of [3H]
proline to acid-insoluble (net collagen synthesis) and acid-soluble
(degradation of newly synthesized collagen) [3H]hydroxyproline was used to assess the changes in collagen metabolism that accompany this
lesion. The rate of collagen synthesis was significantly less on Day 3 in
lung tissue from BHT-prednisolone treated mice compared to BHT alone.
On Day 7 (two days after steroid treatment ceased) there was a
significant increase in the rate of collagen synthesis in BHT-prednisolone
treated mice, compared to both BHT and oil-treated controls. This
increase was maximal on Day 11 and persisted to Day 14. Compared to
oil-treated controls, the rate of collagen synthesis in lung tissue from
mice treated with BHT alone was increased at all times. The percentage
of total protein synthesis committed to collagen was increased from 2 to
4-fold in lung tissue from BHT-prednisolone treated mice compared to
both oil- and BHT-treated controls. A significant increase was evident
at all times and was highly specific for collagen since non-collagen
protein synthesis showed few changes. The percentage of newly
synthesized collagen that was degraded in lung tissue from
BHT-prednisolone treated mice was less than BHT-alone on Days 7 and
11, and than oil-prednisolone on Day 14. These results confirm the
inhibitory effects of steroid treatment on collagen synthesis during the
course of therapy. However, when a high-dose, short-term treatment
regimen is used following the induction of lung damage with BHT there is
an increase in collagen synthesis and a decrease in the degradation of
newly synthesized collagen after steroid therapy is stopped. (This work
was supported by NIH grant HL 29463 and by the Gustave and Louise
Pfaffler Research Foundation. J.P.E. is the recipient of NIH Research
Career Development Award H. 01435.)
137 INDUCTION OF HEPATIC MICROSMAL ENZYMES IN COTTON RATS (Sigmodon hispidus). J.H. Watkins & P.I. Krausa. Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN. 

The effect of microsomal enzyme inducers on bio-transformation was assessed in male cotton rats from Kansas (KSh) or Tennessee (TSh) pretreated daily for 4 days with either 3-methylcholanthrene (3-MC, 20 mg/kg), phenobarbital (PB, 75 mg/kg), pregnenolone-16 alpha-carbonitrile (PCN, 75 mg/kg) or corn oil (5 ml/kg). Benzphetamine N-demethy lase (15 nmol/min/mg protein) and styrene oxide hydroxase (3 nmol/min/mg) were induced by 75% and 60%, respectively, after PB administration. In contrast, the enzymes catalyzing phase II reactions were not inducible. GSH S-trans ferase activity toward chloroform nitrobenzene, ethacrynic acid and sulfobromophthalein was reduced by 25% in TSH rats treated with 3-MC. UDP-Glucuronosyltransferase activity in KSh toward 1-naphthol, morphine, diethylstilbestrol, estrone and testosterone was 5, 0.95, 0.60, 0.01 and 0.04 nmol/min/mg, respectively. Activity toward these substrates was about 2 fold higher in TSH. Sulfortransferase activity toward 2-naphthol was 0.10 and 0.24 nmol/min/mg in TSh and KSh rats, respectively. These data indicate that usually effective doses of microsomal enzyme inducers do not increase the activity of conjugative enzymes in Sigmodon hispidus. (Supported by a PHA Foundation 1984 Research Starter Grant).

139 MIXED FUNCTION OXIDASE INDUCTION AND TOXIC EFFECTS OF THREE COMMERCIAL PCBs AND SELECTED CONGENERS IN THE AVIAN: D. Jones, T.W. Sawyer, K. Rosanoff and S. Safe, Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

The avian appears to be one of the most susceptible species to the adverse effects of PCBs. The sensitivity of White Leghorn cockerels to i.p. injections of Aroclor 1016, 1242, 1254 and selected congeners has been studied with respect to the induction of cytochrome P-450 dependent mixed function oxidases. Induction of the Bursa of Fabricius was also examined as a measure of toxicity. Ethoxyresorufin-O-deethylase (EROD), aryI hydrocarbon hydroxylase (AHH), and aminopyrine N-demethylase were all found to be increased by Aroclor 1254 and 1242. Aroclor 1016 which is a relatively poor inducer of EROD and AHH in the rat dramatically increased these enzymes in the chicken. The PCB congener 3,4,3′,4′, was found to increase aminopyrine N-demethylation in the avian but not in the rat while 2,4,5,2′,4′,5′ was found to increase both EROD and AHH activity in the avian but had little effect in the rat. Induction of the Bursa of Fabricius was found to occur following administration of Aroclor 1254, 1242, 3,4,3′,4′, and 3,4′,2′,3′,4′,5′, but not following Aroclor 1016 or 2,4,5,2′,4′,5′. (Supported by the Texas Agricultural Research Station No. H-6617 and the Texas A&M Organized Research Reserve No. 16-84).

138 HEPATIC EFFECTS OF INTERFERONS IN MALE CD-1 MICE. M.R. Franklin, Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112, U.S.A.

Hepatic effects of multiple (1-5) daily i.p. injections of E. coli derived murine γ and human leukocyte hybbrid (aAIP:Bgl) interferons (IFN) at doses of 1 x 10^9 units/kg were investigated 24 hr after the last dose. Elevated serum ALT values were observed with both interferons, more so with IFN α than IFN γ. The toxicity increased with the number of daily injections. Accompanying the toxicity was a decrease in microsomal cytochrome P-450 concentration (50% after 5 days of daily IFN αAIP:Bgl) and an associated activity (p-nitroanisole N-demethylation) but not in the NADPH-cytochrome c reductase activity. There was no concomitant change in microsomal UDP-glucuronosyltransferase activity (p-nitrophenol:acceptor) or in the cytosolic GSH-S-transferase activity (1-chloro-2,4-dinitrobenzene; acceptor) while producing no change in liver weight for up to 3 daily injections the interferons caused an increased yield of microsomal protein. This effect appeared to offset the decline in cytochrome P-450 concentration since no change in hexobarbital sleep time was observed. By five daily injections decreases in both cytochrome P-450 concentration and microsomal yield resulted in an increase (40%) for IFN αAIP:Bgl in hexobarbital sleep time. This study was supported by Genentech Inc.

140 THE METABOLISM OF BENZ[a]PYRENE BY PCB- AND PBB-INDUCED MICROSMAL ENZYMES: J. Haake, J. Merrill, and S. Safe, Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

The regioselectivity and rate of benzo[a]pyrene metabolism by halogenated biphenyl-induced rat hepatic microsomes was directly related to the formation of 4,5-dihydro-4,5-dihydroxybenzo[a]pyrene; in contrast, microsomes from MC and 3,3′,4,4′-tetrachlorobiphenyl pretreated rats greatly increased the formation of the 7,8- and 9,10-dihydrodiol metabolites of benzo[a]pyrene, while substantially inhibiting microsomal formation of the 6,12-quinone metabolite of this hydrocarbon. Mixed-type inducers caused substantial increases in the formation of all the benzo[a]pyrene metabolites. The results confirm the utility of benzo[a]pyrene as a substrate for determining monooxygenase induction patterns. (Supported by the Center for Comparative Medicine and the National Institutes of Health)

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STEREOSELECTIVE GLUCURONIDATION IN INDUCED RABBIT HEPATIC MICROSOMES. B.L. Finley and G.S. Yost, College of Pharmacy, Washington State University, Pullman, WA

Steroselective glucuronidation of the chiral drug oxazepam by hepatic microsomes from rabbits pretreated with various inducers was used to characterize induced UDP-glucuronosyltransferase enzymes. Hepatic microsomes from induced and control animals were incubated with racemic oxazepam and the activator, Lubrol PX. Relative amounts of the diastereomeric β-glucuronides were determined by HPLC. Significant differences were found in enantiomeric selectivity among the microsomes from rabbits pretreated with different inducers. Differences between GT and GT, forms were indicated since inducers of GT forms usually caused large changes in enantiomeric preference (control R/S = .76, α-naphthoflavone-induced R/S = 1.41) without inducing the rate of formation of the glucuronide diastereomers. Conversely, inducers of GT, forms produced no changes in stereoselectivity yet caused increases (30% over controls for phenobarbital) in the activities. Thus, enantiomeric preference in the glucuronidation process provides a useful tool in the characterization of inducers.

Supported by the State of Washington Initiative Measure No. 171.


The major metabolic pathway of the organochlorine pesticide, chlordecone (CD), both in man and in the gerbil, involves bioreduction of CD to chlordecone alcohol (CDOH) in the liver. We incubated cytosolic extracts of gerbil and human liver with CD and NADPH and found formation of CDOH in vitro. The reaction follows typical enzyme kinetics (K =26 μM, v = 0.14 mmol/min) and proceeds optimally at pH 6.5. "CD reductase" appears to be a member of the "aldo-keto reductases" in that it is a cytoplasmic enzyme, is highest in activity in the liver and kidney, requires NADPH, is sensitive to thiols reagents and reduces a carbonyl containing substrate to its corresponding alcohol. Moreover, a purified enzyme preparation had an apparent molecular weight of 41,500 as judged by SDS-polyacrylamide gel electrophoresis. However, unlike typical "aldo-keto reductases", CD reductase is not found in rats, mice, hamsters or guinea pigs and is insensitive to the model aldehyde and ketone reductase inhibitors phenobarbital and quercetin. In addition, CD reductase activity in liver was increased by 30X following treatment of gerbil with CD. We conclude that CD reductase is a novel aldo-keto reductase that is uniquely inducible by its substrate.

EFFECT OF GLUTATHIONE DEPLETION ON THE PHARMACOKINETICS AND METABOLIC FATE OF ACETAMINOPHEN IN THE RAT. R.E. Galinsky, Department of Pharmacetics, College of Pharmacy, University of Utah, Salt Lake City, UT. Sponsor: M.R. Franklin

Acetaminophen (A) overdose produces acute hepatic and renal necrosis following glutathione (GSH) depletion. Large doses of A concomitantly saturate glucuronide and sulfate conjugate formation. Sulfation of A is rate- and capacity-limited by the availability of inorganic sulfate which derives mainly from the oxidation of cysteine, the rate-limiting precursor for GSH. This study examined the effect of GSH depletion on the elimination kinetics of A. Pretreatment with diethylmaleate (DEM), 3 mmol/kg ip at minus 30 min significantly decreased the total plasma clearance of A following either an iv dose of 150 mg/kg (6.4 vs 4.2 ml/min/kg) or 30 mg/kg A (30.6 vs. 19.3 ml/min/kg). GSH depletion decreased the fraction of dose recovered in urine as A-Sulfate following 150 mg/kg A only. The partial clearance and recovery in urine of A to A-glucuronide was unchanged by DEM pretreatment, however, the partial clearance of A to A-sulfate was significantly decreased. Decreased sulfation occurred in the absence of inorganic sulfate depletion. The results suggest that GSH is not an important source of inorganic sulfate and unless DEM has a direct effect, these results suggest that altered GSH status affects either PAPS formation or sulfotransferase activity.

MONOCHLOROACETIC ACID: TISSUE DISTRIBUTION IN MICE AND RATS. M. R. Berardi, K. R. Cooper, and R. Sneider, Joint Graduate Program in Toxicology, Rutgers University and UMDNJ/Rutgers Medical School, Piscataway, NJ 08854.

Upon observing that monochloroacetic acid (MCA) causes front paw paralysis in mice but not in rats, we studied the tissue distribution of radioactivity in mice and rats following the administration of 1-14C-MCA. Male mice were orally administered a tracer dose, qg LD01 (150 mg/kg), or an LD40 (250 mg/kg) of 1-14C-MCA (1.0 μCi), while male rats received a tracer dose (1.0 μCi). Groups of five or six animals were sacrificed over 48 hours for assay of 14C in plasma, liver, kidney, spleen, heart, lung, and testis, and in 5 brain regions. In both mice and rats radioactivity was persistent in brain tissue, and monophasic elimination rates were 40-50% greater in cerebellum than in hypothalamus, brainstem, cerebral cortex, and whole brain. In the rat but not the mouse, hypothalamic 14C reached higher concentrations than the rest of the brain. Elimination from the remaining mouse tissues was biphasic, while elimination from rat tissues was monophasic and very rapid. Histologic examination of brains from mice treated with a single oral dose of 380 mg/kg MCA reveals loss of Purkinje cells in the cerebellum. In conclusion, the front paw paralysis observed in mice may be related to persistence of MCA in the cerebellum with damage to neurons involved in motor function.

Since the irreversible events leading to the development of Organophosphorus-induced Delayed Neuropathy may take place within a few hours after exposure, the early events in the distribution of neurotoxicants may be the most critical. Previous work has suggested that the delayed neurotoxicant leptophos undergoes biphasic elimination from the hen, with a bimodal half-life of 13 days. Colostomized hens were treated i.v. with neurotoxic doses of $14C$-leptophos or desbromoleptophos and sacrificed after 24 hours. Through intensive early blood sampling the alpha phase, previously designated as distribution into the tissues is shown to be itself biphase and consist of a considerable amount of elimination as well as distribution. The majority of the radioactivity excreted in 24 hours was in the urine, with only small amounts excreted in the feces. Much of the radioactivity in the urine is present in the form of water-soluble metabolites.

ENHANCED LIVER UPTAKE OF LIPOSOMES DUE TO INCORPORATION OF DIGALACTOSYLDIGLYCERIDE (DGDG) IN THE LIPID BILAYER. D. B. Mitchell, T. Baker, C. Marlowe, and W.J. Waddell. Procter & Gamble Company, Cincinnati, OH and University of Louisville, Louisville, KY.

Experiments were designed to investigate the effect of DGDG incorporation into vesicle formulations of cholesterol, dipalmitoyl lecithin, and sphingosine to target the vesicle to galactose receptors on the hepatocyte and allow the hepatic specific delivery of encapsulated drugs. $14C$-carboxynulin (C1) was used as an encapsulated marker. Rats were injected through the tail vein and sacrificed at 15, 30, 60 120 and 240 minutes postinjection. Liver uptake of the 0% DGDG preparation was 20% of total $14C$ dose by 60 minutes postinjection. 40% of the dose was recovered in the liver 60 minutes postinjection of the 4% DGDG vesicle preparation. The 15% DGDG vesicles were cleared more rapidly from the blood with $<65%$ of the label recovered in the liver 60 minutes postinjection. Autoradiographic experiments were performed in mice dosed with the 15% DGDG preparation. The total liver uptake 60 minutes postinjection was marked and represented 80% of the injected dose. Additional experiments were performed to determine the hepatic cell type responsible for the rapid clearance of the 15% DGDG vesicles. Radiochemical analysis of the liver cell fractions indicated that hepatocytes were primarily responsible for the hepatic clearance. Hepatic-specific delivery of drugs is attainable.


Theobromine (TBR) absorption, metabolism, and toxicity were evaluated in weanling male Sprague-Dawley rats fed various fiber diets. Five types of diets with or without 0.6% TBR were fed for 28 days: standard 22% casein (2% cellulose) (S), S + 5% cellulose (C), S + 16% oat bran (5% crude fiber), S + 5% pectin (P), and control (5% crude fiber). Body weight, weight gain, and food intake were decreased in TBR fed rats with the largest effects present after S + P, S + C, and S diets. Testicular atrophy was induced by TBR in all diets except chow, which corresponded with the lowest serum TBR level. Thymic atrophy occurred in all TBR fed rats. Rats dosed with 5 mg/kg TBR (10 μCi $8^{14}C$-TBR) were placed in metabolism cages for 48 hr. Fecal $14C$-TBR elimination was generally reduced in rats fed TBR. Urine metabolites were determined by HPLC with radioactivity detection. Less unchanged TBR (24 hr) was excreted in TBR fed rats (20.2 - 53.8%) than those fed diets without TBR (37.3 - 73.3%). Increased amounts of 6-amino-$5N$-methylformylamino-$1$-methyluracil, 7-methyluric acid, 7-methylxanthine, 3-methylxanthine, and 3,7-dimethyluric acid were detected. These data show induction of TBR metabolism in rats consuming TBR at 250x maximum human consumption. Also, diet composition directly influences serum levels of the test compound, an effect which can modify toxicity.


In order to elucidate the metabolism and toxicity of gentian violet (GV), a mycostat used in poultry feed, the metabolite profiles were determined in tissues and excreta of rodents. Fischer 344 rats and B6C3F1 mice of both sexes were gavaged for 7 days with $14C$-GV. Feces, liver, kidneys, gonads, muscle tissue and fat were collected. Metabolites in extracts were resolved by chromatography in two different hplc systems and confirmed by mass spectrometry and chromatography with authentic standards. Demethylated dye metabolites of $14C$-GV, which had previously been identified by in vitro microsomal incubations, were found in feces and in solid tissues of both rats and mice. The reduced metabolite leuco gentian violet (LGV) was identified in feces and liver of both species. Ratios of the demethylated homologs of LGV, leuco tetramethylpararosaniline (LTM) and leuco pentamethylpararosaniline (LPM) were different in fat of male and female rats, perhaps indicative of the female/ male ratio of 5 in total residue. These differences were less pronounced in mice. LGV, LTM, and LPM were the only metabolites found in fat. These results show the complexity of dye metabolism in mammals and present us with a number of metabolites of possible toxicological significance.
104  EFFECTS OF BUTYLATED HYDROXYANISOLE (BHA) ON HEPATOBILIARY DISPOSITION OF AFLATOXIN B_1 (AFB) IN THE RAT. D.H. MONROE, C.J. HOLESKI AND D.L. EATON, DEPT. ENVIRONMENTAL HEALTH, UNIVERSITY OF WASHINGTON, SEATTLE, WA.

BHA and related dietary antioxidants reduce the carcinogenic activity of a variety of chemical carcinogens. To differentiate between a direct antioxidant effect and enzyme induction effects, BHA was administered to rats for 9 days (500 mg/kg/day, s.c.) or a single time (500 mg/kg p.o.). Eleven days (long-term, LgT) or 4-7 hrs (short-term, S-T) following BHA, "L-AFB (0.25 mg/kg, 30 μCi/kg) was given i.p. Bile was collected at 0.5 hr intervals, and the liver was removed and homogenized after 2 hrs. L-T treatment with BHA increased bile flow to 200%, glutathione S-transferase (G-S-Tase) activity to 165%, and epoxide hydrolase (EH) to 230% of control values. No residual BHA was detected in livers of L-T rats. L-T treatment reduced the amount of AFB remaining in the liver to 50% of control and reduced binding to DNA to 16% of control. S-T treatment increased binding, but had no effect on biliary excretion or binding of AFB to hepatic macromolecules, even though high concentrations of BHA remained in the liver. These results suggest that the anticarcinogenic effect of BHA may be due to an induction of protective enzymatic pathways such as G-S-Tase and/or EH, which act to reduce the amount of AFB-epoxide available for binding to DNA.
(Supported by Am. Cancer Soc. grant IN-26Y).

161  ACCUMULATION OF ETHYLATED GUANINES IN DNA OF HAMSTER ORGANS DURING CHRONIC DIETHYLAMINE (DEN) EXPOSURE. A.T. Fong and R.E. Rasmussen, Dept. of Community and Environmental Medicine, University of California, Irvine, CA 92717. Sponsor: A.R. Buckpitt.

The accumulation of 6-ethylguanine (6E G) and 7-ethylguanine (7E G) in lung, liver, kidney and tracheal DNA was determined in hamsters exposed chronically to DEN (20 mg/kg BW, SC 2/Wk for up to 8 wk). This exposure schedule has been shown to be strongly carcinogenic for the respiratory tract. Liver tumors have also been reported following this treatment. Ethylated guanines in DNA, measured at 24 hr after the SC injections were fractionated by HPLC and quantitated optically by fluorescence spectrophotometry. The concentration of 6E G in DNA increased from single injection levels (liver, 85 μmol/mol G; lung, 42 μmol/mol G; trachea, 65 μmol/mol G) to maximum levels at wk 8 (liver 565 μmol/mol G; trachea, 502 μmol/mol G) or wk 4 (lung and trachea 50 μmol/mol G). 6E G concentrations measured at 3 hr after the SC injections were at least 10X higher than at 24 hr, suggesting the active removal of 6E G from DNA. No accumulation of 7E G was seen in liver, lung or tracheal DNA. Neither 6E G nor 7E G was detected in kidney DNA during the exposure period. The result supports the hypothesis that 6E G, a mis-coding DNA base, accumulates in the target organs of DEN carcinogenesis. (Supported by Air Force Contract F 33615-80-C-0512.)

150  CUMENE HYDROPEROXIDE (CYP) MEDIATED DENITRIFICATION OF 2-NITROPROPANE (2NP) IN UNINDUCED MOUSE LIVER MICROSOMES. E.K. Marker, A.P. Kulkarni, Toxilogics Program, Dept. Environ. and Industrial Health, The University of Michigan, Ann Arbor, MI 48109.

The metabolism of 2NP to nitrite and acetone has been reported previously in an NADPH dependent microsomal reaction (Ullrich, et al., Bioch. Pharmacol. 27:2301, 1978). The involvement of cytochrome P-450 (P450) was clearly shown but the oxidative/reductive nature of the reaction was unresolved. We have investigated peroxide mediated metabolism of 2NP and report, for the first time, oxidative denitration of 2NP in CYP supplemented CO-1 mouse male liver microsomes.

An enzyme mediated process is supported by a decrease in nitrite release to background levels in the presence of boiled microsomes and by linearity of the reaction with time and protein concentration. Two pH optima at 7.6 and 8.6 were apparent differing from each other in optimum substrate concentration suggesting involvement of at least two P450 isozymes. Response to various modifiers of P450 function were also examined. N-octylamine was the most effective inhibitor at both pis. Imidazole, alpha-naphthoflavone and metyrapone were ineffective. Differential patterns of inhibition dependent on pH were seen for SKF 522A and butylated hydroxyanisole providing further support for the involvement of multiple isozymes. The reaction does not appear to involve a chain reaction mechanism.


Recently, a high incidence of mesothelioma, attributed to zeolite (erionite), was reported in Turkey. Zeolite, also found in the Western United States, is an industrial mineral whose threat to human health is not fully recognized. Alarming results are noted from the preliminary evaluation of the ongoing study. A single intrapleural (IP) injection of 0.5 mg of erionite to Fischer 344 rats produced mesothelioma within one year. At 9 mg the latent period was 8 months and out of 22 animals that died 16 had well-differentiated mesotheliomas. No tumors were observed in the amosite- and crocidolite-treated rats nor in 8 of 10 chrysotile-treated rats. In comparison, our earlier studies showed that at a high dose of 20 mg amosite, the average latent period was 26 months. In vitro preparations of these sarcomatous and epitheliod mesotheliomas showed both cell types persisted for 9 or more passages. Cytogenetic analyses indicated a significantly high number of polyploid cells in erionite but not in chrysotile tumors. In the V79 and CHO cytotoxicity assays, however, erionite did not show higher toxicity, as might have been expected from the in vivo studies, suggesting different mechanisms of action in vivo and in vitro systems.
Protein synthesis in tracheal explants from mice treated with the chemical irritant and tumor promoting agent 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was monitored using one and two dimensional polyacrylamide gel electrophoresis (PAGE). Mice were treated with TPA (40 mg) by intratracheal instillation. 24 hr later the mice were sacrificed and the tracheas excised and cultured in medium containing 35S-methionine. After 4 hr the explants were rinsed and lysed for PAGE. One dimensional gels of the tracheas revealed 12-14 major bands of protein from both control and TPA treated animals. After protein blotting and using an antikeratin antibody and immunoperoxidase staining, three of the bands in each of the gels were identified as keratins. TPA treatment of the mice resulted in a decrease in the production of two non-keratin protein bands (MW 45kd and 70kd). Using two dimensional PAGE over 100 individual proteins from the tracheas could be resolved. At least six of these proteins were keratins as determined by immunoblot analysis. TPA treatment produced no alterations in the keratin protein profiles. However, at least 10 proteins in the 40-80 kd MW range were found to be altered after TPA treatment. These results demonstrate that TPA elicits changes in specific marker proteins in the trachea.

The quadratic relationship between administered dose and the number of lung tumors induced by urethane in Swiss mice appears to be related to dose dependence of urethane metabolism (Sichak, S.P. and O'Flaherty, E.J., Tox. Appl. Pharmacol., in press). These investigators found that reduction in total internal exposure to urethane (AUC) caused by induction of urethane metabolism was associated with a disproportionately large decrement in tumor number, suggesting that a metabolite of urethane, rather than urethane itself, is the tumorigenic agent. We have found that changes in the relationship between urethane AUC and tumor number can also be induced without appreciably altering urethane AUC's. Groups of mice were pretreated with either indole-3-carbinol at 150 mg/kg 2 hours before urethane administration or with 80 mg/kg phenobarbital for five consecutive days before injection of urethane on the 8th day. Both pretreatment regimens caused decreases in tumor numbers relative to those found in non-pretreated mice given identical doses of urethane, without significantly altering urethane AUC's. This is further evidence that a metabolite of urethane is responsible for the tumorigenesis associated with urethane exposure. Alternative indices of exposure to the tumorigenic agent which are potentially capable of resolving these conflicting data sets are being explored. Supported by PHS Grant ES07073.

A study was designed to investigate whether a hyperoxic environment would enhance the development of lung tumors following urethane injection. Male A/J mice were injected with urethane (1000 mg/kg) or saline and one week later were placed into either a 70% O2 atmosphere or kept in room air. Animals remained exposed to hyperoxia for 1, 2, 4, 8, or 16 weeks. All animals were killed 17 weeks after urethane injection and the number of visible tumors counted. Urethane-room air mice had an average of 16.9 tumors/lung while both air and oxygen exposed control animals (saline) averaged less than 0.3 tumors/lung. Urethane-oxygen groups at 1, 2, 4, 8, and 16 weeks averaged 19.3, 16.9, 11.4, 9.7, and 3.1 tumors/lung respectively. This indicates that tumor growth was inhibited by 70% O2. One explanation is that high O2 inhibits pulmonary type II epithelial cell proliferation, the cell which is the progenitor of most adenomas following urethane injection. Another possibility is that high O2 produced elevated superoxide dismutase levels which would decrease the likelihood of free radical formation, one of the proposed mechanisms of tumor promotion. (Oak Ridge Graduate Fellow supported by ORAU. 2 Operated by Martin Marietta Energy Systems, Inc. with US Dept. of Energy.)

Telone II, a soil fumigant, was tested in chronic studies using Fischer 344 rats and B6C3F1 mice. Doses administered were 0, 25 or 50 mg/kg to rats and 0, 50 or 100 mg/kg to mice. Telone II was given in corn oil by gavage three times per week for 104 weeks. The primary organs affected were the forestomach (rats and mice), urinary bladder (mice), lung (mice) and liver (rats). Compound-related non-neoplastic lesions included basal cell or epithelial hyperplasia of the forestomach (rats and mice), epithelial hyperplasia of the urinary bladder (mice) and hydrenephrosis (mice). Neoplastic lesions associated with administration of Telone II included squamous cell papillomas of the forestomach (male and female rats, female mice), squamous cell carcinomas of the forestomach (male rats, female mice), transitional cell carcinomas of the urinary bladder (female mice), alveolar/bronchiolar adenomas (female mice) and neoplastic nodules of the liver (male rats). The study in male mice was considered inadequate due to reduced survival in the vehicle control group. CI- and trans-1,3-dichloropropene are the principal components in Telone II, but the 1% epichlorohydrin, a direct-acting mutagen and carcinogen added as a stabilizer, may have influenced the development of forestomach lesions.

The carcinogenicity of benzyl acetate (a fragrance and flavoring agent) was studied in F344 rats and B6C3F1 mice. The chemical was given, in corn oil by gavage once daily, five days per week for 103 weeks, to groups of 50 male and 50 female rats at doses of 0, 250, and 500 mg/kg body weight and to groups of 50 male and 50 female mice at doses of 0, 500, and 1000 mg/kg. There were no adverse effects of benzyl acetate on the survival or body weight of rats or mice during the study. A genital tract infection was probably responsible for the reduced survival observed in all groups of female mice. Under the condition of the present study, benzyl acetate caused an increased incidence of acinar cell adenomas of the exocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. No evidence of carcinogenicity was found for female F344/N rats. For male and female B6C3F1 mice there was some evidence of carcinogenicity, in that benzyl acetate caused increased incidences of hepatocellular adenomas. The increased incidence of squamous cell papillomas or carcinomas and hyperplasia of the forestomach of mice of either sex may have been associated with the administration of benzyl acetate.

159 TRANSFER OF DIMETHYLAMINOSALAMINE (DMN) FROM LACTATING RATS TO NEONATES VIA THE MILK AND INDUCTION OF DNA DAMAGE/REPAIR IN NEONATAL LIVER. D.J. Kornbrust, B.A. Gillis, T. Townsend, *L. Timmons, D. Dietz and B.A. Schwartz. NIEHS, and Thes. Triangle Inst., Research Triangle Park, NC; and *Midwest Research Inst., Kansas City, MO.

The concentration of DMN in the milk of lactating rats, determined by a GC/MS method, was found to vary with the administered dose and to be nearly identical to the DMN concentration in plasma. Following a 20 mg/kg dose, the milk and plasma levels remained high (20-30 ppm) for 4-6 h. Based on the concentration in milk and predetermined rates of consumption (i.e., suckling) by rat pups, it was estimated that the 10-12 day old pups may have received ~2-4 mg DMN/kg body weight via the milk. Microsomal DMN demethylase activity (DMNdi), believed to be responsible for metabolic activation of DMN to a genotoxic form, was slightly higher in neonatal than maternal liver. Using the in vivo/in vitro hepatocyte primary culture/DMN repair assay, a positive DNA repair response was observed in hepatocytes from neonates as early as 2 and as late as 20 h following a 20 mg/kg dose to the maternal animals. Pre-treatment of the neonates with pyrazole 24 h prior to isolation of their hepatocytes increased DNA repair (3-fold) as well as the DNA repair response to DMN received via the milk (2-fold). These results indicate that secretion of DMN into milk may produce genotoxicity in neonatal liver which may be modified by agents that alter DMN activity.


Trichloroacetic acid (TCA) is a major non-volatile product formed during chlorination of water that contains organic material. TCA has recently been reported to induce hepatic peroxisomomas and it is metabolically related to several hepatotoxins and hepatocarcinogens. The ability of TCA to initiate and/or promote hepatic carcinogenesis was assessed by using an in vivo hepatic initiation-promotion model. 5 to 6 week old, male Sprague-Dawley rats underwent a 70% partial hepatectomy (PH) and were then "initiated" with either 10 mg/kg diethylnitrosoamine (DEN) by a single oral dose, or with TCA at 1500 mg/kg (single oral dose) or 500 mg/kg in the drinking water for 10, 20 or 30 days. Two weeks after "initiation," "promotion" was begun with 500 ppm phenobarbital (PB) or TCA at 50 ppm, 500 ppm, or 5000 ppm in the drinking water. Animals were then tested for 3 months following PH for the development of iron resistant and/or gamma glutamyl transpeptidase (GGT) positive altered hepatic foci as a measure of hepatic carcinogenic potential. Each rat was also tested for immune responsiveness utilizing the "traitor" model system. Natural killer (NK) cell mediated cytotoxicity, antibody levels, delayed-type hypersensitivity, interleukins 1 and 2, and prostaglandin activity were all assessed.


Some substances that cause chronic irritation of the gastric mucosa are considered risk factors for gastric cancer in humans. These include sodium chloride (table salt), bile salts, aspirin and other substances commonly encountered in the environment. We have used the MNSG model for gastric carcinogenesis in the rat and shown that sodium chloride and bile salts enhance tumor incidence. Cell kinetics, using flow cytometry and other techniques; histochemistry; autoradiography and light microscopy have been used to identify early changes in the mucosa which correlate with tumor induction at a later time. Following exposure, changes which appear to be significant during incipient stages of carcinogenesis and suggest risk include bursts of DNA synthesis, an increase in acid mucopolysaccharides in focal areas of glandular stomach mucosa, aberrant and irregular glands, focal necrosis, and changes in GSM content of the mucosa. These may be useful in screening for potential risk factors. (Supported in part by PO1-CA02731).
RAPID INDUCTION OF FORESTOMACH CARCINOMA IN PARTIALLY HEPATECTOMIZED Wistar Rats GIVEN BUTYLATED HYDROXYANISOLE. R. Abraham, K.-F. Benitz, R. Hanke, G. Patil and R. Lyon. Department of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y.

BHA (Butylated hydroxyanisole), a synthetic product, is one of several phenolic compounds used in foods because of its antioxidant properties. BHA is readily absorbed by the gastrointestinal tract, induces hepatic microsomal enzymes and undergoes metabolism in animals and man. BHA is also reported to inhibit neoplasia. In partially hepatectomized (PH) rats we were surprised to note that BHA hastened the onset of forestomach tumorigenesis in a remarkably short period of time. Rats, subjected to two-thirds partial hepatectomy (PH) and given 2% butylated hydroxyanisole in the feed for 3 months developed forestomach tumors. Histologically, these lesions were classified as hyperplasias, dysplasias of the basal cell, papillomas and squamous cell carcinomas. Histochemical studies of tumors revealed a marked increase in the phenotypic expression of the onco-fetal enzyme, gamma-gutamyl transpeptidase as well as acid phosphatase. The \([H]\) Tdr labelling index of epithelial cell nuclei and the number of cells in the forestomach were significantly increased in a 100 sq. area closest to the margo placenta. To our knowledge, this is the first report of an experiment, whereby subjecting rats to PH influenced tumorigenesis at an extrahepatic site, decreasing the latent period of neoplastic induction.

[Sponsor for this Research was provided by the American Cancer Society Institutional Grant number 15A.]

HYDROGENATED BENZO(2)PYRENE DERIVATIVES AND SKIN TUMORIGENESIS. D.A. Mahlum; T.J. Wozniak; R.A. Hites; D.W. Lerner; 1Pacific Northwest Laboratory, Biology & Chemistry Department, Richland, WA, 99352. 2University of Indiana, School of Public and Environmental Affairs, Bloomington, IN.

High boiling complex mixtures from coal suppress the skin tumor initiating activity of benzo(a)pyrene (BaP). To determine whether hydrogenated compounds might be responsible for this suppression, we tested the effect of hydrogenated BaP derivatives on the expression of BaP activity. A single 25 ug dose of BaP alone or with 125 ug of 4,5-dihydro BaP or, 7,8,9,10-tetrahydro BaP or a mixture containing several hydrogenated species of BaP was applied to the backs of female Charles River CD-l mice. Other mice were initiated with either 25 or 125 ug of the respective hydrogenated product. After two weeks, all mice were promoted twice weekly with 5 ug of TFA. Significant initiating activity was found for both the dihydro BaP and mixed product as well as for BaP. Marginal activity was found with both doses of the tetrahydro BaP. Application of 25 ug of BaP and 125 ug of dihydro BaP together resulted in the same tumor yield as with either alone. The tumor yield when the BaP was applied with either the tetrahydro or mixed hydrogenated product was slightly lower than for BaP alone. These data show that some hydrogenated derivatives of BaP have tumor initiating activity and may also suppress BaP activity to a small degree. (Supported by U.S. Department of Energy Contract No. DE-AC06-76RL01830.)

ZINC-DEFICIENCY, METHYLBENZYLITROSAMINE (MBN) CARCINOGENESIS: DNA SYNTHESIS AND ADDUCT FORMATION. P.M. Newbome and T.F. Schragel. Massachusetts Institute of Technology, Cambridge, MA.

Esophageal cancer is not amenable to satisfactory chemotherapy or surgical intervention and must be addressed by devising preventive measures. These require a better understanding about etiology and mechanisms. We have observed sharply lowered zinc concentrations in tissues of esophageal cancer patients and developed an animal model for studying the disease. Zinc deficiency significantly enhances MBN-induced esophageal cancer in rats if the deficit is applied through dosing period only; for entire period to termination; or after dosing is complete, implying an effect during different stages of the process. We have observed different patterns of DNA synthesis with different periods of deficit which correlated with tumor incidence. These were also related significantly to DNA adduct formation and persistence, particularly the promutagenic base 6-methylguanine. Comparing the target organ (esophagus) to the non-target organ (liver) we have found that adduct formation is similar but persistence longer and repair slower in zinc deficient esophagus. Timing of these effects, while not entirely clear, suggest effects during (initiation) and after (promotion) of MBN exposure. (Supported in part by grant DMS-CA25382).


A diet high (23% by wt.) in lard enhances DMBA mammary tumorigenesis in rats compared to a control (4%) lard diet. Both diets contain 13% corn oil and are calorically balanced to contain all nutrients in amounts recommended for rats. The high lard (HL) diet decreased tumor latency when it was fed before and after a single dose of DMBA, 2.5 mg given by gastric gavage or when it was fed only before DMBA (Cancer Res. 43:2477s, 1983). The timing and site of action of the dietary effect were studied further. Rats were fed the HL or control (C) diet from weaning to 53 days; all were then fed the C diet and given DMBA at 55 days and then returned to their original diet or fed the other diet to termination of the experiment. DMBA was given by subcutaneous (sc) injection into one mammary gland (0.25 mg) or intravenously (i.v.) (2.5 mg). Rats given DMBA sc and fed HL diet for any period had decreased tumor latency compared to rats fed C diet throughout. Rats given DMBA iv showed a similar result initially but tumors then developed rapidly in all diet groups. The HL diet influences DMBA tumorigenesis by acting at or before initiation as well as during the period following carcinogen exposure. Its effect is exerted entirely or in part at the mammary gland. (Supported in part by NIH Grant CA039194).
An animal host was examined for the development of an antibody towards self components through conjugation of these substances to a halothane biotransformation intermediate. Using an ELISA assay to a hapten-carrier complex [trifluoroacetyleylated rabbit serum albumin (TFA-RSA)], rabbits that were exposed repeatedly to halothane (1% for 2 hr in various O2 atmospheres) were assessed for antibody against the trifluoroacetyl (TFA) group. Multiple exposures generate the induction of an antibody that cross reacts with TFA-RSA. Both phenobarbital-induced and non-induced rabbits developed equivalent high titers of antibody. The appearance of antibody, however, was enhanced if the halothane exposure was conducted under oxidative conditions (40% O2). Immunization of rabbits with TFA-RSA prior to halothane exposure did not result in elevated SGPT. In fact, no correlation was observed between antibody titers and post halothane exposure SGPT values. These studies suggest that halothane exposure under an oxidative atmosphere generates an antibody that cross reacts with a covalently bound oxidative reactive intermediate of halothane. Its role in mediating halothane hepatotoxicity remains unclear.

(NIH AM16715)

Rabbits exposed to halothane under oxidative conditions produce an antibody that cross reacts with the trifluoroacetyl group on trifluoroacetylated rabbit serum albumin (TFA-RSA). The specificity of this antibody induced after multiple halothane exposures was compared to the specificity of anti-TFA-RSA antibody by blocking antibody binding with TFA-lysine. Rabbits were immunized with 1 mg TFA-RSA/rabbit followed by a booster 4 wk later. These animals were pre-treated with phenobarbital and exposed to 1% halothane for 1 hr. After 5 exposures, plasma from these animals was compared to plasma obtained prior to any halothane exposure. Using an ELSA assay with TFA-RSA as antigen, 1 mM TFA-lysine blocked 50% of the anti-TFA-RSA binding in the pre-exposure plasma. However, following 5 exposures to halothane, 24 mM TFA-lysine was required for equivalent blocking of similar amounts of antibody. There was no change in specificity of the antibody over the same period of time in TFA-RSA immunized rabbits not exposed to halothane. These studies suggest that multiple halothane exposures produce a population of antibodies whose specificity is similar, but not identical to the antibody resulting from TFA-RSA immunization.

(NIH AM16715)

In order to ascertain the immunomodulatory effects of dimethylvinylchloride (DMVC), female B6C3F1 mice were given, by the intragastric route, corn oil alone or oil containing 50, 100, 200 or 400 mg/kg of DMVC for 14 days. Twenty-four hrs following the last dose, various parameters of immunity were measured. Exposure to DMVC significantly increased T- and B-cell blastogenesis to the polyclonal mitogens PHA, Con-A and bacterial LPS; while suppressing specific T-cell recognition of alloantigens, delayed hypersensitivity and the antibody response to a T-cell dependent antigen. Macrophage cytostasis and natural killer cell lysis of tumor cells were not profoundly altered. In related studies, increased susceptibility to primary challenge with infectious agents requiring intact T-cell immunity for host protection supports these data. The results of this study show that DMVC, a potential carcinogen, suppresses specific T-cell function without altering natural tumor surveillance and macrophage function.

(Studies supported by NEHS contract No. NO1-ES-1-5000).

To extend the data base on DMVC, our host resistance panel consisting of bacteria and viruses, was used to examine potential immunotoxic effects. Since T- and B-cells, interferon, and macrophages protect the host against these infectious challenges, any changes in these mechanisms could cause increased susceptibility to infection. Female B6C3F1 mice were given 50, 100, 200, or 400 mg/kg of DMVC, dissolved in corn oil. The dosages were administered daily for 14 days by gavage. Challenge with infectious agent occurred 2 days after the last dosage with DMVC. After primary infectious challenge, DMVC had no effect on mortality or survival time of infections due to Streptococcus zooepidemicus, whereas a significantly increased host susceptibility was found to Listeria monocytogenes, influenza A2 Taiwan, and Herpes simplex types 1 and 2 viruses. In addition increased susceptibility was dose related in those infections with the exception of Listeria. After secondary infectious challenge, no effect on host resistance to Listeria, Streptococcus, HSV-1 and HSV-2 was found. However, a dose related increase in susceptibility was noted with secondary infectious challenge with influenza virus. These studies show that more than one assay is needed to properly assess the overall effects of test agents on the various immune mechanisms. (These studies were supported by NIEMS Contract NO1-ES-5000.)
169 SUPPRESSION OF MOUSE SERUM COMPLEMENT LEVELS FOLLOWING SUBCHRONIC EXPOSURE TO DIOXINS. K.L. White, Jr., H.H. Lysy, and A.C. Anderson. Depts. of Biostatistics and Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA 23298. (Sponsor: A.E. Munson)

Exposure to dioxins has been shown to suppress many of the acquired immune responses; however, the effects of these compounds on the complement system have not been investigated. Exposure of female B6C3F1 mice to 2378-terachlorodibenzodioxin (TCDD) for 14 days by gavage at concentrations of 0.01, 0.05, 0.1, 0.5, 1 and 2 μg/kg produced a dose dependent decrease in serum complement levels which ranged from 60 to 18% of vehicle control levels. Animals exposed to 125678-hexachlorodibenzodioxin (HCD) at doses of 0.1, 0.5, 1, and 5 μg/kg showed a dose dependent decrease in complement levels which was significant at the two highest dose levels (38 and 34% of controls). Exposure to either TCDD or HCD decreased the C3 component of complement and increased susceptibility to Streptococcus pneumoniae. Recovery studies demonstrated that, following exposure to low doses of HCD (0.1 and 0.5 μg/kg), the complement system returns to normal levels and, by day 28, recovers to 200% of controls, while animals receiving 10 μg/kg of HCD or 1 μg/kg of TCDD recovered to only 64 and 56% of control. These studies indicate that the dioxins are capable of suppressing innate immunity as well as acquired immune responses. (Supported by NIEHS ES-1-3001.)

170 DIRECT SUPPRESSION OF ANTIBODY PRODUCTION BY CULTURED MOUSE SPLEEN CELLS BY 2,3,7,8-TCDD. M.P. Holsapple, P.J. McNerney, A.N. Tucker* and M.L. Luster*. Dept. of Pharmacology & Toxicology, Medical College of Virginia, Richmond, VA, and *NIEMS, Research Triangle Park, NC.

The objective of this investigation was to determine if dioxin could directly suppress antibody production in vitro by mouse spleen cells. Preliminary results indicated that dioxin could be coupled to serum and that the concentration could be determined by using 14C-2,3,7,8-TCDD of a known specific activity. Dioxin in serum was added to cultures of spleen cells from female B6C3F1 mice which were stimulated with LPS (polyclonal), DNP-Ficoll (T-independent), or SRBC (T-dependent). All three responses were suppressed in a dose-related fashion (50-20 nM) and the curves were parallel. Approximate IC50 concentrations were 15 nM, 16 nM, and 5 nM for the responses to LPS, DNP-Ficoll, and SRBC, respectively. The temporal relationship of the suppression was determined by adding 10 nM dioxin to cultures of spleen cells from female B6C3F1 mice at various times during the culture period with SRBC. Significant suppression (71%) was only observed when dioxin was added at the beginning of the culture period. The genetics of the suppression were tested by adding dioxin to spleen cells from either female B6C3F1 mice (AH-+) or female DBA/2 mice (AH-) which were stimulated with LPS. Preliminary experiments have indicated a comparable suppression of these two strains by dioxin. (Supp. by NIH ES-02520).

170 CHLORINATED DIBENZO-P-DIOXIN (CDD) AND DIBENZO-PURAN (CDF)-INDUCED HUMORAL IMMUNE SUPPRESSION. N.L. Kerkvliet and J.A. Brauner. College of Veterinary Medicine and Environmental Health Science Center, Oregon State University, Corvallis, OR.

The immunosuppressive potencies of octaCDD: 1,2,3, 6,7,8-hexaCDD: 1,2,3,4,6,7,8-heptaCDD and 1,2,3,4,6,7,8-heptaCDF, isomers, major contaminants of commercial grade pentachlorophenol, to suppress the primary splenic anti-SRBC response were assessed in C57B1/6 mice. Linear regression analysis of dose-response curves following a single oral exposure to the various isomers indicated a highly significant linear relationship between the log of the dose and percent immune suppression for all isomers except octaCDD, which was not immunosuppressive. The 50% immuno-suppressive dose (ID50) was 7.1, 85 and 208 μg/kg for hexaCDD, heptaCDD, and heptaCDF, respectively. Comparison of the 50% ID50 of 0.65 μg/kg for TCDD. Results of testing studies indicated that dioxin exposure two days prior to or after antigen was more immunosuppressive than dioxin exposure 7 or 14 days prior to antigen, suggesting some degree of recovery over time. If dioxin exposure was delayed until 4 days after antigen, the antibody response on day 5 was not significantly altered, suggesting that antibody production per se was not affected by dioxin. The ability of dioxin to suppress antibody responses of animals thymectomized at 8 weeks of age was not compromised, indicating no direct influence of the thymus on dioxin-induced humoral immune suppression. (Supported by EPA grant RB81157 and NIH ES00210.)

171 EFFECTS OF GIBBERELIC ACID AND 3',4',4'-TETRACHLOROAZOXYBENZENE ON IGG ANTIBODY RESPONSES IN DEER MICE AND WHITETAIL. L.J. Olson, D.S. Feenstra, R.D. Hinadill, and M.T.S. Hais. Envi-ronmental Toxicology Center, Depts. of Bacteriology and Entomology, Univ. of Wisconsin, Madison, WI 53706.

The total and DNP-specific IgG responses were assayed by an indirect enzyme-linked immunoabsorbant assay (ELISA) in wild deer mice (Peromyscus maniculatus) and Swiss Webster mice treated with gibberelic acid (GA, 17.5, 87.5, 350 μg/kg/injection) or 3',4',4'-tetrachloroazoxybenzene (TCAOB, 1, 5, 25 μg/kg/injection). Other parameters were body, thymus, spleen, liver, kidneys, and heart weights, bone marrow cellularity (BMC), lymphocytes/g of spleen and lymphocytes/g of thymus. Deer mice given 350 μg/kg of GA had significantly lower IgG levels (p < .05). Lymphocytes/g of spleen and BMC increased. Swiss Webster mice treated with GA had lower increases in lymphocytes/g of spleen, with BMC reduced. TCAOB mice had significant suppression of specific IgG levels at all TCAOB doses. Total IgG was less affected, with only TCAOB white mice significantly suppressed. Thymus weights were reduced. Lymphocytes/g of spleen or thymus, and BMC were lower in some TCAOB treated groups. This study shows that the use of TCAOB-contaminated herbicides should be closely monitored. Supported by EPA Grant FR807540.
The altered immune responses of deer mice (Peromyscus maniculatus) fed daminozide and gibberellic acid. L.J. Olson, R.D. Hindeall and R.C. Biddiss, Environmental Toxicology Center and Dept. of Bacteriology, Univ. of Wisconsin, Madison, WI 53706. Sponsor: R.T. A. Haas

Two plant growth regulators, gibberellic acid (GA) and daminozide (DZ) were tested for selected immunomodulatory and toxic effects by feeding to wild deer mice, Peromyscus maniculatus, for 28 days. These two compounds are widely used in agriculture and the deer mouse is common across North America. Compounds and doses were, at 12 mice/dose: saline (negative control); cyclophosphamide (positive control), 20 mg/kg mouse/day; GA and DZ at 25, 50 and 100 mg/kg mouse/day. Among the parameters and assays measured or examined were: splenic plaque forming cell (PFC) assays, hemolysis titers, WBC counts and differentials; bone marrow nucleated cell (BMNC’s) counts; hematoctrits; plasma proteins; and selected organ weights, including the spleen and thymus. GA fed mice had elevated body, heart, kidney, spleen and liver weights, with thymus weights reduced by 12 to 20%. GA decreased PFC/gm of spleen by up to 66%. DZ increased body, kidney and liver weights, and lowered spleen and thymus weights by up to 47%. DZ decreased BMNC’s, PFC’s (by 86%), total lymphocytes (by 28%) and PFC’s/10⁶ lymphocytes (by 87%). The results demonstrate strong immunosuppressive effects from DZ, even at 25 mg/kg. Supported by EPA Grant #R807540.

Effects of post-weaning and perinatal TCAOB exposure on immunity and reproductive efficiency in mice. M.R. Beevins, R.D. Hindeall, and M.T. J. Haas, University of Wisconsin, Madison, WI.

TCAOB (3,3',4,4'-tetrachloroazoxybenzene) is an approximate sterioisomer of TCDD and results in similar toxic effects. Tests were initiated to examine the effects of TCAOB on the immune system of young mice and to monitor the reproductive efficiency of adult female mice. When weaning mice were fed 40 ppm TCAOB, thymic atrophy and a severe reduction in the number of plaque-forming cells (PFC) following sheep red blood cell (SRBC) immunization were seen. No change was noted in the lymphocyte blastogenic response to Con-A (T-cell nitogen) or LPS (B-cell mitogen). A decline was seen in the percentage of Lyt-1 cells (T-helper cells) following TCAOB exposure. In a second experiment adult female mice were fed TCAOB throughout gestation and lactation. Birth and weaning weights of pups produced by TCAOB dams were normal, but total litter mass was depressed for females consuming 10 ppm TCAOB. A reduction was seen in the number of pups per female weaning (at birth & weaning) on this treatment. The 10 ppm dams produced offspring with reduced thymus weights and PFC responses, but normal blastogenic reactions. These studies indicate that TCAOB is immunosuppressive following direct (diet) and indirect (via the dam) exposure. Results of the reproductive study show TCAOB to be fetotoxic, but also suggest a protective maternal influence may be involved.


Reports of Benzo(a)pyrene (BaP) but not Benzo(e)-pyrene (BeP) immunosuppression characterized by a downward modulation of humoral immune response of mice in the absence of overt toxicity showed that immunological assays could be used to demonstrate altered immune function. The current studies were performed to determine if observations issuing from immune function assays correspond to altered host resistance. Following 14 daily dosings of 5, 20, or 40 mg/kg BaP or 40 mg/kg BeP, B6C3F1 mice were exposed to one of five microbial or tumor cell challenges. Within each assay, four challenge levels and three control groups were employed. The challenge agents were: L. monocytogenes, S. pneumoniae, Herpes Simplex Type 2, Influenza A2, and a B16F10 melanoma. A turbidometric technique was developed to ensure precise preparation and delivery of the Strep challenge. BaP modulation of the immune system produced an increased host susceptibility to Herpes, Strep and B16F10 melanoma. BaP increased host resistance to Listeria. There was no change in resistance to Influenza A2. BeP did not modulate the immune system in these tests. These results indicate that alterations in selected immunological assays can predict changes in host resistance and assist in the interpretation of the biological effects of benzo[pyrenes. (Supported by AIPL)

Immunotoxic effects of benzo-(a)-pyrene in vivo and in vitro. R.H. Blanton, M. Lyte and P.H. Bick, Dept. of Microbiology and Immunology, Medical College of Virginia/VCU, Richmond, VA. Sponsor: S.G. Bradley.

The immunotoxic effects of Benzo-(a)-pyrene (BaP) have become apparent during the past few years. Our goal has been to identify the cellular target and mechanism of BaP-induced immunosuppression. We have found that in vivo exposure to 40 mg/kg BaP for 7 consecutive days causes a significant reduction of host spleen cells to produce antibody in vitro when stimulated with the polyclonal activators lipopolysaccharide (LPS) or purified protein derivative (PPD) or when stimulated with the T-dependent antigen sheep erythrocytes (SRBC). Incorporation of BaP, or its non-carcinogenic congeners benzo-(e)-pyrene (BeP), directly into cultures of normal spleen cells suppressed the antibody response to SRBC and the polyclonal activators in a dose and time-dependent manner. Further, addition of BaP to cultures as little as 30 minutes prior to assay caused inhibition of the antibody forming cell response. The above experiments confirm and extend previous reports of BaP-induced suppression of T-dependent responses. In addition, immunosuppression following short term BaP exposure in vitro supports a possible macromolecular binding mechanism for BaP suppression in vitro systems. Supported by NHLBI grant ES-03566 and Training Grant CA-09210.
Differential Immunotoxic Effects of Benzo-(A)-Pyrene in Young and Aged Mice. M. Lyte and P.H. Bick, Dept. of Microbiology and Immunology, Medical College of Virginia/VCU, Richmond, VA. Sponsor: S.G. Bradley.

The aging process is accompanied by a gradual decline in immune competence, especially among T cell-mediated responses. We propose that immunodeficient animals are more susceptible to the immunotoxic effects of environmental chemicals, such as benzo(a)pyrene (BaP), than are young immunologically competent adults. The ability of splenocytes from 4-5 month and 23-26 month old 86C3F1 mice to generate in vitro antibody responses to the presence of increasing concentrations of BaP was tested. Splenocytes from aged mice displayed greater susceptibility to BaP-induced suppression at BaP concentrations 1-1.5 logs lower than observed with spleenocytes from young mice. In vivo experiments utilized 4-6, 16-18 and 24-26 month old mice which were intraperitoneally exposed to BaP for 4 days prior to and following sheep red blood cell immunization. The rank order of immunosuppression was 24-26 > 16-18 > 4-6 months of age. We conclude that (1) aged animals are more susceptible to the immunotoxic actions of BaP than are young adults and (2) evaluation of potential hazards posed by environmental chemicals should include immunodeficient animal models in which immunotoxicity may be more fully and realistically evaluated. Supported by PHS grant ES-03366, ES-05323, and Training Grant CA-09210.


A previous study indicated that high levels of chlorine (25 to 30 ppm) in drinking water of mice was related to a decreased luminescent function of macrophages. Because of the important role of the macrophage in body defense reactions, it is imperative that the significant effects be further investigated. A rat model to determine the implication to human health, especially in regard to the almost universal practice of disinfection of drinking water by chlorination. Some chlorination by-products have been implicated as carcinogens (i.e., trihalomethanes) or promoter-carcinogens (i.e., chlorophenols). It is more than plausible that an epigenetic mechanism of this effect could be related to chemical-induced adverse effects on immune function. The purpose of this research was to determine if chlorine-based chemicals, namely sodium hypochlorite, chlorine dioxide and monochloramine, alter immune functions of Sprague-Dawley rats. Immune parameters assessed were humoral immunity (antibody synthesis); cell-mediated immunity (delayed-type hyperresponsitivity); macrophage function (phagocytosis, oxidative metabolism, and production of prostaglandins and interleukin 1); natural killer cell cytotoxicity (in vivo immunoreactivity to virus and tumors); and lymphocyte production of interleukin 2, a basic immunoregulatory lymphokine.

The hypothesis tested was that since macrophage function is apparently impaired following in vivo exposure to hyperchlorinated drinking water; other immune functions are probably also altered either by direct toxic effects on immunocytes or indirectly via compromised macrophage-mediated participation in these immune responses.

The results suggest that chlorine-based water disinfectants do alter various immune functions in rats. Antibody responsiveness, NK cell cytotoxicity and macrophage-derived TNF were generally increased while O/N reactions and macrophage oxidative metabolism were generally decreased. Various effects were observed on the synthesis of IL-2 and IL-1 and phagocytosis by macrophages depending on the type and dose of disinfectant.


Previous studies have demonstrated that subcutaneous (sc) exposure of mice to DMBA persistently suppresses both humoral and cell-mediated immunity (Ward, et al., Cancer Res., In Press). It was unclear, however, if this immunosuppression was due to the continual release of DMBA from residual corn oil in the sc tissue of these mice or due to a persistent effect on the functional immune cells or their precursors. To address this question, 86C3F1 mice were exposed by gavage to the same doses as sc exposed mice (5, 50 or 100 µg DMBA/g body weight in 10 equal doses over 2 weeks). Antibody plaque forming cell (PFC) responses to the T-dependent antigen sheep red blood cells (SRBC) were quantitated at 3 days and 4 weeks following cessation of the DMBA exposure. Gavaged DMBA produced a significant depression in the SRBC PFC response at the 50 (87%) and 100 (98%) µg dose at 3 days. This was comparable to the level of suppression seen 3 days after sc exposure to 50 (76%) and 100 (97%) µg DMBA/g. Suppression in the gavaged mice was persistent since the SRBC PFC response was still significantly decreased (21 to 87%) at all 3 DMBA doses 4 weeks following exposure, which was comparable to the decrease (20 to 95%) noted following sc exposure. These results indicate that DMBA has persistent effects on the immune system, and that the suppression is not due to the continual release of DMBA in the mice.
181 TUMOR PROMOTER INDUCED IMMUNOSUPPRESSION IN MICE AFTER TOPICAL APPLICATION OF PHORBOL ESTERS.
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It has been suggested that agents which suppress the tumoricidal capacities of cells involved in cell-mediated immunity may promote tumor growth. Various investigators have studied the effects of 12-O-tetradecanoylphorbol-13-acetate (TPA) or similar tumor promoters on natural killer (NK) cells, in vitro, but conflicting results have been obtained. NK activity was therefore assessed after topical dosing. CBA mice (5-6 wks) received TPA or other phorbol esters in acetone via application to shaved dorsal skin. Animals were sacrificed and splenic NK activity was measured against YAC-1 murine lymphoma cells in a 51Cr release assay. At doses of 5 and 10 ug/dose TPA (6 doses) showed significantly suppressed NK activity (44% and 64% suppression, respectively). Animals initiated with a single DMBA dose and promoted with acetone did not exhibit any reduction in NK activity. Significant inhibition was observed in animals initiated with DMBA but promoted with TPZ. PDD, an active promoter (5 and 10 ug/dose) also suppressed NK activity, while non-promoters such as phorbol 1 and 4-O-Me-TPA did not. Suppression of NK function appeared to be decreased if adherent cells were removed from splen cell preparations. These studies indicate that chronic topical exposure to phorbol ester promoters can suppress cell-mediated immunity. Supported by NIHES Grant ES-07073.

182 IMMUNOTOXICOLOGICAL EVALUATION OF CHLORDANE.

Various toxicological and immunological parameters were assessed after exposure of female B6C3F1 mice to 0.1, 1.0, 4.0, and 8.0 mg/kg body weight via oral gavage. Variables evaluated included periodic body weights, terminal organ weights, gross pathology on selected organs, histology, spleen, IgM antibody response to SRBC, lymphoproliferative response to the mitogens PHA, Con A, and LPS, and the mixed lymphocyte response to allogeneic spleen cells. When compared to the corn oil (vehicle) controls, CLD produced a significant, dose-dependent increase in liver weights. Treated groups also demonstrated a significant (p<0.05), but not dose-related, decrease in spleen weights. Total leukocytes were significantly increased in CLD-treated mice (60% at 8 mg/kg). Spleens from CLD-treated groups demonstrated a dose-dependent and significant enhancement of "H-Tdr incorporation at all concentrations of Con A with a 90% increase for the high dose group at 5 ug/ml Con A (optimal concentration). PHA responsiveness was also increased (107% for 4 mg/kg group at 5 ug/ml mitogen). Responsiveness to LPS was unaltered as was the day 4 (peak day) IgM antibody response to SRBC. Spleens from CLD-treated mice also demonstrated a trend towards hyperresponsiveness to allogeneic cells. (Supported by NIHES 1T52ES07087).

183 IMMUNOMODULATORY EFFECTS OF SUBACUTE TREATMENT WITH LOW DOSES OF OOS-TRIMETHYL PHOSPHOROTHIOATE.
K.E. Rodgers, T. Imamura, and B. Devens, Div. of Biomedical Sciences and Div. of Toxicology & Physiology, University of California, Riverside, CA 92521.

The effect of subacute (fourteen day) treatment with oos- trimethyl phosphorothioate (OOS-TMP), an impurity in technical malathion, on the generation of an immune response was examined in female CS781/6 mice. At a dose of 0.5 mg/kg/day OOS-TMP for fourteen days, the antibody response of splenocytes from treated animals was significantly increased. In contrast, the cytotoxic T lymphocyte (CTL) response of these splenocytes was unchanged. The Interleukin 2 response and mitogenic response to Concanavalin A was also elevated at this dose. At 5.0 mg/kg/day OOS-TMP for fourteen days, the ability of the splenocytes from treated animals to generate either a CTL or specific antibody response was unchanged. However, Interleukin 2 production and mitogenic response to Concanavalin A was also elevated at this dose. In addition, the mitogenic response of splenocytes from treated animals to lipopoly- saccharide was also elevated. Cell separation and reconstitution experiments indicate that macrophages were the immune cell population most affected by subacute treatment with OOS-TMP. However, 8 lymphocytes were also affected by this treatment regime. These data suggest that long-term exposure to low levels of OOS-TMP enhance the ability of an organism to generate an immune response. (supported by ES03105)

184 TIME COURSE OF IMMUNOSUPPRESSION FOLLOWING ACUTE OOS-TRIMETHYL PHOSPHOROTHIOATE ADMINISTRATION.
K.E. Rodgers, T. Imamura and B. Devens, Div. of Biomedical Sciences and Div. of Toxicology & Physiology, University of California, Riverside, CA 92521.

The time course of immunosuppression induced by acute treatment with oos-trimethyl phosphorothioate (OOS-TMP), an impurity in technical malathion, was examined in female CS781/6 mice. The immune parameters assayed included the generation of a cytotoxic T lymphocyte (CTL) response and specific antibody production. In addition, the proliferative response to mitogen and the production of Interleukin 2 was examined. On day 1, the CTL and antibody response was suppressed at all doses of OOS-TMP administered (from 1 to 40 mg/kg). However, this immunosuppression was reversible. By day 7, the CTL response of the splenocytes from treated animals was comparable to control levels. The humoral response of the splenocytes from treated animals was recovering by day 7. The mitogenic response of treated splenocytes to Concanavalin A was not significantly decreased by OOS-TMP pretreatment at any time point. The mitogenic response of treated splenocytes was slightly decreased on days 1 and 3, but had recovered by day 7. In contrast, Interleukin 2 production was significantly elevated on days 1 and 3, but had returned to normal by day 7. These data suggest that OOS-TMP was immunosuppressive at nontoxic doses of compound and this suppression was reversible. (supported by ES03105)
PRODUCTION OF IMMUNOSUPPRESSIVE FACTORS BY MACROPHAGES FOLLOWING OOS-TRIMETHYL PHOSPHOROTHIOATE TREATMENT. K. E. Rodgers, T. Inamura, and B. H. Devens, Div. of Biomedical Sciences and Div. of Toxicology & Physiology, University of California, Riverside, CA 92521

OOS-Trimethyl phosphorothioate (OOS-TMP) is an impurity present in commercially available organophosphorus pesticides. The mechanism of immunosuppression resulting from acute administration of 10 mg/kg OOS-TMP to female C57Bl/6 mice for one day was examined. Cell separation and reconstitution experiments indicated that splenic macrophages from OOS-TMP treated animals were most affected. Macrophages from OOS-TMP treated animals were less efficient at presenting antigen to immune T cells. Further studies indicated that phagocytic capability and interleukin 1 production of treated macrophages was elevated. However, the percentage of Ig positive splenic macrophage was decreased in OOS-TMP treated animals. Macrophages from OOS-TMP treated animals released factors that block tumor cell proliferation and mitogenic response to Concanavalin A and lipo polysaccharide at suboptimal concentrations of mitogen. Fresh supernatants, but not frozen, from splenocytes or splenic macrophages from animals treated with OOS-TMP were shown to block the generation of an antibody response. These data suggest that the mechanism of immunosuppression resulting from OOS-TMP treatment may be due to a decrease in Ig position macrophages and the release of cytostatic factors. (Supported by ES03105)

TEMPERATURE DEPENDENT PHAGOCYTOSIS OF BACTERIA IN VIVO: EFFECTS OF THE ORGANOPHOSPHATE DFP
D.R. Steup, G.A.W. Waterhouse, and R.B. Forney Department of Pharmacology and Toxicology Indiana United States of America, 101 South Maloney Street, 46223

Reticuloendothelial cell phagocytosis was evaluated in male Sprague-Dawley rats (150-200 g) by measuring the organ uptake of intravenously administered, 35S-labeled E. coli (10^9 organisms per 150 g body weight). All animals were sacrificed 10 minutes after bacterial challenge with specimens of liver, spleen, lung, kidney and blood being assayed for radioactivity. Exposure of rats to elevated environmental temperatures (32-36 °C) for two hours prior to administration of E. coli produced significant increases in average body temperature (2.1 °C) and hepatic uptake of bacteria. Both of these increases were antagonized by SC injection of 1.0 mg/kg DFP 1 hour prior to the bacterial challenge. Effects of drug-induced hyperthermia were evaluated following SC injection of 5 mg/kg phenylephrine in conjunction with exposure to ambient temperatures of 27-29 °C. These animals demonstrated significant hyperthermia (1.8 °C) and a striking (>90%) increase in pulmonary uptake of bacteria, neither of which was antagonized by administration of DFP. The finding that body temperature can influence in vivo phagocytosis may have important implications regarding the role of fever in the immune response and the possible immunologic consequences of antipyresis.

SUBACUTE EXPOSURE OF RATS TO THE ORGANOPHOSPHATE DFP: LACK OF EFFECT ON MITOGENESIS IN VITRO OR ON ANTIBODY PRODUCTION IN VIVO. C.A.W. Waterhouse, B.S. Martin, R.B. Forney. Dept. of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46223.

Male Sprague-Dawley rats (250-300g) were injected with DFP (sc) daily for up to 21 days at a level which produced only salivation and movement, transient tremors (0.1-0.5 mg/kg). Animals were sacrificed at 1, 14, or 21 days with splenic lymphocytes being isolated and incubated in vitro for up to 4 days with PHA (10-100 µg/ml), Con A (0.1-1.0 µg/ml), or LPS (0.1-1.0 µg/ml). Cells from DFP treated animals demonstrated no significant decreases in response to the mitogens (as measured by 3H thymidine uptake) despite an overall decrease in splenic cell number (when expressed per gram of tissue or per spleen). Antibody titer (to SRBC) was measured in animals exposed to DFP for 10 days prior to inoculation as well as throughout the afferent (induction) and efferent (antibody producing) phases of the primary immune response. Titers of the DFP treated animals were indistinguishable from those of saline treated controls. These data suggest that repeated exposure to moderate levels of DFP do not halt the animals’ ability to launch a humoral immune response, even though spleen cell number may be altered. Similarly, DFP given in vivo does not decrease the responses of cells to T or B cell mitogens in vitro, thus implying that long term effects in these cells are less likely.

Although epoxides are in general highly toxic, little is known about their effect on the immune system. Thus we examined the effect of a variety of epoxides on cell-mediated immunity both in vitro and in vivo. Standard assays using appropriate targets were utilized to monitor cytotoxic T lymphocyte (CTL), natural cytotoxicity (NC), and natural killer (NK) cell activity. Spleen cells from C57Bl/6 mice were exposed to nontoxic doses of propylene oxide (PO), glycidol, cyclohexene oxide (CO), styrene, styrene oxide (SO), styrene glycol (SG), and trichloropropene oxide (TCP0). None of these compounds showed any great suppression or stimulation of CTL response or NK activity at maximal doses. PO, glycidol, CO and SG, also did not suppress natural killer cell activity. In contrast, styrene, SO, and TCP0, were strong suppressors of NK activity with suppression as follows: styrene - 68% and 96% at 75 and 100 µg/ml, respectively; SO - 57% and 89% at 50 and 100 µg/ml, respectively; and for TCP0 - 75% and 88% at 2.5 and 5 µg/ml, respectively. in vivo, SO at 100 mg/kg suppressed CTL response, however, NK activity was slightly elevated. The mechanism of CTL or NK cell suppression by these compounds is not known.
THE LACK OF AUTOIMMUNE RESPONSE IN A.SW/SNj MICE TREATED WITH PROCAINAMIDE AND HYDRAZINE. X. Joseph, C. J. G. Robinson and T. Bales, Center for Drugs and Biologics, FDA, Washington, DC.

The major histocompatibility complex (MHC) controls susceptibility to some chemically induced immunologic reactions. Previously, ten inbred mouse strains bearing different H-2 (murine MHC) haplotypes were screened for autoimmune responses to HgCl2, gold sodium thiomalate (GST) and D-penicillamine. Only one of these strains, A.SW/SNj (H-2k), developed antinuclear antibodies (ANA) in response to treatment with all three agents. In the present study we examined the ability of this strain to develop ANA in response to treatment with procainamide (P) and hydrazine (H), two drugs which frequently induce ANA in humans. Groups of 4- to 5-month-old A.SW/SNj and C3H/HeSnj (a resistant strain) mice of both sexes were given P (200 mg/kg) or H (40 mg/kg) in drinking water, or GST (10 mg/kg im; positive control) with appropriate controls. After 4 months of treatment, A.SW mice were given the lipid A portion of lipopolysaccharide, a polyclonal B cell activator, 25 μg ip 2x weekly for an additional 4 months along with the drug treatments. Periodic screenings were done for proteinuria, ANA, BUN and total immunoglobulin. No significant changes in any of these parameters occurred due to treatment with the exception of induction of ANA directed against the nucleoli in GST-treated A.SW mice. These results suggest that H-2 controlled sensitivity is antigen-specific.


With repeated administration to animals, chlorphenamine (CP) has been shown to induce a phospohodiosis in lymphocytes. Studies were initiated to determine if a functional toxicity occurred to mouse and human lymphocytes as a result of in vitro exposure to CP during 3 days in culture. The effects on the blastogenic response to the following T-cell mitogens, phytohemagglutinin (PHA) and concanavalin A (Con A) and the B-cell mitogens lipopolysaccharide (LPS) and pokeweed mitogen (PWM), were examined. The responses of mouse splenic lymphocytes to PHA, Con A and LPS were inhibited by 30%, 20% and 20%, respectively, after 1 day in culture at 10-5M CP with the inhibition increasing on days 2 and 3. Human peripheral blood lymphocyte blastogenic responses were minimal on day 1. Responses to PHA, Con A and PWM were all inhibited after 2 days at 10-5M CP (by 30%, 50% and 30%, respectively). By day 3 the responses to PHA and PWM had recovered to control levels while there was a partial recovery in the Con A response. These results show that CP exposure can alter the functional response of lymphocytes to mitogenic stimulation, and based upon their recovery, human lymphocytes appear less susceptible than mouse lymphocytes to CP-induced toxicity. (Supported by the Center for Alternatives to Animal Testing)

SUPPRESSION OF NATURAL KILLER CELL ACTIVITY BY ANTISEIZURE DRUGS. N.C. Margareten and R.P. Warren, Utah State University, Toxicology Program, Logan, UT. Sponsor: R.P. Sharma.

Natural Killer (NK) cells are a discrete subpopulation of lymphoid cells with the innate ability to recognize and lyse certain tumor and virus-infected cells. There is evidence in rodents and man that NK cells have a role in resistance to growth and metastatic spread of tumors. Therefore depression of NK cell activity in humans could have deleterious effects. Phenytoin, a widely used antiseizure drug, depressed NK cell activity of mononuclear cells isolated from healthy human subjects (p<0.01) in the normal serum dose-dependent and was found at concentrations of phenytoin considered to be therapeutic. Propylene glycol, used frequently as a diluent for drugs, also depressed NK cell activity (p<0.01 at 1.4%v/v) but the antiseizure drug carbamazepine did not. Lymphocytes isolated from epileptic children currently receiving antiseizure medication were incubated in normal serum and sensitivity was found as compared to age-matched control subjects suggesting a long-term deleterious effect of therapy on NK cell function. Supported in part by USPHS ES07097.


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Host resistance models provide a complex challenge to immunologic mechanisms, giving a comprehensive evaluation of chemical effects. Five host resistance models are presently being used in B6C3F1 mice to assess the effects of xenobiotics on host resistance. *Listeria monocytogenes* (LM) and *Streptococcus pneumoniae* (SP) are the bacteria used. LM is primarily defended by macrophages and T-lymphocytes. 1-3x10^6 organisms given IV are lethal to 50-40% of the mice. SP is primarily defended by PMNs, antibody, and complement. 1-3x10^7 organisms are lethal in 3 days. Herpes simplex type 2 is the virus used. All components of the immune response are used for defense against this DNA virus. IV inoculation of 5-10x10^3 infectious particles produces paralysis by day 4 and death between days 7 and 14. *Plasmodium berghei* is the protozoan used and host resistance mechanisms are primarily antibody-mediated. Infection and death are elicited by as few as 5x10^5 parasitized erythrocytes. The pulmonary metastatic B16F10 melanoma is the tumor model in which NK cells, macrophages and T cells are important in implantation and growth. The IV injection of 1-3x10^7 tumor cells results in 20-200 visible tumor nodules in 20 days. These models have been used for 3 years as indicators of chemically induced immuno-toxicity. (Supported by NIEHS NO1-ES-5001).

**ASSESSMENT OF CELL-MEDIATED IMMUNITY.** J.A. Munson, M.P. Holsapple, K.L. White, Jr., D.L. Musgrove and A.E. Munson. Depts. of Pharmacology & Toxicology, and Biostatistics, Medical College of Virginia, Richmond, VA.

Reported here are the baseline studies on assays performed in B6C3F1 female mice over the last 3 years that are used for evaluating the effects of xenobiotics on the afferent and efferent arms of cell-mediated immunity. Delayed hypersensitivity (DHR) to keyhole limpet hemocyanin is measured by a stimulation index based on the influx of radiolabeled monocytes into the sensitized ear. Control values for the DHR range from 3.14 to 5.72. The mixed lymphocyte response to allogeneic spleen cells has control values on peak day (Day 4) which range from 20,000 to 45,000 cpm. Spleen cell response to the T cell mitogens, phytohemagglutinin (PHA) and Concanavalin A (Con A) has control values between 85,000 and 170,000 for PHA (Day 3) and between 90,000 and 190,000 for Con A (Day 3). Natural killer (NK) cell evaluation is performed on spleen cells and the baseline values are 10-15% for the 100:1 effector:target cell ratio. Macrophage assays are performed on peritoneal adherent cells and on the fixed cells of the liver, spleen, and lungs. Other assays used for assessment of cellular immunity include T cell enumeration and bone marrow analysis of cell number, DNA synthesis and CFU/GM. These assays have been validated and have been shown to detect immuno-toxicity. (Supported by NIEHS NO1-ES-1-5001).

**ASSESSMENT OF HUMORAL IMMUNITY.** A,E. Munson, H.L. Lysy, A.C. Anderson, M.P. Holsapple, and K.L. White, Jr. Depts. of Pharmacology & Toxicology, and Biostatistics, Medical College of Virginia, Richmond, VA.

As part of the National Toxicology Program, a series of assays has been established, capable of evaluating the effects of drugs and chemical agents on the humoral immune response in female B6C3F1 mice. The assays presented here represent refinements of previously established procedures, as well as recently developed methodology. The validation of the assays has been conducted over a three year period in several laboratories. The plaque forming cell (PFC) response to sheep erythrocytes (sRBC) is a functional assay which measures the ability of test animals to produce antibodies to a specific antigen. Control values range between 1600 and 2500 PFC/10^6 spleen cells. The effects of agents on serum complement have been studied using a microtiter hemolytic assay. CH50 values of untreated mice range between 45 and 90 U/ml. The baseline spleen cell response to the B cell mitogen, lipopolysaccharide (LPS) has a peak value of mean cpm's of between 40,000 and 105,000. Accurate risk assessment of the immuno-toxic potential of drugs and chemicals can only be made when interpretable data are obtained from validated assays for which historical control data have been well established. (Supported by NIEHS NO1-ES-1-5001).

**VALIDATION OF THE MOUSE EAR SWELLING TEST (MEST) AS AN ALTERNATIVE DERMAL SENSITIZATION MODEL;** B.J. Dunn, S.C. Gad and D.W. Dobbs. Department of Toxicology, AT&Ted Corporation, Morrisstown, N.J.

A reproducible test design for evaluating the potential of a chemical to induce a dermal sensitization response (using swelling of the mouse ear as an end point) was developed as a cost effective and reduced animal useage alternative to the guinea pig maximization test (GPMT).

Once a seemingly optimized general test design was developed, the sensitivity and validity of this design was evaluated utilizing a panel of known positive sensitizers and a smaller number of known negative compounds. Positive compounds were selected to represent as wide a variety of structural groups as possible, while the negatives were selected to include both the most common vehicles and types of compounds which would have the greatest potential for giving false positive reactions. The relative sensitivity and practicality of the MEST and GPMT were compared and are presented. Criteria for evaluating results were also developed and are presented. Test design variations were developed to allow evaluation of problem compounds and materials.
Traditionally, the predictive tests for dermal sensitization in man have been performed using the albino guinea pig (G.P.) as a model. There are a number of factors which make the prospect of an alternative model attractive. The best G.P. designs use a significant number of animals and are labor intensive. G.P.'s require significant caging and husbandry resources, and are becoming increasingly expensive. The results from G.P. studies are not truly quantitative due to the nature of the measured end point (subjective assessment of skin erythema and edema).

Following up on earlier reports in the literature which describe a wide variety of study designs, we have spent the last two years in extensive evaluation, development and validation of an alternative test using swelling of mouse ears as a measured end point. As part of the process we evaluated 8 strains, 8 age groups, sexes, induction forms (number, route and timing), use of adjuvants, interval to challenge, and vehicles. A methodology for preparing induction sites to give increased absorption of and test sensitivity to test compound was developed. A small battery of standard positive and negative controls was used to compare all these design variables, and the results were used to modify test design. The resulting optimized study design is markedly superior to the existing G.P. tests in terms of cost, time to perform, number of animals used, and amount of test substance needed.

The use of corn oil (CO) as a carrier vehicle for lipophilic chemicals is a common procedure in toxicology studies. In such experiments the CO is assumed to exert a negligible effect on the test animal. This may not be true when the impact of a toxicant involves the immune system. Adult mice were injected i.p. with CO or mineral oil (MO) and the peritoneal exudate cells (PEC) collected 5 days later. The CO and MO treated mice produced 76.6±10^6 and 17.7±10^6 PECs per mouse, respectively (uninfected mice yield 2-5x10^6 PECs). The total quantity of oil contained in the PECs was significantly higher in CO mice than in MO animals (8.7±mg vs. 0.33 mg). The amount of oil taken up per 6x10^6 PECs was also elevated in the CO treated mice (CO=0.62±mg, MO=0.11±mg). Overall, PECs from mice receiving CO contained 26.5 times as much oil as the PECs from mice given MO. When PEC suspensions from the CO, MO, and uninfected mice were diluted to 2x10^6 cells/ml, the CO cells showed a higher chemiluminescence value than MO or control cells. This suggests a greater proportion of "activated" macrophages to be present in the animals injected with CO. The change in PEC numbers and increased percentage of activated macrophages following CO stimulation seen in these studies indicate that the actions of the carrier vehicle may be significant, particularly when assessing immunocompetence.


A very rapid, versatile and inexpensive in vitro assay for screening the effects of xenobiotics on the phagocytosis of mononuclear phagocytes has been developed. Phagocytic cells may be treated with test materials once or repeatedly in vivo or in vitro and, if desired, the cells can be maintained for extended culturing before determining their phagocytic activity. This assay utilizes stained yeast cells as the phagocytic particle and quantitation is performed spectrophotometrically.

Increased macrophage activation and elicitation following intraperitoneal corn oil injection. P.E. Tan, R.D. Hindell, and M.R. Bleavins, Univ. of Wisconsin, Madison, WI. Sponsor: M.T.S. Haie

In vitro dose and time related effects of T-2 toxin, a trichothecene mycotoxin, on murine splenic and thymic cell responses. M.J. Taylor, B.J. Hughes, and R.P. Sharma. Toxicology Program, Utah State University, Logan, UT.

Studies were undertaken to investigate the direct effect of T-2 toxin on various populations of lymphatic cells. Cells, prepared from syngeneic male NFS mice, were cultured with three concentrations of either lipopolysaccharide (LPS), pokeweed mitogen (PWM), phytohemagglutinin (PHA), or concanavalin A (Con A) for 48 hours. T-2 toxin (10^-4 - 10^-7M) was added at 0 or 24 hr. Exposure to T-2 toxin (10^-11 - 10^-7M) after 24 hr caused an increase in H-thymidine uptake by splenic cells. PWM stimulation increased in this system, the response to LPS was increased to a lesser extent. However, T cell responses to PHA and Con A decreased. Forty-eight hr exposure to T-2 toxin (10^-11 - 10^-12M) decreased both the PWM and LPS responses. Thiolic cells (immature T cells) were even more sensitive to T-2 toxin; Con A responses decreased at 10^-1 M. The increased PWM responses correlated with previously observed effects of in vivo exposure. The results suggest a direct action of T-2 toxin on murine lymphocytes, perhaps via a lack of cell-mediated regulation by T suppressor cells. (Supported in part by USPHS ES07097.)
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. Enhancement of NK activity was found to be mediated by the induction of interferon. We report here that murine spleen cells exposed to MnCl₂ for 18 hr in vitro display increased NK cell activity. Enhanced NK activity by MnCl₂ in vitro was accompanied by interferon induction. Optimal enhancement of NK activity by MnCl₂ was observed at a concentration of 10 μg/ml. No difference in the number of viable cells was observed between control and MnCl₂-treated cultures. Treatment of spleen cells with anti-asialo GM₁ antibody and complement completely abrogated the enhancement of NK activity by MnCl₂ in vitro. MnCl₂ enhancement of NK activity does not appear to be mediated by endotoxin contamination of culture medium. Similar enhancement of NK activity by MnCl₂ was observed using a conventional source of fetal bovine serum and “endotoxin-free” fetal bovine serum. Results suggest that an adherent population of spleen cells is required during in vitro MnCl₂ exposure for enhancement of NK activity by MnCl₂ to occur. These results indicate that enhancement of NK activity by Mn Cl₂ is similar to that of more complex molecules.

Human fibronectin (HF) has been found to be a key factor in host defense mechanisms. Acting as an opsonic protein for the reticuloendothelial system, plasma fibronectin stimulates phagocytic uptake of some bacteria and other non-bacterial foreign matter in the blood. The purpose of this study was to evaluate the safety of HF following iv administration in rats and rabbits. In acute studies, HF was found to be well tolerated after a single iv administration of 45 mg/kg in rats and rabbits at a rate of 0.5 ml/min. In a 5 day repeated dosing study in rats, 5 animals/group were administered iv HF at 0, 15 or 45 mg/kg/day. No toxicity was found and no irritation was observed at the injection site. Similarly, rabbits (3/sex/group) also tolerated the above doses well with no systemic toxicity after 5 days of repeated iv administration. In vitro compatibility study of HF with human whole blood from donors revealed no evidence of toxicity. In conclusion, heterologous fibronectin was well tolerated by rats and rabbits, and in vivo studies, it was compatible with therapeutic blood. Therefore, this potential therapeutic agent is considered safe for clinical trial in man.

Nine monkeys (Macaca fascicularis), previously shown to have pulmonary and/or dermal hypersensitivity reactions from soluble platinum salt (Pt) inhalation exposures (Fed. Proc. 43:1936, 1984), were held at the NIOSH animal facility for a period of 15 months with no further Pt exposure. These animals' pulmonary and dermal responses to Pt were subsequently re-evaluated. One of five monkeys retained positive Pt dermal hypersensitivity while three of nine animals were still hypersensitive to a Pt pulmonary bronchoprovocation challenge. Dermal Pt hypersensitivity was examined by direct cutaneous sensitivity while Pt pulmonary hypersensitivity was examined by analyses of each individual animal's pulmonary function responses to a serial Pt bronchoprovocation challenge. Results were compared to each animal's pre-exposure baseline values. These data indicated that Pt pulmonary and dermal hypersensitivity reactions are long lasting in this primate model. The findings are consistent with long-lasting hypersensitivity reactions observed in terminated, sensitive Pt refinery workers.

The development of a model of experimental silicosis in the mouse is useful for studying the role of the immune system in modulating pulmonary inflammation and collagen deposition evoked by the instillation of silica crystals. A survey of six different strains of mice that differed at the H-2 and IgH loci demonstrated a significant variability in the level of response in both silica-induced inflammation and fibrosis. Female mice from 6 different strains were injected intratracheally with silica crystals in sterile saline (α-quartz, <5 μm) at 5 mg/mouse. Four wk post silica instillation, animals were sacrificed and evaluated for inflammatory and fibrotic changes. Pulmonary inflammation was monitored by increases in wet lung weight (indices) and cell number and protein content of the lung lavage fluid; fibrosis was assessed by increased hydroxyproline content of the lungs. DBA/2, Balb/c, C57BL/6 and C57BL/10 were significantly above C3H/He and CBA/J mice in hydroxyproline content, protein in the lung lavage and lung indices. DBA/2, Balb/c and C57BL/10 were also significantly above CBA/J animals in cell number and lung lavage fluid. This variability suggests an immune directed modulation of particulate-induced pulmonary damage.

(8H ES05259)
Acrolein (AC) is a highly reactive aldehyde. Possible immune alterations attributed to AC include weak sensitizing potential, suppression of antibacterial defenses, and immunosuppression by virtue of being a metabolite of cyclophosphamide. Four groups of 83 male rats each were exposed to 0, 0.1, 1.0, and 3.0 ppm of inhaled AC 6 hrs/day, 5 days/week for 3 weeks. Selected rats were then evaluated for changes in local pulmonary or systemic immunity by quantitating the number of antibody-plugging cells in the mediastinal lymph nodes or spleen following i.p. or i.t. immunization with sheep erythrocytes. T and B cell blastogenesis to phytohemagglutinin P and Salmonella antigen in the same organs and in vivo host resistance to Listeria monocytogenes were also measured. The splenic antibody response increased slightly with increasing doses, whereas the pulmonary antibody response was unaffected. Lymphocyte blastogenesis and resistance to Listeria challenge were not affected. Body and spleen weights were depressed in the 3 ppm group. Histopathologic examination of 5 transverse sections of the nasal turbinates revealed AC induced exfoliation, erosion, and necrosis of the respiratory epithelium, accompanied by inflammatory infiltrates and squamous cell metaplasia. Lung histology was unaffected.

In utero exposure to a single injection of the adenostine deaminase inhibitor 2'-deoxycoformycin (2dCF) has been reported to dramatically affect lymphoid tissues and immune function of offspring. Administration of 2dCF to adult mice results in lymphocytotoxicity and suppression of responses to T cell mitogens. We have employed 2dCF in our investigation of periods of sensitivity of the developing immune system to exogenous agents. On days 7 or 15 after breeding, plug positive female mice were given i.p. injections of 0.5 or 2.0 μg/g of 2dCF in saline or saline only. Male offspring of mice treated on d 7 had similar responses to control progeny. Female offspring of day 7-treated mice demonstrated higher levels of NK activity and had a greater percentage of antibody producing cells than controls, whereas blastogenic responses to PMA were reduced. Both male and female progeny of dams injected on day 15 also had increased numbers of antibody producing cells to SRBC. No other effects were observed in offspring of treated or control mice. Exposure of adult mice to 2dCF altered splenic and thymic weights and immune function.

The effects of subcutaneous zinc(II)acetate (ZnAcet) treatment on the acute toxicity of intraperitoneally injected nickel(II)acetate (NiAcet) in rats were studied. Male Fischer F-344 rats, 200-250 g, received either NiAcet alone, ZnAcet alone, NiAcet plus ZnAcet, or saline. In the lethality study the dose of NiAcet was 115 μmol/kg; for the other tests the dose was 95 μmol/kg. ZnAcet, body weight, and body weight gain were unaffected. No significant differences were noted in renal or hepatic function. The results indicate that ZnAcet inhibits some of the toxic effects of NiAcet in rats including lethality, hyperglycemia and nephropathy. The mechanism of this action does not appear to involve alterations in the pharmacokinetics of nickel(II),

The effect of AT-125 on urinary excretion of methymercury (MM) in the mouse. Kathleen M. Muler and Paul J. Kostyniak Department of Pharmacology and Therapeutics, SUNY at Buffalo, School of Medicine, Buffalo, NY 14214.

Previous experiments in this laboratory suggested that elevated glutathione concentrations (GS) and reduced N-glutamyltransferpeptidase (N-GTP) levels in urine may contribute to a strain difference in the urinary excretion of MM observed in the mouse. In the present studies, the N-GTP inhibitor AT-125 was administered to CBA/J mice pretreated with 50 μg/kg of MM (50 μg/kg) to determine whether increasing urinary GS in this strain would result in a significantly increased urinary excretion of MM. Doses of AT-125 ranging from 3.0 to 30 mg/kg increased urinary GS in a dose-related fashion. The peak effect was attained at two hours after injection. 7.5 mg/kg AT-125 increased urinary GS to a peak value of 250 ± 40 μM or 311 ± 63 mmol GS/mg creatinine. When this dose was administered to mice 24 hours after MM, either the rate of decline in body burden of MM, or the rate of 50% output into urine or feces, varied significantly from control values. Two hours after administration of 30 mg/kg AT-125, urinary GS was 129 ± 46 μM or 1.50 ± 0.13 μmol/mg creatinine. The dose of AT-125 resulted in a two-fold increase in urinary GS output and significantly increased the rate of decline in MM body burden during a four-hour period following AT-125 dosing. The results suggest that urinary GS must be in the μM range to affect a redistribution of MM across the luminal membrane.
Histopathological examinations of the liver and kidney have been carried out on mice with chronic cadmium intoxication subsequent to treatment with a variety of dithiocarbamates and BAL, all of which are able to mobilize cadmium from its aged deposits in vivo. The examinations carried out on animals sacrificed after 43 and 95 days, revealed that the observed pathological changes were dependent on the structure of the chelating agent and the time. Untreated animals showed, at 95 days, kidneys with hypocellular glomeruli with segmental sclerosis and lymphocytic infiltration. Animals treated with the more efficacious chelating agents did not show such features. The difference between the pathology of the liver of treated and untreated mice varied with the antidote.

Inhibition of Chromium-Induced Acute Nephrotoxicity by Ascorbic Acid. W.J. Power, Jr., K.M. Sliwko, and S.C. Cad. Allied Corp., Dept. of Toxicology, Morristown, NJ 07960

Previous work in this laboratory evaluated the efficacy of six potential therapeutic agents to protect against the acute nephrotoxic effects of chromium. Of these, only ascorbic acid (vitamin C) was successful. This protective action of vitamin C was examined further in this study. Vitamin C was thought to exert its protection against chromium by reducing Cr$^{6+}$ to the less toxic Cr$^{3+}$. EDTA, having a greater affinity for Cr$^{3+}$, could therefore enhance the effects of vitamin C. Three therapeutic regimens were used to treat chromium poisoning: 1) Vitamin C, 150 mg/kg/24 hours + EDTA, 25 mg/kg/12 hours administered concurrently immediately after chromium exposure. 2) One week pretreatment with vitamin C (30 mg/kg/24 hours) then vitamin C, 150 mg/kg/24 hours immediately after chromium exposure. 3) One week pretreatment with vitamin C, then vitamin C + EDTA immediately after chromium exposure. Acute mortalities were significantly reduced by all three treatment regimens following exposure to 80 mg/kg sodium dichromate. Acute administration of 20 mg/kg sodium dichromate produced proteinuria, glycosuria, phosphaturia, enzymuria and electrolyte imbalance. In each of the three therapeutic treatment groups these signs were significantly improved over the animals receiving only chromium. These findings strongly support our earlier recommendation of ascorbic acid use as an emergency therapy following accidental chromium exposure and now supports prophylactic treatment as well.


Ultrastuctural morphometric analyses conducted on proximal tubule cells of rats given a single i.v. injection of Pb (3.0 mg Pb/kg) and killed 0, 4, 8, 16, 24, 48 or 72 hr later showed that the overall volume density of both nuclear and cytoplasmic inclusions in proximal tubule cells increased after 8 hr to reach a maximum at 24 hr. After this point, the volume density of nuclear inclusions rapidly decreased but those in the cytoplasm remained on a plateau, indicating that formation of nuclear inclusions is a reversible process, and movement of Pb back into the cytoplasm may occur during this treatment regimen. The above changes were accompanied by 1.5- to 2.5-fold increases in nuclear volume densities at the 8-72 hr time points but little change in other organelles. Studies of renal protein synthetic patterns conducted using 2-D gel electrophoresis and monitoring incorporation of $^{35}$S methionine/cysteine showed the appearance of 3 new proteins and the disappearance of 2 proteins in Pb-injected rats relative to controls at the 24 hr time point. Results of these studies indicate intranuclear movement of Pb results in expression of specific new gene products and apparent repression of others at a dose below which overt cell injury occurs.


Urinary enzymes have been used to study the nephrotoxicity of chemicals. The mechanism of the acute renal toxicity induced by Cd-metallothionein (CDMT) was studied in rats using urinary enzyme markers. Different doses (0.05 to 0.4 mg Cd/Kg) of CDMT were injected to rats (250g) and 24 hr urine samples were collected in the cold; 3 days before and after injection. Urine samples were processed by centrifugation and dialysis. Urinary enzymes, N-acetyl-$eta$-D-glucosaminidase (NAG), a lysosomal enzyme; alkaline phosphatase (AP) and y-glutamyl transpeptidase (GGT), brush border enzymes were estimated in urine samples. The urinary excretion of these enzymes were significantly increased after CDMT injection, showing a peak at 1-2 days and then decreased. In vitro addition of Cd$^{2+}$ as CdCl$_2$, or CDMT to enzyme preparations had different effects; addition of CdCl$_2$ (>10 mg/ml) inhibited NAG and CDMT did not show an inhibition; GGT was inhibited by CdCl$_2$ (12.5 mg/ml) and CDMT (>250 ng/ml); whereas AP was inhibited by CdCl$_2$ (>25 ng/ml) but enhanced by addition of CDMT (962.5 ng/ml). These results suggest that although different forms of Cd may have different effects on certain urinary enzyme activities in vitro, they may be useful as non-invasive markers to study the CDMT induced nephrotoxicity. (Supported by MRC, Canada).
ASSSESSMENT OF NEPHROTOXICITY IN RENAL CORTICAL SLICES EXPOSED TO MERCURIC CHLORIDE (HgCl₂) AND POTASSIUM DICHROMATE (K₂Cr₂O₇). J.H. Smith and M.K. Boyd. NCI, Bethesda, MD

Recently, the mechanism of CHCl₃ nephrotoxicity was determined in an in vivo model where organic ion accumulation was monitored after in vitro exposure of renal cortical slices to CHCl₃. The purpose of this investigation was to adapt this model to evaluate other acute nephrotoxicants. Male Fischer 344 rat renal cortical slices were preincubated with HgCl₂ or K₂Cr₂O₇ (1.4 x 10⁻⁶ to 1.4 x 10⁻⁴M) under O₂. Following preincubation, slices were removed and incubated for physiological assessment of proximal tubular functions during a subsequent 90 min incubation. Slices were monitored for organic ion accumulation, gluconeogenesis, lipid peroxidation and glutathione (GSH). Preincubation and incubation media were assessed for the presence of renal enzymes originating from brush border, cytosol and lysosomes. HgCl₂ and K₂Cr₂O₇ (2.5 x 10⁻⁵M) produced similar decreases of organic ion accumulation, gluconeogenesis and GSH. Enzyme release (alkaline phosphatase, lactate dehydrogenase, β-acetyl a-glucosaminidase) was detected only in slices incubated with HgCl₂, though enzymuria is observed after in vivo administration of both HgCl₂ and K₂Cr₂O₇. Malondialdehyde was detected in slices preincubated with HgCl₂, which indicates lipid peroxidation. This model may be useful in characterizing the nephrotoxicity of other nephrotoxicants.

COBALT CHLORIDE EFFECTS ON RENAL FUNCTION. N.E. Davies. West Va U Med Ctr, Morgantown, WV

Cobalt chloride (CoCl₂) can protect against the nephrotoxic effects of hexachlorobutadiene (HCBD). In the present studies CoCl₂ effects on renal function were characterized in conscious male rats. Four groups of rats were used. CoCl₂-6H₂O (60 mg/kg) or NaCl vehicle was administered via sc injection (2 ml/kg) daily for 2 days. On day 2 HCBD (300 mg/kg) or peanut oil vehicle (1 ml/kg) was given by ip injection. Clearance studies were performed 1 day after HCBD. Glomerular filtration rates (clearance of inulin) was comparable in all groups, and decreased from untreated rats. CoCl₂ increased the urine concentration and excretion of K⁺. HCBD increased urine volume, whether expressed as flow rate (0.0045 vs 0.0015 ml·min⁻¹·100g BW) or as % of volume filtered (7% vs 1.4%). Urine K⁺ concentration decreased but in the HCBD group K⁺ excretion exceeded filtration. Glucosuria accounted for 20% of the urine output and 1.7% of the filtered load of water. CoCl₂ blocked effects of HCBD: urine flow rate was decreased (0.0021 vs 0.0014 ml·min⁻¹·100g BW) and glucosuria accounted for only 10% of urine output or 0.5% of volume filtered. These results indicate that CoCl₂ pretreatment has several actions to block tubular toxicity of HCBD. Supported by a grant from the West Virginia Heart Association, West Virginia Affiliate.


The mercuric ion is a potent nephrotoxin in the adult rat, but has little effect on neonates. Nephrotoxic response increases as the rat matures. The distribution and elimination of trace quantities of Hg are dissimilar in immature and adult rats (Thomas & Smith, Toxicol. Appl. Pharmacol. 43:43). Our study assessed the distribution of high levels of Hg in the maturing rat. CD rats received sc 5 mg/kg 203-HgCl₂ on postnatal day 1,8,15,22 or 29. Hg concentration was measured in the entire body, renal cortex, medulla, papilla, liver and subcellular fractions of hepatic and renal cortical cells 24, 48 and 120 hours post-injection (p.i.). Whole body elimination of Hg was slow in rats injected on day 1,8 or 15. Only 20% of the dose was excreted by 5 days p.i. Excretion was far more rapid in the day 22 and 29 rats, which had about half the initial body burden by 5 days p.i. Concentration of Hg in the renal cortex (the main site of Hg toxicity) 24 hours p.i. was higher than in any other tissue, and there was an age-related increase in this parameter. Increased distribution of Hg to the renal cortex may explain the increased response of maturing rats to Hg toxicity. There was an age-related decrease in the distribution of Hg to the liver. In subcellular fractions, Hg was found in greatest concentration in the 100,000 x g supernatant. The hepatic level of Hg decreases, and renal cortical content increases with postnatal age, corresponding to the increased toxic response.

AUTORADIOGRAPHIC DISTRIBUTION OF HEXACHLOROBUTADIENE. Study in the rat after single epicutaneous application. F. Duprat, D. Gradiski I.N.R.S., 54500 Vandoeuvre-les-Nancy (France)

The distribution of hexachloro-1,3-butadiene (HCBD) was studied after epicutaneous application of 14C-HC BD in male rats. A whole-body autoradiographic technique was used and in this way distribution pictures of non-volatile substances were obtained. The HCBD was rapidly absorbed through the skin and was shown to distribute very quickly throughout the body. The liver and the kidneys were organs which first had a high concentration of 14C. HCBD was eliminated by urine, bile and pulmonary routes. Later, labelled compound(s) can be detected in orbital glands and keratinized structures of upper GI tract. These later localisations are discussed (accumulation or excretion/secretion processes).
EFFECT OF 2-HEXANONE (HX) UPON 1,2-DIBROMO, 3-CHLOROPROPAINE TOXICITY M.F. Raisbeck, E.M. Brown W.R. Hewitt, University of Missouri-Columbia & SmithKline & French Lab.

The soil fumigant DBCP is a potent nephrotoxicant in the rat. Considerable evidence exists that it must be activated to a proximate intermediate, and that chemicals which modify this process modify the toxic effects of DBCP. Ketonic solvents such as HX potentiate the nephro- and hepatotoxicity of halocarbons such as CHCl₃, thus it is of interest to see if such compounds may also affect the toxicity of more complex, mixed halogen such as DBCP.

F-344 rats were dosed with HX (0, 1, or 10 mmole/kg po.) and, 18 hr later, DBCP (40, 80, or 100 mg/kg ip.) in a 3x4 factorial experiment. HX pretreatment potentiated DBCP induced lethality, body weight loss, and nephrotoxicity. DBCP alone induced necrosis of inner cortical proximal tubular cells and swelling of more superficial tubules. HX pretreatment increased the severity and to some degree, the extent of the lesion, but did not otherwise change the distribution of the lesion. HX pretreatment did not affect the hepatotoxicity of DBCP at the dosages studied.

EFFECT OF 2-HEXANONE (HX) UPON HEPATOCARCINOGENICITY OF 1,2-DIBROMO- AND 1,2-DICHLOROETHANE M.F. Raisbeck, E.M. Brown, and W.R. Hewitt, University of Missouri-Columbia and SmithKline & French Labs.

The ability of the ketonic solvent HX to potentiate the nephro- and hepatotoxicity of the halocarbons chloroform is well established. This experiment examines the effect of HX upon the nephro- and hepatotoxicity of the halogenated analogues EDB and EDC.

Adult, male F-344 rats were pretreated with HX (1 or 70 mmole/kg) po. then challenged at 12 and 24 hours with EDB (40, 70, or 100 mg/kg) or EDC (66, 132, or 200 mg/kg) ip.

HX potentiated the lethality of both EDB and EDC and the nephrotoxicity of EDB (70 and 100 mg/kg) as indicated histologically and by significant increases in BUN and creatinine at 24 and 48 hours post challenge. Interestingly, HX significantly decreased the SGPT response to 100 mg/kg at 24 hr. It was not possible to cause measurable hepatic or renal damage with sub acutely-lethal combinations of HX and EDC. EDC was consistently lethal within less than 24 hr to HX pretreated rats at dosages greater than 350 mg/kg; lethality was apparently due to cardiac dysfunction and pulmonary edema. Naïve rats did not evidence renal or hepatic damage at dosages less than 750 mg/kg EDC.
Isolated cells from target organs offer a potentially valuable approach for mechanistic studies. Hepatocytes and cortical tubules were prepared by concurrent collagenase perfusion of liver and kidney from male Sprague-Dawley rats dosed 0 with either 2,5-hexanediol (HD: 15 mmol/kg in corn oil) or corn oil only (CO: 10 ml/kg) 18 hr prior to cell isolation. Cortical digest contained primarily proximal tubule fragments, as indicated by high alkaline phosphatase (ALP)/hexokinase ratios, and few glomeruli or single cells. Incubations were conducted with hepatocytes and tubule suspensions in culture medium in closed glass flasks under carbon dioxide at 37°C for 4 and 8 hr, respectively. Under these conditions, 2 or 7 mM CHCl₃ and 2 mM 1,1,2-trichloroethane (TCE) markedly enhanced lactate dehydrogenase (LDH) release from HD hepatocytes relative to CO controls as reported by Jernigan et al. (Toxicologist 2, 37). These same haloalkane concentrations were also toxic to kidney tubules. However, there was no clear difference in the suppression of p-aminohippurate uptake and in LDH and ALP release between proximal tubules from HD or CO rats. Only N-acetylglucosaminidase release was somewhat higher in tubules from HD rats. These data suggest that the enhanced nephrotoxicity produced by HD pretreatment may derive at least in part from migration of haloalkane metabolites from liver. (Supported by internal R&D grant).

A subchronic toxicity study of the flame retardant 2,2-bis(bromomethyl)-1,3-propanediol (BMP; dibromonopropyl glycol; FR-1138; CAS No. 3296-90-0) was conducted in male and female Fischer 344/N rats and B6CF1 mice. The chemical was administered by oral gavage in corn oil five days per week for 13 weeks to rats at doses of 0, 50, 100, 200, 400, and 800 mg/kg and to mice at doses of 0, 25, 50, 100, 200, and 400 mg/kg. There was a dose-related decrease in body weight in male and female rats and mice at the top dose. BMP caused dose-related mortality in male rats at 800 mg/kg (2/10) and male mice at 400 mg/kg (3/10). Dose-related histopathologic lesions were seen in male rats and male and female mice. Transitional cell hyperplasia of the urinary bladder was seen in male rats and male and female mice. Lesions of the kidney in mice consisted of atrophy, degeneration, and dilation of the renal tubules. These results indicate that the kidney and urinary bladder are target organs with high doses of BMP.
A method of obtaining positional renal slices and a perfusion system for their incubation have been developed. Fresh rabbit kidneys are decapsulated after retrograde in situ perfusion. Three cylindrical cores of tissue (2.5 cm OD) are taken from each kidney, and reproducible slices are made on a mechanical slicer. (Anal. Biochem. 104:118, 1980). Four defined cell populations are isolated by positional slices: inner medulla contains collecting ducts (CD) and thin limbs (TL) only; inner stripe (IS) contains CD, TL and ascending thick only; outer stripe has all components of IS plus straight proximal tubules, while cortical slices contain all renal cell types except TL. The slice cuts over 20 slices/min in any selected thickness between 100–500 

um ± 10%. Up to 6 slices, set on a 1.25 cm thick sponge with a central hole, are perfused in a 10 ml beaker. Serum free media is added to just above the surface of the apona. Bubbling with O_2-CO_2 (95:5) through PS 20 tubing placed in the center hole causes a circulating current through the apona. Incubation in this system for 6 hr at 25°C produced no histologic damage. This methodology offers a means of studying site specific nephrotoxicity in a controlled environment, while maintaining cellular interactions and integrity. (Univ. of Ariz. Grad. Fellowship and NIH-S07-RRO 

-577777).
COMPREHENSIVE EVALUATION OF THE URINARY TRACT AFTER CHRONIC EXPOSURE TO CYANURATE IN DRINKING WATER. T. Cascieri, FMC, Princeton, NJ; S. Barbee, Olin, CT; B. Hammond, Monsanto, MO; T. Inoue, Nissan, NY; N. Ishida, Shokoku, CA; A. Wheeler, ICI, DE.

Cyanurate (CA) is used in water treatment. Pharmacokinetic data (rat, dog, human) showed CA is well-absorbed orally then rapidly eliminated unchanged in excreta while dermal exposure resulted in minimal uptake. Thus, the relevant exposure approach was ad libitum offering for 24 months to rats (80-100g/excpine) of monosodium CA saturated drinking water (0.7% by wt.) coupled with 3 lower levels (.35, .17 and .07% by wt.), untreated and Na controls. Sacrifices were made at 6, 12, 19 and 24 mos. with emphasis on urinary tract exams. CA acted via a physical mechanism, i.e., precipitation unchanged in urine, resulting in secondary effects (mechanical irritation/physical obstruction) in the urinary tract of a limited number of high dose rats which had received 500-900 mg/kg/day of test material prior to sacrifice. A direct nephrotoxic effect was absent based on light/electron microscopic kidney exams (incl. renal papilla/pelvis) clinical chemistry/urinalysis (incl. anal. for enzymes; potassium and other electrolytes). CA is also noncarcinogenic. A mouse study still in progress at similar levels has revealed no kidney toxicity through 18 mos.


As an aid in the examination of the causal factors of hydrocarbon nephropathy in the male rat, a rapid (2 week) urinary screening procedure was developed. Thirty male F-344 rats (10 animals/treatment) were orally dosed twice per week (2ml/Kg) for two weeks with 2,2,4 trimethylpentane (TMP), 2,3,3, TMP or 2,3,4 TMP. Controls consisted of 10 untreated, sham manipulated animals. 24 hour urine collections were obtained and analyzed immediately following each dose. Increases in four urine parameters (protein, cell counts, NAG activity and GOT activity) which have been shown previously to be reliable indicators of hydrocarbon nephropathy in the male F-344 rat were used to compare the relative nephrotoxicity resulting from each treatment. Significant (p<0.05) nephrotoxicity, relative to controls, was detected following treatment with each of the TMP isomers. Based on the magnitude of changes observed in urine parameters, the relative nephrotoxicity of the TMP isomers was: 2,3,4 TMP > 2,2,4 TMP > 2,3,3 TMP > Control.


Recent interest has been focused toward an understanding of the functional effects of pure and mixed hydrocarbon exposure on the kidney of male rats. As part of an ongoing program to characterize the time course of renal lesion development using biochemical markers, male F-344 rats were exposed continuously to jet fuel vapor (90 days) at 1000 mg/m3. The rats were removed one day/wk for 24 hr urine collections. The standard urine assay included, NAG, Alk Phos, LDH, GOT, creatinine, protein, osmolality and urine volume. Following exposure, the animals were allowed to recover for 9 wks during which a urine concentration test was performed and monitoring of urinary parameters continued. Significant elevations were observed in NAG, LDH, GOT and protein in treated rats relative to controls. Immediately following exposure, a significant reduction in the urine concentrating ability of exposed animals was noted. All biochemical indicators returned to control values following the 9wk recovery period.


Nephropathy has been demonstrated in male rats inhaling JP-5 (150 mg/m³) continuously for 90 days. This study was conducted to investigate the effect of a single 60 min. inhalation exposure to JP-5 at 2500 mg/m³, the current N.A.S. recommended EEL. Groups of 20 exposed and 20 control male F344 rats. Necropsies of 10 rats/wk were conducted at 24 hrs and 28 days postexposure. Urine was collected periodically to evaluate osmolality, volume, protein, NAG, Alk. Phos., glucose, LDH, yGTP, GOT and renal epithelial cell presence. Growth, water consumption, organ weights, and blood parameters were analyzed. Liver and kidney tissues were examined microscopically. Exposure to JP-5 produced eye irritation and mild CNS depression, with symptoms disappearing within 2 hrs postexposure. Significant (p<0.05) elevations in urine glucose, LDH and GOT developed within 24 hrs postexposure, but returned to control levels by day 6. Serum BUN and Alk. Phos. were also increased. Renal epithelial cell exfoliation was noted in exposed rats examined postexposure, without concomitant increases in urinary biochemical markers. Exposed rats displayed mild diffuse hylane droplet formation in the proximal convoluted tubules. Body weights, organ weights, hematology, water consumption and urine osmolality were unaffected.
THE SELECTIVE INDUCTION OF \( \omega \) AND 8-OXIDATION OF FATTY ACIDS IN THE LIVER AND KIDNEY OF MALE RATS ADMINISTERED 2,2',4-TRIMETHYLPENTANE.

E.A. Lock, M.D. Stonard, and C.R. Elcombe, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

2,2',4-Trimethylpentane (TMP) is a component of unleaded gasoline which produces a sex-related increase in renal hyaline droplet formation and tubular necrosis. We have examined the effect of ten daily doses of TMP (2ml/kg, po) on renal and hepatic microsomal and peroxisomal enzyme activities and the concentration of \( \alpha \), \( \beta \), and \( \gamma \) globulins in male rats. TMP treatment increased liver weight (167% of control) and kidney weight (116%). In the liver, the concentration of cytochromes P-450 and b5 and the activities of aldrin epoxidase, ethoxycooumarin-0-deethylase and ethoxyresorufin-0-deethylase were not markedly altered. However both lactic acid hydroxylation (LAD) and cyanide insensitive palmitoyl CoA oxidation (PCoA) were increased to 300% and 320% of control values respectively. Renal cytochrome P-450, LAD and PCoA were also increased to 334%, 1492% and 240% of control values respectively. Renal \( \alpha \), \( \beta \), and \( \gamma \) globulins increased ten fold while other renal microsomal activities were unaffected. TMP appears to selectively induce both \( \omega \) and 8-oxidation of fatty acids, presumably to facilitate its own metabolism.

DISPOSITION AND METABOLISM OF N-METHYLPIRROLDONE IN THE RAT. D.A. Wells and G.A. Degenis, College of Pharmacy and Graduate Center for Toxicology, University of Kentucky, Lexington, KY. 40536.

Sponsor: H.W. Dorough

N-Methylpyrrolidone (NMP) is an aprotic solvent which is widely used in many large scale chemical processing operations. The disposition of radiolabeled NMP was studied in male Sprague-Dawley rats following a single i.v. injection. Urinary excretion accounted for the elimination of 73% and 76% of the administered 5 \( \mu \)Ci dose within 12 hr for [ring-\( ^{14} \)C]NMP and N-[ring-\( ^{14} \)C-methyl]pyrrolidone (N-[ring-\( ^{14} \)C-MIPR], respectively. Only 2.3% of either 10C-dose was found in the feces and less than 1% was eliminated through respired air after 24 hr. Tissue distribution studies showed that 10% of the 14C-dose of N-[ring-\( ^{14} \)C-MIPR remained in the major organs after 6 hr. The liver, small and large intestines and their contents had the highest accumulation of 14C-activity at this time. Radiomonitored HPLC analyses of 12 hr urine samples obtained following an i.v. dose of [4-\( ^{14} \)H]-N-[ring-\( ^{14} \)C-MIPR or [ring-\( ^{14} \)C]NMP revealed one major metabolite, in each case, which represented 96% of the total urinary radioactivity. This was determined to be a conjugate which upon acid hydrolysis yielded a product containing all three radiolabels and tentatively identified as N-methyl-\( \gamma \)-aminobutyric acid. It was concluded that NMP is rapidly metabolized in the rat and eliminated primarily through the urine as one major metabolite.

As previously reported, in rats fed ethyl 5-\text{cyano-3,4-diphenyl-6-oxo-1(6H)-pyridazinylacetate (DS-29399)}, for 30 days (12.5-200 mg/kg/day), pathologic hypertrophy was noted in tubular cells of the inner cortex of the kidney. Cells were greatly enlarged with atypical mitotic figures. To gain more information on structure-activity relationships, a pilot 30-day feeding study in rats was conducted with pyridazinone structural analogs related to DS-29399. The ethyl acetate group of DS-29399 was substituted as follows: R=acetic acid (Compound A); R=methyl propionate (Compound B); R=methyl (Compound C); R=hydrogen (Compound E). Each compound was fed at two dose levels, 10 and 200 mg/kg/day. The most extensive and severe kidney lesions were noted in rats fed Compound B. Hypertrophy of tubular cells of the inner cortex was noted in all animals and atypical mitotic figures were present. In rats fed Compound B a dose related incidence of kidney lesions was noted. Hyperplasia of tubular cells was present only in the high dose group fed Compound C. Microscopic lesions in the kidneys were not present in groups fed Compounds D and E. Results suggested that Compound A is a common toxic metabolite for DS-29399 and Compound B. Compound C appeared to be metabolized by a similar mechanism.


The purpose of this study was to evaluate the effect of surgically isolating the kidney on renal function. Cannulae were implanted in the carotid artery, the left renal vein, and the urinary bladder. This permitted simultaneous sampling of blood entering and leaving the kidney and of the urine for up to 225 min following surgery. Inulin, para-aminohippuric acid (PAH), phenolsulfonphthalein (PSP) and/or normal saline were administered via the jugular vein. The measured renal clearance of inulin and PAH, and the renal excretion rate for PSP were comparable to values obtained in sham-operated rats and to reported literature values. Renal plasma flow and glomerular filtration were calculated as 5.6 ml/min and 1.2 ml/min, respectively. A physiologically-based pharmacokinetic model was developed to simultaneously describe the disappearance of inulin and PAH from arterial and renal venous blood and their excretion into the urine. These data suggest that the surgical procedure did not adversely affect renal function and that this surgical procedure provides a means to directly measure the renal excretion parameters needed for physiologically-based pharmacokinetic models.

Senescent rats (1 and 2.5 years old) are more susceptible to cephaloridine (CPH)-induced nephrotoxicity than young adults (2.5 and 4 months old) following a 24-hour treatment. These experiments were designed to determine if the increased susceptibility of senescent rats to CPH nephrotoxicity is related to an enhanced intrinsic susceptibility of the kidney cells to the toxic effects of CPH. Male F-344 rats (2.5, 4, 10-12 and 33 months) were used. Renal cortical slices from naive rats were incubated in a phosphate-buffered medium containing p-aminohippurate (PAH), tetraethylammonium (TEA), and 0, 1, 5 or 10 mM CPH. Slices were incubated at 37°C for either 90 or 180 minutes. CPH decreased the renal cortical uptake of PAH and TEA in a concentration-dependent manner in all ages studied. Similarly, CPH increased malondialdehyde production, an index of lipid peroxidation, by renal cortical slices from both young and senescent rats. However, an increased susceptibility to CPH nephrotoxicity with age was not observed in vitro. These results suggest that the increased susceptibility of senescent rats to CPH nephrotoxicity in vivo is not related to an enhanced intrinsic susceptibility of the aging kidney.


The nephrotoxic potential of compounds like the aminoglycoside antibiotics and DEAE dextran is related to the cationic properties of the molecule. Vancomycin (VAN) and ristocetin (RIS) are cationic, (pH 7.4 and 11, respectively) glycopeptide antibiotics. VAN is nephrotoxic in animals and humans. If VAN-induced renal injury is related to cationic charge, RIS should possess an equivalent nephrotoxic potential. Female C57BL mice received a single (ip) injection of VAN or RIS and the extent of renal injury determined 48 hr later. Dosages of VAN and RIS ranged from 0 to 750 umol/kg. Renal injury was assessed as alterations in kidney weight, blood urea nitrogen (BUN) concentration and renal cortical slice organic ion accumulation. VAN increased kidney weight at dosages of 225 to 750 umol/kg. VAN depressed slice organic ion accumulation and elevated BUN at dosages of 450 to 750 umol/kg. Renal dysfunction was not produced by 75 umol/kg VAN. RIS increased kidney weight (750 umol/kg) but did not produce renal dysfunction at dosages of 75 to 750 umol/kg. The marked differences in the nephrotoxic potential of VAN and RIS suggest that cationic charge may not account for the nephrotoxic potential of VAN.


Fenoldopam mesylate, a dopamine-ergic vasodilator, was administered orally, twice daily, to Charles River CD rats or beagle dogs for 3 months to assess drug toxicity. Rats (15/sex/group) were administered doses ranging from 15 to 130 mg/kg/day; dogs (3/sex/group) received doses ranging from 6.25 to 48 mg/kg/day. Control animals were dosed with an equivalent volume of distilled water (rats) or empty gelatin capsules (dogs). The principal toxicologic effect in rats was a dose-related increase in renal tubular damage which was associated with slight elevation of BUN and creatinine values, neutrophilia and increased myeloid-erythroid ratios, and the presence of crystals in the urine. The latter were identified as the 8-glucuronide conjugate of the drug. Fenoldopam produced scleral and mucosal congestion in dogs at a dose-related frequency, but was without any other toxicologic effect. The apparent species difference in the oral toxicity of fenoldopam mesylate may be related to metabolic differences, since the glucuronide conjugate of the drug is the major urinary metabolite in the rat whereas the sulfate conjugate is the major urinary metabolite in the dog.

AN IN VITRO NEPHROTOXICITY ASSAY. J.F. Sina, C.L. Bean, C. Noble, and M.O. Bradley, Merck Institute for Therapeutic Research, West Point, PA. Sponsor: Mark Hite

Suspensions of proximal tubules from rabbit kidney have been used to establish an assay with which the inherent nephrotoxicity of chemicals can be determined. The incorporation of H-leucine into protein was used as the indicator of toxicity since protein synthesis is critical for cellular maintenance and function and requires a metabolically competent cell. To validate the assay, the rank order of toxicity of a series of cephalosporins was determined. Based on the D50 (dose required to reduce incorporation to 37% of control), the ranking of these drugs in vitro was similar to that reported in vivo with the exception of cephalothin which was more toxic in vitro than expected. Data in the literature suggested that this discrepancy might be due to variations in drug distribution. We therefore developed an assay to determine the concentration of drug at the target site/unit time. We found that cephalothin is cleared from the kidney more rapidly than are other cephalosporins, thus target organ exposure is reduced. When drug distribution is accounted for, the toxicity of the drugs in vitro parallels that seen in the clinic. The methodology has also been used to address basic research questions such as possible mechanisms of toxicity at the cellular level, effects of drug interactions on toxicity, and whether differences in species susceptibility to chemicals reflect differences in inherent toxicity or variations in drug distribution.
Limited information is available regarding the hepatoxicity of toluene (T). So we have evaluated the subchronic hepatoxic potential of T using male Fisher-344 rats as animal models. T was given orally in corn oil to groups of 6 rats at doses 0, 10 and 20 mmole/kg b.w. each day, 5 days a week for 3 consecutive weeks. Urines were collected for 48 h after the exposure and the rats were killed. Urinary volumes were increased due to T at 20 mmole/kg during 0-24 h. Significantly increased levels of urinary glucose and proteins were observed at both dose levels during 0-24 h; urinary proteins were also significantly elevated at the higher dose during 24-48 h, although an increase but not significant was also found at 10 mmole/kg. Urinary level of N-acetyl-8-D-glucosaminidase was significantly increased at 20 mmole/kg T during 0-24 h but not significantly during 24-48 h. Renal cortical accumulation of para-aminophenol was significantly reduced at 10 mmole/kg T after 48 h. Urinary creatinine and BUN levels were not affected. Serum transaminase activities were modified due to T. A dose-dependent decrease in the urinary excretion of hippuric acid was found. Thus subchronic oral administration of T may produce renal damage preferably at the proximal tubular region in Fisher-344 rats. (Supported by IRSST, Quebec).

Polychlorinated dibenzo furans (PCDFs) and related toxic halogenated are hydrocarbons elicit a number of common biologic and toxic responses which are triggered by their initial binding to a cytosolic receptor protein. These effects include the induction of several cytokine P-450 dependent monoxygenases (eg, aryl hydrocarbon hydroxylase, AHH), body weight loss and thymic atrophy. The dose-response effects of selected PCDFs on AHH induction in rat hepatoma H-4-II E cells and cytosolic receptor binding affinities have been determined. The results of these in vivo and in vitro studies demonstrate the remarkable effects of structure on the activity of PCDFs. A systematic study of each of the four different position for chlorine substitution in the dibenzo furan ring system showed that the toxic and biologic potencies of these compounds varied with respect to differential chlorine substitution at all four position, i.e. C-3(7) > C-2(8) > C-4(6) > C-1(9). The structure-activity relationships for PCDFs were different from the polychlorinated dibenzop-dioxins and this was directly related to the asymmetric structure of the former group of compounds. (Supported by the National Institutes of Health)

Primary hepatocyte cultures prepared from female SD rats were used to further investigate the mechanism(s) by which PCBs are released from hepatic tissue. 1.48 nmol 6-CB was dissolved in dimethylformamide and this solution was mixed with Fetal Bovine Serum (FBS). Following 3 hours of incubation, 43% (0.6 nmol) of available 6-CB was taken up by isolated hepatocytes from FBS. Subsequent release of 6-CB from hepatocytes was examined at 4 and 24 hours of culture in serum- and albumin-free media. At 4 hours, 78% of 6-CB was released from hepatocytes and it was associated exclusively with protein (1.7 pmol 6-CB/mg protein) despite the presence of newly synthesized triacylglycerol (TG)-rich very low density lipoproteins (VLDL) in the culture media. Following 24 hours of culture, no further release of 6-CB from hepatocytes was observed (79%). Although association of 6-CB with VLDL was detected at 24 hours, and its concentration in VLDL (41 pmol 6-CB/mg TG) was greater than that in protein (2.6 pmol 6-CB/mg protein), 97% of total 6-CB released could be accounted for in the protein fraction. These data suggest that the great majority of 6-CB which redistributes from hepatic tissue becomes associated with newly-synthesized protein. (Supported by ES 03493).

Hepatomegaly and Lipid Metabolism in Rats Treated with 2,3,7,8-Tetrachlordibenzo-p-dioxin (TCDD). B.J. Christian, L.A. Menahan, and R.E. Peterson, University of Wisconsin, Madison, WI.

The effect of TCDD on liver mass and lipid metabolism was examined by determining selected plasma and tissue constituents. Mature (450g) Sprague-Dawley rats, adapted to a B+6 feeding schedule, were treated with 75 µg TCDD/kg or corn oil/acetone (vehicle). Tissues were sampled 2, 4, 6, and 8 days posttreatment at 16 hr after feeding. TCDD caused liver enlargement, evident at day 2 and plateaued at 6 days, compared to pair-fed rats which maintained a constant liver weight. The liver enlargement was due to cellular hypertrophy and not hyperplasia since TCDD-treated and pair-fed rats had a similar total DNA content/liver. The hepatocellular enlargement of TCDD-treated rats was partially due to higher levels of phospholipid and protein which increased progressively after treatment and is indicative of membrane proliferation. The primary factor contributing to the liver hypertrophy at day 2 was increased accumulations of glycogen and triacylglycerols (TG). Hepatic and plasma TG were 3X higher in TCDD-treated rats than in pair-fed animals at all times after treatment. Yet, plasma ketone bodies and free fatty acids were lower in TCDD-treated rats than in their pair-fed counterparts. These results suggest that TCDD enhances the esterification of fatty acids at the expense of ketone body production. (Supported by NIH grant ES01332).
Guinea pigs were treated with TCDD (dioxin) in corn oil, corn oil, cleaned soil, TCDD added to clean soil, and soil contaminated with TCDD at a 2,4,5-T manufacturing site. Comparable volumes and doses of soil and vehicles were used in all cases (6 μg TCDD/kg, contaminated soil equivalent to 6, 12, and 24 μg TCDD/kg). Animals were observed for signs of toxicity upon to two months following a single dose, and autopsied after death or at the end of the experiment. Two thirds of the guinea pigs treated with TCDD or TCDD on cleaned soil died within one month, showing the typical TCDD wasting syndrome with typhic atrophy. No control animals or those treated with contaminated soil at any dose showed this syndrome or died after day 1 (gavage error). Analysis of composite liver samples showed high TCDD levels in animals treated with TCDD on cleaned soil (18,000 ppt), much lower levels in animals treated with contaminated soil (97 ppt at 24 μg/kg level), and none in clean soil treated guinea pigs. These data suggest that the bioavailability of TCDD from this soil is less than 0.03%. Supported by contract no. C-29786 N. J. Dept. of Environmental Protection.

**Toxicity of TCDD in Guinea Pigs Fed by Total Parenteral Nutrition (TPN)**


Treatment of animals with TCDD causes a progressive weight loss with parallel mobilization of adipose tissue. This study investigated the effects of continuous TPN feeding on the toxicity of TCDD in male, Harleym strain, guinea pigs. At a dose of 2.0 μg TCDD/kg i.p., the TPN-Fed, TCDD-treated animals demonstrated a weight gain which was slightly below that observed for the TPN-fed controls. 50 percent (5/10) of these animals died between days 6 and 12 following treatment. The remaining TCDD-treated animals appeared moribund and were killed at day 21 following treatment. Morphologically, the TPN-fed animals demonstrated hepatocellular accumulation of lipid and or glycogen as well as fatty alteration in the liver, kidney, and pancreas. However, in all cases no significant shift differences were observed between olive oil-treated or TCDD-treated animals (TPN-fed) were observed. Quantitatively, significant increase in liver weight, hepatic content of total lipid, cytochrome P-450, as well as serum cholesterol and triglyceride were observed in TCDD-treated animals. All of these were also compared to animals fed ad libitum, pair-fed to TCDD-treated groups, and with or without saline infusion. These results suggest that in addition to decreased food consumption and body weight loss, other metabolic alterations significantly contribute to TCDD-induced toxicity and lethality. (Supported in part by NIH Grant ES02069 and EHS Center Grant ES01077).

**The Role of Ornithine Decarboxylase Induction in the Toxicity of 2,3,7,8-TCDD**

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Ornithine decarboxylase (ODC), an enzyme whose activity modulates cell growth and differentiation was induced by 2,3,7,8-TCDD and the role of this activity in mediating the toxicological effects of 2,3,7,8-TCDD was investigated. Male Wistar rats, treated with an acute dose of 2,3,7,8-TCDD (250 μg/kg) exhibited a 6 hour peak of induced ODC activity which preceded the 72 hour maximal induction of microsomal aryl hydrocarbon hydroxylases and cistrons 9-o-deethylating amines. One week following 2,3,7,8-TCDD exposure, the rats exhibited several signs of toxicity which included a dramatic loss of body weight and a severe depletion of lymphocytes in both the thymus and the spleen. The co-administration of p-fluoromethylornithine (500 mg/kg) with 2,3,7,8-TCDD eliminated the induction of ODC and affected the rate, but not the extent of induction, the whole body wasting syndrome and the lymphocytic atrophy of the immunological organs. It was concluded that ODC activity does not mediate the toxicity of 2,3,7,8-TCDD and that its induction in rats, in contrast to mice, is not regulated by the Ah receptor. (Supported by the National Institutes of Health)

**Hydrogen Peroxide and Free Radicals in TCDD Induced Peroxidative and Mitochondrial Malondialdehyde Formation**

S. J. Stich, M. O. Hassan, Z. A. A. Al-Bayati, and W. J. Murray. Department of Biochemical Chemistry, University of Nebraska Medical Center, Omaha, NE

Previous studies have shown that TCDD induces hepatic microsomal lipid peroxidation as measured by malondialdehyde (MDA) formation and content of conjugated dienes. The mechanism by which TCDD enhances lipid peroxidation is not known. Microsomes and mitochondria from control and TCDD treated animals were isolated, and the effects of catalase, superoxide dismutase, and selected free radical scavengers on formation of MDA were determined. MDA formation was inhibited by catalase but not superoxide dismutase with microsomes and mitochondria from both control and TCDD treated animals. In the presence of Triton X-100, hydroxyl radical scavengers also inhibited MDA formation with microsomes from control and TCDD treated rats. Greatest inhibition was observed with microsomes from TCDD treated animals. Single oxygen scavengers also inhibited MDA formation in the presence of Triton X-100 with microsomes from TCDD-treated rats. The results suggest that H2O2, singlet oxygen and hydroxyl radical are involved in the TCDD-induced microsomal lipid peroxidation.
ADENINE (Ad) and ITS ANALOGUE INDUCE CHANGES IN LIPID PARAMETERS IN CONTROL AND 2,3,7,8-TETRA-CHLORODIBENZO-P-DIOXIN (TCDD)-EXPOSED RATS WHICH AFFECT TOXICITY. C.M. Schiller, R. Walden, C.M. Adcock and C.R. Shaof. Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC

TCDD is a potent dioxin that readily increases serum and liver lipids in rats. The Ad analogue 4-aminopyrazolopyrimidine (APP) decreases serum lipids and lipoproteins (LP) by blocking the release of liver triglyceride (TG) as administered 0, 1, 6 or 12 mg APP/kg body wt (i.p., every other day for 3 wk). Examination of serum 4, 8 and 12 hr after dosing, indicated that 12 mg APP/kg was most effective in decreasing serum TG and very low density LP. This dose of APP was given with 325 µg TCDD/kg body wt (2 x the LD50). In a mortality study, groups of rats (N=7) were given vehicle (corn oil) (C), TCDD in vehicle (T), vehicle + APP (C-APP) and TCDD + APP (T-APP). These animals were pair fed, C v. T and C-APP v. T-APP. The T-APP did not prolong life; i.e., mean time to death 10.5 d in T-APP v. no deaths in T within 21 d after exposure. Ad, which lowers liver TG, was given 38 mg Ad/kg body wt (i.p., every other day for 10 d) and induced a decrease in liver TG, 27 and 24% respectively in Ad and T-Ad. In contrast to APP, Ad stimulated food removal and decreased body wt loss in both T-Ad and T-Ad as well as increased the mean time to death. The effects of these agents may be indicative of the critical metabolic breach in TCDD toxicity.


One of the earliest responses observed following TCDD exposure is an increase in serum lipids. To further understand the importance of this increase we investigated the effect of TCDD on the concentration of blood ketone bodies. Male Sprague-Dawley rats were treated with a single 1p dose of TCDD at 25 or 100 µg/kg (both doses being below the LD50); controls, pair-fed (PFC) and ad libitum (ALC), received an equal volume of vehicle only. Whole blood levels of B-hydroxybutyrate (BHB) and acetoacetate (AcAc) were measured at 3, 7 and 10 days after a 24-hr fast. Data was analyzed in a paired t-test. At 25 µg/kg there was no difference in BHB or AcAc levels at all time points. There was a decrease in BHB levels in rats receiving 100 µg/kg compared to PFC at 3 and 10 days, 0.81 vs 1.48 and 0.33 vs 0.51 mM, respectively. Decrease were also seen in AcAc levels at these times, 0.13 vs 0.20 and 0.08 vs 0.12 mM, respectively. In addition, decreases were found in BHB levels as compared to ALC at days 7 and 10(0.56 vs 0.43 and 0.33 vs 0.11 mM) and AcAc levels at all time points(0.13 vs 0.28; 0.11 vs 0.28 and 0.08 vs 0.31 mM). Changes versus ALC may reflect differences in adipose tissue stores, however, decreases as compared to PFC suggests a fundamental alteration in the processing of fatty acids after TCDD treatment. (NIN Grant ES02859, NIN Training Grant 5T32ES07026 and EHS Center Grant ES01247)

RESPONSES OF LACTATING DAIRY COWS TO POLYCHLORINATED BIPHENYLS. L.B. Willett, H.I. Durat, and T.-T. Y. Liu. Ohio State Univ./Ohio Agricultural Research & Development Center, Wooster, OH

Polychlorinated biphenyls (PCB) have entered the feed of some dairy cattle over the last 50 years. Farn silos, coated with a material that contained PCB, have been a major source. Four lactating Holstein cows (mean body weight = 576 kg) were orally dosed with 10, 100 and 1,000 mg/day of Aroclor 1254 in sequential 60-day periods. Six control cows received daily sham doses. The following were recorded: (daily) milk production, feed intake, heat observations (weekly), body weight, heart and respiratory rates; plus 12 determinations of milk PCB residue and blood clinical chemistry (22 components) from predose through the end of dosing. Each cow had a liver biopsy and sulfobromophthalein (BSB) clearance predose and at the end of each dosing period. Average 305-day lactations were not significantly different between control (6,822 kg) and PCB-dosed (7,545 kg) animals. Clinical signs indicating toxicosis were not evident, mean clinical chemistries were within normal range, and liver histologies and BSB clearance were not different for dosed and control cows. Average PCB residue concentrations in milk fat at the end of the three dosing periods were 2, 13, and 91 µg/g, respectively. PCB exposure, resulting in milk fat residues 10 times those typically encountered, had no apparent effect on health and productivity. (Supported in part by Monsanto Contract No. RF 715618.)

DERMAL ABSORPTION KINETICS OF LIQUID BROMO-CHLOROETHANE IN RATS. G. W. Jeppson, J. N. McDougall and M. E. Andersen, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH

Determination of skin permeability constants for chemicals dissolved in vehicles is important to understanding the skin as a barrier. In this study we determined absorption kinetics of bromochloromethane (BCM) in rats treated dermally with neat BCM or BCM diluted (100 to 1000 µg/ml) with mineral oil. BCM was injected into a 1.75 cm² glass chamber glued to skin on the back. Serial blood samples were drawn for 24 hr through a jugular cannula and analyzed for BCM and carboxyhemoglobin (COHb) levels. Permeability constants were determined by accounting for storage in blood and tissues, the amount metabolized and amount exhaled. Permeability constants were 0.0075 (100 µg/ml), 0.0088 (500 µg/ml), 0.0070 (1000 µg/ml), and 0.0074 cm/hr (neat BCM). Steady-state blood BCM increased from 0.6 µg/ml at an applied concentration of 100 µg/ml to 72.2 µg/ml at 1000 µg/ml. COHb levels were related to the extent of microsomal metabolism of BCM. Steady-state COHb level was 2.1% at 100 µg/ml and 16.3% at 1000 µg/ml. Plasma bromide concentrations were determined to quantify total BCM metabolism. Plasma bromide levels ranged from 2.3 ppm at 100 µg/ml to 11.2 ppm at 1000 µg/ml. The permeability constants of BCM were similar regardless of its concentration in a mineral oil vehicle.
Biphenyl derivatives in which neither phenyl ring is symmetrically substituted, may exist as enantiomers. Racemization, due to the rotation around the central C-C bond, can be hindered via the substitution of 3 or 4 bulky groups ortho to the pivot bond and opens the possibility of obtaining biphenyl enantiomers, stable at room temperature. 3,3'-Diamo-2,2',4,4',6,6'-hexachlorobiphenyl (1) was derivatized with (-)-NTPA chloride and separated into diasteromers by HPLC. Cleavage of the HPLC groups gave the optical antipodes which, on the basis of 4 ortho chlorine atoms, were stable against racemization for 90 min at 160 °C. Chlorine substitution for the amino groups in (1) led to the first separated PCB enantiomers. The 2,2',3,3',4,4',6,6'-octachlorobiphenyl enantiomers displayed a specific optical rotation of 15° and a mp 7° higher than that of the racemate. Various biologic effects of the PCB enantiomers will be reported.

(Supported by the DFG. L.W.R. is the recipient of an A. v. Humboldt Research Fellowship)

Four chlorinated hydrocarbons [1,2,3-trichloropropene (3E), 1,1,2,3-tetrachloropropene (4E), 1,2,2,3-tetrachloropropene (4A) and 1,1,2,3-trichloropropene (5A)] were tested for potential toxicity in two freshwater fish species and one aquatic invertebrate species. These materials are used as solvents and as raw materials and intermediates in numerous industrial processes. In static acute toxicity studies with rainbow trout, 96-hour LC50 values were 0.93, 4.2, 6.4 and 13.0 mg/l for 4E, 3E, 5A and 4A, respectively. Static acute 96-hour LC50s in bluegill sunfish were 0.42, 1.3, 3.8 and 13.0 mg/l for 3E, 4E, 5A and 4A, respectively. Thus, the two chlorinated propenes are considered to be moderately to highly toxic to freshwater fish whereas the two chlorinated propenes are considered to be slightly to moderately toxic to fish. Static acute testing in Daphnia magna (water fleas) resulted in 48-hour LC50s of 1.3, 4.7, 13.0 and 37.0 mg/l for 4E, 3E, 5A and 4A, respectively. Therefore, the chlorinated propenes are considered to be moderately toxic and the chlorinated propenes slightly toxic to aquatic invertebrates. The overall order of acute aquatic toxicity for this series of compounds is 4E > 3E > 5A > 4A.

The relative pharmacokinetics of trichloroethylene (TCE) and 1,1,1-trichloroethane (TCA) were studied in male S.-D. rats of 300-350g during and after 2-hr inhalation exposures. Fifty or 500 ppm of each chlorinated solvent was administered to groups of 4 rats via a one-way breathing valve inserted into the trachea of the anesthetized test animals. Samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently and analyzed for their solvent content. Alveolar levels of each test compound were calculated from the exhaled breath concentrations and the respiratory monitoring data. Systemic and cumulative uptake were determined periodically during the 2-hr exposure period. % Uptake, as measured after steady-state was reached, was greater for TCE than TCA at both exposure levels. % Uptake of TCA was inversely related to the inhaled vapor concentration. Although cumulative uptake of TCE and TCA over the 2-hr was similar (1.96±0.01 mg and 1.85±0.23 mg, respectively) in rats inhaling 50 ppm, substantially more TCE (22.4±2.8 mg) than TCA (13.8±1.0 mg) was absorbed in rats inhaling 500 ppm. Steady-state alveolar and exhaled breath levels of TCA were greater than for TCE. Upon cessation of exposure, TCE was eliminated from the blood more rapidly than was TCA. (U.S. EPA CR811215 and R808282)
ALTERATIONS IN FUNCTIONAL VITAMIN A METABOLISM CAUSED BY POLYBROMINATED BIPHENYLS. R.K. Jensen, M.H. Zille, M.E. Cullum, S.D. Sleight and S.D. Aust, Departments of Pathology, Food Science and Human Nutrition and Biochemistry, Michigan State University, East Lansing, MI 48824.

Many xenobiotics cause hepatic depletion of vitamin A. To study alterations of functional vitamin A metabolism in rats fed polybrominated biphenyls, groups of 6 male Sprague-Dawley rats were fed 0 or 1 ppm 3,3',4,4',5,5'-hexabromobiphenyl (HBB) in a semi-purified diet containing 15,000 IU retinyl acetate/kg for 140 days. Rats were given a single ip dose (40μCi) of 3H retinyl acetate 24 h prior to sacrifice. Serum and tissues were analyzed for vitamin A by reversed phase HPLC. The results are presented as X ± SD with control values given first: 2.9 ± 1.8 vs. 0.1 ± 0.1 mg retinol/liver; 16.9 ± 4.5 vs. 0.6 ± 0.3 mg retinyl esters/liver; 9.1 ± 106 ± 3.6 ± 106 vs. 3.3 ± 106 ± 1.2 ± 106 DPM/liver; 1.3 ± 0.6 vs. 13.4 ± 6.4 μg retinol/kidney; 5.8 ± 2.3 vs. 40.5 ± 27.3 μg retinyl esters/kidneys; 1.2 ± 105 ± 7.8 ± 104 vs. 1.3 ± 106 ± 4.3 ± 105 DPM/kidneys; 273 ± 60 vs. 296 ± 113 ng retinol/ml serum; 1.5 ± 106 ± 6.4 ± 105 vs. 3.0 ± 106 ± 8.3 ± 105 DPM/24 h urine; 2.8 ± 106 ± 5.2 ± 105 vs. 4.0 ± 106 ± 1.5 ± 106 DPM/24 h feces. Results indicate that HBB caused marked alterations in functional vitamin A metabolism characterized by hepatic depletion of vitamin A and an increase of renal vitamin A. (Supp. by Michigan Agr. Exp. Station, NIH/ES-02781 and USDA/84-CRCR-1-1377.)

EXTRACTION AND ANALYSIS OF CHLORDIORMINE AND DEMETHYLCHELORDIORMINE FROM MAMMALIAN TISSUES. J.A. Rieger and L.V. Allen. University of Oklahoma College of Pharmacy, Oklahoma City, OK.

A relatively simple procedure was developed for extracting chlordiormine (CDM) and demethylchlorodiormine (DMDM) from mammalian tissues. The time honored method of extracting with an organic, non polar solvent, partitioning into an acid, aqueous, polar solvent, and back extracting into a very small volume of chloroform was utilized. Tests were carried out to determine best organic solvent and optimal volumes and acidity of the aqueous medium.

The mass spectra CDM and DMDM were determined in a Hewlett-Packard 5985A GC/MS instrument. Single ion monitoring was used to measure the amount of CDM and DMDM recovered from several tissues utilizing the extraction method alluded to above. Recovery of CDM and DMDM from deionized water averaged 84% and 72%, from human whole blood 71% and 64%, and from human liver homogenate 64% and 53%, respectively.

Studies were also carried out to determine the feasibility of utilizing HPLC in analyzing CDM and DMDM. Waters, Model 440 HPLC, equipped with a 254 nanometer absorbent detector, and a micro-Bondapack C12 reverse phase column was utilized. A relatively good separation of peaks was achieved and a linear detector response was obtained when 1.0, 2.0, and 3.0 microgram samples were injected onto the column. Sensitivity with HPLC was 10 times greater than GC/MS.

FOOD CONTAMINATION: NEW TOXICOLOGICAL CONCERNS ABOUT AN OLD PROBLEM. A.M. Fan, I. Hertz-Picciotto, and R.J. Jackson, California Department of Health Services (DHS), Berkeley, CA.

Food contamination from chemical residues in food is of increasing concern as evidenced by findings in or affecting California. The fumigant ethylene dibromide (EDB), which was previously thought not to leave a residue, has been detected in grain and grain products. EDB has been found to produce carcinogenic and adverse reproductive effects in animals. Epidemiological data are suggestive of a dose-related response at two chemical plants, but a strong evidence for increased cancer mortality is lacking. Investigation by DHS seems to show that carcinogenic potency of EDB in humans is similar to that seen in test animals. Water contamination, an emerging problem, was identified as the source of trichloroethylene (TCE) which has been detected in infant formulas. There is evidence from animal but not human studies that TCE is carcinogenic. It is also a central nervous system depressant. Measures to prevent the exposures to toxic contaminants in food are being taken as follows: 1) elimination of chemical from dietary sources, such as banning of EDB with a phase-out plan; 2) institution of improved quality control, such as food protection program for closer sampling of processed foods, supported by health risk assessments.

SKIN PENETRATION OF FOURTEEN PESTICIDES IN YOUNG AND ADULT RATS. P.V. Shah1, H.L. Fisher2, M.R. Sumler1, and E.L. Helli2. 1NSI, 2USEPA, HERL/DBR, RTP, Research Triangle Park, NC 27711.

Skin penetration of fourteen pesticides representing eight groups with widely differing physical and chemical properties, was studied in young (33 day old) and adult (75 day old) female Fischer-344 rats. Carbon-14 labeled pesticides were usually applied in acetone to the previously clipped mid dorsal skin. The treatment area was 2.8 cm² in young and 5.6 cm² in adults. The treated area was protected with a perforated plastic blister. The penetration of the fourteen pesticides in young animals ranged from 2.9% to 81.5%, 2.0% to 64.4% and 0.90% to 90.1% at low, medium and high dose respectively. In adult it ranged from 7.7% to 86.4%, 2.7% to 90.5% and 1.0% to 93.3% at low, medium and high dose levels respectively. Penetration of eight of the fourteen pesticides showed significant age-dependent differences. In two of these eight pesticides the penetration was greater in young than adult rat. Four of the fourteen pesticides did not show significant effect of age on penetration. Three of these showed first-order kinetics in both young and adult over the dose range examined. Dose response curve of young and adult were not parallel in eight of the fourteen pesticides. In summary, age-dependent differences on penetration were observed, but no specific trend among the groups of pesticides was identified.
Acute and Subchronic Dermal Toxicity Evaluations of 4,5-dichloro/4 chloro-2-n-octyl 3(2H)-isotha- 
zuolone (DCO1). P. K. Chan, M. E. Murphy, G. P. O'Hara, J. N. Moss, and J. M. Smith, Rohm and 
Haus Company, Syngenta Ag, 1997.

Acute toxicity studies indicated that bicloro 
(DCO1), containing 35% 4,5-Dichloro-2-n-octyl- 
3(2H)-isothaizolone and 5% 4-chloro-2-n-octyl- 
3(2H)-isothaizolone in xylene, was slightly toxic 
after an oral dose (LD50 in rat = 1.89 g/kg), 
moderately toxic after a dermal dose (LD50 in 
rabbit = 1.7 g/kg), severely irritating to the 
skin and eyes of rabbits. Three groups of young 
adult NZW rabbits (6/sex/group) were treated 
percutaneously with DCO1 in acetone at 0 (acetone 
only), 1, and 5 mg/kg at a dose volume of 1 ml/kg 
(i.e., conc. of DCO1 in the low and high dose was 
0.1 and 0.5% W/V, respectively). Higher concen- 
trations were severely irritating to the skin. 

The dose was applied to intact or abraded skin 
daily, except weekends, for a total of 15 days 
over a 21-day period. An untreated control group 
received only hair shaving. No deaths occurred 
and no systemic toxic signs were seen. No 
effects were observed on body weights, routine 
hematology, clinical chemistry and urinalysis 
values, and organ weights. Histopathologic 
examination of tissues revealed no compound- 
related effects but local effects indicative of 
skin irritation on the treated skin. Slight to 
moderate skin irritation was seen in the low and 
high dose groups. The no-observed effect level 
for systemic toxicity of DCO1 after repeated 
dermal exposure was at least 5 mg/kg/day.

**DERMAL ABSORPTION AND DISPOSITION OF CHLORDECONE IN YOUNG AND ADULT RATS.** L.C. Hall, N. Fisher, 
M. R. Sumler, and P. V. Shah. USEPA, HESL, DBD, 
RTB & Northrop Services, Inc., RTP, NC

Skin penetration & disposition of 14C-chlorode-
cone was studied in young (Y) (33 d old) & adult 
(A) (75 d old) female Fischer 344 rats. Acetone 
solutions (100 μl Y, 200 μl A) containing 8μg/ml/ 
ml of chlordecone was placed over the previously 
clipped (24 h) mid-dorsal region of the back. 
The treatment area was 2.3% of the body surface 
area and was protected by a perforated plastic 
basket. Animals were maintained in Nalgene me-
tabolism cages for collection of urine & feces. 

Chlordecone derived radioactivity was determined 
by liquid scintillation counting. Approximately 
14% of the applied dose was absorbed from 
the application site in 120 h at 0.12%/h in both Y & 
A rats. The dose response curves for Y & A at 72 
h were parallel & showed dose-dependent absorp-
tion. Distribution of absorbed chlordecone was 
followed in liver, kidney, blood & carcass. Or-
gan & tissue content increased throughout the 
study period suggesting prolonged absorption. 
Carcass had the highest chlordecone derived con-
tent followed by liver, kidney & blood. Excre-
tion of chlordecone derived radioactivity occurred 
both urine & feces. Fecal excretion was 
greater than urine & displayed an initial lag. 
The urine/feces ratio at 120 h was 0.26 in A & 
0.33 in Y. In summary, chlordecone was slowly 
absorbed following dermal application in Y & A 
rats & no age dependence was found.

**A COMPARISON OF DERMAL PENETRATION OF CERTAIN 
PESTICIDES IN VIVO AND IN VITRO IN MICE.** R. E. 
Grissom & F. E. Guthrie. Interdepartmental 
Toxicology Program, North Carolina State 
University, Raleigh, NC 27695.

The dermal penetration of 2,4-D, cyhexatin, fen-
valerate, parquat, maleic hydrazide and captan 
was determined in vivo and in vitro in female 
mice. The amounts of pesticide were measured in 
vivo at the site of application and in the blood, 
Tiver, kidney, excreta and carcass. In vitro 
study were conducted using a Franz Diffusion 
Cell System. The amount of pesticide was 
measured in the skin patch and in the bathing 
medium. All of the compounds studied penetrated 
slowly both in vivo and in vitro.

Hydrophilic compounds penetrated similarly in 
vivo and in vitro, whereas, lipophilic compounds 
penetrated slower in vitro than in vivo.

Alterations of the constituents of the bathing 
media in vitro did not produce any significant 
changes in penetration.

**FIVE MONTH ORAL(DIST) TOXICITY/INFECTIVITY STUDY 
OF BACILLUS THURINGIENSIS IN SHEEP.** W. W. 
Bailey, S. W. Bohnett, T. D. McIvor, J. P. 
Thistle, C. M. Hiba, J. A. Whorton, P. W. Day, 
and R. E. Stoll, College of Pharmacy and School 
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querque, NM; New Mexico Veterinary Diagnostic Ser-
vices, Albuquerque, NM; Sandoz Research Instit-
ute, East Hanover, NJ. Sponsor: R. C. Schnell

Bacillus thuringiensis [500 mg/kg of Thricide- 
HP (Sandoz) or Dipel (Abbott); Approximately 
10^3 spores/sheep/day] was administered in the 
diet for five months to castrated Mixed Rambou-
lett/Merino sheep (24-34 kg initially). No 
significant treatment effect was seen on weight 
gain, or clinical chemistry parameters. No 
significant gross clinical changes were observ-
ed. Several blood and tissue samples taken just 
prior to the time the sheep were killed or at 
neuropsy were cultured positive for Bacillus 
Thuringiensis. However, no clinical changes or 
lesions thought to be caused by Bacillus thurin-
giensis infection were observed. Detailed gross 
and microscopic pathologic examination of the 
sheep revealed several spontaneous lesions in 
the control and treated sheep. However, the only 
lesion that may have been associated with the 
treatment was lymphoid hyperplasia in Peyer's 
patches of three sheep. Bacillus thuringiensis 
did not cause significant pathologic change of 
intestine when administered in the diet to sheep 
for five months. Bacillus thuringiensis was not 
a pathogen when administered in the diet to 
sheep.
Crude extracts of the protozoan I. thermophila can hydrolyze the potent acetylcholinesterase inhibitors diisopropylfluorophosphate (DFP) and 1,2,2-trimethyl methylphosphonofluoridate (soman). Similar activities have been described from mammalian, squid, and bacterial sources. Characterization and purification of the active proteins from I. thermophila were performed. Extracts were made by freeze-thawing packed cells centrifuged from axenic cultures. Hydrolysis was detected using F- electrode inserted in a buffered salt and DFP solution to which 100 lambda of extract was added. Sephacryl and sepharose-DEAE columns and polyacrylamide electrophoresis were used in the purification process.

Rate constants for the crude extract were K∂m = 5.71 mM and Vmax = 2.145 umoles g protein^-1 hr^-1. Activity is apparent at a range of pH from 5 to 10 and tonic strengths of 0-500 mM NaCl. A 1,000 fold purification has been achieved. Mol. wt. is 75,400. Turnover rate is 8.3 molecules of DFP hydrolyzed per molecule of DFPhase per second. One other DFPhate may exist. Enzymes such as DFPhases may prove useful as means of decontaminating wounds, surfaces, and water supplies.

Carbosulfan is a broad spectrum carbamate insecticide used for the control of soil and foliar insects on alfalfa, citrus, and other crops. The acute toxicity of carnosulfan was investigated since alfalfa is grazed by surface-feeding ducks and carbosulfan, its major environmental degradate, is acutely toxic to birds.

Oral intubation of carnosulfan to Mallard ducks, Bobwhite quail and Ring-necked pheasants resulted in 14-day oral LD50s of 10 mg/kg, 82 mg/kg and 20 mg/kg, respectively. Dietary administration studies in Mallard, Bobwhite and pheasants indicated 8-day LD50s of 304 ppm, 1,100 ppm and 1275 ppm, respectively. In one-generation reproduction studies survival, growth and reproductive performance were not affected in quail up to and including 150 ppm. In ducks, a significant reduction in viable embryos, number of 14-day old survivors, hatching body weights, eggs laid, egg weights, eggshell thickness and adult body weights were observed at 100 ppm. The no-effect level was 30 ppm.

Carbosulfan is less toxic than carbofuran to avian species and the hazard associated with its long-term exposure should be minimal as a result of its rapid degradation in plants and soil.

Methoxychlor and o,p'-DDT, but not chlordane, at very high sublethal levels of exposure in vivo, are weakly estrogenic in the channel catfish, Ictalurus punctatus, as assessed by the induction of the hepatic synthesis of vitellogenin, the egg yolk precursor protein. All three of these compounds are estrogenic in vivo in higher vertebrates. The induced protein was purified from the serum and demonstrated the same molecular weight range, electrophoretic mobility, phosphorus content and lipid content as estradiol-induced vitellogenin. Experiments were designed to determine whether o,p'-DDT, 1,1,1-trichloro-2,2-bis(p-hydroxyphenyl)ethane (HPTE) or chlordane bind to hepatic estrogen receptors. Both high affinity and low affinity specific binding of [3H]estradiol-17β was observed in nuclear and in cytosolic preparations of catfish liver, as assessed by competition for radioligand binding by unlabelled estradiol-17β and by diethylstilbestrol. Competition with specific high affinity [3H]estradiol-17β binding in nuclear preparations was not observed with any of the organochlorine compounds except HPTE when it was in 20,000 fold excess over radioligand concentration. It appears unlikely that binding to estrogen receptors is involved in the mechanism of their estrogenic action in catfish.

The purpose of this study is to examine the effect of particle size on the inhalation toxicity of liquid aerosols. Rats were exposed to mists of chlorpheniramine (CVP), an organophosphorus insecticide, generated by a size-selective inhalation chamber (Isuda et al., FAAT, 4, 378, 1984). Mists of two different particle size distributions were used; mist(S) consisting of particles smaller than 1 μm and mists (L) larger than 1 μm in aerodynamic diameter. The LC50's of CVP after 4 hr. exposure to rats were 0.34 and 0.072 mg/ℓ for mists(S) and (L), respectively. CVP was tightly bound to plasma components(s) up to 0.83 mg/ℓ. The extractable blood CVP concentration was significantly higher in rats exposed to mist(L) than exposed to mist(S) at essentially the same atmospheric concentrations of 0.08 mg/ℓ. When rats were exposed to mist(S) and (L) at their LC50 concentrations, the extractable blood CVP concentration was significantly higher in rats exposed to mist(S) than exposed to mist(L). Thus, the higher blood CVP concentration due to greater retention of inhaled mists may partly relate to the higher toxicity of CVP mist(L). The CVP concentration ratio of target site(s) vs. blood might be varied by the particle size.
OOS-TMP, an impurity in many organophosphorus insecticides, causes a pneumotoxicity in rats at low doses (20 mg/kg) resulting in increases in bronchopulmonary lavage lactate dehydrogenase (LDH) activity and morphological alterations of bronchiolar epithelium. Co-administration of the nontoxic isomer, 0,0,0-trimethyl phosphorothioate (OOS-TMP), has been found to protect against the increase in LDH levels and bronchiolar changes by OOS-TMP. Since OOS-TMP requires metabolic activation for pneumotoxicity, the effect of OOS-TMP on pulmonary and hepatic P-450 content and P-450-mediated monoxygenase were examined as a possible biochemical mechanism of antagonism. Oral treatment with OOS-TMP (4 mg/kg) decreased pulmonary P-450 level by 40 to 45% at 2 and 6 hr while no changes were seen in hepatic P-450 level. Lung microsomal 7-ethoxyco- marin O-deethylase (7-EC) was abolished at 2 hr and inhibited 73% at 6 hr. Liver 7-EC was not altered while p-nitroanisole demethylase activity was decreased 50%. Superoxide dismutase and reduced glutathione levels were not affected. These results support the view that the lung is a target organ of delayed toxicity produced by OOS-TMP, and the antagonistic effect of OOS-TMP may be due to alterations in the metabolic activation processes of OOS-TMP in lung and/or liver. (Supported by PHS grant ES02225)


In an Agent GB (Sarin, isopropyl methylphosphonofluoridate) preliminary teratology study conducted at NCTR, it was observed that the dose response curve of the maternal animals was very steep. The maternal animal either died during the treatment or survived with no observable fetal toxicity. Animals that died displayed many of the classical signs of cholinesterase (C) inhibition toxicity. A study was be conducted to determine if the maternal deaths, clinical observations, and/or weight loss could be correlated with the blood C levels in individual animals. Baseline C levels (plasma & RBC) were obtained prior to treatment by gavage with 380 mg/kg GB for 30 days. There was a definite drop in the plasma C level after the first dose which then remained low throughout the dosing period. There was a highly statistically significant correlation between body weight and plasma C levels. RBC C levels did not change during treatment. Death occurred for 14 of the 20 dressed animals and each had dramatically lower plasma C levels.

However, the 6 surviving animals also had dramatically lower plasma C levels during dosing which recovered along with body weight on the cessation of dosing.

EFFECT OF IMPUERITIES OF MALATHION ON REMOVAL OF 5-HYDROXYTRYPTAMINE (5-HT) IN ISOLATED PERFUSED RAT LUNG. E. Rosso and T. Immura. Div. of Toxicology and Physiology, University of California, Riverside, CA 92521

0.0,0-trimethyl phosphorothioate (OOS-TMP) and 0.0,0-trimethyl phosphorodithioate (OSS-TMP) are impurities present in organophosphorus insecticides which have been shown to cause damage in bronchiolar epithelial Clara cells, but little is known about their effect on lung capillary endothelial cells. We examined removal of 5-HT, an index of pulmonary endothelial cell functions in isolated perfused lung following oral treatment of rats with OOS-TMP or OSS-TMP. Removal of 5-HT was decreased in perfused lungs from rats treated with these compounds (40 mg/kg) 3 days following the treatment and less metabolite 5-hydroxyindoleacetic acid (5-HIAA) was recovered in the effluent of lungs. Dose-response studies indicated that 40 mg/kg had a maximal inhibitory effect on removal of 5-HT. To examine if the decrease in 5-HIAA in the effluent is due to decreased enzyme activity or diminished uptake of 5-HT into lung tissue, the deamination of [14C]-5-HT by 3000g supernatant fractions of lung homogenates from treated rats were measured. The monoamine oxidase activity per mg protein was lower than control 1 day following 40 mg/kg. These data indicate that OOS-TMP and OSS-TMP affect capillary endothelial cells in rat lungs. (Supported by PHS grant ES02225)
277 EFFECTS OF PERINATAL EXPOSURE TO ETHYLENETHIOUREA ON THE THYROID OF RATS AND MICE EXPOSED AS ADULTS. R.S. Chhabra, G.E. Wilkinson, B.D. Carlton, F.J. Kurtz, S.L. Grumbein and J.D. Toft. National Toxicology Program, NIEHS, Research Triangle Park, NC and Battelle Columbus Labs, Columbus, OH.

Effects of exposure to ethylenethiourea (ETU) in adult rats and mice after perinatal dosing were examined. Rat and mouse dams received ETU in feed during breeding, gestation, and lactation. Pup exposure continued at maternal dose levels until assignment to chronic dosing groups at 8 weeks of age. Fertility, pup survival, and litter weights during the first postnatal week were similar in treated and control groups. Perinatal exposure had only a slight influence on the degree of weight reduction in mice exposed to ETU as adults. Preliminary histopathology (9 mos. necropsy) revealed thyroid follicular cell hyperplasia (rats) and diffuse cytoplasmic vacuolization (mice) in groups exposed as adults; the former lesion was slightly more pronounced in rats also exposed perinatally. Decreases were observed in serum thyroxine (rats and mice) and triiodothyronine (rats) levels; increases in thyroid-stimulating hormone produced by adult exposure to ETU were greater in perinatally exposed rats. Thus, while adult exposure to ETU appeared necessary to elicit thyroid toxicity, perinatal exposure made these effects more severe. (Supported by NIEHS Contract No. NO1-ES-8-2151).


Bauer et al. recently reported (Amer Rev Resp Dis 1990;A151,1984,abst) that a 10-min exposure to 0.3 ppm nitrogen dioxide (NO2) by mouthpiece caused decrements in pulmonary function in exercising asthmatics. To investigate further, we exposed 12 mild asthmatics (male, 18-35 yrs., hyperreactivity to methacholine) in a chamber (natural breathing, 20°C, 40% RH) for 110 min to clean air and to 0.3 ppm NO2 on separate days. They performed intermittent treadmill exercise (3 cycles of 20 min rest followed by 10 min exercise, V̇e=42 L/min). Forced vital capacity (FVC) and 1-sec expiratory volume (FEV1) were measured before exposure and after each exercise. After the 1st exercise, NO2 exposure compared to air exposure produced significantly greater reductions in FEV1 (-12% vs -6%, P<.02) and possibly in FVC (-6% vs -3%, P<.1). The reductions in FEV1 were similar to those reported by Bauer. With repeated exercise, FEV1 returned toward baseline values. There was a wide range of subjects' responses: compared to the changes seen in clean air, 1 subject had a slight increase in FEV1 in NO2, 5 subjects had little or no change, and 6 had 5-18% decrease in FEV1 greater than the decrease seen in clean air. We conclude that inhalation of 0.3 ppm NO2 causes decrements in pulmonary function in moderately exercising, naturally-breathing asthmatics.

278 MECHANISM OF ACTION OF U-40481A ON SKELETAL MUSCLE CONTRACTILITY. V.C. Ravikumar and J.A. Rieger. Dept. of Pharmacodynamics & Toxicology, University of Oklahoma College of Pharmacy, Oklahoma City, OK.

The mechanism by which the pesticide metabolite U-40481A (\( N^2-(2,4\text{-xylyl})\text{-N-methyl formamidine hydrochloride}\)) decreases skeletal muscle contractility was investigated using the isolated rectus abdominis and sartorius muscles from Rana pipiens. U-40481A, at a high concentration (5x10^{-3} M), by itself produced a slow contraction of the rectus muscle, taking 20-30 minutes to reach near maximum. At a lower concentration (5x10^{-4} M), U-40481A non-competitively inhibited both ACh- and KCl-induced contractures of the rectus muscle. The same concentration of U-40481A also decreased the peak tension elicited by 8 mM caffeine (bolus) by >50% of control responses in both the rectus and sartorius muscles. Finally, when caffeine was added cumulatively to the bath, U-40481A (5x10^{-4}; added 5 min before caffeine) seemed to potentiate the contractures at the lower caffeine cumulative concentrations while it decreased the contractures at the higher caffeine cumulative concentrations and also the maximum tension produced in the rectus muscle. It is postulated that in addition to decreasing both sensitivity of the receptors to ACh and excitability of the muscle membrane, U-40481A also interferes with the E-C coupling mechanism and/or some step beyond and thereby decreases skeletal muscle contractility.

280 RELATIONSHIP BETWEEN LUNG FUNCTION AND COMPOSITION IN RATS SUBCIRHONICALLY EXPOSED TO CHLORINE. D.L. Costa, R.S. Kutzman, and R.T. Drew. Medical Department, Brookhaven National Laboratory, Upton, NY.

Lung function was compared to structure and composition in rats after subchronic challenge with chlorine (Cl2) gas. Groups of 24 male, Fischer-344 rats (SFPR) were exposed to 0, 0.5, 1.5, or 5.0 ppm Cl2 for 6 h/day, 5 days/wk for 62 days. Six days postexposure, each rat underwent a series of static and dynamic lung function tests, after which the lungs were divided for histopathologic and connective tissue analyses. Lung compliance was increased in a dose-dependent manner while air-flow dynamics were slightly, but not significantly, impaired. No appreciable differences in lung morphology were found although loss of tracheal cilia and focal epithelial erosion were evident in the 5.0 ppm rats. Connective tissue changes were limited to the lung collagen accumulations at 1.5 and 5.0 ppm. Thus, a conclusion that excessive lung collagen suggests in vivo pulmonary fibrosis would conflict with the functional data which indicated loss of elastic recoil. The mild pathology associated with these apparently divergent lesions suggests a degeneration of lung tissue with consequent repair-related increases in connective tissue. Supported by U.S.D.O.E. No. DE-AC02-76CH00016 and N.T.P. Intergency Agency No. 222-Y02-ES-9-0043.

Twenty guinea pigs were exposed to particles of cotton dust (average size 2.5 μm AWD) for 52 weeks. The exposure was: 20.8 mg/m³, 6 hours/day, 5 days/week. Control animals received identical treatment except for dust exposure. The dust was generated by sonicating bulk cotton dust obtained from a mill. Daily measurement was made of respiratory frequency (f) and tidal volume (Vₚ) before and after exposure while animals were breathing room air (f air and Vₚ air) or air containing 10% CO₂ (f CO₂ and Vₚ CO₂). During the first 6 weeks of exposure, animals responded with an increase in f air and f CO₂ and a decrease in Vₚ air and Vₚ CO₂. The response was always more pronounced on the first day of each week. From week 6 to 14 there was little change in respiratory pattern between pre and post exposures. From week 16 on, differences were observed again with greatest response on the first day of the week.

At termination there was a highly significant difference between the control and exposed group respectively in lung weight (7.02 g ± 0.8 vs. 9.42 ± 1.5) and lung volume (9.75 ml ± 2.0 vs. 14.99 ml ± 3.3). We conclude a definite chronic effect resulted from inhalation of cotton dust. Supported by a USDA Coop. Agric. and NIEHS I-R01-ES02747.

CONCURRENT AEROSOL EXPOSURE POTENTIATES LUNG RESPONSE TO OZONE. D.L. Warren, D.J. Guth and J.A. Last. CPCR, Univ. of Ca., Davis, Ca. 95616.

Previous work from this laboratory has demonstrated that exposure to respirable acidic sulfate aerosols potentiate the response of the lung to oxidant gas exposure as evaluated morphometrically and biochemically. We have extended these studies by measuring lung permeability as radioactivity recoverable in lung lavage after intravenous injection of various radioactively labelled tracers. Male Sprague-Dawley rats were exposed continuously for 7 days to various levels of O₃ with and without concurrent exposure to respirable acid aerosols. For example, recovery of tracer, normalized to serum level, was twice the control values (p<0.05) in rats exposed to 0.64 ppm O₃. In rats exposed to 0.64 ppm of O₃ + 5 mg/m³ ammonium sulfate aerosol (<1 μm MMAD), the recovery of tracer was 70% higher than values in animals exposed to O₃ alone (p<0.05). No effect is observed in rats exposed to aerosol alone. Changes in lung permeability correlate with changes in morphometric parameters in exposed rats. We conclude 1) changes in epithelial permeability accurately reflect damage occurring in lungs exposed to oxidant gases; 2) such permeability changes precede changes in lung fibroblast proliferation and collagen synthesis; and 3) synergy between oxidant gases and respirable acid aerosols involves a mechanism that includes increased lung edema.

MEASURING NASAL AIRWAY RESISTANCE (NAR) DURING INHALATORY EXPOSURES. J.S. Martin and M.P. Tansy, Temple University, Philadelphia, PA.

The toxicological assessment of the respiratory consequences of exposures to airborne substances usually involves a histomorphological examination of the nasal mucosa. There are usually no quantitative measures applied to determine the effects of inhalatory exposures to the passage of air through the nares. The purpose of this report is to describe a technique for the measurement of NAR in the anesthetized rat that can be used for inhalatory exposures. Essentially, a T-tube is inserted into the trachea to monitor pressure via a differential pressure transducer. A close fitting oro-nasal cone is attached to a pneumotachograph and to the pressure transducer. Thus, the differential pressure between external nares and trachea can be recorded along with measured nasal airflow. The animal is placed into a plexiglass chamber. Air and air-vapor mixtures are then drawn into the animal chamber from a large vaporization chamber where precise air-vapor mixtures can be produced and which can be sampled for analysis. Preliminary results indicate that the animal preparations are stable for up to 2 h of continuous recording. We conducted test exposures to measured concentrations of methyl methacrylate vapor (MMA) in air to determine if the model would respond to gasses. MMA (250 ppm) resulted in an 18% increase in rat NAR within 10 minutes. It is concluded that this model is of value in assessing the effects of airborne substances on upper NAR.

MUCOUS GLYCOPROTEIN CONTENT OF PULMONARY LAVAGE FLUID OF THE RAT. J.B. Morris, School of Pharmacy, Univ. of Connecticut, Storrs, CT.

The overproduction of mucous glycoproteins (MGP) represents a common response of the pulmonary airways to injury, and is also the hallmark of chronic bronchitis. To assess the feasibility of employing pulmonary lavage to quantitate the airway MGP content of the rat, the MGP removed from the lungs of male Sprague-Dawley rats by 3 successive lavages with cold isotonic saline were solubilized with urea and mercaptoethanol, and were purified by ultrafiltration and Sephacryl CL-6B gel chromatography. MGP, which elute with the void volume of this gel, were quantitated by their carbohydrate content. Initial studies revealed that the 2nd and 3rd lavages removed 3.5- and 5.3-fold less MGP than the 1st, suggesting that some pool of MGP was being washed out by this procedure. In a second study, isoproterenol was administered by a regimen known to produce MGP-hypersecretion in the rat as assessed by histologic criteria (100 mg/kg/day for 6 days, Am. J. Path. 95: 407-421, 1979). Lavage MGP content of isoproterenol-treated rats averaged 450 μg compared to 210 μg in vehicle-treated controls (p<0.01). In summary, it is possible to purify and quantitate the MGP removed from the airways of the rat by pulmonary lavage and the amount of lavageable MGP is doubled by isoproterenol, an agent known to induce goblet cell hyperplasia.
The role of lipid peroxidation in acute NO2 toxicity was studied in rat lung. After 4 hr exposure to 40 ppm NO2 there was no increase in lipid conjugated dienes in lung homogenate, lung lavage, or alveolar macrophages. Thiobarbituric acid reactive materials were not increased in lung homogenate or lavage and slightly increased in free cells. In rats maintained on a semipurified diet with 0, 10 or 1000 ppm Vitamin E (VE) for 11 weeks, the lung content of VE was 3.6, 17.4 and 87.7 μg VE/lung, respectively. Groups of 5 or 6 rats were exposed to 0, 10, 20, 30 or 40 ppm NO2 for 4 hr and NO2 toxicity was measured by increase in lavageable protein, sialic acid, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (GODH), acid phosphatase (AP), and aryl sulfatase (AS). Increases in lavageable protein, sialic acid, AP and AS were not affected by VE (p > .05). Increases in lavageable LDH, MDH, and GODH after NO2 exposure were significantly attenuated by 1000 ppm VE (p < .05), consistent with a lipoperoxidative mechanism. (Supported by US DOE contract no. DE-AC02-760E03490 and NIH Training Grant No. 5 T32 HL07216-08).

EVALUATION OF THE PULMONARY EFFECTS OF HDI TRIMER AEROSOLS IN GUINEA PIGS. Ferguson, J.S., Schaper, N.M. and Alarie, Y., Dept. Ind. Env. Hlth. Sci., Univ. of Pittsburgh, Pittsburgh, PA

Hexamethylene diisocyanate trimmer (HDT) is a viscous liquid and used in spraying operations for durable coatings. It has been shown to be a pulmonary irritant following a single exposure. A group of guinea pigs was exposed to 65 to 74 mg/m² for 3 hour/day on 11 consecutive days to establish if a cumulative pulmonary toxic effect would occur. The particle size was around 0.7 μm MMD. Prior to and following exposure, each animal was exposed to 10% CO2 in 20% O2 and 70% N2 to evaluate their pulmonary performance. Following the first exposure, the respiratory frequency of these animals was increased and frequent apneic periods were observed as well as coughing. Also their ventilatory response to 10% CO2 was highly abnormal. However, with repeated exposures a tolerance began to develop as indicated by a return toward normal of their ventilatory response to 10% CO2. The tolerance occurred within the first 5 days of exposure. From day 6 to 11 there was a demonstrable effect but the level of effect was much less than following the first exposure. Supported by NIEHS Grant 1 RO1 ES02747.

TOLUENE DIISOCYANATE (TDI) INDUCED AIRWAY HYPERRESPONSIVENESS AND INFLAMMATION IN GUINEA PIGS. T. Gordon, D. Sheppard, D.M. McDonald, S. Distefano, and L.A. Scypinski. Cardiovascular Research Institute, UCSF, San Francisco, CA

We examined the changes in airway responsiveness to increasing doses of an acetylene (Ach) aerosol in anesthetized and ventilated guinea pigs 2, 6, or 24 hr after exposure to 2 ppm TDI or 2 hr after exposure to air or 1 ppm TDI. The concentration of Ach calculated to cause a 200% increase in pulmonary resistance was significantly lower for animals studied 2h (6.8μg/ml) or 6h (7.7μg/ml), but not 24h (23.9μg/ml), after TDI than for air animals (30.7μg/ml). Exposure to 2 ppm TDI caused a patchy loss of cilia, shedding of epithelial cells into the lumen, and an influx of inflammatory cells into the trachea and other airways. In the lamina propria of the trachea, the concentration of extravascular polymorphonuclear leukocytes (PMN's) was 13 to 26 fold greater in animals studied 2 or 6h after exposure to 2 ppm TDI or 2h after 1 ppm TDI than in animals exposed to air. The concentration of PMN's in the epithelium was significantly increased only in animals examined 2h after 2 ppm TDI. Exposure to TDI also caused an influx of eosinophils into the tracheal mucosa. This influx occurred later and was more persistent than the influx of PMN's. These results indicate that a single exposure to TDI can cause an increase in airway responsiveness that is associated with epithelial injury and acute airway inflammation.
289 EFFECT OF CONCENTRATION ON THE DISPOSITION OF INHALED 14C-BUTADIENE IN BGCF mice. J.S. Dutcher, J.A. Bond, and R.F. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; L.S. Birnbaum, NIJEHS, Research Triangle Park, NC.

Butadiene (BD) is used primarily in the production of elastomers, polymers, and other chemicals. BD is carcinogenic in mice with malignant neoplasms observed in a variety of tissues. The purpose of this investigation was to determine the effect of different concentrations of inhaled 14C-BD on the disposition of 14C-BD in mice. Male BGCF mice were exposed nose-only to 13.5, 175, and 1390 μg 1,3-butadiene-1-14C/l air (8 to 800 ppm, 25°C, 620 torr) for 6 hr. Urine, feces, and expired air were collected for 65 hr. Excretion patterns were independent of dose with 50% of the 14C excreted in urine, 11% as 14CO2, 7% in feces, and 5% as BD or a volatile metabolite. Excretion of 14C in both urine and as 14CO2 was biphasic. At all exposure concentrations, half-times (mean ± SD) for elimination of 14C in urine were: first phase - 2.1 ± 0.8 hr; 2nd phase - 16.3 ± 5.4 hr, and for CO2: first phase - 1.0 ± 0.5 hr; 2nd phase - 13.8 ± 1.0 hr. The results of this investigation indicate that rates of excretion of 14C are relatively constant throughout the dose range used. (Supported by NIEHS through an Interagency Agreement under U.S. Department of Energy Contract No. DE-AC04-76EV01013.)

290 DISPOSITION OF NICKEL SULFATE SOLUTIONS FOLLOWING INTRARACHINAL INSTILLATION. M.A. Medinsky and C.H. Hobbs. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Both epidemiology and laboratory studies have shown nickel (Ni) compounds to be carcinogenic. The purpose of our experiments was to determine the target organs for Ni absorbed from the respiratory tract into the blood. Male and female Fischer-344 rats were given 1, 10, or 100 μg of Ni as nickel sulfate, containing trace amounts of 63Ni, by intratracheal instillation. Urine and feces were collected, and rats were necropsied at predetermined times up to 96 hours after instillation. At all times, lungs, trachea, larynx, kidney, and urinary bladder contained the highest concentrations of Ni as determined by liquid scintillation spectrometry. Urine was the major route of excretion of Ni accounting for 50% of the dose after instillation of 1 or 10 μg Ni and 80% of the dose after instillation of 100 μg Ni. Fecal excretion accounted for 20% (1 and 10 μg doses) or 13% (100 μg). Of the Ni remaining in the body at the end of 96 hrs, 90% was in the lungs. The long-term half-time for clearance of Ni from lungs was approximately 40 hrs. Results suggest that respiratory tract tissue and tissues associated with urinary excretion of Ni may be the target organs for inhaled Ni compounds. (Research performed for the National Toxicology Program under Interagency Agreement 22-Y-01-ES-82108 under DOE Contract No. DE-AC04-76EV01013.)

291 METABOLISM AND DISPOSITION OF 14C-METHYL BROMIDE IN FISCHER-344 RATS AFTER INHALATION. J.A. Bond, J.S. Dutcher, M.A. Medinsky, and R.F. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; L.S. Birnbaum, NIJEHS, RTP, NC.

The major use of methyl bromide (MB) is as a disinfectant to fumigate soil and stored food commodities. Human exposure occurs during the manufacture and use of the chemical. The purpose of this investigation was to determine disposition and metabolism of 14C-MB in rats after inhalation. Male Fischer-344 rats were exposed nose-only to 337 nmol 14C-MB/L air (9.0 ppm, 25°C, 620 torr) for 6 hr. Urine, feces, expired air, and tissues were collected for up to 65 hr after exposure. By 65 hr after exposure, about 75% of the absorbed MB was excreted. The major route of excretion of 14C was as 14CO2, with about 47% (3900 nmol) of the total 14C-MB absorbed excreted by this route. Eighty-five percent of 14CO2 was excreted with a halftime of 3.9 hr. Half-times for elimination of 14C in urine and feces were 9.6 hr and 16.1 hr, respectively. Lung, adrenal, kidney, liver, and nasal turbinates contained the highest concentrations (100-250 nmole/g tissue) of 14C immediately after exposure. Elimination half-times of 14C from tissues were between 1.5 and 5.5 hr. In all tissues examined, less than 10% of 14C in tissues was MB. The data indicate that after inhalation, MB is rapidly metabolized in tissues and readily excreted. (Supported by NIEHS through an Interagency Agreement 22-Y-01-ES-20092 under U.S. DOE Contract No. DE-AC04-76EV01013.)


A physiological model was used to describe the DBM metabolism to carbon monoxide (CO) in the presence of the ISO. In the Iso model ISO was a competitive, non-metabolized substrate with an inhibitory binding constant, Kml. Gas uptake studies were conducted with rats for each chemical and for mixtures: 200 ppm (DBM):10000 ppm (ISO) and 200 ppm (DBM):20000 ppm (ISO). The data for chamber DBM concentration versus time were best fit with Kml of 0.2 mg/L. Four hour exposures to a constant concentration were modeled to predict blood carboxyhemoglobin (HbCO). Exposures were 200 ppm (DBM):0 ppm (ISO), 200 ppm (DBM):20000 ppm (ISO), and 200 ppm (DBM):6000 ppm (ISO). The complex time course of HbCO was well-represented by the model. With DBM alone, HbCO was highest at the end of exposure (~15%) and decreased exponentially. With mixed exposure, HbCO was low at the end of exposure (~2% with 10000 ppm ISO) but increased to a maximum at 2 to 3 hrs after exposure. The decreased post-exposure inhibition occurs because the poorly soluble ISO is more rapidly lost by exhalation than is DBM. In complex mixtures where competitive substrates have markedly different tissue solubilities the maximum rate of metabolism of a particular chemical in the mixture may occur in the post-exposure period.
MORPHOMETRIC ANALYSIS OF ERYTHROMYCIN ESTOLATE-INDUCED CHANGES IN CULTURED HEPATOCYTES. E.M.B. Sorensen and Dania Acosta. Department of Pharmacology and Toxicology, The University of Texas, Austin, TX

Parenchymal hepatocyte cultures were exposed to a series of concentrations of erythromycin (EE), a well-known hepatotoxin. In order to compare data collected using morphometric analysis and enzyme leakage as monitors of hepatotoxicity. As the concentration of EE was increased from 10 to 100 μM, the relative volume percentage (RVP) of Type I cells (morphologically similar to control cells) was reduced. Analysis of variance indicated that cultures exposed to 75 or 100 μM EE had a significantly lower RVP (p<0.01) than did cultures exposed to lower concentrations of the hepatotoxin. In contrast, the RVP of the other cell types, which showed increased expression of cell damage (i.e., types II, III, IV), increased to statistically significant levels following exposure of hepatocyte cultures to 75 and/or 100 μM EE. These results paralleled those obtained from lactate dehydrogenase leakage and demonstrated the applicability of this methodology in the general assessment of cytotoxicity following xenobiotic challenge. Planimetric analysis of the four cell categories was conducted using the Cambridge Q10 Image Analysis System. Area estimates of over one hundred hepatocytes showed that Type I and Type II cells were not significantly different from one another. Type III cells, however, were significantly larger (p<0.05) and Type IV cells were smaller (p<0.001) than cell types II and III. These results demonstrated that data collected from morphometric analyses paralleled those obtained from the assay of LDH release from cultured hepatocytes. This work was supported by a grant from the Johns Hopkins University Center for Alternatives to Animal Testing.

INTERFERENCE OF INTRACELLULAR CALCIUM DYNAMICS IN CCl₄ INJURY OF CULTURED RAT HEPATOCYTES. K.S. Santone and D. Acosta, College of Pharmacy, The University of Texas, Austin, TX 78712

We have developed a system of primary cultures of postnatal rat hepatocytes to investigate the role of intracellular Ca²⁺ in carbon tetrachloride (CT)-induced cell injury. Previously, we have shown that CT-induced toxic injury was not dependent upon extracellular Ca²⁺. Equilibrating levels of ¹⁴C-CT to hepatocyte protein was determined as an indicator of CT metabolism by the cultured hepatocytes. Following a 15 min treatment of 2 mM CT, 1.70 mmoles of ¹⁴C-CT was found bound per mg of cell protein. This level did not increase with time of treatment, although equilibrant binding was dose-dependent with a two-fold increase in binding occurring at the 4 mM CT treatment concentration. ATP-dependent microsomal Ca²⁺ uptake was quickly and dramatically inhibited following both 2 mM and 4 mM CT treatment to cultured hepatocytes, reduced to 62% and 18% of the levels of untreated control values, respectively. ATP-dependent mitochondrial Ca²⁺ uptake was only significantly inhibited with 4 mM CT, and both microsomal and mitochondrial Ca²⁺ uptake were not altered when Ca²⁺ was removed from the culture treatment medium. The changes listed above have been found to occur earlier, and to a greater degree than other CT-induced damage observed in the hepatocytes. These results provide further evidence to the hypothesis that changes in intracellular Ca²⁺ homeostasis may be a vital step linking CT activation to CT-induced cell death.

MULTIPLE DRUG METABOLISM: A MODEL FOR DRUG TOXICITY USING HEPATOCYTES PREPARED FROM A PERCOLL GRADIENT. R.C. Brown and W.R. Biplack, Department of Pharmacology and Nutrition, USC School of Medicine, Los Angeles, CA 90033

Hepatocytes prepared by collagenase perfusion were purified using a self-generating Percoll gradient, which provided an efficient recovery of cells with high viability. Aniline (AN) and p-nitroanisole (pNA) were selected as representative xenobiotic agents, since they serve as precursors in the synthesis of dyes, pesticides and drugs. These substrates are metabolized by cytochrome P-450 to more polar metabolites, p-amino phenol (pAP) and p-nitrophenol (pNP), respectively, followed by conjugation with sulfotransferase and glucuronoyl transferase. To evaluate the efficiency of both phases of drug metabolism, the nonconjugated, sulfate and glucuronide products were determined. AN inhibited pAP metabolism, while pNA had little effect on AN metabolism. With less p-AP formed, lower sulfate and glucuronide metabolites also resulted. However, the effect on p-Ap distribution was seen as a specific increase (3-fold) in the nonconjugated metabolite. Thus, the model system indicates not only that one drug may alter the metabolism of another, but also that a metabolite even if present at low concentrations may be preferentially cleared allowing another metabolite to accumulate. If that metabolite were toxic or carcinogenic, the cell would then be placed at greater risk for molecular damage. (supported in part by a grant provided by Carnation Research Laboratories)

COMPARATIVE CYTOPATHOLOGY OF MONOLAYER RAT HEPATOCYTES EXPOSED TO LETHAL CONCENTRATIONS OF AFLATOXIN B₁ AND ACETAMINOPHEN. M.A. Hayes and D.B. Pickering. Department of Pathology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1. Sponsor: I.C. Munro

Primary monolayer cultures of hepatocytes from F344 rats were exposed to aflatoxin B₁ (AFB₁) or acetaminophen (AA) for 48 hours. AFB₁ at 1 μM or 10 μM, which produced 67% and 97% killing at 48 hours respectively, caused numerous large finger-like attached cytoplasmic projections (blebs) observed on almost all hepatocytes between 6 and 24 hours. Formation of these blebs was dose-dependent, and preceded detachment, lactate dehydrogenase (LDH) release and trypan blue uptake. Occurrence of blebs was independent of serum concentration or collagen coating of dishes. Blebs produced by AFB₁ were morphologically different from those produced by cytochalasin B and phalloidin, but were similar to blebs produced by N-hydroxy-2-acetylaminofluorene, cycloheximide and amiloride. By comparison, AA at equilivalently lethal concentrations of 4 mM or 16 mM did not generate peripheral blebs, but caused trypan blue staining while cells were attached to the culture dish. This toxic response to AA resembled that to CCl₄ and methotrexate. These studies demonstrate at least two distinct modes of lethal injury of cultured hepatocytes exposed to xenobiotics. Supported by NSERC Canada (#A2761)

Treatment of female Sprague-Dawley rats with acetaminophen (AA, 1.2g/kg) results in a loss of sinusoidal cells, glycogen depletion and an infiltration of macrophages (MF) into the pericentral region of the liver. To study the mechanism of MF accumulation and their potential role in AA-induced hepatotoxicity, we examined the chemotactic and activating properties of conditioned medium (CM) from isolated rat hepatocytes. Cultured hepatocytes were treated with AA (1-500 μM) for 1-8 hr. MF were isolated from rat livers by collagenase/pronase perfusion and differential centrifugation on a metrizamide gradient. The chemotactic response of MF to CM was assayed by the Boyden chamber technique. 25% CM produced a 2-3 fold stimulation of MF chemotaxis. The maximum response was observed with CM from cells treated with 50-100 μM AA for 2-4 hr. This same dilution of CM also produced a 2-3 fold enhancement of phagocytosis of 51Cr-labeled superoxide anion production by liver MF. AA alone had no effects on chemotaxis, phagocytosis or superoxide anion release. These results demonstrate that AA damaged hepatocytes can release factors which attract and activate liver MF. Once localized at an injured site, MF may contribute to the hepatotoxicity of AA. (Supported by NIH grants AA3048 and AI20183).

299 HALOTHANE HEPATOTOXICITY IN SEVERAL STRAINS OF GUINEA PIG. R.C. Lind, A.G. Gandolfo, I.C. Sipes, Dept. of Anesthesiology, Univ. of Arizona, Tucson, AZ 85724.

Previous animal models for halothane induced liver injury are in rats and have required extensive manipulation of the animals; hepatic enzyme induction, hypoxia, transient ischemia, etc. Hall et al (Flinders University, Adelaide, Australia) have reported that halothane is hepatotoxic in the guinea pig without manipulation or pretreatment. We have examined the strain specificity of this response. Albino outbred Amans and inbred Hartley strains, and colored inbred strains 2 and 13 (400-600g) were anesthetized for 4 hr with 1% halothane at 21% O2 without pretreatment. At 48 hr post exposure the animals were terminated for measurement of SGPT and histopathological examination of liver sections. Preliminary results with each strain indicate a diffuse coagulative hepatic necrosis in approximately 50% of the animals exposed with associated elevations of SGPT of 2 to 10 fold above pre-exposure values. Panlobular fatty vacuolization of hepatocytes was also present. Animals that did not develop an elevation in SGPT beyond the normal range (20-30 W-L units/ml) at 48 hr post H, still demonstrated multiple small foci of necrotic hepatocytes throughout the hepatic sections sampled. This guinea pig model of halothane associated hepatoxicity is viable in the four strains evaluated and may well prove superior to and more realistic than current rat models. (NIH AN 16715).

298 CCl4 INDUCED CHANGES IN HEPATIC DNA, RNA, LIPID, PROTEIN AND GLYCOCEN IN CHLOROCON المتواتر الاعتداءات BROMOBENZENE AND ACETAMINOPHEN INDUCED HEPATOTOXICITY. Z. Liu and M.R. Franklin, Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112, U.S.A.

Previous work has shown that dietary chlordane (CCl4) results in a powerful potentiation of hepatotoxicity and lethality of an ordinarily nontoxic dose (100 μl/kg) of CCl4. Time course morphometric analysis of this interaction indicated that hepatitis cell death was suppressed with CD + CCl4 but not with CCl4 alone. The present study was designed to estimate a number of biochemical parameters of cellular metabolism. Male rats were maintained on a normal powdered diet or on a similar one containing 10 ppm CD. On day 15, the animals received a single ip injection (100 μl/kg) of CCl4. Hepatic protein, DNA, RNA, lipid and glycogen were determined at 0, 1, 4, 6, 12, 24 and 36 hr after CCl4. Hepatic protein decreased (18%) 24 hr after CCl4 challenge with CD + CCl4 but not with CCl4 alone. Hepatic RNA was decreased (37%) with CD + CCl4 36 hr after treatment; no changes were observed with DNA. Both DNA and RNA were increased (20 and 16%, respectively) 6 hr after exposure to CCl4 alone. Lipid content was increased for all CCl4 groups after 4 hr with CD + CCl4 in comparison to CCl4 alone. Glycogen was depleted in both groups, that in the CD + CCl4 being greater. The changes observed in the CD + CCl4 rats were indicative of a lack of metabolic order needed for hepatic regeneration in contrast to those observed with CCl4 alone. (Supported by EPA-R810702 and ES-07045.)

300 THE PROTECTIVE ACTION OF SKF 525-A TOWARDS BROMOBENZENE AND ACETAMINOPHEN INDUCED HEPATOTOXICITY. Z. Liu and M.R. Franklin, Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112, U.S.A.

The protective action of a single dose of SKF 525-A (80 mg/kg) on bromobenzene (0.2 ml/kg - i.p.) and acetaminophen (2.0 g/kg - i.p.) toxicity was examined in phenobarbital induced adult male rats. The hepatotoxins were given 0.5, 8 or 12 hrs after SKF 525-A administration (i.p.) and toxicity determined by serum ALT elevations and cytochrome P-450 destruction 24 hr (acetaminophen) and 48 hr (bromobenzene) later. Protection was evident for all the time intervals between SKF 525-A and hepatotoxin administration although only complete protection was seen with the 0.5 hr interval. Hepatic SKF 525-A concentrations were 280, 38 and 26 mmol/g liver at the 3 time intervals. The secondary and primary amine SKF 525-A metabolite concentrations were 89, 7 and 3 and 83, 32 and 14 respectively for the 3 time intervals. The amount of cytochrome P-450 sequestered as a metabolite-intermediate complex at the time of hepatotoxin administration was similar at all time intervals. Thus, the greater protection at the short time interval (0.5 hr) appears to derive from the ability of SKF 525-A and/or its metabolites to block hepatotoxin bioactivation in a manner other than cytochrome P-450 metabolic-intermediate complex formation. Supported by USPS Grant No. CA 15760.
301 POTENTIATION OF ACETAMINOPHEN TOXICITY IN MICE BY DFM0, AN ORTHINE DECARBOXYLASE INHIBITOR.
W. Dairman and L.P. Juhasz, Hoffmann-LaRoche Inc., Nutley, NJ.

We have previously reported that the oral administration of the polyamines, spermidine or spermine, protected mice against acetaminophen (ACTM) induced hepatotoxicity (Fed.Proc. 66: 371, 1964). In continuation of our studies on the possible physiological function of polyamines in prevention and/or repair of chemically induced cellular injury, we have studied acetaminophen toxicity in mice in which polyamine biosynthesis has been inhibited. Polyamine biosynthesis was inhibited via inhibition of the rate limiting enzyme, orthinine decarboxylase (ODC), by a-difluoromethyl-ornithine (DFMO). Young adult male C57 mice were given 1% DFMO in the drinking water starting 18 hours prior to ACTM (200 mg/kg p.o.) then sacrificed 24 hours following ACTM. These mice also were given 100 mg/kg of DFMO s.c. 30 minutes prior to and at 3 and 6 hours following ACTM. Cytotoxic activity was assessed by determination of GPT and GOT serum levels in the serum obtained at sacrifice. In the group of mice which received only ACTM, GPT and GOT serum values were moderately elevated in comparison to vehicle or DFMO treated mice but were about 3 or 4 fold lower than those found in mice treated with both DFMO and acetaminophen. These results support the hypothesis that endogenous polyamines play a protective role against chemically induced cellular injury.

303 SPECIES DIFFERENCES IN THE INHIBITION OF ACETAMINO-
PHEN-GLUCURONYL TRANSFERASE (ACET-GT) BY DIAZEPAM
AND ITS METABOLITES.

We have observed that the toxicity of acetaminophen is potentiated by the coadministration of diazepam (DZ) (1.54 mg/kg) in dogs, but was not potentiated in other species tested even at higher doses of DZ (see McClain et al of this volume). Since glucuronidation is a major pathway for the detoxification of acetaminophen, alterations in glucuronidation of acetaminophen may result in potentiation of toxicity. Previous studies (Levin, et al '64) The Toxicologist 4 demonstrated that benzodiazepines inhibit the activity of microsomal glucuronosyltransferases (GT1 and GT2) with markedly different I50's and Ki's. Glucurontransferase-activity from hepatic microsomes of the dog was more sensitive to the inhibition by DZ than GT activity in microsomes from the other species tested. In this study the effects of DZ, nardazepam (NDZ) and oxazepam (OX) on ACET-GT activity were investigated in hepatic microsomes from dog, rat, squirrel monkey, and human. Microsomes (0.5 mg protein/ml) were incubated with 2 to 24 mM ACET, 5 mM UDPGA in 66 mM Tris (pH 7.8) and 0.05% Brij 58. The IC50's for the inhibition of ACET-GT by DZ were 2.5, 70, 45 and 70 µM for the dog, rat, monkey, and human, respectively. The IC50 for the inhibition of ACET-GT from dog by NDZ was 45 µM compared to values greater than 100 µM for other species tested. OX was less potent as an inhibitor that was DZ or NDZ, with IC50's for all species greater than 85 µM. These results demonstrate that ACET-GT is inhibited by DZ, and NDZ. Hepatic microsomes from the dog were markedly more sensitive to these inhibitory effects than were the microsomes from other species tested. These in vitro results correlate well with in vivo results in that the dog exhibited the most prominent acetaminophen-diazepam interactions. On the basis of the in vitro results, one would predict that the dog might be greater than ten fold more sensitive to potential drug interactions with diazepam. In conclusion, since the inhibitory effects of diazepam on the dog were only observed at relatively high doses and considering the 10 fold higher IC50's observed for ACET-GT from human liver, it is most likely that the inhibition of ACET-GT has any clinical significance for humans. (The authors wish to thank Dr. D.S. Sipes of Univ. of AZ for the gift of human liver microsomes.)

302 POTENTIATION OF ACETAMINOPHEN TOXICITY IN DOGS TREATED WITH DIAZEPAM.

During the course of toxicity studies in the dog with a combination of diazepam and acetaminophen, it was noted that diazepam potentiated the toxicity of acetaminophen at relatively high dosages. To explore this effect, a six week toxicity study was conducted in male beagle dogs (5/group) with a formulation of acetaminophen (A) and a formulation of diazepam plus acetaminophen (D + A administered orally at a dosage of 1.54 mg/kg/day of diazepam and/or 250 mg/kg/day of acetaminophen. Almost all parameters of acetaminophen toxicity were more severely affected in the diaze-

304 EFFECT OF CARBON DISULFIDE AND PHENOBARBITAL ON HEPATIC CHOLESTEROL METABOLISM IN THE F344 RAT.
J.E. Simmons, R.A. Sloane, E.W. Van Stee, National Institute of Environmental Health Sciences, RTP, NC and Dept. of Environmental Sciences and Engineering, UNC-Chapel Hill, NC. Sponsor: D.J. Hoobrock, Jr.

The effects of acute exposure to carbon disulfide (CS2) on hepatic cholesterol metabolism were studied to distinguish between the abilities of CS2 and its oxidative metabolites to alter hepatic cholesterol metabolism. Male F344 rats were exposed either to 600, 300, 150, 75, or 30 ppm CS2 for 6 hr by inhalation, or to 0.1% phenobarbital (PB) in the drinking water starting 5 days before exposure to CS2, or both. Exposure to 600 ppm CS2 after pretreatment with PB resulted in a decrease in the rate of hepatic cholesterol synthesis (in vitro synthesis of cholesterol from radiolabeled acetate) and an increase in the concentration of hepatic cholesterol. A concentration-response relationship between hepatic cholesterol metabolism and exposure to CS2 was determined in rats that had been pretreated with PB. These observations are consistent with the theory that metabolism of CS2 is necessary for the expression of CS2-mediated alterations of hepatic cholesterol metabolism and provide support for the idea that metabolism of CS2 is necessary for the expression of hepatotoxicity.
1,4-Bis-(3,5-dichloropyridyloxy)benzene (TCPBOP) produces a pleiotropic response in mouse liver similar to phenobarbital. This response includes: induction of select microsomal monooxygenase activities, increase in liver weight and proliferation of the smooth endoplasmic reticulum. Compared to phenobarbital, TCPBOP has been shown to be a much more potent agonist for this response. Moreover, a structure-activity relationship has been demonstrated among a group of five TCPBOP analogs with respect to microsomal monooxygenase induction. This report demonstrates that this structure-activity relationship can be extended to encompass: zonal cellular hypertrophy, proliferation of the smooth endoplasmic reticulum and cellular toxicity. Quantitative evaluation of cellular hypertropic changes, utilizing image analyzing techniques, indicates a good correlation between hepatocyte size and induction of microsomal monooxygenases for TCPBOP and four analogs. The presence of a structure-activity relationship, based on both biochemical and morphologic criteria, among a group of analogs closely related to TCPBOP supports a receptor-mediated mechanism of action. (Supported by the Texas Agricultural Experiment Station, No. 6376)

Epichlorohydrin has been implicated (Jones et al. 1979) to be the metabolite of DBCP responsible for its toxic effects in rats. This was based on the reported finding of identical metabolites in the urine of rats administered non-radioactive ECH or DBCP. In the present study male Fischer 344 rats were administered orally 10 mg/kg of (1,2,3,14C)-DBCP. Within 3 days 55% of the radioactivity was excreted in urine, 18% in feces and 20% as exhaled carbon dioxide. The remainder was recovered in tissues. At least 20 radioactive metabolites were found in urine. N-acetyl-S-(3-chloro-2-hydroxypropyl)-L-cysteine (ACPC) was identified at only 3% of the dose, although it was identified previously as the major urinary metabolite of ECH at 36%. In vitro studies with rat liver microsomes showed that ECH was metabolized to aliphachlorohydrin (ACH), but under similar conditions neither ECH nor ACH were formed from DBCP. Binding of radioactivity to liver microsomal proteins occurred for both substrates, but was less for (14C)-ECH than for (14C)-DBCP. Thus, both in vivo and in vitro metabolic data indicate that ECH is at best a minor metabolite of DBCP in rats, and therefore is not likely to account for the toxicity of DBCP.
A QUANTITATIVE POST-LABELLING ASSAY FOR THE PRINCIPAL VINYL CHLORIDE-DNA ADDUCT.  

N\textsuperscript{7}-(2-Oxoethyl)guanine is the principal DNA adduct formed in the livers of rats exposed to vinyl chloride. A sensitive assay of this adduct has been developed with a view to determining the quantitative correlation between exposure dose and DNA dose in the tissues of experimental species exposed to unlabelled vinyl chloride. DNA samples are spiked with N\textsuperscript{7}-(2-oxoethyl)\textsuperscript{[\textsuperscript{14}C]}guanine. After reduction with NaBH\textsubscript{4}, the product is released by heating to 100°C. The N\textsuperscript{7}-(2-hydroxyethyl)guanine is converted into its N\textsuperscript{7}-OH-0-acetate by reaction with \textsuperscript{[\textsuperscript{14}C]}acetate anhydride. The acetoxyl groups are then cleaved using n-propylamine and the products are resolved by hplc. The amount of radioactivity recovered in n-propyl acetamide provides a measure of the total N\textsuperscript{7}-(2-oxoethyl)guanine in the sample and the radioactivity associated with N\textsuperscript{7}-(2-hydroxyethyl)guanine permits correction for losses. This method has advantages over simpler procedures relying on radiolabelling of N\textsuperscript{7}-(2-oxoethyl)guanine by reduction with Na\textsubscript{2}B\textsubscript{4}H\textsubscript{4}. This latter approach overestimates the amount of adduct due to incorporation of tritium by exchange mechanisms. The new dual label approach provides a strategic basis for the quantitation of DNA adducts in general.

PURIFICATION AND CHARACTERIZATION OF HUMAN AND RAT LIVER MICROSMAL CYTOCHROMES P-450 RESPONSIBLE FOR 4-HYDROXYLATION OF MEPHENYTOIN, A PROTOTYPE OF GENETIC POLYMORPHISM. T. Shimada and F.P. Guengerich, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37232

The hydantoin derivative mephenytoin has been used extensively as an anticonvulsant agent. In order to address the basis of the genetic polymorphism of mephenytoin 4-hydroxylation, attempts were made to purify the cytochrome P-450 responsible for this substrate from human and rat liver microsomes. To measure the hydroxylation activities of liver microsomes we developed a simple and rapid method using \textsuperscript{14}C-mephenytoin as a substrate. The reaction was a typical microsomal monoxygenase activity involving cytochrome P-450, and the activities were found to vary from 5.6 to 156 pmol product formation/min/mmol cytochrome P-450 in several human liver microsomal preparations. The cytochrome P-450 was purified from human liver microsomes by using amine-ocetyl Sepharose 4B, hydroxylapatite and ion-exchange chromatography. The antibody raised to this P-450 preparation completely inhibited mephenytoin 4-hydroxylation activity in human liver microsomes. The purified cytochrome P-450 had an apparent molecular weight of 48,000 daltons and was identified as the P-450 which we had previously purified from human liver microsomes. In rat liver microsomes, we found that pregnenolone 16a-carboxonitrile significantly induced 4-hydroxylase activity. (Supported by USPHS grant CA 30907.)

XENOBIOTIC METABOLISM BY PROSTAGLANDIN SYNTHETASE S.A. Lacy and J.G. Babish. Dept. of Preventive Medicine, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

The prostaglandin synthetase (PGS) hydroperoxidase utilizes in vitro a range of xenobiotics as cofactors, many of which are converted to toxic and mutagenic metabolites. PGS cyclooxygenase activity (measured as the initial velocity of oxygen uptake upon arachidonic acid addition to ram seminal vesicle microsomal preparations) gives a relative comparison of a chemical to act as a hydroperoxidase cofactor. Twenty compounds were assayed from 1\muM to 5\muM at a minimum of six concentrations each.

Results indicate xenobiotic cofactors fall into four groups: (1) chemicals increasing oxygen uptake greater than 100% above control (no cofactor) at least one concentration, including aminopyrine, benzidine, diethylstilbestrol, phenacetin, and phenylbutazone, (2) cofactors such as 9-aminocadinine, benzo(a)pyrene, and dimethylbenzanthracene, weakly stimulatory to O\textsubscript{2} consumption (<50% above control at at least one concentration), (3) compounds slightly inhibitory to O\textsubscript{2} uptake at all concentrations tested (e.g., aniline, chrysene, 2-acetamidofluorene, 3-methylcholanthrene), (4) strongly inhibitory xenobiotics, such as 1-aminopyrine (K\textsubscript{f} 15\muM), 2-aminofluorene (K\textsubscript{f} 5\muM), 2-aminoanthracene (K\textsubscript{f} 2\muM), and 3,6-diaminocadinine (K\textsubscript{f} 5\muM).

EVIDENCE TO SUGGEST THE PRESENCE OF AN ACTIVE CELLULAR ONCOGENE (c-onc) IN THE B6C3F1 MOUSE LIVER. T. R. Fox and P. G. Watanabe, Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, MI 48640.

The present study was conducted to determine whether the high spontaneous liver tumor (SLT) incidence in B6C3F1 mice might be associated with an active c-onc. DNA isolated from SLT's of 24 mo. old male B6C3F1 mice was evaluated for transforming activity in NIH 3T3 fibroblasts using standard transfection methodology. A transfection freq. of 0.005-0.02 transformants/\mu g tumor DNA was observed with 9 of 11 mice (84%) showing positive transforming capabilities. This incidence was considerably higher than that reported (10-20%) with DNA from human tumors. In contrast, hepatic DNA isolated from ten, 24 mo. old and two, 3-5 mo. old non-tumor bearing male B6C3F1 mice did not elicit any transforming activity in the 3T3 assay. These results strongly suggest the presence of an active c-onc in the SLT DNA of B6C3F1 mice. Identification of the apparent c-onc and evaluation of factors which might modify its expression should increase our understanding of potential mechanisms which enhance hepatic tumor formation. This should allow for more informed estimates of risk in man from data generated in this strain of mouse.

It has been postulated that, since gap-junctional intercellular communication is involved in embryogenesis, cell differentiation, proliferation, germ cell development, immune and neurological function, disruption of this form of communication might be involved in teratogenesis, tumor promotion, reproductive dysfunction, and immune- and neurotoxicology. Many naturally occurring chemicals are known to have multiple toxic effects and are carcinogenic to animals and human beings. Since some of these toxins have been shown to be tumor promoters and since many tumor promoters seem to be able to inhibit intercellular communication by either cytotoxic or noncytotoxic mechanisms, we tested a series of these biological toxins in the Chinese hamster V79- metabolic cooperation assay. Teleocidin, dehydroaplysia toxin and anhydrodebroaplysiamethaplysia toxins, palytoxin, T-2 toxin, vomitoxin and aflatoxin B1, were tested for their cytotoxicity and ability to inhibit metabolic cooperation. Our results demonstrated a wide range of cytotoxicity and of their ability to inhibit metabolic cooperation. Their ability to inhibit intercellular communication by cytotoxic or noncytotoxic mechanisms would be consistent with the hypothesis that methemoglobin produced by NaNO2 (5.0 mM) or dimethylaminophenol (0.10 mM) reversed cyanide-induced ATP suppression in rat HFs; no reversal occurred in the absence of RBCs. In contrast, Na, S, O2 (10 mM) reversed ATP suppression by cyanide (1.0 mM) without cytochrome oxidase inhibition by converting it to CNS-. However, RBCs were found to increase both the rate of recovery in ATP and CN-→CNS- conversion by an additional 60 to 70% after 60 min at 37°C. The demonstration that RBCs contribute to reversal of CNS toxicity by Na, S, O2 is new and studies to identify the enzyme system in RBCs involved in this reaction are in progress. (Supported by USAMRC DAMD17-82-C-2111.)


A combined hepatocyte(HP)-erythrocyte(RBC) system has been developed for the study of cyanide and antodicote action at the cellular level (Gee et al., The Toxicologist 4:127). Further studies with this system revealed cyanide produced by NaNO2 (5.0 mM) or dimethylaminophenol (0.10 mM) reversed cyanide-induced ATP suppression in rat HFs; no reversal occurred in the absence of RBCs. In contrast, Na, S, O2 (10 mM) reversed ATP suppression by cyanide (1.0 mM) without cytochrome oxidase formation by converting it to CNS-. However, RBCs were found to increase both the rate of recovery in ATP and CN-→CNS- conversion by an additional 60 to 70% after 60 min at 37°C. The demonstration that RBCs contribute to reversal of CNS toxicity by Na, S, O2 is new and studies to identify the enzyme system in RBCS involved in this reaction are in progress. (Supported by USAMRC DAMD17-82-C-2111.)


TCDD and related compounds exert their toxicological effects via binding to the Ah receptor. Our data indicate that TCDD's potent toxicity may be partly due to the slow reversibility of this binding reaction. However, measurement of dissociation rate has been hampered by receptor stability in cytosol preparations. We examined the ability of various compounds to stabilize the rat liver Ah receptor or alter the specific binding (SB) of TCDD. Several protease inhibitors were unable to protect native receptor from thermal inactivation at 20°C. 20mM molybdate likewise failed to protect, implying that this receptor may differ from steroid receptors in some fundamental way. Binding of TCDD confers stabilization at all temperatures examined. The presence of CaCl2, affords slight protection at 15°C-25°C, but lowers recovery of SB. The importance of sulfhydryl groups in TCDD binding is evidenced by a 50% reduction in SB when diithiothreitol is deleted from the buffer. Serine protease substrates and the sulfhydryl reagent mersaryl increase TCDD binding at low concentrations, but at higher levels SB can be completely inhibited. Mersaryl at high concentrations can also cause rapid dissociation of previously bound TCDD. Our data show that the high affinity interaction of TCDD with the Ah receptor is persistent, enhances thermostability and entails reduced sulfhydryl groups. (Supported in part by NIEHS Grant ES02025 and EHS Center Grant ES01247.)

Phorsol Myristate Acetate (PMA) and Metabolic Cooperation: Examination of Cell Pre-Treatment, DOSE, DURATION and CEL DENSITY PARAMETERS. T.G. Hartman and J.D. Rosen. Department of Food Science, Cook College, Rutgers University, New Brunswick, NJ. Sponsor: M.A. Cello.

The effect of cell density, exposure time, PMA concentration and pre-exposure on V-79 HGPRT+ cells in the metabolic cooperation assay was determined. PMA inhibited metabolic cooperation at a dose as low as 0.1 ng/ml final media concentration. An exposure period of only 1 minute resulted in maximum recovery of HGPRT+ cells. Recovery with and without PMA exposure varied according to cell density. Pre-exposure of cells to PMA increased the recovery of both post PMA-treated and non-treated HGPRT+ cells in a dose-dependent manner. These results suggest that PMA affects metabolic cooperation by causing rapid physico-chemical changes in cell membranes. (NJAES #D-10201-1-85)

In bench scale pyrolysis studies tetrabrominated dibenzo-\textit{p}-dioxins (TBDDs) were obtained by pyrolyzing a number of different materials including 2,4,6-trichlorobenzene, as well as a flame retardant additive Tetrabromobisphenol A, and an epoxy mixture containing the latter flame retardant. The TBDDs and related pyrolysis products were characterized by high resolution gas chromatography-mass spectrometry and the enzyme inductive effects of the TBDDs and pyrolysis products containing TBDDs were assessed using an in vivo chick embryo model and compared with 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-tetra-CDD). On the basis of aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin deethylase (7-ER) induction the TBDDs tested (including 1,3,6,8/1,3,7,9-TBDDs) were considerably more potent than corresponding TCDDs yet less potent (by a factor of about 150) than 2,3,7,8-tetra-CDD. Dose response curves for the induction of AHH and 7-ER were parallel to the dose response curves obtained for 2,3,7,8-tetra-CDD. The results raise the prospect that mixtures containing TBDDs may be more toxic than mixtures containing corresponding TCDDs.

Apothetized rats were exposed by respirator to 1802 (21 Iz/v, 1 hr) following I.P. injection with CCl4 (1 ml/kg bw). Livers were lyophilized, converted to CO2 using a Schutte-Unterzaucher procedure, and analyzed by isotope-ratio mass spectrometry to determine the fractional abundance of 18O. Total oxygen content of the samples was used to convert fractional 180 to molar excess 1802. Control rats exposed only to 1802 showed an excess 180 of 650 moles/g liver compared with rats breathing normal air. CCl4-treated animals exposed to 1802 had an excess 180 of 1230 moles/g liver which was further elevated to 1980 moles/g by phenobarbital pretreatment. Rats pretreated with piperonyl butoxide showed excess 1802 similar to groups not treated with CCl4. A significant portion of the excess 180 was found in each of the major liver fractions examined: methanol-water soluble, chloroform soluble, and pellet. These results demonstrate that CCl4 initiates autoxidation of non-lipid as well as lipid constituents of liver, and establish a useful technique for direct quantification of these effects. (This abstract does not necessarily reflect EPA policy).

CHOLESTEROL EPoxide HYDrolase ACTIVITY IN RAT LIVER MICROSOMES. A. Sevanian and L. McLeod. Institute for Toxicology, University of Southern California.

Cholesterol epoxide (CE) occurs as a common membrane lipid peroxidation product. It has been reported that free radical reactions involving lipids yield alpha (a) and beta (b) CE in varying amounts as influenced by the radical system in question. In the present study we examined the kinetics of epoxide hydrolase with respect to the conversion of (a) and (b) CE to cholestene triol (CT). Liver microsomes were incubated with 1 - 35 uM (a) or (b) IAC-CE, or combinations of (a) and (b) CE, and rates of hydration measured by a previously reported chromatographic method. Various epoxide hydrolase inhibitors were tested as well as some common cholesterol oxidation products in the search for specific and nontoxic inhibitors. We determined the Km for (a) and (b) CE to be 3.2 and 4.2 uM, respectively. The KI for (a) CE, vs. (b) CE, was 13.6 uM while the KI for (b) CE was 7.3 uM. Apparent product inhibition was found with the KI for CT being 14.4 uM for (a) and 4.7 uM for (b). No inhibition was found using cyclohexene oxide, styrene oxide, isoquinoline or benzil, consistent with previous findings suggesting that the epoxide hydrolase for xenobiotic epoxides differs from CE hydrolase. Among the cholesterol oxidation products 7-keto cholesterol exhibited the lowest KI (4.6 uM). Apparent hydration rates for (a) and (b) CE suggest differences in the "productivity" of the reaction and the effect of CT on the kinetics of hydration.


Males帅 Sprague-Dawley rats (300g) treated with TCCD (6.25 to 100ug/kg) were compared seven days after treatment to ad libitum-fed control (ALC) and pair-fed control (PFC) rats. Body weight and total and resting oxygen consumption in TCCD-treated animals were reduced in a dose-dependent fashion. Similar reductions were observed in PFC rats. Body temperature remained similar in ALC, PFC and TCCD-treated rats, but circulating concentrations of thyroid hormones were different. T4 levels were reduced (50%) by all doses of TCCD. In PFC rats, T4 levels were decreased to a lesser extent (10%). Circulating concentrations of T3 tended to be similar to those of ALC and PFC rats at low doses of TCCD, but were higher at high doses. TSH levels were generally greater in TCCD-treated rats than ALC and PFC rats. When rats were challenged by cold exposure on week 7 (4°C, 23 hr), circulating concentrations of T4, T3 and TSH were all increased above the levels observed for each group at 22°C. However, differences in thyroid hormone levels between groups remained. These results suggest that hypophagia and weight loss following treatment with TCCD played a greater role in decreasing oxygen consumption in TCCD-treated rats than the altered circulating concentrations of thyroid hormones. (NIH Grant ES01332).

Visual impairments have been demonstrated behaviorally and electrophysiologically in rats and monkeys neonatally exposed to lead (Pb). Little evidence exists on auditory and visual effects of Pb exposure in humans, although we observed increased latencies of brainstem auditory evoked potentials (BAEP) in children with histories of undetected Pb absorption (Otto et al. Environ. Res., in press). BAEPs and pattern-reversal visual evoked potentials (PREP) were recorded in 80 children aged 3 to 7 with blood lead (Pb) levels ranging from 6 to 47 μg/dl. Elevated hearing thresholds were observed in children with higher Pb levels. The latencies of BAEP waves I and III varied inversely with Pb level, contrary to prediction, a possible result of adjusting stimulation level for hearing threshold. Pb latency, the most clinically relevant PREP measure, did not vary significantly with blood lead (Pb) level. An exploratory analysis of PIN2 amplitude, however, varied inversely with Pb consistent with other data suggestive of Pb-related visual impairment. Since the PREP reflects cone-mediated photoreceptor function, other measures of scotopic visual function may provide more sensitive indices of lead exposure effects in humans.


Our in vitro electrophysiological studies show that Pb depresses the amplitude of rod, but not cone, photoreceptor potentials (Science 206: 78, 1979). To determine if this selective effect occurs in vivo, rod and cone ERGs were measured in adult rats developmentally exposed to Pb. Analysis of rod (cGMP) and cone (cAMP) cyclic nucleotide systems and quantitative ultrastructural analysis of retinal morphology were performed as correlating measures. Single-flash rod ERG V-log I functions revealed a 25% and 15% reduction in a- and b-wave max. amplitude, respectively, and a 1 log unit decrease in absolute sensitivity. Cone ERGs were unchanged. Pb increased the levels of cGMP of dark- and light-adapted retinas by 40% and 25%, respectively, whereas cAMP levels were unchanged. Morphological analysis revealed necrotic and vacuolated rod outer segments and a decreased thickness of the outer nuclear layer which is accounted for by a decreased number of nuclei. Also there was a large increase in the number of membrane-bound glycogen containing vacuoles mostly located in the rod inner segment. Thus developmental Pb exposure caused selective longterm functional, biochemical and morphological rod photoreceptor deficits. Supported by ES 03183 (DAF) and SNE 02651 and RCOA SE 00144 (DBF).

EFFECTS OF DEVELOPMENTAL AND CONTINUOUS LEAD EXPOSURE ON THE FUNCTIONAL PROPERTIES OF RETINOCOLLICULAR (RG) AND -COLLICULAR (RC) RESPONSES IN ADULT HOODED RATS. D. Impelman, R.D. Wilson and D.A. Fox. U. Houston, College Optometry, Houston and USDA, College Station, TX.

We previously showed that neonatal Pb exposure produces a visual acuity loss correlated with excitability changes in RG and RC pathways which mediate spatial vision (Toxicologist 3: 69 and 70, 1983). Central changes in their electrophysiological properties include: (1) decreased conduction velocities (CVS) in large (tg) and increased chronaxies in tg and medium (tg) diameter optic axons, (2) decreased postsynaptic depression in tg RC recovery functions and (3) depression of geniculocortical recovery function. The effects of continuous (neonatal + postweaning) exposure to 0.2% PbAc on RG and RC functions were studied to compare with neonatal only (0.2% PbAc) effects. In continuous Pb rats, tc CVS were now decreased while chronaxies remained increased. Tg postsynaptic responses were decreased approx. 20% in dLGN and SC. Tg CVs were normal but SC response recovery was decreased approx. 30%. Tg SC recovery recovery was less depressed than in controls. These results show that a continued Pb exposure (blood Pb approx. 55 μg/dl) produces further pre- and postsynaptic deficits in the RG and RC pathways that result in depressed conduction in tg, depressed postsynaptic responses in dLGN and altered SC responses to the three OT conduction groups. Supported by ES 03183 (DAF).

SYNAPTIC FUNCTIONS IN RETINOCOLLICULAR (RC) PATHWAY OF RATS WITH 2,5-HEXANEDIONE (HD) NEUROPATHY: A PREFERENTIAL SMALL FIBER SYSTEM EFFECT. R.D. Wilson, D. Impelman and D.A. Fox. USDA, College Station and U. Houston, Coll. Optometry, Hou, TX.

In rats with HD neuropathy pre- (optic tract;OT) and postsynaptic (superior colliculus;SC) components of RC pathway develop similar morphological alterations. To differentially analyze functional effects of this neuropathy on large (tg) and medium (tg) diameter OT axons and on their corresponding SC components, synaptic I/O functions were studied. Although both fiber groups showed changes in amplitude, latency and recoverability, t2 response properties were more affected at all stages of HD neuropathy than t1. Also t2 response amplitudes were dispersed and amplitude-intensity functions were depressed. The SC response to t1 and t2 activation were studied by simultaneously recording from OT and SC while stimulating optic chiasm. The two SC components (C1 and C2) were differentiated from their OT inputs and from each other by their functional characteristics. In rats with advanced HD neuropathy, C2 amplitudes were decreased, synaptic delay was increased, recovery functions lacked postsynaptic inhibition and were characterized by a prolonged period of hyperexcitability. Only slight changes in C1 were observed. These data in the RC pathway clearly demonstrate greater functional changes in medium, compared to large, diameter OT fibers and in their corresponding SC components in HD neuropathy. Supported by ES 03183 (DAF).

Five macaques were exposed to 256 ppm CS2 over a 7-27 week period (6 hrs/day, 5 days/week). Filamentous swelling and degeneration in peripheral and central axons were confirmed in one animal sacrificed immediately after intoxication. The affected axons were particularly numerous in pyramidal, dorsal spinocerebellar and optic tracts. Except in the visual system, there was little permanent degeneration. The basal ganglia necrosis described by Richter (1945) was not observed. All dosed monkeys had a marked loss of central retinal ganglion cells. This loss was found with anterograde HRP tracer studies to spare the representation of peripheral vision. Motor coordination deficits were reversible with the termination of repeated exposure. However, visual studies revealed initial signs of reduced acuity and contrast sensitivity with sparing of flicker-fusion thresholds. All four tested monkeys suffered permanent contrast sensitivity loss at all spatial frequencies. Special tests may be needed to detect this visual dysfunction in humans. Support: R509555010, ES01247, DA00623.


Among the reported actions of chloridine-form (CDM) insecticide/acidicide is that it acts as an agonist/antagonist on octopaminergic or a-adrenergic receptors. Acute CDM HCl (5-40 mg/kg) exposure also produces a large enhancement of rat pattern reversal visual evoked potentials (PREPs; Boyes and Dyer, Exp. Neurol. in press). The possibility that CDM actions on rat visual function were mediated through a2-adrenergic receptors was investigated. In the first experiment, rats were treated with either the a2-agonist clonidine HCl (0.05-0.5 mg/kg i.p.), or the a2-antagonist yohimbine HCl (0.5-2.0 mg/kg i.p.). The PREP P13 component was enlarged by clonidine in a dose-related fashion, resembling in character the effect of CDM. Also similar to CDM action, component N2P2 was suppressed by clonidine. In contrast, N2P2 was enhanced by yohimbine. Preliminary results suggest that yohimbine (2 mg/kg) pretreatment diminished the effects of both CDM (40 mg/kg) and clonidine (0.1 mg/kg), although additional dose-response data are required. These results support the hypothesis that CDM alters visual function through a2-adrenergic receptor actions.

*Supported by an NRC research associateship.

327 EFFECTS OF A TYPE I OR TYPE II PYRETHROID ON PAIRED VISUAL EVOKED POTENTIALS. R.S. Dyer and W.K. Boyes, Neurophysiology Branch, NTD, USEPA, Research Triangle Park, NC 27711.

Type II pyrethroids (e.g., deltamethrin, DEL) prolong sodium action potential times longer than Type I pyrethroids (e.g., permethrin, PER), and may block activity at GABA receptors as well. Type I and Type II pyrethroids are rarely compared in neural systems more complex than single nerves or synapses. We report here the effects of presenting paired flash stimuli to unanesthetized rats upon the evoked potentials following treatment with DEL, PER or vehicle. Rats with electrodes implanted over the visual cortex were treated by gavage with either vehicle (corn oil), 4 mg/kg DEL, 16 mg/kg DEL, or 200 mg/kg PER. Following 2 hrs, flash evoked potentials (FEPs) to paired flash stimuli (181-200 msec) were recorded. DEL produced an increase in FEP latencies, with no differential effect upon the second flash of the pair. PER did not alter peak latency, but produced larger amplitude second flash responses than controls. We conclude that the two compounds produced differential effects upon the FEP. Contrary to expectation, the Type I rather than the Type II compound appeared to increase excitability at the relatively lower ISI of 200 msec, thereby raising questions about the way in which findings at the ionic level can be generalized to the functioning organism.

328 POSTRADIATION BLOOD FLOW IN THE VISUAL CORTEX OF PRIMATES. L. G. Cockerham, E. L. Pautler, and J. D. Hampton. Physiol Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD.

Supralethal doses of radiation have been shown to produce a marked drop in blood pressure and a decrease in total cerebral blood flow. In an attempt to delineate some factors associated with the early transient incapacitation observed in complex visual discriminatory behavior in primates following large doses of radiation, the blood flow in the visual cortex of monkeys was determined by the hydrogen clearance method. Compared to control animals, the monkeys exposed to 100 Gy, whole-body, gamma radiation exhibited an abrupt decline in blood pressure within 10 min postradiation and a transient increase in hematocrit which persisted for 15 min and then declined steadily. The blood flow in the visual cortex decreased by about 85% within 10 min postradiation and then remained relatively stable for the next 50 min although the means suggested a slight recovery followed by a slow decline to 75% below the baseline by 60 min postradiation. The magnitude of the change is sufficient to partially account for the deficits in visual discriminatory behavior in monkeys reported by others. The tendency for a partial recovery in blood flow was noted at a time when some monkeys exhibited a temporary recovery of visual discriminatory ability following radiation. The variability of blood flow and behavioral proficiency noted in both types of studies suggests the interplay of multiple regulatory mechanisms and their differential susceptibility to ionizing radiation.

Brain wave measures have been used to analyze central nervous system function and cognition. Otto et al. (Neurophysiol. Clin. Neurophysiol., 1981) reported significant changes in slow, event related, brain waves in children exposed to lead levels ranging from 6 to 59 ug/dl. Many of these children have been retested in a 5 year follow-up study. Reaction time (RT) and P3 were measured using a discrimination task in which children were asked to respond to a rare tone, embedded in a sequence of common tones, by pressing a button quickly. Data were analyzed from 38 children, age 6 to 12 years, with lead exposures of 6 to 30 ug/dl. No significant results were obtained when RT and P3 were compared to original lead values; however, complex interactions resulted when RT and P3 were compared to current lead values. Using a multiple regression model, we found that RT was significantly related to the interactions of blood lead (PbB) X maternal IQ and of PbB X HOME scores (a measure of caregiving). Also, when the latency of the P3 frequent peak was subtracted from the latency of the P3 rare peak, the result was found to be related to PbB X HOME scores as well as PbB X Socio-Economic Status. Thus, limited evidence was obtained of cognitive impairment as a result of lead exposure.

330 CIRCADIAN RHYTHM (CR) IN AMINOGLYCO-SIDE-INDUCED OTOTOXICITY: W. McKinney, B. Evans, A. Yonovits and M.H. Smolensky. Graduate School of Biomedical Sciences and School of Public Health, University of Texas Health Science Center at Houston, TX. Sponsor: Jeffrey C. Theiss

Toxicity studies are affected by CR. Our interest focuses on the role of CR on antibiotic-induced ototoxicity. Sprague-Dawley female rats were investigated for CR in aminoglycoside-induced ototoxicity evaluated as altered threshold to acoustic stimuli (8, 16, 24 and 32 kHz) using auditory brainstem responses (ABR). Different groups of 25-30 comparably aged rats, all housed under a CR synchronizing light (0600-1800) and dark (1800-0600) schedule, received daily subcutaneous injections of 225 mg/kg kanamycin sulfate (K), study 1, or 100 mg/kg gentamycin (G), study 2. In each 6-week long study, separate subgroups were treated once daily at times corresponding either to 0800, 1600, 2000 or 0200. For K and G, large and statistically significant differences in ototoxicity resulted depending on the daily circadian cycle. Rats treated during nocturnal activity (2000 or 0200) were minimally affected while those treated at 0800 or 1400 exhibited severe ototoxicity. The difference in ABR threshold over all the test frequencies for treatment during the nighttime vs daytime was <30db.


The purpose of this investigation was to evaluate the rat as a model in studies of aminoglycoside ototoxicity. Two strains of rats, Sprague-Dawley and Fisher-344, were used. Eight rats of each strain were dosed subcutaneously for 14 days with either 100 or 80 mg/kg of gentamicin-sulfate in saline. Four control rats of each strain received saline sub-cutaneously. Brainstem auditory evoked response (BAER) thresholds were recorded at 32, 16, 8, 4, 2, 1 and 0.5 kHz from 4 treated and 2 control rats of each strain on day 1 and 11 post administration. BAER tested rats were then sacrificed and temporal bones were removed. After fixation of inner ear tissues by routine methods, the basilar membrane was microdissected. Hair cell counting was performed and results were graphed in a cytocochleagram. Hair cell loss was observed in the base and hook regions of all Sprague-Dawley and Fisher-344 rats treated with 100 mg/kg of gentamicin. This loss was more severe in the Fisher-344 rats. Greater BAER threshold changes in the Fisher-344 rats also suggest the Fisher-344 strain as being more sensitive. We conclude that Fisher-344 rats are a useful model to study aminoglycoside ototoxicity as they exhibit both functional and morphological changes and are more sensitive than Sprague-Dawley rats. (Supported by NIEHS Training Grant # 5T32ES07062 and Program Project Grant #ES-OS05785)

332 SUBCHRONIC METHYLPYRIDINE (MP) EXPOSURE DOES NOT MINIMIZE ACUTE MP EFFECTS ON BRAINSTEM AUDITORY EVOKED RESPONSES OF RATS. R. Janssen and R. S. Dyer. Neurophysiology Branch, Neurotoxicology Division, U.S. EPA, RTP, NC.

In prior experiments in this laboratory, acute ip administration of 2-, 3-, and 4-methylpyridine (2MP, 3MP, 4MP) and of the parent compound, pyridine, produced changes in the brainstem auditory evoked responses (BAER) of rats. Specific effects differed across compounds, but included prolonged latencies and increased amplitudes of peaks, reflecting changes in peripheral and/or central auditory function. The following three experiments were conducted to determine whether there are effects of subchronic exposures to 2MP, 3MP and 4MP. Male Long-Evans rats were given oral doses of an MP compound at 0 (saline control), 50, or 100 mg/kg bw for 30 consecutive days. One week before testing, rats were implanted with skull screw electrodes. Two rats were tested unanesthetized 48 hours after the last dose. Responses to 512 click stimuli were averaged, and latencies and amplitudes of the resulting waveform peaks were analyzed. None of the three compounds produced significant effects on peak latencies or amplitudes, although there was a trend (p<.09) towards longer latencies in the 3MP-treated animals. The lack of effect seen in these data may be due to one or more of the following: (1) tolerance to acute MP effects; (2) differences in toxicity between oral and ip routes; (3) lack of any persistent effect.

Recent reports suggest an interaction of the Type II pyrethroids at or near the picrotoxinin binding site on the GABA receptor complex (Lawrence and Casida, Science 221:1399, 1983). Using these data we predicted that deltamethrin (DLT), a Type II compound, and not cismethrin (CSM), a Type I compound, would interact with picrotoxin (PTX) to produce additive effects in vivo. Dosage-effect functions for DLT, CSM, and PTX were determined for both figure-8 maze activity and the acoustic startle response using adult male LE hooded rats (8-10/group). For interaction studies PTX (0.25-2.0 mg/kg) was administered i.p. 0.5 hr prior to either DLT (1.0-6.0 mg/kg) or CSM (3.0-15 mg/kg). Both compounds were given p.o. 1.5 hr prior to motor activity and acoustic startle testing. All compounds produced a decrease in motor activity. Both DLT and PTX decreased latency of the startle response, while CSM increased latency and had no effect on latency. Results of these studies indicate that there is an additive effect of PTX and DLT, but not of PTX and CSM, on both motor activity and the acoustic startle response. These data demonstrate another difference in the in vivo effects of DLT and CSM, which is consistent with the reported differential effects of these pyrethroids on the GABA receptor complex.


Sponsor: P.A. Watanabe.

Myelinic edema was induced in male Fischer 344 rats by daily ingestion in food of 25 mg/kg of hexachlorophene (HCP). The rats appeared to be clinically normal at one week. Mild myelinic edema, however, was present in the sensorimotor pathways, and there were significant decreases in grip strength and accelerating rod performance. Sensory evoked responses, measured from the cerebellum (CER's) and from the sensory cortex (SER's), were not detectably changed. HCP was removed from the diet of some rats at this time. Grip strength and accelerating rod performance returned to normal after one recovery week. Histology was performed after two recovery weeks, and the edema was no longer evident. Other rats, given HCP for two weeks, moved normally in their cages or on a table top. Grip strength and accelerating rod performance were worse than at one week of treatment, and CER's and SER's were now abnormal. Myelinic edema was moderate to severe along the sensorimotor pathways. Three weeks of recovery after two weeks of treatment resulted in reduced edema, improvement in CER's and SER's, and normal grip strength and accelerating rod performance.

334 TOluene-I nduced OTOTOXICITY BY SUBcutaneous ADMINISTRATION. G. T. Pryor and R. A. Howd. SRI International, Menlo Park, CA.

We have found that inhalation exposure of rats to toluene causes irreversible hearing loss (Neurobehav. Toxicol. Teratol. 5;53-62, 1983; 6;111-119, 1984). Although we have assumed that this effect was caused by systemic toluene and/or its metabolites, the possibility of a peripheral locus could not be excluded because the toluene vapors had access to the auditory meatus and associated auricular structures. Therefore, an experiment was done in which groups of 12 weanling male Fischer rats were injected subcutaneously with PEG300 (control) or 1.5 or 1.7 g/kg of toluene for 7 days. The rats were then trained to perform a multifrequency conditioned avoidance response (CAR) task. When the frequency of the tone stimulus was increased from 4 (used during training) to 20 kHz, the rats treated with 1.7 g/kg toluene were significantly impaired, but there was no effect on performance of the shock and light CARs. Tone intensity-response functions were generated at 4, 8, 12, and 20 kHz, and auditory response thresholds were estimated. There were no differences among groups at 4 kHz, but the toluene-treated groups had dose-related elevated thresholds at 8 kHz and above. Therefore, the ototoxic effect of toluene seen with inhalation exposure was of systemic origin. These results also rule out noise associated with inhalation exposure as the main causative factor. Supported by NIDA Contract No. 271-80-3712.

336 CUTANEOUS SENSORY RECEPTORS ARE REDUCED IN NUMBER FOLLOWING ACRYLAMIDE ADMINISTRATION. B.D. Goldstein. Dep. of Pharmac. and Toxicol., Medical College of Ga., Augusta, GA.

Acrylamide monomer (ACR) produces a central-peripheral distal axonopathy. It has been shown that ACR affects proprioceptive receptors in muscle. The purpose of this study was to determine whether ACR affects more slowly conducting cutaneous sensory mechanoreceptors. Cats were administered 30 mg/kg/day ACR for 10 days. On the 11th day the animal was anesthetized, the tibial nerve dissected and functionally isolated mechanoreceptors arising from the hindpaw were obtained. Four types of mechanoreceptors were characterized: field, rapidly adapting, slowly adapting I, and slow adapting II. There was no change in the frequency-response curve of the slowly adapting I receptors. However, the total number of receptors obtainable was reduced to 40% of control. The distribution of the receptor types also changed. There was an increase in the rapidly adapting receptors from 16% to 33% and a decrease in the slowly adapting receptors from 17% to 3% (type I) and from 5% to 0% (type II). These data suggest that ACR does affect cutaneous receptors. There appears to be a transformation of slowly adapting receptors to rapidly adapting receptors. Supported by NS18664.
Electrophysiological Changes in Peripheral Sensory Receptors Following Sub-Acute Administration of Soman. B.D. Goldstein, Dept. of Pharmac. and Toxicol., Medical College of Ga., Augusta, GA.

Soman (GD) is an organophosphorous agent which is highly reactive. The purpose of this study is to determine if sub-acute administration of GD has any neurotoxic effects other than cholinesterase inhibition. Cats were administered either 2.5 mg/kg/day (10 days) or 5 mg/kg/day (5 days) GD. Electrophysiological studies were performed the day after the last injection. Cutaneous sensory receptors and muscle spindles were tested to determine whether they respond properly to their adequate stimulus. Four types of cutaneous receptors were isolated: field, rapidly adapting, slowly adapting I, and slowly adapting II. There was no difference in the frequency-response curves of slowly adapting mechanoreceptors. However, the total number of cutaneous receptors of all types were reduced to 34% of control with no change in the distribution of these receptor types. There was no difference in the frequency-response or the number of obtainable muscle spindles in either treatment groups. These data suggest that GD affects non-cholinergic systems. It is a nonspecific effect since it reduced the number of receptors regardless of its type. However, GD spared muscle spindles. Muscle spindles are recoupled and it is possible that the GD could not reach the vulnerable portion of the muscle spindle. Supported by USAMRDC DAMD 17-82-C-2217.


Because lindane is used in human and veterinary medicine, its effects on various indices of toxicity were determined. Lindane was dissolved in oil and administered per os in a single dose. After 40 mg/kg, clonic seizures appeared as soon as 25 min, were maximal at about 1 hr, and had usually disappeared by 2 hr. Colonic temperature was decreased 2°C at 45 min, was maximally decreased at about 2 hr, and was still decreased at 3 days. Body weight was increased for 4 days and was associated with increased food intake. After 30 mg/kg, seizure activity was less severe, body temperature was not affected, but body weight was reduced for 3 days. The effects of 30 mg/kg on limbic evoked potentials were evaluated in rats with chronically implanted electrodes. Lindane increased the amplitude of the response evoked in the dentate gyrus (DG) by stimulation of the prepyriform cortex, even in the absence of seizures. Long-term potentiation (LTP) of this response lasted as long as 2 weeks in some rats. This exceeds the half-life of lindane in brain, which is less than 24 hr. Recurrent GABAergic inhibition, tested by paired pulse stimulation, remained functional in both the DG and the CA3 subfield of the hippocampus during LTP. LTP produced by 30 mg/kg lindane lasted longer than effect on seizures, colonic temperature and body weight produced by the higher dose. LTP may be a particularly significant effect of lindane, perhaps related to its proconvulsant effects.


The effect of lindane on GABA-mediated inhibition was tested in the dentate gyrus (DG). Rats, chronically implanted with stimulating electrodes in the perforant path (PP) and recording electrodes in the DG were administered lindane, 10 mg/kg, i.p. in DMSO. This is a dose sufficient to produce myoclonic jerking in all subjects and clonic seizures in 20%. Inhibition was assessed by using paired-pulse stimulation of the PP. The amplitude of the population spike (PS) evoked by the second stimulus was used to estimate the extent of inhibition at time intervals between 10-400 ms. GABA-mediated recurrent collateral inhibition was tested in two different situations, anesthetized and unanesthetized. Recurrent collateral inhibition is augmented in the anesthetized state approximately two-fold. In neither the unanesthetized nor the anesthetized situation did lindane alter inhibitory function. In the unanesthetized state the time to 50% recovery of the second PS was increased from 27 ± 5 msec to 31 ± 8 msec. In the anesthetized state the time to 50% recovery was increased from 49 ± 4 msec to 54 ± 10 msec. These data provide no evidence that a reduction in GABA-mediated inhibitory function plays a role in the hyperexcitability response to lindane exposure.


Histochromical and electrophysiological methods were used to study cholinergic mechanisms of TMT toxicity in the hippocampus. Long-Evans rats were dosed po with TMT either in a single (high) dose of 12.25 mg/kg or 3 mg/kg on 3 consecutive days (low dose). Animals were sacrificed 4 days later and the brains processed for acetylcholinesterase (AChE) histochemistry. High dose TMT elicited marked pathology in the granular cell layer while pyramidal cells were spared. AChE histochemistry revealed loss of staining in the CA1 region. Low dose animals had necrosis of the CA1 region while granular cells were unaffected; no loss of AChE staining was seen. Quantitative electroencephalograms (EEG) from low dose rats implanted with bipolar electrodes revealed significant increases in hippocampal slow theta rhythm (4-6 Hz) with corresponding decreases in delta (0-4 Hz) and beta (16-50 Hz) activity 7 days after dosing. The theta increase was blocked by atropine and EEG changes preceded damage to the CA1 region. These results suggest that increased theta activity is involved in CA1 pyramidal cell death which does not occur without an intact cholinergic presynaptic pathway.
Soman (GD) is an organophosphorous agent which has a high affinity for the central nervous system. In an effort to determine what non-cholinergic systems are affected by GD, we studied the effect of GD on spinal cord reflexes, in particular, the monosynaptic (MSR) and dorsal root reflex (DRR). Adult mongrel cats were injected subcutaneously with 2.5 μg/kg/day (10 days) or 5 μg/kg/day (5 days) GD. A third group of animals were pre-treated with physostigmine and atropine and the administration of a single dose of 1 mg/kg (a.c.) GD. Some of the animals in this group were observed for up to 45 days.

Spinal cord function was assessed in the remaining 1 mg/kg GD cats 14 days after the injection. The 2.5 GD and 5.0 GD groups were studied one day after the last injection. The MSR and DRR were recorded in unanesthetized spinal cord transected animals. The MSR was depressed in the 2.5 μg GD and 1 mg GD groups but not the 5 μg GD group. The DRR appeared normal. Excitability of the reflexes was increased with quipazine. The MSR showed normal excitability but the DRR was depressed. No treatment resulted in clinical signs of delayed neurotoxicity. These data suggest that GD affects spinal cord synapses without producing neurological symptoms.

Supported by USAF RDC DAMD 17-82-C-2217.

**EXTRACELLULAR CALCIUM-DEPENDENT AND INDEPENDENT EFFECTS OF METHYLMERCURY ON SPONTANEOUS RELEASE OF ACETYLCOLINE.**


Acute administration of methylmercury (MeHg) produces stimulation followed by cessation of spontaneous release of acetylcholine (ACh) at the neuromuscular junction. To determine whether this effect was independent of extracellular Ca²⁺ concentration and whether MeHg increased spontaneous ACh release by intracellular mechanisms, we conducted experiments using conventional intracellular microelectrode recording techniques in the rat hemidiaphragm. Increasing bath Ca²⁺ concentration from 1 to 2 or 4 mM decreased the latent period required by 100 μM MeHg to increase spontaneous ACh release as measured by miniature end-plate potential (MEPP) frequency from 48 to 34 to 22 min, respectively. Depolarization with elevated extracellular K⁺ (15 mM) shortened the latent period from approximately 30 min to 1-5 min and also shortened the time required for MeHg to cause complete cessation of MEPPs. In experiments conducted in K⁺ depolarized preparations to which no Ca²⁺ was added MeHg increased MEPP frequency, although not as much as in solutions containing Ca²⁺. An unexpected finding was that when MEPP frequency was reduced to zero by MeHg, washing with elevated K⁺ solutions containing no MeHg caused a return of MEPPs. Results of this study indicate that MeHg has an intracellular action on spontaneous ACh release, that extracellular Ca²⁺ contributes to, but is not required for this effect, and that under some conditions the block of spontaneous release can be relieved. (Supported by NIH Grant ES03199 and a grant from Mich. State Agric. Experiment Station.)
345 EFFECTS OF CADMIUM ON CHOLINERGIC TRANSMISSION IN THE RAT DIAPHRAGM. B.V.R. Saxty and L.K. Owens, Department of Pharmacology, Vanderbilt University, Nashville, TN

The symptoms of acute cadmium poisoning both in animals and men suggest transient facilitation followed by depression of cholinergic transmission with death due to respiratory failure. Therefore, the effects of cadmium chloride were studied in phrenic nerve-hemidiaphragm of Fischer 344 rats. Each hemidiaphragm preparation was mounted in an organ bath containing Kreb's bicarbonate buffer (pH 7.4, 37°C), and the nerve and muscle were alternately stimulated electrically using platinum electrodes. A continuous tracing of muscle contractions was obtained. Cd++ decreased contraction heights upon both stimulation of the nerve (ED50, 2 μM) and the muscle (3.1 μM). The responsiveness of the muscle (80%) and the nerve (30%) were partially restored by washing the tissue and continued stimulation. At doses lower than the ED50, the maximal blockade was observed within 30 min, and at doses higher than the ED50, the maximal blockade was observed within 10 min of electrical stimulation. At all doses, the responsiveness of the nerve was affected before the muscle. Stimulation of the CdCl2-treated preparation in the presence of ZnCl2 restored the responsiveness of the muscle but not the nerve. These observations indicate that Cd++ is more toxic to the nerve than to the muscle. (Supported by grants from US PHS-NIH, ES-03172, HD-10607 and The Council for Tobacco Research, U.S.A., Inc.)


Previous work from our laboratory has suggested that organophosphorus agents (OPs) which produce delayed neuropathy also induce electrophysiological changes in rat peripheral nerve. The purpose of this experiment was to determine the effects of pyridostigmine (which protects against acute OP toxicity) on nerve and muscle function. Rats were dosed once daily with 2 mg/kg ip and measurements were made 24 hours after 1,4,10 or 20 consecutive doses. Pyridostigmine did not change the amplitude, duration, area conduction velocity or relative refractory period of either the sural or sciatic nerve. There was no change in the depolarization of the sciatic nerve in response to potassium and no effect on muscle twitch tension. Pyridostigmine produced an immediate inhibition of erythrocyte acetylcholinesterase and plasma cholinesterase, both of which returned near control values 24 hrs later. These results are consistent with our previous reports (Arch Toxicol 52: 157, 1983; Neurosci Abstr. 9: 421, 1983) showing that neuropathy-inducing OPs produce electrophysiological changes but that non-neuropathic agents such as parathion do not, despite a marked, acute effect on esterase activity. (Sponsored by USAMRICD)

346 THE NATURE OF THE TOXIC ACTION OF BACILLUS THURINGIENSIS VAR. ISRAELENSIS δ-ENDOTOXIN. G.J.P. Singh and S. S. GTI, Div. of Toxicology and Physiology, University of California, Riverside, CA 92521

Physiological lesions leading to the poisoning syndrome of insecticidal parasporal crystal of Bacillus thuringiensis var. israelensis (BTI) were studied using standard electrophysiological techniques. In the cockroach, Periplaneta americana, the alkaline-solubilized BTI crystal toxin exerts both myotoxic and neurotoxic effects. Of these, the action of BTI on muscle function is detected soon after treatment, whereas the neurotoxic effects are observed with a considerable delay. Examination of BTI-poisoned cockroaches suggest that the myotoxic effects of the toxin may be responsible for the initial manifestation of its poisoning syndrome in vivo. The neurotoxic activity of BTI appears to be due to its ability to block synaptic transmission. Results of experiments performed on the central nervous system indicate that the action of BTI is localized presynaptically, BTI does not interfere with the intracellular transmitter-release mechanisms. It may, however, inhibit transmitter release by interfering with the influx of Ca++ into presynaptic nerve terminals. Such an action of BTI may also extend to the peripheral nervous system. At the insect neuromuscular junction, however, BTI impairs the muscle well before its effects upon transmitter release could be detected.

348 INFLUENCE OF METAL ION CONCENTRATIONS ON SPONTANEOUS MOTILITY OF THE RAT DUODENUM. M. Herley and M.F. Taney, Temple Univ., Philadelphia, PA.

The literature contains several references to observations indicating that various metal ions have effects on gastrointestinal motor activity. However, the experimental models are rarely the same and there is no means of making quantitative comparisons of results. The purpose of these studies was to compare the effects of heavy and trace metal ions upon in vivo spontaneous motor activity of the duodenum of young adult male Sprague Dawley rats. The heavy metals were CdCl2 (0.5), Pb(CH3COO)2(0.8), and HgCl2(0.1). The trace metals were AlCl3(0.05), CoCl2(2.96), CuCl2(0.32), and ZnCl2(0.1). Rats were given daily l.p. injections (mg) of these metal ions for 7 consecutive days. Longitudinal force was acquired via a transducer-polygraph and recorded on magnetic tape. No less than 400 contractile events were recorded from the duodenum of any rat. Experiments were replicated 4 times. Motor activities were quantitated by means of measurement of intercontractile intervals. It was determined that the trace metals were not associated with significant effects upon average spontaneous contractile frequencies. Mercury and lead significantly increased the average frequency of motor activity while cadmium decreased the spontaneous average contractile frequency. We concluded that the pacemaker activity of the rat small intestine can be influenced by the exogenous administration of these heavy metals.
349 EFFECT OF CHRONIC EXPOSURE TO LEAD ON IN VITRO CONTRACTILE RESPONSE OF THE RAT FORESTOMACH. C.T. Walsh, E.B. Ryden and K.M. Harnett. Dept. of Pharmacology, Boston University Medical Center, Boston, MA. Sponsor: J.K. Marquis.

Previous studies have demonstrated that chronic exposure to lead in rats slows gastric emptying. The contractile response of the forestomach from lead-treated rats was examined in vitro. Male Wistar rats were fed 4% lead acetate in their diet (NTH-07); controls were pair-fed. After 7 weeks, blood was collected (180-220g lead/dl) and the forestomach dissected. Tissue was suspended in a physiologic saline which for lead-treated rats contained 1.2x10^-5 M lead. Chronic lead exposure had no effect on the maximum tonic contraction induced by KCl, methacholine or serotonin. Lead-treated tissue showed enhanced sensitivity to methacholine (1.6x10^-7 M), as has been shown previously. Physostigmine-induced increase in tension was also significantly greater in tissue from lead-treated rats. Electric field stimulation (1Hz, 1sec, 100V) produced a contraction attributable to postganglionic acetylcholine release. This response was unaltered in lead-treated tissue. These results indicate that lead intoxication did not impair the contractile apparatus of the forestomach smooth muscle. The lack of net effect on activation of intramural cholinergic neurons, despite the enhanced sensitivity to cholinergic agonists, may indicate a reduction in acetylcholine release in lead-treated tissue. (Supported by NTM Grant 02665.)

351 SERUM BRODIFACOUM CONCENTRATIONS AND COAGULOPATHIC EFFECTS IN ANTICOAGULANT POISONED DOGS TREATED WITH VITAMIN K1. M.J. Murphy, A.C. Ray, B. Woody and J.C. Reagor. Texas Veterinary Medical Diagnostic Laboratory, College Station, Texas.

In order to correlate coagulopathy with respect to serum brodifacoum (SF) concentrations, four mixed-breed dogs were given a divided LD50 dose (1 mg/kg) over a three-day period (0.33 mg/kg/day p.o.). Vitamin K1 therapy (0.8 mg/kg, 7ID for 5 days) was initiated at the onset of life-threatening elevations of clotting parameters. Coagulopathy was monitored using packed cell volume, total protein, activated clotting time (ACT), prothrombin time (PT), and partial thromboplastin time. Following ether and ether:acetonitrile extractions of serum, SF concentrations were determined using reverse phase chromatography with U.V. (254 nm, 313 nm) and fluorescent detection (excite 313 nm, emit 357 nm) detection. Recovery from spiked samples was 75% in the 1 μg/ml range and 65% in the 25 ng/ml range. The lower limit of detection was 2 ng/ml. Serum concentrations peaked at 1.15 μg/ml on day 4, following initial exposure. Significant coagulopathy (ACT, 190; PT, 61) was apparent by days 8-10. SF concentrations at the time of initiation of therapy (day 10) were 37-83 ng/ml. Clotting factors returned to normal within 24 hours of Vitamin K1 therapy. Serum concentrations below 12 ng/ml (after day 13) caused no measurable coagulopathic effect, following cessation of therapy. Elimination of SF followed a classical exponential decay with a distributional phase 1/2 of 1.4 days and an elimination phase 1/2 of 8.7 days.


Exposure to styrene (S), trichloroethylene (TCE) and carbon tetrachloride (CT) is known to produce hepatotoxic effects in animals and humans. Warfarin (W), the coumarin anticoagulant, is mostly eliminated by hepatic biotransformation and the site of its anticoagulant action is located in the liver. Therefore, the effects of above solvents on the anticoagulant response to W were studied in male Sprague-Dawley rats. Groups of rats were given i.p. injections of either S (0.6 and 1.2 g/kg) or TCE (5.6 and 11.1 mmole/kg) or CT (1 mmole/kg) in corn oil 24 h prior to or simultaneously with W (1 mg/kg, s.c.) and the animals were sacrificed 24 h after W. Doses of solvents used in this study showed hepatotoxic effects as verified by significant increases in serum transaminases response. A significant increase in prothrombin time (P.T.) was seen when W was treated simultaneously with S or TCE at any dose level, but not so with CT. An increase in the P.T. of W was also noticed in the groups pre-treated with S or TCE and with CT group. Increase in serum transaminases activities due to each solvent was not further increased due to W. Solvents alone had no effect on the P.T. In vivo metabolism of S or TCE was not modified by W. So, acute exposure to organic solvents may lead to enhanced anticoagulant response to W. (Supported in part by IRSST, Québec.)

352 SPECIES COMPARISON OF BONE MARROW PERTURBATIONS IN MICE AND RATS EXPOSED TO ETHYLENE OXIDE. S. Lock, R.E. Hand, Jr. & F. Stenglein, Biol. Div. Oak Ridge National Laboratory, Oak Ridge, TN.

Previous studies have shown that exposure of mice (C57B/6j) to 255 ppm of ethylene oxide (ETO) causes an initial loss of granulocytic elements from the bone marrow followed by replacement and hyperproliferation with an associated deficit in the lymphocyte population. In the first of this series of experiments male F344 rats and male BALB/c mice were exposed to 255 ppm ETO for 5 days/week for 2 weeks. Body and spleen weights were recorded for all animals. Bone marrow was counted and aliquots were stained with propidium iodide (PI) for flow cytometric (Ortho 500) analysis. Forward and 90° scatter parameters allowed quantification of the granulocyte and lymphocyte populations. Cell cycle information was obtained from PI fluorescence histograms. In mice total bone marrow cellularity, lymphocytes and granulocyte populations were depressed by 29%, 8% and 30% respectively following exposure to ETO. Cells in the G1 phase were most severely affected. Conversely rats exposed to ETO had slight increases in total marrow cellularity with both the granulocytic and lymphocytic populations contributing to this increase. It is tentatively concluded that the hematopoietic system of the mouse is more sensitive to ETO than that of the rat. (Research sponsored by OHER, USDOE under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems Inc.)
THE USE OF FETAL TISSUE TO DEMONSTRATE THE CYTOSTATIC EFFECTS OF ETHYLENE OXIDE (EO). D. M. Popp, S. Lock, and R. A. Popp. Oak Ridge National Laboratory, Oak Ridge, TN

We have shown that inhalation of EO affects the hematopoietic system. Hematocrit, red cell number and hemoglobin are generally depressed and the pluripotent hematopoietic stem cells (CFU-S) are reduced. This study was designed to assess the effect of EO on the developing hematopoietic system in the fetal liver of embryos. C57BL/10 females were mated and copulation plugs were noted. The pregnant females (13.5 and 14.5 days post copulation) were exposed to 255 ppm EO, 6 hr/day for 4 days. The fetuses were removed and weighed and the fetal livers were dissected out. The fetal livers were carefully teased and the released cells were suspended in phosphate buffered saline. Nucleated cell counts were made and aliquots were injected into lethally irradiated recipients to assess the CFU-S number. At either time period there was a 60% reduction in fetal weight, 65% reduction in cellularity, 68% reduction in incidence of CFU-S and an absolute reduction of hematopoietic stem cells of 43%. The exposure of pregnant females is a sensitive indicator of the cytostatic effects of EO.

(Relationship sponsored by the Office of Health and Environmental Research, U.S. Dept. of Energy under contract DE-AC05-84OR21400 with the Martin Marietta Energy Systems, Inc.)


TDC is an organometallic salt reported to induce transplantable fibrosarcomas, hepatomas, and malignant lymphomas of the spleen with leukemic manifestations. Data presented was obtained on the tissue distribution and accumulation of TDC and was needed to evaluate chronic toxicity. In a 90d study TDC residues were measured by plasma emission spectrometry in target organs of mice and rats gavaged with 31 or 125 mg/Kg B.W. In both species there were dose-related accumulations of TDC that were greater in males than females with levels in spleen > liver > lungs > heart. After 90d the mean concentration (ppm) of TDC in vehicle, low, and high dose groups were:

<table>
<thead>
<tr>
<th>Organ</th>
<th>VM</th>
<th>LM</th>
<th>HM</th>
<th>VT</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>0.00</td>
<td>17.17</td>
<td>63.11</td>
<td>0.00</td>
<td>18.52</td>
<td>52.00</td>
</tr>
<tr>
<td>Liver</td>
<td>0.14</td>
<td>5.08</td>
<td>22.54</td>
<td>0.11</td>
<td>6.23</td>
<td>18.06</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.02</td>
<td>3.82</td>
<td>12.48</td>
<td>0.02</td>
<td>3.68</td>
<td>14.72</td>
</tr>
<tr>
<td>Heart</td>
<td>0.00</td>
<td>2.24</td>
<td>7.25</td>
<td>0.00</td>
<td>2.25</td>
<td>5.92</td>
</tr>
</tbody>
</table>

A 2yr carcinogenesis test is now underway in F344 rats dosed with 25 or 50 mg/Kg. After 6wk there has been a dose-related incidence of rales associated with mortality and a 10-20% decrease in B.W. gain. The association between chemical residue accumulation and histopathological lesions in the target organs are currently being investigated.


Polythemia is a hematological disease characterized by erythrocytosis and occasionally leukocytosis. Various types of polythemia occur in man and animals as the result of either hemococoncentration, elevated erythropoietin, or more rarely, autonomous myeloproliferation. A closed colony of Wistar rats developed about a 40% incidence of polythemia following transplacental exposure to methylmercury (MeHg), ethylthiourea (EU), and nitrite (NO). Dams were 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. Alternatively, dams were gavaged on days 17-19 with 60 mg/kg of ethylthioinc (EU). EU and NO2 spontaneously form Eu in the mammalian stomach. Transplacentally exposed offspring developed clinical signs of polythemia at 2-6 months of age with hematocrits ranging from 60-84%. Death resulted in 1-4 months after onset depending on the severity of the condition and secondary complications. Previous investigators noted several features of this disease in rats that were similar to human polycythemia vera, a fatal myeloproliferative disorder involving enlargement of bone marrow stem cells. Determinations of red blood cell mass and narrow cell culture characteristics from recent experiments indicate that disease is an absolute polycythemia that is erythropoietin sensitive. Serum from polycythemic rats is currently being analyzed to determine erythropoietin levels. Elevated levels would indicate secondary polycythemia, while depressed levels would be diagnostic of 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. The results of the erythropoietin analysis will define the usefulness of this potential animal model for the study of human polycythemia.

356 PYRIDIMINE 5'-NUCLEOTIDASE ISOENZYME CHARACTERISTICS IN LEAD EXPOSURE. C.R. Angle, S.J. Stobs, L.R. Cook, and M.A. Mitchell. University of Nebraska Medical Center, Omaha, NE.

Red blood cell (rbc) pyridimine 5'-nucleotidase (PS'N) is an enzyme system that dephosphorylates pyridimine 5'-nucleotides. PS'N is sensitive to low lead levels and inhibition of PS'N has been implicated as a mechanism in the toxicity of lead within the rbc. Recent evidence indicates multiple forms of the enzyme within red cells. Since PS'N isozymes have different substrate specificities, HPLC assay was made of rbc PS'N activity in individuals with high and low lead exposure using CMP, UMP, dUMP and (d)TMP as the substrates. There was no difference in activity of rbc PS'N between control and lead exposed subjects when using dCMP, dUMP and (d)TMP as the substrates. Up to 40-50% inhibition of PS'N activity was observed in lead exposed subjects when using UMP and CMP as the substrates. The addition of 10-4 M lead to rbc enzyme preparations from control subjects inhibited the activity for dUMP and UMP by 84.6 and 88.2%, respectively. Thus, the effect of lead exposure on PS'N activity is not duplicated by direct addition of lead to rbc PS'N preparations. These results support the hypothesis that multiple forms of rbc PS'N exist and lead exposure does not inhibit all forms of the enzyme. Only the isoenzymes utilizing UMP and CMP as the substrates provide a sensitive index of lead exposure.
Previously, Pt and P2 (primary metabolite of Pt) were shown to suppress murine immunologic functions. In this study, we assessed the effects of Pt and P2 on bone marrow hematopoietic cells. C57Bl/6 males were given Pt (4 mg/kg, po, daily) or corn oil (5 ml/kg, po, daily) for 14 days. Mice showed no cholinergic signs. Brain and plasma cholinesterase activities were respectively, 52% and 32% of control, 1 day after Pt, and 72% and 97% of control, 7 days after Pt. In vitro colony-forming units (CFU) were assayed on days 2, 5, 7, 12 and 14 after Pt. CPU-S (stem cell) were unaltered through day 9, then declined to 41% of control on day 12 and 45% on day 14. CPU-E (erythroid), BFU-E (erythroid) and CFU-Str (stromal) were 91% of control, 7% on day 9 (13% respectively), then rose to 71%, 137% and 37% of control respectively, then rose to 71%, 137% and 37% of control by day 14. CPU-MK were temporarily elevated to 142% of control on day 7. After in vitro exposure of normal marrow to 10% Mx, CPU-Str, CPU-E, and BFU-E were 30%, 55% and 75% of control, respectively. CPU-MK increased markedly to 300% of control. Our results indicate that Pt altered hematopoietic cells, perhaps, in part, via direct action of P2.

It has recently been proposed that muconaldehyde (MUC), a six carbon diene dialdehyde, may be a hematotoxic metabolite of benzene. Previous studies have shown that MUC is very reactive towards sulphydryl groups and is hematotoxic in mice. MUC was found to react with 2-chlorobarbituric acid (TBA), forming a chromogen with a 492 nm absorption maximum in methanol. A high pressure liquid chromatography (HPLC) method for separation and determination of MUC, as the TBA chromogen, in biological materials has been developed, using absorbance detection at 492 nm. The method utilizes a C-18 reverse phase column and a mobile phase consisting of methanol:phosphate buffer, pH 6.5 (40:60). A second HPLC method involving ion-pairing conditions was also developed. A developed 24 h after methylamine hydroxide is present in the methanol:phosphate buffer (50:50) mixture. The chromatographic peaks were characterized using a stopflow, on-line scanning technique. Preliminary experiments involving TBA trapping of MUC added to mouse liver microsomes followed by protein precipitation and HPLC analysis of the supernatant, show that this method is feasible for detecting MUC or similar α,β-unsaturated aldehyde from benzene in vitro.

The effects of toluene and benzene on 59Fe distribution in 10-30 g rainbow trout were investigated. Intraperitoneal administration of toluene, at a dose which resulted in no histological lesion (866 mg/kg), caused a significant (p<0.05) increase in the percentage of 59Fe incorporated into RBC's (86.9% vs. 67.0% in controls). A significant increase in 59Fe incorporation into the head kidney (5.13% vs. 3.25 in controls) and a significant decrease in incorporation into the spleen (1.73% vs. 2.44% in controls) were observed. There was no significant change in 59Fe incorporation in the liver. Benzene (866 mg/kg, ip), a hematopoietic toxin, resulted in a significant decrease in 59Fe incorporated into the RBC's (52.2% vs. 60.5% in controls). A decrease in the percentage of 59Fe incorporated into the head kidney following benzene treatment was observed. There was no significant change in the WBC count, WBC count, and hematocrit in either the benzene or toluene treated animals. The sensitivity of this assay is demonstrated by the detection of statistically significant alterations in the percentage of 59Fe incorporated at a dose below that which causes visible lesions both histologically or by peripheral cell counts.

F-344 rats exposed to NB exhibit a variety of abnormalities including methemoglobinemia (Hgb), proliferative splenic capsular lesions and testicular atrophy. Mgb is dependent on metabolism of NB by intestinal microflora and is decreased in rats fed pectin-free diets. To determine if splenic and testicular lesions were also dependent on microfloral metabolism, male F-344 rats were fed AIN-76A (a purified diet containing no pectin) or AIN-76A plus 5% pectin and exposed to 50 ppm NB or ambient air via inhalation for 90 days. Evaluation of spleens from NB-treated animals revealed diet-related differences in the frequency and severity of capsular lesions. Capsular lesions were encountered in 3/10 animals receiving the pectin-free diet and in 10/10 animals receiving the diet containing pectin. Capsular lesions in pectin-fed rats were of greater severity and consisted of multifocal fibroblastic hyperplasia and mesenchymal cell proliferation. All NB-treated animals exhibited testicular lesions. These were more severe in pectin-fed rats. These results are consistent with the involvement of intestinal microflora in the production of metabolites toxic to the spleen and testes and suggest that the testis is the more sensitive target organ.

363 ACUTE TOXICITY AND METABOLISM OF 3,4-DICHLOROBENZYLXYACETIC ACID (3,4-DCBAA), A POTENTIAL ANTI-SICKLING AGENT. R. C. Peffer, A. S. Mehanna, J. D. Alvin, D. J. Abraham, and M. A. Zemaitis. University of Pittsburgh School of Pharmacy, Pittsburgh, PA 15261.

3,4-DCBAA was synthesized as a potential drug for the treatment of sickle-cell anemia. It has shown anti-sickling properties in vitro tests (Abraham et al., J. Med. Chem., 25, 9). As a prelude to pre-clinical testing, the drug's acute toxicity in mice and metabolism in rats have been studied. IP administration of 3,4-DCBAA to Swiss-Webster mice (male and female) at dose levels of 100, 200, 300, 400, and 500 mg/kg indicated an LD$_{50}$ of 364 mg/kg. Oral administration of 3,4-DCBAA to Swiss-Webster mice (male and female) at dose levels of 800, 1200, 1600, and 2000 mg/kg revealed an LD$_{50}$ of 1246 mg/kg. Extraction of urine samples from male Wistar rats dosed IV or IP with 14C-labelled 3,4-DCBAA and subsequent analysis by HPLC demonstrated at least three metabolites along with the unchanged drug itself. Mass spectra of the eluted peaks suggested that one metabolite is a glycine conjugate. Results of comparative incubations with beta-glucuronidase/aryl sulfatase and 0.2N NaOH supported this suggestion. Mass spectra of other metabolites suggest ester formation with subsequent cleavage to form 3,4-dichlorobenzoic acid and a highly polar acetic acid derivative.


CGS 15975A, 8-fluoro-2-[3-pyridyl]-pyrazolo[4,3-c]quinolin-3-one hydrochloride, potently displaces labeled benzodiazepines from brain binding sites and antagonizes the pharmacological effects of benzodiazepines. Suspensions of CGS 15975A in 2% cornstarch were administered by gavage to Sprague-Dawley CD rats at doses of 5, 25, 50, or 100 mg/kg/day for at least 13 weeks, followed by a 4-wk recovery period. Results indicate that daily doses $\geq 5$ mg/kg of CGS 15975A induced marked signs of toxicity in the rat. Toxicity was characterized by the presence of mild, overt symptomatology at doses $\geq 25$ mg/kg; reductions in body weight measurements or transient reductions in food consumption at doses $\geq 25$ mg/kg; and indications of drug-induced hemolytic anemia in all dose groups. The hemolytic anemia was characterized by reduced erythrocytic parameters, reticulocytosis, red cell dyscrasias, leukocytosis, altered white cell differential, hyperbiliurubinemia, altered electrophoretic profile, increased splenic weight, and microscopic evidence of splenic congestion and hemosiderosis in renal tubular epithelia and hepatic Kupffer's cells. These data indicate that CGS 15975A induces pronounced but reversible changes that are consistent with a drug-induced hemolytic anemia at oral doses as low as 5 mg/kg.


Several heme derivatives with thiolate and terminal thiol side chains have been synthesized. The thiolate or thiol moieties can serve as internal fifth ligands to the heme iron in the presence of a Lewis base in aqueous and non-aqueous solutions. When these compounds are combined with various inhibitors of cytochrome P-450 in the presence of excess reducing agent and the absence of oxygen they form the six coordinate complexes analogous to those formed in the active enzyme. These complexes were characterized spectrophotometrically in an attempt to explain features observed in the difference binding spectra of cytochrome P-450 reacted with these same inhibitors.
Severe anemia results by day 14 postpartum in offspring of Long-Evans rats receiving NaN₃ in the drinking water throughout gestation and lactation. This anemia is characterized by low RBC counts and hemocrits and decreased MCV's. Blood smears demonstrate microcytosis and hypochromasia. Also observed are growth retardation, serum lipemia, fatty liver degeneration and decreased hematopoesis in bone marrow and spleen. This clinical picture is consistent with severe iron deficiency in the neonate. Serum iron values of pups from dams drinking 1 gm/l NaN₃ and 2 gm/l NaN₃ are reduced from control in a dose responsive manner. 0.5 gm/l NaN₃ is at or near the no effect level when dams are maintained on standard lab chow containing 250 ppm Fe. Dams on 2 gm/l NaN₃ are slightly anemic and show low serum iron values compared to control. Total iron binding capacities of these dams and their pups are significantly elevated at day 14 pp, a compensatory change due to their iron deficient state. Pups have enlarged hearts (a response to hypoxia) and small spleens. These data indicate an inadequate iron status of the treated dam resulting in her inability to provide an adequate lactational iron supply. (This abstract does not necessarily reflect U.S. EPA policy).

Thioridazine 5-sulfoxide (T5SO), a major oxidative metabolite of the neuroleptic thioridazine, exists as 2 dia stereoisomeric pairs of enantiomers. T5SO isomers are identified by HPLC as fast eluting (RSF) and slow eluting (RSS) ring sulfoxide. Racemic T5SO is a more potent cardiotoxic agent than thioridazine. An acute pharmacokinetic study of T5SO was performed in rats (40 mg/kg ip) to determine the appropriate dilution for each isomer. The serum data were fitted to a 2 compartment model. Clearance was 17 l/kg/hr for RSF and 16 l/kg/hr for RSS. The volume of distribution was 65 and 64 l/kg, respectively. Both isomers underwent a rapid distribution and then slower elimination. The elimination rate constant was 0.387 for RSF and 0.337 for RSS. Serum and urine samples from 28 thioridazine treated patients were also examined for RSF and RSS. Total T5SO concentration ranged from 9 to over 900 ng/ml yet RSF and RSS concentrations were equal in all samples. In both the rat and man, elimination of T5SO appears to be a nonstereoselective process.

The objectives of this study were to develop and evaluate a technique for routine, noninvasive, measurement of BP in conscious beagle dogs for toxicologic research. Heart rate (HR), systolic (SYS), diastolic (DIAS), and mean arterial (MAP) pressures were measured with a DYNAMAP Research Monitor Model 1255 (Critikon, Inc.) as follows: Dogs were restrained in a Harvard Dog Sling, a neonatal cuff was wrapped around the base of the tail and BP was determined once a minute. Initially, normal values were obtained, 5-10 trials/session, 1-3 sessions/day for 15 days in 6 dogs. The day to day, session to session and trial to trial variabilities were determined and were found to be minimal. The day to day MAP ranged from 93.9 ± 3.4 - 112.7 ± 1.3, HR L11.1 ± 1.9 - 125.7 ± 4.1, SYS 125.5 ± 4.4 - 156.3 ± 2.0 and DIAS 73.9 ± 3.0 - 90.7 ± 1.2. The effects of various drugs on BP were determined. Noradrenalin increased SYS, DIAS and MAP by 75-110 mm Hg and decreased HR by half. Epinephrine increased HR by 20 bpm. Phentolamine decreased SYS, DIAS and MAP by up to 25 mm Hg. Isoproterenol increased HR by up to 130 bpm and decreased SYS, DIAS and MAP by 20 mm Hg. Thus the above technique is suitable for routine measurement of BP in conscious dogs.

We have previously reported that induction of myocardial necrosis by I produced resistance to the necrogenic effects of subsequent doses of the drug and that the preexistence of lesions was necessary for its development. In the present study we examined whether pretreatment with other agents which protect against the necrogenic effects of I and vice versa. Groups of male Sprague-Dawley rats (500-600 g) were given either D, 0.3 mg/kg po, for 3 days and E, 25 mg/kg ip, for 2 days starting on the third day of D treatment or saline (S). Half of the rats from both groups were killed 48 hr after the last treatment. All the remaining rats were challenged with a single dose of I (0.05 mg/kg sc) 10 days later and were killed 48 hr thereafter. In another experiment, rats that were pretreated with I were challenged with D-H as above. Myocardial lesions were graded on a scale of 0 to 3 with 3 being most severe. Rats that were pretreated with D-H and challenged with I had significantly lower scores (0.75) of fore myocardial lesions than rats pretreated with S and challenged with I (2.1). Similarly, rats that were pretreated with I and challenged with D-H had lower lesion scores (0.87) than rats that received S pretreatment and D-H challenge (2.3). These data indicate the development of cross-resistance between I and D-H-induced cardiac effects.
371 ELEVATED BLOOD PRESSURE IN RATS EXPOSED TO SOLVENT REFINED COAL-III (SRC-III) HEAVY DISTILLATE. L.B. SASNER, D.L. LUNDSTROM, D.L. SPRINGER, & D.D. MARGULL. Pacific Northwest Laboratory, Biology & Chemistry Department, Richland, WA 99352.

The susceptibility of the cardiovascular system to solvent refined coal-III process (SRC-III) heavy distillate (HD) was studied in the rat using an isoproterenol (IP) myocardial infarct model. Male Fisher rats were exposed to HD by inhalation (0.7 mg/L), 6 hours/day, 5 days/week, for 6 weeks. After a 10-20 day recovery period, control and HD exposed rats were injected (SC) with 0, 20, 40 or 60 mg of IP per kg and heart rate, blood pressure, EKG and 


Bevantolol is a beta-adrenoceptor blocking agent with a high degree of cardioselectivity. In a 80 week study, 50 CD-1 mice/sex/group were given 0, 25, or 100 mg/kg/day of bevantolol hydrochloride in diet. The study terminated at 60 weeks due to high mortality resulting from amyloidosis. In a repeat study, 50 CF-1 mice/sex/group received bevantolol in diet at 0, 100, 300, 600, 900, and 1200 mg/kg for 80 weeks followed by a 13-week observation period. The groups given 1200 and 900 mg/kg terminated after 13-weeks of treatment because of high mortality. Animals were observed daily for signs of toxicity, body weights and food consumption were monitored weekly, and ophthalmic examinations were done periodically. In the first study, mortality rates were 36, 26, and 48% for females, and 30, 32 and 48% for males at 100, 25 mg/kg and controls, respectively. In the second study, mortality rates were 38, 28, 16 and 22% for females and 52, 70, 50 and 52% for males at 600, 300, 100 mg/kg and controls, respectively. The high dose group in each study showed 10 to 19% body weight gain suppression. No drug-related gross or histopathologic changes were observed. Common neoplasms were liver cell adenomas, alveolar cell adenomas, adrenal cortical adenomas and generalized lymphoid tumors. Tumor incidences or other parameters of tumorogenesis were not different from controls.
A lipid regulating agent (CI-924), chemically described as $5,5'$-[1,1'-Biphenyl]-2,2-diylbis-(oxy)]bis[2,2-dimethylpentanoic acid] was evaluated in acute single dose rodent studies, in repeated dose studies in rats, dogs, and monkeys and for genotoxicity in a bacterial plate assay. Single oral doses were essentially non-toxic with LD50 values greater than 5000 mg/kg in mice and rats. In escalating dose studies, CI-924 was clinically well tolerated by dogs and monkeys at doses up to 800 mg/kg for 14 days. Slightly lower RBC counts and hemoglobin values occurred in dogs, while in monkeys, emesis and body weight loss were noted. Serum CPK, ALT and AST levels were increased slightly, and mild fatty change was observed in the liver. In two week studies in dogs given doses up to 600 mg/kg, no significant changes in body weight, food consumption, clinical laboratory parameters, or pathologic findings were found. Significant drug-related findings in rats given up to 1000 mg/kg for 2 weeks were limited to the liver and consisted of increased liver weights, correlating with hypertrophy, and increased granular eosinophilia. CI-924 was not mutagenic when tested in several strains of Salmonella typhimurium and in one strain of yeast.

The initial toxicologic profile of the novel cardiotonic agent CI-930, was determined in rodents, dogs, and monkeys. Following single oral doses, LD50 values were 167 mg/kg and 94 mg/kg in mice and rats, respectively. In a two-week oral toxicity study, rats received daily doses of 4.5 - 70 mg/kg. Body weight gain and food consumption were suppressed at 70 mg/kg. Fatty change was noted in the liver of animals at 35, or 70 mg/kg. In dogs given 3, 7.5, 15 mg/kg/day during a two week pilot study, anorexia was seen at 15 mg/kg. Hemorrhages were seen grossly in the heart at 15 mg/kg and in a male given 7.5 mg/kg. Multifocal arteritis/periarteritis was seen in the hearts of most animals and myocardial necrosis with fibrosis was evident in the female given 15 mg/kg. Monkeys received escalating doses of 1 to 12 mg/kg over a two week period. The male died after a dose of 10 mg/kg and the female became moribund and was terminated after a dose of 12 mg/kg. Hemorrhages in the heart were evident in the female. Myocardial necrosis and inflammation, and hepatic fatty change was also seen in monkeys. These findings indicate that the heart is the principle target organ of toxicity as indicated earlier with this class of compounds.

Celliprolol, a new cardioselective beta-receptor antagonist, has demonstrated therapeutic activity in the treatment of hypertension and angina. Studies were conducted in different animal species to evaluate the safety of this agent following iv administration. In acute studies, I.D.50 values in mice were approximately 64 mg/kg and in rats 70 mg/kg. Signs of intolerance in both species were lethargy, disturbed motor coordination, absence of Finna reflex, exophthalmos, lacrimation, pilorecursion and accelerated breathing. Gross necropsy of survivors after 14 days in mice and rats revealed no drug related changes. In the acute dog study, celliprolol at 30 and 70 mg/kg iv induced no mortality, whereas 100 mg/kg dose was lethal. Signs of intolerance were retching, vomiting, marked agitation, deep apopnic respiration and reddening of the skin. In subacute safety studies, the iv administration of celliprolol to rats at doses of 5, 10 or 20 mg/kg/day and to dogs at 2, 4 or 8 mg/kg/day for 30 days elicited no clinical or pathological drug related toxicity, establishing adequate margin of safety for clinical trials via this route of administration.
Carcinogenicity of Captafol in B6C3F1, Mice.
M. Hirose, Y. Kurata, T. Ogiso, S. Fukushima, and N. Ito. 1st Department of Pathology, Nagoya City University Medical School, Nagoya, Japan. Sponsor: J.R.P. Cabrel.

The potential carcinogenicity of captafol, a fungicide, was examined in B6C3F1, mice. Captafol was given at levels of 0.0, 0.0.015, or 0.3% in diet to a total of 203 males and 203 females for 96 weeks after which time the animals were returned to basal diet for a further 8 weeks. Mice surviving 42 weeks or longer were included in the effective numbers. Males and females given 0.3% captafol showed increased cumulative mortalities in the final quarter period of the experiment. Significant increases in the development of neoplastic lesions were found in the heart, spleen, forestomach, small intestine, and liver of mice of both sexes treated with captafol. Tumors induced by captafol were, histologically, hemangioendothelioma in the heart, hemangioma or hemangiopericytoma in the spleen, papilloma and squamous cell carcinoma in the forestomach, adenoma and adenocarcinoma in the small intestine, and hyperplastic nodule and hepatocellular carcinoma in the liver. The incidences of hemangioendothelioma of the heart in high dose groups and adenocarcinoma of the small intestine in middle dose groups were 20/47 with males and 11/51 with females, and 32/46 with males and 13/49 with females, respectively. These results demonstrate wide spectrum carcinogenicity for captafol in B6C3F1 mice.

Profile: A Computerized System for Toxicological Information and Biological Potency Evaluation.

A computerized toxicological data base is developed which utilizes a hierarchical format for data entry and access. The format specifies distinct experimental parameters such as dose, substance identification (CAS number, chemical name), types of biological effects (pathological, physiological, biochemical), test animals (species, sex, age), exposure conditions (group size, dosing regimen) and dose-response information (numerical tabulation of results, statistical significance).

Studies were selected for the data base using these criteria: 1) contained a test of the subject chemical for biological activity, 2) test was performed in a mammalian or sub-mammalian system, 3) more than one dose level was used, and 4) numerical presentation of data. Information can be accessed at various levels which can be identified in the standard format; it allows the selection of studies that are concerned with any combination of the particular parameters used as selection criteria for the retrieval. The system also provides the capability to perform data correlation with the inclusion of regression analyses (linear, logarithmic, polynomial, exponential, saturation) and slope for dose-related responses. No-observed-effect levels and minimal-effect levels can be determined from the data output.

Effects of Chlorodimeform on Cardiovascular Functional Parameters in the Postweanling Rat. W. P. Watkinson. USEPA, EBD, RTP, NC 27711 (Sponsor: R. Chadwick)

Chlorodimeform (CDM), a formamidine pesticide, is acutely toxic to mammals, producing a variety of adverse cardiovascular (CV) effects. To further clarify these CV actions, studies were conducted to investigate the differential effects of intravenous (IV) and intraperitoneal (IP) administration of chlorodimeform in 22-30 day old pentobarbital-anesthetized Sprague-Dawley rats. The first group of animals (N = 25) were given sequential IV injections of 5, 10, 30, 60, and 120 mg/kg CDM, or normal saline vehicle followed by a single injection of 60 mg/kg CDM. Heart rate (HR), arterial blood pressure (BP), and electrocardiogram (ECC) were monitored for all animals. Acutely (0-3 min), CDM produced profound depressive effects on all CV parameters monitored. In addition, multiple arrhythmias and alterations in ECC waveforms and intervals were observed. Following a transient recovery period, there was a persistent, delayed (2-15 min) depression of HR and BP. These effects were similar to but less severe than those observed in a previous study using geriatric animals. The second group of animals (N = 32) received a single IP injection of either 10, 30, or 60 mg/kg CDM, or normal saline vehicle. The decrease in HR produced by CDM in these animals was comparable to the delayed HR effect seen in the first group despite the differences in dosing regimens and routes of administration.

Comparative Toxicity of Dimethyl-stilbene Acid and 6-Methyl-Dimethyl-stilbene Acid in Fisher 344 Rats. D. Emmerling and F. Kurtz. Battelle Memorial Institute, Columbus, OH.

Certain anorexenoid analogs possess potent biological activity and have been proposed as possible cancer chemopreventive agents. The toxicities of two such synthetic analogs, dimethyl-stilbene acid (DS) and 6-methyl-dimethylstilbene acid (6-MDS), and the naturally occurring retinoid, all-trans-retinoic acid (RA), were compared. Groups of ten Fisher 344 rats of each sex received daily doses of DS (0.006 to 0.10 umole/kg), 6-MDS (0.62 to 10.0 umole/kg) or RA (12.5 and 50.0 umole/kg) for 91 days. The major signs of intoxication with 6-MDS and DS were similar to those seen with RA in this and previous studies and included dose-related reduction in body weight, anemia, long-bone fractures, elevation in serum alkaline phosphatase activity, dermal lesions, and mortality. Despite their similarity in chemical structure, it was necessary to increase the 6-MDS dose levels 100 times over those used for DS to achieve the same severity of toxic response. Equitox dose of RA were approximately 10 times higher than those required for 6-MDS. Thus, while 6-MDS was considerably less toxic than DS, both synthetic arotinoids were more toxic than RA. In addition, with all three compounds, females were generally more sensitive to intoxication than males. (Supported by NCI Contract No. N01-CP-85656).
Nizatidine (N), a new histamine H₂-receptor antagonist, is being evaluated for the treatment of duodenal ulcer (anticipated human dose = 5-6 mg/kg/day). Toxicity observed in rats after six month studies with N (dietary dose ca equivalent to 30, 135, and 600 mg/kg/day) included slight reduction in body weight gain and efficiency of food utilization, minor hematologic changes, and increased relative liver and kidney weights. Histopathological examination of stomach and pylorus of rats revealed no adverse effects. In addition, serum androgen levels and secondary sex organ weights of males were unaffected by treatment. In dogs, toxicity evident after chronic oral N administration of 50, 140, and 400 mg/kg/day included slight reduction in body weights, increases in thrombocyte counts, and overt effects associated with weak cholinerenic activity of N. Endoscopic evaluation of stomach and biopsies of the gastric mucosa of dogs after nine months of treatment revealed no gross or histopathologic alterations. The results of these studies support the clinical safety of N for the treatment of acute duodenal ulcer.

Carbosulfan is a broad spectrum carbamate insecticide used for the control of soil and foliar insects. Since its major environmental degrade, carbofuran, is acutely toxic, the aquatic toxicity of carbofuran was investigated.

Exposure of bluegill sunfish, rainbow trout and carp to carbofuran in a static system resulted in 96-hr LC50s of 15 ug/l, 42.4 ug/l and 55 ug/l, respectively. For daphnids, the 48-hr EC50 was 1.5 ug/l. In a 60-day rainbow trout study, the percentage of survival of fry at 9.11 and 19.2 ug/l was significantly reduced after 30 days. After 60 days, fry length was reduced at 9.11 ug/l. The MLC5 was 2.09-4.37 ug/l. In a 30-day fathead minnow study, bodyweight and length were reduced at 18.4 ug/l. The MLC5 was 8.28-18.4 ug/l.

Exposure of channel catfish and bluegill sunfish to dibutylamine (DBA)¹⁴C carbofuran showed little accumulation of radioisotope since the majority of ¹⁴C-residues were incorporated into natural products (e.g., fats).

Carbosulfan is acutely toxic to aquatic organisms but based on its short half-life and immobility, the hazard associated with its long-term exposure to aquatic species should be minimal.

Triprolidine was administered in the feed to male and female B6C3F1 mice and Fischer 344 rats at dose levels of 4000, 2000, 1000, 500, 250 and 0 ppm. Animals were observed for clinical signs of toxicity, body weights taken, and food consumption measured weekly. All animals were necropsied at the end of 90 days and organ weights taken; serum AST and ALT enzyme levels were measured in the high, medium, low, and control groups. Gross and microscopic pathology was performed. In mice, weight gain was depressed greater than 10% for the 4000 ppm dose group for male and females. There were no treatment-related lesions in mice. Clinical pathology was inconclusive. In rats, mean body weight gain was depressed greater than 10% in the 4000 and 2000 ppm dose groups for male rats and in the 4000, 2000 and 1000 ppm dose groups for female rats. The parotid salivary gland in both sexes exhibited a dose-response effect for cytoplasmic alterations of the acinar cells. Dose-related fatty change in the liver occurred in 4000, 2000, 1000 and 500 ppm dose levels in male rats and in the 4000 ppm dose level in female rats. Clinical pathology indicated elevated enzyme levels (ALT) at the 4000 ppm dose level for female rats.

Administration of powdered rat chow suspension containing radiodiode (¹²⁵I) and pretreated with 100 ppm chlorine dioxide to rats has been shown to result in the elimination of over 40% of the dietary iodine in the feces. Furthermore, we have also shown that this radiodiode is mostly in a non-extractable form, and it is covalently bound to fecal particulate matter. We, therefore, investigated the hypothesis that ingestion of ClO₂ in drinking water could accelerate fecal loss of dietary iodide. For this purpose fasted rats were fed ad lib. with powdered chow in which NaI⁵I was mixed in calibrated doses. The animals received distilled water on the first day, followed by 100 ppm ClO₂ on the second through the fourth day consecutively. Twenty-four hour fecal samples were analyzed daily to establish stable iodine loss bound covalently to fecal particulates. Our preliminary data indicate that there is a near linear relationship between iodine loss and length of exposure to ClO₂. This may imply that chronic ingestion of this water disinfectant induces iodine deficiency by loss of dietary iodide via the fecal route. (This abstract does not necessarily reflect EPA policy.)
Subchronic administration of chlorine dioxide (ClO₂) in drinking water has been previously shown to depress serum thyroxine in the African Green monkey (C. aethiops). One possible mechanism may be that ClO₂ causes iodine deficiency. Alternately ClO₂ may also affect the iodine concentrating mechanism in the thyroid gland. In either case, exposure to the test chemical should change glandular radiiodine uptake. To test this hypothesis we measured the basal radiiodine uptake of monkeys, followed by a 60-day subchronic exposure to 100 ppm ClO₂ in drinking water. After exposure the Ⅰ uptake was measured again. Our findings show that the iodine uptake in female monkeys increased by 8%, and 3 months after cessation of exposure the uptake values tended to return to near normal. This may suggest that exposure to the test chemical induced an overall iodine deficiency which resulted in an increased compensatory iodine uptake. Such compensatory acceleration of iodine uptake is known to occur in early phases of iodine deficiency goiter. (This abstract does not necessarily reflect EPA policy.)

**A 90-DAY SUBCHRONIC TOXICITY STUDY WITH THYENIDIANINE ADMINISTERED TO MALE AND FEMALE B6C3F1 MICE AND FISCHER 344 RATS.**


Thyendiainine was administered in feed to male and female B6C3F1 mice (4000, 2000, 1000, 500, 250 and 0 ppm) and Fischer 344 rats (2000, 1000, 500, 250, 125 and 0 ppm). Animals were observed for clinical signs of toxicity, body weights taken, and food consumption measured weekly. Animals were necropsied at the end of 90 days and organ weights taken; serum AST and ALT enzyme levels were measured in the high, medium, low and control dose groups. Gross and microscopic pathology was performed. In mice, weight gain was depressed greater than 10% in dose groups greater than 250 ppm. Necrosis of individual parotid acinar cells occurred in the two highest dose groups of both sexes while hepatocellular cytomegaly and karyomegaly occurred primarily in the two highest dose groups of male mice. In rats, weight gain was depressed greater than 10% in the 2000 and 1000 ppm dose groups for male rats and in the 2000, 1000 and 500 ppm dose groups for female rats. Clinical pathology results were inconclusive. Multifocal lesions of the parotid gland (both sexes) were seen at all doses plus control. Fatty change in liver (male only) occurred in all doses plus control. Food consumption patterns were similar in all groups for both rats and mice.

**A 90-DAY SUBCHRONIC TOXICITY STUDY WITH CAFFEINE ADMINISTERED TO B6C3F1 MICE AND FISCHER 344 RATS.**

William T. Allaben and Paul Ross, National Center for Toxicological Research, Jefferson, Arkansas 72079

Caffeine was administered in the drinking water to male and female Fischer 344 rats at dose levels of 3000, 1500, 750, 375, 188, and 0 ppm, and to male and female B6C3F1 mice at dose levels of 1500, 750, 375, 188, and 0 ppm. Animals were observed for clinical signs of toxicity, weighed, and food and water consumption measured weekly. All animals were necropsied at the end of 90 days and organ weights taken. Microscopic pathology was performed routinely in all major organ systems in the high dose and control group and on all lesions observed grossly. In rats, mean body weight gain was depressed more than 20%, food consumption slightly depressed, and water consumption decreased by more than 10% in both males and females in the 3000 ppm dose group. The parotid and submaxillary salivary glands of rats exhibited dose-related alterations in acinar and parenchymal cells. In mice, weight gain was depressed more than 10% for the 188, 375, and 750 ppm dose groups in males only. There was little variation in food consumption but greater than 15% decrease in water consumption in the 750 and 1500 ppm dose groups in male and female mice. There were no treatment-related lesions in mice. Data from these studies indicates minimal toxicity from caffeine at dose levels administered.

**SUBCHRONIC TOXICITY STUDY WITH TRIBUTYL PHOSPHATE IN RATS.**


Tri-n-butyl phosphate (TBP) is used in industrial fluids. To evaluate the toxicologic effects associated with continuous administration of TBP, Sprague-Dawley rats (15/sex/group) were fed diets containing TBP at levels of 0, 6, 40, 200, 1000 or 5000 ppm for 90 days. Significantly depressed red blood counts were seen in males at 5000 ppm at termination (but not at lower levels in males or at any level in females). Significant increases in prothrombin and act. part. thromboplastin times were also seen at termination in the 5000 ppm males. No effects were seen on white blood cell counts (total or differential, including monocytes). Clinical chemistry changes included increased serum gamma-glutamyl transpeptidase levels in both sexes at 5000 ppm. Increased absolute and relative liver weights were seen in both sexes at 5000 ppm. Histopathologic studies indicated compound-induced transitional cell hyperplasia in the urinary bladders of males and females at the 5000 ppm level and males only at the 1000 ppm level. No microscopic changes in nerve tissues, bone marrow or liver, or remarkable changes in ChE levels (brain, plasma or RBC) were seen. TBP exhibited a low level of toxicity for an industrial chemical.

During munitions manufacturing processes workers may be exposed to the dyes CI Solvent Yellow 33 (2-[2-(quinolinyl)-1,3-indadione], a mixture of CI Solvent Green 3 (1,4-di-p-toluidinoanthraquinone) and CI Solvent Yellow 33 (70:30) or to an explosive manufacturing by-product 1-acetyl-3,5,7-trinitro-1,3,5,7-octahydroxetrazocine. Dermal, eye or oral toxicological evaluations were performed in New Zealand white rabbits and Fischer 344 rats according to standard protocols. The three materials caused little or no irritation to the skin (0.5g/site) or eye (0.1g/eye) in the rabbits. The dermal toxicity tests in rabbits indicated LD₅₀'s greater than 2000 mg/kg for all three materials; oral toxicity in rats demonstrated LD₅₀'s greater than 5000 mg/kg. The results of two-week multiple-dose dermal toxicity tests of CI Solvent Yellow 33 in rabbits at concentrations of 50, 200, and 1000 mg/kg showed mild to moderate hyperkeratosis, acanthosis and adnexal hyperplasia when compared to untreated skin sections.

(Supported by the US Army Medical Research & Development Command under contract No. DAMD 17-82-C-2301.)


Acute toxicity studies in mice, rats and dogs and three-month oral toxicity studies in rats and dogs were conducted to determine the toxicity of SCH 33844, an orally absorbed ACE inhibitor which is pharmacologically active in rats and dogs. The oral LD₅₀ in mice and rats was > 2500 mg/kg and the intraperitoneal LD₅₀ was approximately 400 mg/kg in mice and 600 mg/kg in rats. In dogs the median non-lethal dose was 2000 mg/kg and the no effect dose was 500 mg/kg.

Dogs dosed orally with 20, 60 or 250 mg/kg daily for three months had no changes in physical condition, behavior, heart rate, blood pressure, EKG, food consumption, body weight, ocular structure, hematology, blood chemistry, and urine composition.

In a three-month toxicity study in rats at doses of 30, 150 and 600 mg/kg, a reversible dose-related decrease in food consumption and body weight gain occurred in all male rats and the 600 mg/kg-dosed females. All 150- and 600 mg/kg-dosed rats had a reversible dose-related decrease in hematocrit, hemoglobin and erythrocyte count and all males and the 600 mg/kg-dosed females had a reversible increase in blood urea nitrogen.

In these studies dogs dosed with 60 or 250 mg/kg and all rats had a reversible dose-related increase in the granularity of the renal juxtaglomerular cells.


The preclinical toxicity of ABBOTT-49816 was evaluated in two studies in Sprague-Dawley rats. In the first study rats were treated po at 4, 15 and 60 mg/kg/day for 90 days. Twelve drug-related deaths (1 low-dose, 1 mid-dose and 10 high-dose) occurred. Hypochloremia, hypokalemia and increased relative kidney and testis weights occurred at all doses. Dehydration, decreased activity, emaciation, weakness and nasal discharge were observed in rats given 60 mg/kg/day. Nephrocalcinosis, renal tubular epithelial regeneration, myocytolysis and myocardial calcification were the major microscopic lesions. These lesions were more severe in males than females. Testicular degeneration also occurred in high-dose males. Subsequently, a follow-up 90-day oral study was conducted with and without electrolyte supplementation. Doses of 0.3, 1.0 and 4.0 mg/kg/day were used. No deaths occurred. Microscopic lesions occurred in unsupplemented rats given 1.0 and 4.0 mg/kg/day. Lesions were not observed in supplemented rats at 1.0 mg/kg/day. Electrolyte supplementation appeared to lessen the toxicity of ABBOTT-49816. A dosage of 1 mg/kg/day for 90 days was considered to be the "no-toxic-effect" dose in rats given ABBOTT-49816 with electrolyte supplementation.

SEPARATION AND IDENTIFICATION OF ENOLASE ISOZYMES BY HIGH-PRESSURE ANION-EXCHANGE CHROMATOGRAPHY. A.I. Solefer, M.S. Miller, M.I. Sabri, P.S. Spencer. Institute of Neurotoxicology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

A rapid technique for separating and quantitating the three enolase isozymes present in rodent brain and nerve was developed using high-pressure anion-exchange chromatography. At pH 7.9, one cationic and two anionic enzyme forms were separated with baseline resolution in an imidazole buffer containing ethylenediaminetetraacetic acid and magnesium. Chromatography was performed at room temperature with optimal separation being achieved by several steps including an isocratic buffer wash and a two-phase linear sodium chloride gradient (0-450 mM, 450-500 mM). The recovery of enolase activity was 90% or greater for brain and 85% for sciatic nerve. Chromatography of liver and axon-free (degenerated) sciatic nerve allowed the identification of non-neuronal, hybrid, and neuron-specific enolase isozymes. These enzyme forms respectively constituted 40%, 29%, and 19% of total activity in brain, and 63%, 13%, and 4% in normal sciatic nerve. These data provide a starting point from which to investigate the action of selected neurotoxins (e.g., acrylamide) which have been suggested to inhibit neuron-specific enolase and thereby precipitate axonal degeneration. Supported by grants NIOSH 0085/1,2063, NS 19611.
REFERENCE RANGE VALUES FOR ROUTINE CLINICAL PATHOLOGY DETERMINATIONS ON LABORATORY ANIMALS.
R.A. Schroer, F. Cohn, P. Gallo, and M. Brodeck. Medical Research Division, American Cyanamid Company, Pearl River, NY. Sponsor: F.J. Mecler

Reference range intervals for routine serum chemistry and hematology parameters have been defined for rats, mice, rabbits, dogs and monkeys (cynomolgus). The ranges to be presented are summarized by sex for all species and for several different age groups in the more commonly used rats and dogs.

The summary ranges list the sample size (N), mean, standard deviation as well as the 10th, 25th, 75th, and 90th percentiles. In all cases, the minimum sample size is 50 and frequently exceeds 100.

The data compiled into these ranges consisted of that which was generated during safety assessment studies. Only data from control animals or from pre-treatment periods were used. No animal contributed more than one set of values per range.

Plots will also be presented showing the age related changes that occur, particularly in the rat and dog.


Capsaicin and synthetic capsaicin (N-vanillinylnonanamide, VN) are pungent compounds utilized as spices in the human diet. Several studies have shown that these compounds cause destruction of fine unmyelinated fibers in rats injected as neonates or adults. However, relatively few studies have been conducted to evaluate their effects on other organ systems. We evaluated the toxicity of VN in a 2 week study in which adult male and female Sprague-Dawley rats received a total of six subcutaneous injections of either saline, vehicle control (propylene glycol) or VN at doses of 200 or 400 mg/kg. Results showed that VN treatment caused a significantly decreased food consumption in males and females, and a significant decrease in body weight gain only in males. Organ weights were not affected in either sex. Hematology revealed slight increases in WBC's only in males at the high dose. Serum chemistry showed changes in several parameters, most of which were related to vehicle. Histopathology demonstrated that the only VN-related lesion was a slight enhancement of vehicle-induced necrosis at the injection site. This study shows that the more routine toxicity parameters are not dramatically changed following administration of VN.


The acute toxicity of the carbamate pesticide carbofuran technical (2,3-dihydro-2,2-dimethyl-7-benzofuranyl[1-1-butyloxy][thio)methylcarbamate] has been determined in mice, rats, rabbits and guinea pigs.

Signs of toxicity observed in these studies are those commonly associated with cholinesterase inhibitors. Carbofuran was found to be moderately toxic when administered orally to rats and mice, practically non-toxic when administered dermally to rabbits, a minimal eye irritant, non-irritating to the skin and a sensitizing agent.


To examine the impact of hazardous chemicals on soil ecosystems, thirty-nine EPA priority chemical pollutants were evaluated by a 48 hr earthworm toxicity test at Cornell University. Approximately 300 earthworms per chemical (10/dose) were used to calculate earthworm LC50 values by placing individual adult Eisenia fetida in capped glass vials lined with filter paper to which various concentrations of test chemical solutions were applied. Results from the Cornell study indicated that toxicity in Eisenia fetida was influenced by chemical class, functional group, solubility and vapor pressure. To test whether these results had application to mammalian toxicity, earthworm LC50 determinations generated by Cornell were compared with the available literature LD50 values for the following mammalian test systems: oral (rat, mouse, rabbit), dermal (rat, rabbit), IP and SQ (rat). Significant correlations were found with the following mammalian LD50 comparisons: oral (rabbit>mouse>rat) and IP (rat). Although the number of chemicals tested in each comparison varied, preliminary results generated at Union Carbide suggest that the earthworm toxicity test may be an alternative for mammalian range-finding tests.
The objective of these studies was to assess the acute and subacute toxicity of oral TRI. Male S.-D. rats of 200-250 g were gavaged with 0, 0.5, 1.0, 2.0 or 4.0 g TRI/kg bw in corn oil and sacrificed 24 hr later. Serum enzyme assays, organ weight measurements, and histopathological examination of the liver and kidney did not reveal any treatment-related effects. In a short-term study, rats of 230-260 g were gavaged with 0, 0.5, 5.0 or 10.0 g TRI/kg once daily for 5 days, rested for 2 days, and dosed once daily for 4 more days. The 5 and 10 g/kg doses caused transient hyperexcitability and protracted narcosis, as well as fatalities. There was relatively little evidence of toxicity however in the survivors or in the 0.5 g/kg group. In a long-term study, rats of 200-250 g were gavaged 5 times weekly for up to 12 weeks with 0, 0.5, 2.5 or 5.0 g TRI/kg. Blood samples were taken at 2-week intervals for 6 weeks from male rats of each age group. Blood was similarly taken for an additional 6 weeks from the controls and 0.5 g/kg group. Rats given 2.5 or 5.0 g/kg exhibited reduced body weight gain and CNS effects like those seen in the short-term study. Some 35% of these rats died during the first 50 days of experiment, but only the 5.0 g/kg group showed an increase in serum enzyme levels. Ingestion of 0.5 g/kg for 12 weeks did not result in alterations in indices of toxicity. (Supported by EPA CR 811215)

DEPRESSION OF RAT ADRENAL MICROSOMAL CYTOCHROME P-450 AFTER ADMINISTRATION OF 2,3,7,8-TETRAChLORODIBENZO-P-DIOXIN (TCDD). C.A. Mebus and W.N. Piper. Dept. of Pharmacology, Univ. of Nebr. Med. Ctr., Omaha, NE 68105

As an undesired by-product in the manufacturing of phenoxyherbicides, TCDD has been released into the environment through industrial accidents and spraying of contaminated herbicides. Although exposure to TCDD has been associated with chloracne, skin hyperpigmentation, hirsutism, alopecia and porphyria, the exact mechanisms by which TCDD toxicity remain unknown. Effects such as a wasting syndrome and hypoglycemia in animals exposed to TCDD suggest possible alterations of adrenal function. This study was designed to examine adrenal microsomal cytochrome P-450 levels in TCDD-treated rats. Male Sprague-Dawley rats (220-240g) were given a single, oral dose of TCDD (50ug/kg; 1:2 v/v, acetone/corn oil). Adrenal microsomal cytochrome P-450 levels were determined seven and fourteen days after administration of TCDD. Seven days after TCDD administration, rat adrenal microsomal cytochrome P-450 levels were decreased to 72 percent of control values. Fourteen days following TCDD administration, adrenal microsomal cytochrome P-450 levels were decreased to 62 percent of control values. These decreases in adrenal microsomal cytochrome P-450 levels in response to TCDD exposure may be responsible for alterations of adrenal steroidogenesis, and suggest decreases in the activity of adrenal microsomal 21-hydroxylation. (Supported by NIH Grant ES-02423)

TOXICITY TESTING PROGRAMS SUPPORTING PRODUCT DEVELOPMENT ACTIVITIES IN THE PHARMACEUTICAL INDUSTRY. M. Palese Lund, Syntex Pharmaceuticals, Palo Alto, CA. Sponsor: D.W. Hallesy

A significant portion of any pharmaceutical company's R & D investment is directed toward conducting toxicity programs to establish the safety of new drugs and support FDA and international regulatory filings. These toxicology programs can be regarded as part of an "R & D investment portfolio," each with an associated financial risk. A number of strategies were developed for sequencing tox studies which both fulfill regulatory requirements and meet product development timetables, in addition to defining the financial investment and risk. This presentation analyzes 4 tox scenarios supporting registration of a hypothetical "typical" new systemic drug for chronic therapy. These strategies range from Scenario I, a conservative, sequential approach to use of tox resources prior to determination of clinical efficacy, to Scenario IV, an accelerated parallel-track tox program requiring major upfront expenditures prior to knowledge of clinical efficacy. The financial risks associated with these strategies are described, considering such variables as initial cash outlay to clinical definition, probability estimates of achieving a CO for project decision points (results of IND-enabling tox, teratology, chronic tox studies), time to achieve registration and return on investment.
The purpose of this study was to recommend drinking water standards for several common contaminants to which military populations may be exposed for periods of up to 1 year. The recommended standards are based on the highest dose that could be documented as having no adverse health effects or on the highest concentration that would not cause an objectionable taste. Criteria documents prepared for each substance describe the rationale for the recommended standard and state the important assumptions and uncertainties that are incorporated into the recommendations. A steering committee of military personnel responsible for environmental health was established to review and to decide how to treat the assumption and uncertainties. The scientific content of the documents was also given critical evaluation by experts not involved in the preparation of the criteria documents. We believe this explicit treatment of assumptions and uncertainties and separation of responsibilities for value and scientific judgments represents a refinement in the process of standard setting based on health-related considerations, which would be appropriate for all public and occupational health standards.

A common problem encountered when evaluating oncogenic potential is the evaluation of tumor frequencies in treated animals when incidences are different in corresponding control animals. In an effort to address this problem, tumor profiles have been produced for rats supplied by Charles River Laboratories Inc. (CER®CD®, CER®CD®GS®). The tumor profiles are based on histopathology data from 680 control rats used in three oncogenicity studies. An across-study diagnosis dictionary has been created associating and labelling similar tumor types across the three studies. Using these data, tumor profiles have been produced indicating the frequency of tumors occurring in each tissue. The results show tumors occurred most frequently (in descending order) in the pituitary gland, mammary gland (females), endocrine tissues, and reproductive tissues. For the most commonly occurring tumors, curves have been constructed relating probabilities of tumor occurrence to time and histograms have been generated relating tumor frequencies to age. The tumor profiles are routinely updated as additional histopathology data become available. The tumor incidence data for each animal is stored in SAS® data sets on computer tapes and the across study diagnosis dictionary is stored on a DEC10® data set. These data sets are easily updated as each oncogenicity study is completed.

Chinese hamster cells (V79) were treated with ethylnitrosourea (ENU) and cis-platin alone and in combination. At the completion of drug exposure, cells were rinsed and incubated with bromodeoxyuridine for 26 hours, then stopped in metaphase by adding colchicine. Sister chromatid exchanges (SCEs) were quantitated on trypsinized cells after staining with Hoechst 33258 (bisbenzimide) and Giemsa. Cis-platin produced 19 to 55 SCEs per cell (control) over the concentration range of 1 to 10 μM, respectively. ENU produced 25 to 72 SCEs per cell (control) over the range of 200 μM to 1 mM, respectively. The combination experiment employed a factorial design in which cells were treated with both agents simultaneously in various concentration combinations; it is analyzed with response surface methodology using a polynomial model. 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 in part by NIH ES02992).
Beginning in utero and continuing for 24 months postweaning, CD-1 mice were exposed to rodent chow (N) or mixed diets of 65% N and 35% chicken meat. Meat was either frozen (F), thermally sterilized (T), gamma-irradiated using 60Co (G), or electron-irradiated using a linear accelerator (E). Both G and E received c. 5.9 Mrad in vacuo at -25 ± 15°C. Compared to N, meat-fed groups had higher body weights, higher mortality, and higher incidences of myocardial degeneration and immune glomerulonephropathy. Decreasing mean body weights in the second year in males fed G were caused by poor survival among heavier weight mice, although overall mortality among F, G, and E males was similar. Males fed T survived better than F, G, and E males, due to less heart and kidney disease. Virgin females fed G had significantly higher mortality than F, T, and E, but no link was found to any specific pathological condition. Incidence of neoplasms in both sexes of mice fed F, lowest among males fed G, and lowest among females fed G (significant). Individual neoplasm types were not affected by treatment, except for interstitial (Leydig) cell tumor. This was highest in the two groups of males fed irradiated chicken meat: N - 0/115, F - 1/175, T - 0/115, G - 4/115, E - 4/115. Historically, this tumor occurs at 0.2% incidence in CD-1 mice. (Supported by USDA Contract No. 53-3K06-1-29.)

Streptozotocin (STZ) damages the pancreatic beta cells producing experimental diabetes. Signs of STZ induced diabetes include loss in body weight (BW) and organ weight and histopathological changes. To assess if these changes are due to STZ or a BW decrease, a control group was fed restricted for 20 days to match the BW of STZ animals. Male (Dunkin-Hartley) guinea pigs were given a single intracardiac injection of either 1) STZ 150 mg/kg (STZ-TX), 2) vehicle and food restricted (FR), or 3) vehicle and fed ad libitum (control). BW of FR and STZ-TX groups were the same during the study and were less than control. Food intake in FR animals was lower than both STZ-TX and control animals. Organ/BW ratios and histopathology were evaluated 20 days after treatment. Heart and liver ratios were lowest in FR animals. Kidney and spleen ratios were lower than STZ-TX animals, while adrenal weights were elevated above control. No significant histological changes were observed in the FR group. Proximal tubular degeneration, splenic hematopoiesis and a decrease in pancreatic beta granules were observed in STZ-TX animals. This study shows pathological differences between FR and STZ-TX animals. (Supported by the Coronary Heart Disease Research Project of the American Heart Assistance Foundation, Washington, D.C.).

Comparative acute toxicity studies with caffeine in B6C3F1 mice and Fischer 344 rats. William T. Allaben, National Center for Toxicological Research, Jefferson, AR 72079

Acute toxicity studies were performed in B6C3F1 mice and Fischer 344 rats preliminary to 90-day subchronic toxicity studies. Young adult male and female rats and mice were administered a single oral dose of caffeine in the morning after a 16 hour period of fasting. Caffeine was prepared as an aqueous Na benzoate solution, or as a suspension in tricatanon. Rats were dosed at 800, 400, 200, 100 or 50 mg/kg body weight, and mice at 1500, 750, 500, 250 or 125 mg/kg body weight. Animals were observed, clinical signs recorded, and dead animals removed during the first 8 hours after administration of the compound and in the morning and afternoon of each following day for a period of 14 days. Dead animals and all surviving animals were subjected to routine necropsy. There were no significant lesions observed in animals surviving 14 days. The estimated LD50 for caffeine, as a solution, was 400 mg/kg body weight for male and female rats and 180 mg/kg body weight for male and female mice. The estimated LD50 for caffeine, as a suspension, was 300 mg/kg body weight for male and female rats and 420 mg/kg body weight for female rats and 370 mg/kg body weight for male and female mice.


BALB/c and B6C3F1 mice of both sexes were divided into test groups and fed either a semipurified diet (AIN-76A) or a natural ingredient diet (NIH-07) containing graded levels of 2-acetylaminofluorine (2-AAF) for 90 days. 2-AAF was fed to males at 0, 25, 50, 75, or 100 ppm, and to females at 0, 50, 100, 200 or 250 ppm. A large number of dead or moribund B6C3F1 males fed the AIN diet were removed from the study prematurely. AIN fed mice removed early as well as some sacrificed at the end of the study showed myocardial damage with hemorrhaging. A much smaller number of BALB/c males fed the AIN diet also exhibited these observations, while none of the females from either stock were affected. Increased levels of serum aspartate aminotransferase (GOT) (P<.01) occurred in the B6C3F1 male mice that were sacrificed, supporting the histopathological changes that occurred. There was no other significant difference in the GOT between diets, or 2-AAF doses. Microbiological and chemical analysis of the diet showed no evidence of pathogenic organisms or nutritional deficiencies. We postulate that myocardial damage was due to dietary factors, probably associated with fat decomposition. These data indicate that myocardial damage in mice caused by semipurified diet can vary dramatically between strains as well as sexes.
410 CHANGES IN PULMONARY PROTEINASE INHIBITOR AND ELASTASE ACTIVITY FOLLOWING ACUTE BCNU ADMINISTRATION. B. J. Jarvis and R. K. Largen. College of Pharmacy, Oregon State University, Corvallis, OR.

The systemic administration of the carcinostatic drug BCNU can result in an increased incidence of pulmonary fibrosis at a total cumulative dose in manum of 1500 mg/m². The mechanism involved in the development of BCNU-induced pulmonary fibrosis is not well defined. Studies using male Sprague-Dawley rats treated with a single i.p. dose of BCNU have shown biochemical changes in the pulmonary system consistent with a possible mechanism for BCNU-induced fibrosis. Lavage levels of a major protective protein in the lung, α-1-proteinase inhibitor (PI), were monitored at various intervals after BCNU administration (15 or 20 mg/kg). PI levels increased in a time and dose dependent manner to 366 ± 71 mcg/ml (control 62 ± 8 mcg/ml) at 28 days. Of greater significance is the finding that PI activity (nmole PI/mole trypsin inactivated) was decreased bimodally to 10% of control at 24 hours and to 48% at 28 days (control: 0.48 ± 0.03 nmole/mg). This was accompanied by a simultaneous rise in PAM-generated elastase activity which may account for increased tissue damage and result in the deposition of the collagen matrix responsible for fibrosis.

411 PATHOGENESIS OF 4-IPOMEANOL-INDUCED PULMONARY EDEMA IN MICE AND INHIBITION BY PIPERONYL BUTOXIDE PRETREATMENT. S.K. Durham, W.L. Castleman. Dept. of Pathology, Cornell University, Ithaca, NY 14853. Sponsor: J.C. Babish

The morphogenesis of pulmonary injury and associated edema induced by 4-IPOMEANOL (4-IP) was studied by combined light and electron microscopy. Weanling mice received 4-IP by intraperitoneal injection and were studied at intervals from 2 to 360 hours after treatment. Interstitial edema associated with damaged endothelium was observed as early as 2 hours whereas injury to bronchiolar epithelium was not observed until 4 hours after injection. The most severe damage to endothelium was observed from 12 to 24 hours and was characterized by marked dilation of the perinuclear envelope and separation of cytoplasmic processes from the basal lamina. The most severe damage to bronchiolar epithelium occurred from 36 to 48 hrs. Cell repair and resolution of the edema was complete by 240 hours. In additional experiments, pretreatment by piperonyl butoxide resulted in a marked decrease in the severity of the pulmonary edema and cell injury. It is concluded that the capillary endothelium, in addition to the nonciliated bronchiolar epithelium, is an early cell target in the mouse and that endothelial cell injury plays a major role in the development of pulmonary edema in this species. The results further suggest that pulmonary endothelial cells may have the capacity to metabolize xenobiotics, such as 4-IPOMEANOL, to form ultimate toxins.

412 PATHOGENESIS OF LUNG INJURY PRODUCED BY O,0,0-S-TRIMETHYL PHOSPHOROTHIOATE (OOS-TMP): ROLE OF PROTEASE AND ANTIPROTEASE. L. Hasegawa and T. Imamura. Div. of Toxicology and Physiology, University of California, Riverside, CA 92521

OOS-TMP is an impurity found in widely used organophosphorus insecticides. Although OOS-TMP has been shown to be a pneumotoxin, little is known about its pathogenesis. Since the imbalance in protease-antiprotease has been proposed as a mechanism of various lung injuries, the effect of OOS-TMP on the activity of pulmonary alveolar macrophage (PAM) esterases and on specific esterase inhibitors in the bronchopulmonary lavage fluid and serum were measured. OOS-TMP (20 mg/kg) administered orally to rats produced an increase in debris in the lavage fluid as early as 4 hr and a 27% increase in PAM esterase activity 6 hr after treatment. The activity then declined to 63% of control value on day 3 and had not recovered to any significant extent on day 7. Chymotrypsin inhibitory capacity (CIC) of the lavage fluid was decreased by 45% at 6 hr but recovered rapidly and was at control levels by 24 hr. Trypsin inhibitory capacity (TIC) of serum was affected to a lesser extent such that no change was detected after 6, 12 or 24 hrs, but there was a 17% decrease on day 3 which had recovered to 95% of control value by day 7. These data, early elevation of PAM esterase levels and decreased CIC, support the view that pathogenesis of OOS-TMP produced lung injury could be due to increased protease levels. (Supported by PHS Grant ES03105)
COMPARTMENT SPECIFICITY OF SILICA-INDUCED PHOSPHOLIPIDOSIS IN THE LUNGS OF RATS.

Intratracheal injection of silica into the lungs of rats causes marked increases in their phospholipid content. The compartmental specificity of this increase in phospholipids is not known. Twenty-eight days following a single 50 mg intratracheal dose of silica, total pulmonary phospholipids were increased 16.1-fold, but DNA and protein were increased only 3.4-fold and 3.5-fold, respectively. The intracellular compartment of pulmonary surfactant phospholipids was increased 52.4-fold from 2.8 ± 0.3 to 146.8 ± 23.4 mg per pair of lungs. The extracellular compartment of pulmonary surfactant phospholipids was increased 28.2-fold from 2.2 ± 0.6 to 62.1 ± 14.1 mg per pair of lungs. These increases in the phospholipid content of the intra- and extracellular surfactant accounted for 59.1 and 24.6% of the total increase in pulmonary phospholipids, respectively. All other subcellular fractions, including microsomal, mitochondrial, and plasma membrane combined accounted for 13.0% of the total increase in pulmonary phospholipids. These data indicate that the effect of silica on pulmonary phospholipids is confined primarily to the surfactant system.
*Supported by National Research Service Award, ES-07126 from NIEHS/NIH.


This laboratory has previously shown that dibutyryl cAMP (DcAMP) produces a 66% increase in benzo(a)pyrene (BP) metabolism to aequous metabolites (AM) by rat lung slices. This effect occurs beyond 6 hrs and is maximal at 12 hrs. We now present evidence that the DcAMP-dependent increase in BP metabolism may involve the induction of the MFO system. Male Sprague-Dawley rats (175-250g) were given DcAMP i.p. (50 mg/kg) along with either 4mg/kg i.p. of the translation inhibitor, cycloheximide (CH) or 3.75 mg/kg i.p. of the transcription inhibitor, actinomycin D (AD). Twelve hrs later, lung slices were incubated for 90 min with 4mM 3H-BP. Incubation media was extracted with ethyl acetate:acetone and aqueous 3H was assayed. The DcAMP-induced increase in AM was eliminated by both CH and AD. Several microsomal MFO activities were also examined from the liver and lungs of rats 12 hrs after DcAMP treatment. Lung coumarin hydroxylase, BP monoxygenase and benzenethamine demethylase (BD) were increased by DcAMP (102%, 89% and 30% respectively). Ethylmorphine demethylase, ethanolamin deethylation and cytochrome P450 content were unchanged. Of the five MFO activities, only BD was elevated (22%) by DcAMP in the liver. These results indicate that cAMP induces specific MFO activities in the lung. (Supported by ES 07067 and 00454).

5-HYDROXYTRYPTAMINE RECEPTOR ANTAGONISTS AND MONOCROTALINE PYRROLE-INDUCED CARDIOPULMONARY TOXICITY. P.E. Ganev, K.H. Sprugel, K.E. Boner and R.A. Roth. Dept. of Pharmacology, Toxicology, Michigan State University, East Lansing, MI 48824.

Monocrotaline pyrrole (MCTP) causes endothelial cell damage, pulmonary hypertension, and right ventricular hypertrophy in rats by an undetermined mechanism. A role for 5-hydroxytryptamine (5HT) in the cardiopulmonary response to MCTP has been suggested. To investigate the role of 5HT, the effect of two 5HT receptor antagonists was examined in MCTP-treated rats. Co-treatment with metergolin (MTG), an antagonist which binds to both 5HT1 and 5HT2 receptors, did not alter MCTP-induced elevation of lung weight or right ventricular hypertrophy. 5HT-induced vascular smooth muscle contractions are mediated by 5HT2 receptors; therefore, MCTP-treated rats were co-treated with ketanserin (KET), a specific 5HT2 receptor antagonist. At a dose of KET which inhibited 5HT-induced platelet shape change in platelet-rich plasma, KET did not affect the elevation in lung weight or the increased accumulation of radio-labeled albumin in the lung tissue of MCTP-treated rats. Moreover, MCTP-induced right ventricular hypertrophy was not attenuated by KET. These results suggest that interaction of 5HT with its receptors is not involved in the cardiopulmonary response to MCTP. Supported by NIH grant ES05318.
PASTY ACID SYNTHESIS IN BCNU-INDUCED PULMONARY INJURY. A.C. Smith, R.A. Gram and M.R. Boyd, NCI, Bethesda, MD.

BCNU causes pulmonary injury in patients treated with high doses of the drug. We have described a multi-dosing animal model to study the mechanism of BCNU-induced pulmonary toxicity. After 2 doses of BCNU (10 mg/kg), electron microscopic studies have shown changes in lung Type II cells which are associated with cell toxicity. Type II cells are responsible for surfactant production and are an active site for phospholipid synthesis, therefore we measured fatty acid synthesis as a marker for Type II cell injury after BCNU. Fatty acid synthesis was measured in vitro by 14C-acetate incorporation into lipids in lung slices made from control and BCNU treated rats. Lung slices from rats treated with a single dose of 30 mg BCNU/kg incorporated 15% less 14C-acetate into lung lipids 2 hr after BCNU. At 4 hr, lung slices from BCNU treated rats synthesized 25% less fatty acids than slices made from control rat lungs. This depression in fatty acid synthesis lasted up to 24 hr after BCNU. The multi-dosing model for BCNU-induced pulmonary fibrosis involves treating rats with 5 mg BCNU/kg/week for 6 weeks. Three doses of BCNU (15 mg BCNU/kg) resulted in a marked 40% decrease in 14C-acetate incorporation. After 6 doses of BCNU (50 mg BCNU/kg) fatty acid synthesis was depressed by 64%. Use of fatty acid synthesis rates may be a useful tool to study BCNU-induced pulmonary toxicity in rats.

TOXICOLOGY AS "INTEGRATED" MOLECULAR BIOLOGY. S. J., Rutgers University, Piscataway, N.J. 08854.

Toxicology is concerned with the question of life and death at various levels of biological organizations. Future progress of toxicology, therefore, may depend on our ability to define life and death in molecular terms. To define life it is essential to integrate experimental information of molecular biology into a coherent system of concepts and theories. Such synthetic approach contrasts with the more "analytical" strategy employed in classical molecular biology. The basic assumption of the "integrated" molecular biology is that the cell possesses a set of properties not revealed by individual cellular components but originating only as a consequence of coherent interactions among appropriate components. Many such examples are known in physics and chemistry, dissipative structures of Prigogine being only one of them. Recently a molecular model of the living cell (the Bhopolator) was proposed on the basis of a new concept called conformors, packets of energy and genetic information stored in transient conformational strains of biomolecules. According to this model, life at the cellular level can be defined as a set of coupled physical and chemical processes that form a dissipative structure capable of self-production.


Pretreatment of rats with acetone results in both enhanced metabolism and alterations in the toxicity of certain chemicals. The mechanism(s) of these acetone-induced changes is(are) not well characterized. The lipid composition of membranes can significantly influence chemical metabolism catalyzed by membrane-bound enzymes. The purpose of this investigation was to determine whether acetone exposure alters the lipid composition of hepatic microsomal membranes. Hepatic microsomes were prepared from female Sprague-Dawley rats 24 hr after a single po dose of acetone (0.5, 1, or 2.5 ml/kg) or water, or 24 hr following the third daily dose of sodium phenobarbital (100 mg/kg). Microsome suspensions were extracted with chloroform/methanol (2:1) and the extracts were assayed for cholesterol and phospholipid. Acetone treatment did not significantly alter lipid composition. Sodium phenobarbital (positive control) treatment resulted in a significant decrease in cholesterol content /mg protein (16.4%, p<0.02) and a significant decrease in cholesterol/phospholipid molar ratios (p<0.01). These data suggest that acetone-induced alterations in the metabolism and toxicity of other chemicals are not associated with changes in the lipid composition of microsomal membranes.


DECALIN (Decahydronaphthalene) is a common component of many industrial solvents. Groups of male and female Fischer 344 rats were administered pure samples of cis- or trans-decalin (3g/kg) intragastrically on alternate days over a 14 day period. At the conclusion of dosing, male rats showed reduced weight gain relative to controls while female rats showed no weight change relative to controls. Male rats also showed renal tubular necrosis at the corticomediulary junction. Urine studies in both male and female rats showed that cis-decalin was metabolized to cis, trans-1-decalol, cis, cis-1-decalol, and cis, trans-2-decalol, while trans-decalin was metabolized to trans, trans-1-decalol and trans, cis-2-decalol. Kidney extracts from decalin-treated male rats contained 2-decalones. No ketone formation was found in kidney extracts of either female or control rats. Research sponsored by the Air Force Office of Scientific Research under contract AFOSR 84-0152.
The urinary excretion of nitrosoproline (NPRO) was used to assess the role of dietary factors in the endogenous formation of N-nitrosocompounds. We have investigated the role of orally administered nitrate on this process. Male rats were fed a purified diet and deionized water ad lib for 4 wk, and, after an 18 hr fast, given oral nitrate (1.25g/kg) followed, after 45 min, by oral [15N]-proline (250mg/kg, 60µCi/kg). Urine was then collected via a bladder catheter for 6 hr. The rate and total amount of [15N]NPRO formed was similar in conventional microflora, germ-free and antibiotic treated rats (equivalent to nitrosation of approx. 0.01% of the initial dose of [15N]-proline) suggesting no involvement of the intestinal microflora in this reaction. Furthermore, conventional and germ-free rats excreted similar amounts of NPRO after administration of nitrate and [15N]-proline or [15N]proline alone, demonstrating no involvement of exogenous nitrate in the nitrosation process under the conditions of the experiment. The results suggest that nitrate/nitrite reserves in the body are important in the formation of NPRO in vivo.

We thank U.K. Ministry of Agriculture, Fisheries & Food for financial support.


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 (0.5/8/treatment) on gestation days 8-10 at doses of 40,000, 80,000, or 160,000 I.U./kg/day. Dams that received 160,000 I.U./kg/day showed reduced weight gain on days 8-11. Sixty-seven of 81 pups (82.7%) from the 160,000 I.U./kg/day group had external craniofacial malformations. This dosage also caused a significant reduction in the live birth index and 24 and 48-hour survival indices. Survival was also significantly reduced over days 3-7 and 8-10. The following showed no treatment effects: length of gestation, total litter size, first occurrence of pinnae detachment, incisor eruption or eye opening, surface righting, cliff avoidance, negative geotaxis, swimming development, open field activity, swimming maze performance or operant learning ability. No gross or histopathologic changes were attributed to treatment in surviving animals. Maternal administration of vitamin A acetate produced craniofacial malformations, neonatal death and decreased survival. Reflex development, motor coordination, and maze and operant learning were not affected.


Benzodiazepines are widely used and have been shown to produce physical dependence (PD), but the influence of dose and duration of exposure on PD has not been established. To begin studies in this area we have developed a model of benzodiazepine PD in mice. Diazepam (D2) was administered in lab chow for eight weeks; we observed drug consumption as high as 1600 mg/kg/day. Blood assays indicated low levels of D2, but very high levels of the active metabolites nordiazepam and oxazepam, generally totaling 8,000 to 10,000 ng/ml. Animals appeared healthy throughout the treatment phase with the exception of deaths due to apparent drug-induced aggression. The behavioral and physiological state of each animal was scored during treatment and withdrawal phases; tests included piloerection, tremor, pelvic elevation, tail elevation, body tone, abdominal tone and pupil size. A composite withdrawal score was plotted against time; this score increased significantly (p<0.01) one day after withdrawal and remained elevated for up to fourteen days. This model provides a quantitative method to study the effect of various doses and durations of exposure to D2 and similar benzodiazepines.
425 BEHAVIORAL EFFECTS OF CHRONIC MANGANESE (Mn) ADMINISTRATION IN RATS: LOCOMOTOR ACTIVITY STUDIES. J.P. Nachtmann, R.E. Tubben and R.L. Commissaris, Dept. Pharmaceut. Sci., College of Pharmacy & AHP, Wayne St. Univ., Detroit, MI Mn is an industrially important metal which produces lesions in the basal ganglia of rats and humans. Humans poisoned with Mn often exhibit an initial hyperactivity ("Mn madness") followed by a parkinsonian-like syndrome. The present studies examined the effects of chronic Mn exposure on locomotor activity in rats maintained on 0.0 or 1.0 mg Mn(Cl)₂. 5H₂O/ml drinking water. No differences in food/water consummation or body weights were observed at this level over three months treatment. Locomotor activity was tested weekly in 15 min sessions. Mn treatment produced a 50% increase in activity on weeks 5-7 before returning to control values. Habituation measured within a test session was not affected at any time. At 14 weeks, Mn animals were found to be more responsive than controls to the effects of the locomotor stimulant, d-amphetamine (1.25 mg/kg, IP). Consistent with clinical reports, these results suggest that Mn may produce a transient increase in dopaminergic function, as measured by both spontaneous and amphetamine-stimulated locomotor activity.

(Supported in part by WSU Grants-in-Aid #306-6183 to JPN and #306-6179, 167-1713 and 367-1321 to RLC)

426 EFFECT UPON OPERANT BEHAVIOR OF RATS FROM INHALING SUB-LETHAL LEVELS OF PYROLYSATES FROM A POLYIMIDE AND POLYURETHANE FOAM. W.H. Lawrence, John Autian and Caroline Sanford, Materials Science Toxicology Labs., Dept. of Medicinal Chemistry, Colleges of Pharmacy and Dentistry, Univ. Tenn. Center for the Health Sciences, Memphis, TN 38163.

In the toxicological evaluation of combustion/pyrolysis products of materials, not only is the lethality of the products important, but in many circumstances disruption of logical behavior may result in increased hazards to the exposed individuals.

Acute lethality (LD₅₀) was determined for each sample by pyrolyzing varying weights of the material in the chamber with rats and exposing them for 30 minutes post-pyrolysis. Other rats were trained on a fixed-interval, shock-avoidance schedule. Replicate experiments were conducted exposing individual trained rats to pyrolysis products from one-half of the LD₅₀ sample weight. The pyrolysis and exposure procedures used in the acute lethality studies were duplicated for the behavioral studies.

A dramatic difference was noted in the rats' shock-avoidance lever pressing activity when exposed to pyrolysates from these polymers. The polyimide produced essentially no change in pattern, while the polyurethane produced a major disruption.

(Supported in part by NASA Contract NAS 9-15670.)

427 COMPARATIVE EFFECTS OF FOUR ORGANOPHOSPHATES ON OPEN FIELD BEHAVIOR IN THE RAT. F.O. Riedinger and W.M. Bourn, School of Pharmacy, Northeast Louisiana University, Monroe, LA. Sponsor: R. Don Brown.

The purpose of this research was to assess the dose-related effects of 4 organophosphate insecticides on various behavioral parameters measured in an open field. Rats were dosed orally with either malathion, parathion, dimethoate, or abate at 4 dose levels. Behavioral responses were measured both in an open field and in an electronic activity monitor. Large and small body movements were quantified in the activity monitor while ambulation, rearing, grooming, and inner square penetration were measured in an open field. Animals receiving the high doses of parathion and dimethoate demonstrated severe deficits in large body movements, open field activity, and cholinesterase activity. Lower dose levels produced variable effects on open field activity while not influencing movements measurable by the activity monitor. In addition, an experiment was conducted using 2 different open field conditions (dim illumination with no auditory stimulus vs bright illumination with a 100 db tone). After exposure to parathion, animals in the dim illumination demonstrated a decrease in open field activity while animals tested in the bright condition responded similarly to controls. These results show (1) that differences should be expected in behavioral studies involving different organophosphates, (2) environmental conditions alter the impact of organophosphates on behavior.


Although there are many human inhalation exposures to organophosphates, most pharmacological work involving DFP has not been performed by this route. Mice were exposed to the vapor from the volatilization of 4 mg of DFP or 3H-DFP for 5 min which resulted in a dose of 2 mg/kg. DFP treatment reduced rectal temperature with the maximum effect observed between 15 and 60 min, which did not return to control levels until 10 hr. Motor incoordination (rotarod test) produced by DFP treatment had a rapid onset, with complete recovery by 10 hr. Determination of the time course of tissue levels of 3H-DFP showed that 3H-DFP rapidly penetrated all tissues and was quickly hydrolyzed to free diisopropylphosphoric acid (DIP) or was covalently bound to tissue. Cholinesterase inhibition in brain, lung, diaphragm and plasma was temporally related to levels of bound 3H-DFP between 1 and 8 hrs, but not at earlier time points which may be due to noncholinesterase binding. The time course of hypothermia and motor incoordination was correlated with neither free 3H-DFP nor bound 3H-DFP levels in the brain nor with cholinesterase inhibition in brain, which suggests that noncholinesterase bound 3H-DFP may contribute to these effects.

(Supported by USAMRDC Contract #DAMD 17-82-C-2212).
Three behavioral tests (locomotor activity, rotorod, treadmill) were performed to assess the effects of physostigmine salicylate on neuromuscular function in rats. Animals received either vehicle, 0.05, 0.1, 0.2, 0.4, or 0.8 mg/kg physostigmine IP (N=6-8/group). In Experiment 1, spontaneous locomotor activity as determined by the number of photobeam interruptions, was reduced from vehicle control levels at a dose of 0.8 mg/kg. In Experiment 2, motor coordination was evaluated by placing rats on an accelerating rotorod (0-14 rpm in 120 sec). Doses of 0.2 mg/kg or greater produced performance decrements that differed significantly from control values. Locomotor endurance was measured using a treadmill device that accelerated to 1 mi/hr at a 17% grade (Experiment 3). Compared to vehicle control levels, the high dose (0.8 mg/kg) significantly reduced running time. Based on these behavioral measures, the rotorod appears to be the most sensitive test for detecting decrements in motor performance.

The behavioral effects produced by acute exposure to sublethal doses of soman were examined in Sprague-Dawley rats. Soman (100-150 µg/kg I.M.) produced a dose-related decrease in spontaneous motor activity (SMA), fore- and hindlimb grip strength, heat reactivity and rectal temperature, effects which were well developed by 2 hr after exposure. In addition, acoustic startling response amplitude decreased, while response latency increased. Soman also decreased the percentage of conditioned avoidance and escape responses and increased the response latency. In both the 103 and 116 µg/kg groups, effects on hindlimb grip strength persisted up to 14 days after exposure, while effects on hot plate response lasted for 7 days. A biphasic change in motor activity was seen in the 103 and 116 µg/kg groups: Initial SMA depression during the first 24 hr after exposure was followed by SMA increases which persisted up to 21 days. Animals that showed this delayed hyperactivity often exhibited seizure activity and increased irritability when handled. The results of these studies show that sublethal doses of soman can cause marked and often long-lasting changes in behavior in the rat. Supported by Contract DAAH01-81-C-A277, Mod. P00072.

Tricresyl phosphate (TCP) is used commercially as a plasticizer and a flame retardant. TCP is composed primarily of the tri-para, tri-meta, and tri-ortho (TCOPE) isomers. TCOPE is a neurotoxin, producing a delayed peripheral neuropathy. A subchronic toxicity study of TCP (containing less than 0.1% TCOPE) was conducted in both sexes of F344 rats and B6C3F1 mice. Animals received TCP at dose levels of 800, 400, 200, 100, 50, or 0 mg/kg by gavage in corn oil, 5 days per week for 13 weeks. Reductions in body weight were seen in male rats and mice (800, 400, and 200 mg/kg) and in female mice (800 and 400 mg/kg). Serum cholinesterase activity was decreased in rats and mice at all TCP dose levels. Decrements in grip strength were seen in male (800 and 400 mg/kg) and female (800, 400, and 200 mg/kg) mice and in female rats (800 and 400 mg/kg). Male and female mice (800 mg/kg) exhibited a significant decrease in spontaneous motor activity and a significant increase in paw lick latency. Sperm motility, concentrations, and morphology were adversely affected by TCP in both rats and mice. Microscopic lesions in rats and mice were seen in the lumbar spinal cord, adrenal cortex, ovaries, and testes. Degeneration of the sciatic nerve was seen in mice only. (Supported by Contract N01-ES-59563-03 from NTP)

The hepatotoxicity of bromobenzene is increased or decreased by various ortho-substituents. In this study the total metabolism (MET) and protein covalent binding (CVB) of six [99mTc]-o-substituted bromobenzene [α-Brc3H4]-X; X = H, Me, OMe, Br, Cfg or CN were examined with liver microsomes from phenobarbital (PB) treated and untreated (UT) rats. With UT microsomes, total MET was generally linear with time, with relative rates decreasing in the order of Me > OMe > Br > Cfg. PB induction increased MET for all except Cfg. The increase was due primarily to a short-lived "burst" of activity in the first 5 min of incubation. The time courses for CVB paralleled those for MET, but the relative binding efficiencies (pmol CVB/nmol MET) of these compounds varied widely, from 230 for CN to 0 for OMe. The rank order for total CVB was CN > H > Br > OMe > Me > Cfg, which corresponds reasonably well to the relative order of in vivo hepatotoxicity. These results suggest that o-substitution influences toxicity by altering the relative yield of reactive metabolites as well as the total rates of metabolism of these compounds. Supported by NIH-GM-21794.
The chemistry of CUB of BB metabolites was investigated by observing in vitro the effects of 26 chemical or enzymic probes on the total metabolism (MET) and CUB of [3,5-3H]-BB with liver microsomes from phenobarbital pretreated rats. P-450 inhibitors decreased CUB and MET in parallel, while H2O, H2O2 and H2O2 scavengers had no effect on MET or CUB. At the other extreme, sulfonilureas profoundly reduced CUB with little or no effect on MET. Ascorbate, diaphorase and bromophenols reduced CUB significantly more than they reduced MET, but UTPS did not. Free radical inhibitors reduced CUB only slightly more than MET, while tri-chloropropene oxide reduced MET somewhat more than CUB. The effects of ascorbate, BSH, HS03- and TCPD on the MET and CUB of 5 a-Brc,H4X derivatives of BB (X = MeO, Me, Br, CF3, ON) were generally similar to those observed with BB. Overall these results provide some support for epoxides in CUB, but are more consistent with a major role for quinone metabolites of BB. (Supported by NIH-GM-21784).

The hepatotoxicity of BB is attributed to the CUB of reactive metabolites to cellular macromolecules with subsequent loss of function. Considerable evidence has implicated BB-3,4-oxide as the reactive (and toxic) metabolite; other evidence has suggested that bromoquinones are involved in the CUB of BB. To define better the chemistry of the CUB process we prepared two forms of tritium/carbon-14 (T/C) dual-labeled BB: BB1 = 2,4,6-7T/C; BB2 = 3,5-7T/C. Time course studies (0-60 min) of their total metabolism (MET) and CUB were performed using liver microsomes from phenobarbital (PB) pretreated and control rats. By C-14 counting, both MET and CUB (ca. 30-35% of MET) were identical for BB1, BB2 and C-14-BB alone. PB increased both MET and CUB by ca. 45-50% at 45 min. By 3-H counting, MET and CUB of BB1 and BB2 were essentially identical, but total CUB of 3-H was only 29% that of C-14; N-acetyl cysteine (MAC, 1mM) reduced CUB by 65% without affecting MET or the T/C ratio of CUB material. 3-H water was a major metabolite of BB1 and BB2. These results strongly implicate a major role for reactive metabolites more highly oxidized than BB-epoxide in the CUB process. (Supported by NIH-GM-21784).

Chlorophenols (CPs) are prominent environmental contaminants which can be formed in potable water by, or as a result of, the chlorination process. In order to assess the degree of hazard of these compounds we have studied the distribution, metabolism and excretion of 2- and 4-CP in rats. Male rats received 14C-labeled CP (50 mg/kg in corn oil) by gavage, killed at various times, tissues removed and examined for radioactivity. CPs were rapidly absorbed with peak plasma concentrations occurring at 2 hours. Significant amounts of label were also found in the GI tract and in fat, but by 4 hours the amount in fat had decreased markedly. By 24 hours 91% of the administered dose had been excreted in the urine and 4% had appeared in the feces. HPLC analysis of the urine indicated that less than 2% of the recovered label was present as the parent CP. The majority of the material (greater than 90%) was present in 2 peaks subsequently shown by GC-MS to be the glucuronide and sulfate conjugates of CP. These results are consistent with a very rapid absorption, metabolism and excretion (primarily by the kidney) of the CPs and their metabolites; and with the exception of fat, very little of the material is distributed to the various organs. (Supported by EPA Cooperative Agreement No. CR-809385-02.)

The administration of 1 unit of bleomycin (BLM) with 1 : 6Cl of [3H]-BLM produced a biphasic disappearance curve from the lung. The Tf1 of the a phase of the curve was 2.7 hr while the 8 phase Tf2 was 33.7 hr. Most of the radioactivity (RA) in lung was associated with the soluble fraction (SF). Specific RA was decreased for the SF from 8.7 x 104 dpm at 0.5 hr to 9.6 x 103 dpm at day 6 hr. Thereafter, it was similar to that of mito and micro. The specific RA in the nuclear fraction (NF) decreased from 7.2 x 103 dpm at 0.5 hr to 1.5 x 103 dpm at 24 hr. Distribution of RA in the subcellular fractions (SCF) of the liver was similar to the lung and a major % of RA was associated with the SF. The % RA in the SF decreased slowly from 82% at 0.5 hr to 60% at 24 hr. The remaining three fractions contained nearly the same % of RA at all time points except the NF at 24 hr. The specific RA in all SCF of the liver increased gradually with time. Lung, plasma and liver contained a significant amount of RA covalently bound to proteins. The binding was the highest for the lung followed by the plasma and liver. The SF of the lung covalently bound an increasing % of the total RA. Most of the RA in plasma was covalently bound whereas a small % of total RA was bound to RBC. The RA recovered in 24 hr urine averaged 75% of the administered dose. (NRLBI Grant #2R01 HL 2735-04).
There is a paucity of information concerning the biotransformation of cyanide to cyanate. Because this is a well-characterized chemical reaction, it has been generally presumed that cyanate also would be formed biologically. The present drug metabolic studies were conducted using carbon-14 labeled cyanide in the isolated perfused rat liver system. Hemodynamic pressure was maintained at 120 mm Hg. Blood was infused into the portal vein and the effluent perfusate exited by the inferior vena cava. The perfusate was collected, partially purified and subjected to anion exchange chromatography. The radioactive components were separated and analyzed. Cyanide is rapidly metabolized by the isolated perfused rat liver system and three major radioactive fractions were observed. These fractions were isolated and the properties of these three components were found to be consistent with the properties of cyanide, cyanate and thiocyanate. Furthermore, the predominant pathway for the formation of cyanate appears to be from cyanide rather than thiocyanate. (This work supported by funds from NIGMS, NIHES and USAMRDC).

Ethylene carbonate (EC) is a material with a toxicity profile which resembles that of ethylene glycol (EG). To determine whether the toxicity of EC could be explained on the basis of its metabolism, male Fischer 344 rats received 200 mg/kg of uniformly labeled 14C-EC in water by gavage and the radiolabel was followed for 72 hr. EC was rapidly metabolized, with approx. 57% and 27% of the administered dose eliminated in the expired air as 14CO2 and in the urine, respectively, with the remainder found in the carcass. Separation of the urinary metabolites using HPLC revealed a single major peak which was identified by GC/MS as ethylene glycol. In a separate group of animals administered an equimolar dose of 14C-EC (141 mg/kg), approx. 37% of the dose was expired as 14CO2 and 42% was excreted in the urine as EG. When expressed on the basis of the ethanediol moiety, the disposition of EC was identical to that of EG. Peak blood levels of EC (2.5 ppm in 15 min) and EG (140 ppm in 1 hr) measured in EC-treated rats revealed that EC is rapidly converted to EG. Thus the toxicity of EC may be explained on the basis of its rapid metabolism to EG, and utilization of the extensive EG toxicity data base for EC appears justified.

A single physiologically-based pharmacokinetic model has been used to describe the disposition of inhaled MC in humans (35 & 350 ppm), rats (150 & 1500 ppm) and mice (150 & 1500 ppm). The model accurately described the time course of MC in blood and expired air as well as the body burden, amount metabolized, and distribution of MC to other body compartments (e.g. rat and mouse fat & liver). The same model also correctly described the expiratory and metabolic rates after administration of 143 mg/kg 14C-MC to rats in drinking water over an eight hr period.

The consistency of this model supports its use for predicting the disposition of MC in humans consuming water with trace quantities (10 ppb) of MC throughout their lifetime. The model predicts that steady state levels of MC would be achieved within a few days, and that the ultimate body burden in humans would be approximately 10° lower than those present in rats at the NOEL (875 ppm) or mice at the NOEL (1500 ppm) in chronic studies.

The absorption of 1,3-dichloropropene (DCP), DCP effects upon respiratory physiology, and blood concentrations of DCP were determined in rats exposed to 30, 90, 300 or 900 ppm TELONE II vapors (91% DCP) for 3 hrs. The absorption of DCP by rats was not observed to increase proportionally with increasing exposure level. An observed 40-50% depression in the RMV of rats exposed to 300 or 900 ppm TELONE II was partially responsible for this finding. Steady-state blood levels of cis-DCP attained in exposed rats were 20% lower than levels of trans-DCP even though the cis:trans isomer ratio in the TELONE II used was 1:1.2. Post-exposure elimination curves for both isomers displayed an initial rapid and dose-dependent elimination phase (2-3 min half-life) followed by a slower elimination phase (38-44 min half-life). The DCP dosages calculated from the areas under these blood concentration curves (AUC) were roughly proportional to all but the high exposure levels. However, DCP dosages calculated from AUC were 8-13% lower than those determined by vapor uptake measurements, suggesting a significant portal-of-entry effect. Overall, these data demonstrate that a complex interaction of DCP-induced changes in respiratory physiology and isomer-specific, saturable, elimination mechanism(s) determine the ultimate body burden of DCP in rats exposed to TELONE II vapors.
Seneconine (Sn) is one of the most toxic representatives of pyrrolizidine alkaloids (PAs). Using a new HPLC method involving a PRP-1 column and direct injection of the deproteinated reaction mixture we have examined the in vitro metabolism of Sn. The major metabolites formed upon metabolism of Sn by rat and mouse liver microsomes were 6,7-dihydroxy-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP) and Sn N-oxide (SnNO). The assay was optimized with respect to pH, time, Sn, and protein concentration. Km values for DHP and SnNO formation were 0.24±0.05 and 0.10±0.05 mM, respectively. In optimal condition specific activities of the microsomes from phenobarbital induced rat associated with DHP and SnNO formation were 1742 and 841 nmol/min/mg protein, respectively. Activities of uninduced microsomes were about 4 times lower (4.3±0.5) in the case of DHP formation and remained unchanged for SnNO. The time course for conversion of Sn to DHP and SnNO remained linear for 20 min but biotransformation still occurred after 60 min. The analytical method has also been extended to the investigation of the in vitro metabolism of other PAs. (Supported by NIH grants CA-22524 and ES-00210).

Blood carboxyhemoglobin (COHb) has long been recognized as an index of CO poisoning. Our previous study, however, indicates that comparable COHb levels resulting from different routes of CO administration have drastically different effects on circulating levels of glucose, lactate and pyruvate. Heart rate, respiration rate, blood gases, and nitrogen stress were studied in rats at comparable COHb concentrations elevated by inhalation (inh) or intraperitoneal (IP) injection. Two CO concentrations, 5000 ppm and 2000 ppm, were administered by inh, and 100% CO at a dose of 30 mL/kg was administered by IP injection. While an increase in heart rate and a decrease in arterial PO2, PCO2 and pH were observed in the inh group, no changes occurred with the IP group. At the end of 60 min of exposure, the following observations were noted: venous PO2 dropped from 45 mm Hg to 20 mm Hg in both groups; inh group had higher venous PCO2 and lower pH than the control group. After 40 min of CO administration, the heart and respiration rates declined rapidly and equally under nitrogen stress. These studies suggest that the formation of COHb which consequently reduces the oxygen-carrying capacity of the blood may not be the major reason for CO toxicity. It appears that COHb formation should be considered only as an index of inhalation exposure rather than as a general criterion to account for the process responsible for CO poisoning.

CI-925, a nonsulphhydryl angiotsin converting enzyme (ACE) inhibitor, is an orally active anti-hypertensive agent in animal models. The short term toxicologic profile was assessed in rats, mice, and dogs and the mutagenic potential in standard bacterial assays for reverse point and frameshift mutation. Single oral doses were not highly toxic in rodents, with LD10 values of 4290 and 4015 mg/kg in female and male rats and 2510 and 2210 mg/kg in female and male mice, respectively. In dogs no clinical or pathological changes occurred at escalating doses up to 900 mg/kg. CI-925 was well tolerated in two week studies at doses up to 150 mg/kg in rats and 30 mg/kg in dogs. Hyperkeratosis of the skin was noted on the feet of rats especially at high doses and BUN was slightly to moderately increased in females given 700 mg/kg and above and males at 350 mg/kg and above. Amenorrhea and diarrhea resulted in electrolyte imbalance and dehydration in one dog each given 300 or 600 mg/kg. Pathologic findings of gastric ulcers or erosions in some dogs given 300 or 600 mg/kg and prominent juxtaglomerular apparatus in dogs given 300 mg/kg and higher and rats given 350 mg/kg and above were similar to those noted with other drugs of this class. CI-925 was considered nonmutagenic and has an adequate safety margin.

The toxicity of a novel cardiotonic agent 4,5-dihydro-6-([4-[(1H-imidazol-1-yl)phenyl]-5-methyl-3(2H)-pyridazinone, monohydrchloride (CI-930) was evaluated following IV administration to mice, rats, dogs and rabbits. Single IV doses resulted in LD50 values of approximately 160 mg/kg in mice and 120 mg/kg in rats. Dose levels up to 25 mg/kg/day were well tolerated following repeated IV administration to rats for 4 weeks. At 50 mg/kg, post-dose depression was observed. Saliary gland and liver enlargement were drug-related pathologic changes. In dose range studies in Beagle dogs, the maximum tolerated intravenous dose level was estimated as 2 mg/kg. Higher dose levels resulted in clinical signs of toxicity and pathologic changes in the heart. Following repeated daily IV administration for 4 weeks in dogs, heart murmurs were noted together with drug-related increases in heart rates and decreases in PR and QT electrocardiographic intervals. At 2 mg/kg/day 2 of 6 dogs had hemorrhage in the arterioventricular valve; one of these dogs also had focal myocardial degeneration and necrosis and arteriolar medial hyperplasia. No pathologic changes were observed in the heart at 1 mg/kg/day, a dose level several times higher than the proposed human dose. CI-930 showed no venous irritation potential at concentrations 10 fold higher than the proposed clinical formulation.
ROLE OF PROSTAGLANDINS IN RETINOID TOXICITY.
C.J. Viau and S.D. Harrison, Jr. Grad. Ctr. for Toxicology, University of Kentucky, Lexington, KY

A fetal mouse bone culture assay was used to assess the role of altered arachidonate disposition in retinoid toxicity. The release of pre-incorporated $^{45}$Ca and $^{3}$H-arachidonate (AA) from bones to culture media was monitored by liquid scintillation spectrometry following 24 hr treatments with all-trans-retinoic acid (RA, 0.01 to 100mM) AA, prostaglandins (PG) and inhibitors of AA disposition. PG in ethyl acetate extracts of media were separated by TLC in two solvent systems (benzene:dioxime: acetic acid, 20:10:1; chloroform: methanol: acetic acid:water, 4:5:4:0.0:0.4) identified by coelution with standards in both solvent systems and quantified by liquid scintillation spectrometry. At doses exceeding 10mM RA toxicity, manifested as a dose-dependent (p=0.004) decrease in $^{45}$Ca release, was accompanied by a dose-dependent (p=0.002) increase in 3H-AA release as compared to paired diluent (1% DMSO) controls. PGD$_2$ (50mM), PGE$_2$ (50mM) and PGF$_{2\alpha}$ (10mM) also decreased the $^{45}$Ca released from bones to 69.2±2, 67.2±25 and 71±8% of paired controls, respectively (p<0.01, p=0.02). Indomethacin (0.5 and 5.0mM) had no effect alone but blocked the RA-induced $^{3}$H-AA release and the RA toxicity. AA, PGD$_2$, PGE$_2$ and 6-keto-PGF$_{1\alpha}$ were detected in all media extracts. No treatment-related alterations in the PG profile were detected. These results implicate altered AA disposition as a mechanism of RA toxicity in bones.

EXAMINATION OF POTENTIAL REPRODUCTIVE EFFECTS OF AQUEOUS ETHYLENE DIBROMIDE SOLUTION ADMINISTERED TO LONG-EVANS RATS BY GAVAGE. B.D. Carlton, D.L. Nabash, K.A. Colling, A.H. Basaran, I.D. Osis, and M.K. Smith, Battelle Columbus Labs, Columbus, OH, and USEPA, HERL, Cincinnati, OH. Sponsor: G.L. Fisher

Aqueous solutions of ethylene dibromide (0.4, 4.0, or 40.0 mg/kg) were administered to male and female Long-Evans rats for approximately 66 days. Males were dosed for 66 days prior to breeding and throughout the 10-day breeding period. Females received EDB for 14 days prior to breeding and throughout breeding, gestation and lactation. Selected pups were dosed following weaning until day 40 or the day of vaginal opening. Animals in the high dose group were more irritable when handled and showed nose-shoveling behavior following dosing. No clinical signs of toxicity or body weight depression were observed. Fertility, fecundity, and litter weight were also unaffected. Parturition was delayed in 80% of high dose F0 females. No alteration in estrous cyclicity was observed, but vaginal opening was delayed among EDB-exposed F1 females. F0 males showed no adverse effects of EDB exposure when sperm count, sperm morphology, motility, or velocity were evaluated. Some high dose animals showed gastric irritation at necropsy. No histopathological lesions of the reproductive tract were observed. (Supported by EPA Project No. CR810862-01. This abstract does not represent the policy or opinion of the USEPA.)

ETHYLENE DIBROMIDE (EDB) RESIDUES IN CHICKENS DURING AND AFTER FEEDING OF CONTAMINATED DIET. E. Lehning, T. Whalen, and D. Polin, State of Michigan (Geigley) Lab and Dept. of Animal Science, Michigan State University, E. Lansing, MI.

Flour containing EDB at 500 ppb was confiscated by the State of Michigan authorities. It was fed to laying hens for 21 days. Eggs were collected daily during the feeding period and for 21 days after the feeding period. Hens were killed for tissue samples on days 0 and 21 after withdrawal of EDB-contaminated diet. A GC procedure was developed to detect EDB in the gaseous phase of broken-out egg samples in a closed container. Based on the dose response of EDB in eggs, detection sensitivities were 3.7 to 4.5 ppb depending on the statistical methodology. Eggs had EDB by day 6 of feeding EDB diets, reached a plateau value of 30 ppb by day 8, and no longer contained detected EDB by day 6 of withdrawal.


Limited data have been reported on the male reproductive effects of EDB which is structurally similar to the testicular toxin dibromo-chloropropane (DBCP). The majority of data regarding EDB's spermatoxotoxicity has been obtained in bulls, an unconventional test species. As such, the present study was conducted to determine the effects of EDB upon rat sperm. Long Evans hooded male rats were intubated with 0.22, or 44 mg/kg/day EDB for 12 or 30 days and sacrificed 24 hours after the last dose. Cauda epididymal sperm counts, spermatozoon enumeration from homogenized testes, and sperm morphology was assessed. Body, liver, kidney, and reproductive organ weights were also measured and histopathological evaluations conducted on the epididymis, testes, and liver. The males treated with 44 mg/kg/day showed an increase in liver and kidney weight and a decrease in epididymal weight, cauda weight, epididymal sperm count, and spermatozoon enumeration by 12 days of treatment. There was a significant decrease in spermatozoon enumeration after 30 days of treatment in the 22 mg/kg/day group while the other parameters measured in this group were not significantly affected. These results suggest that EDB may affect male reproductive function through a testicular effect and/or a post-testicular effect at the level of the epididymis. This research was supported by March of Dimes Grant 59-104 and NIEHS ES-07073.
RELATIONSHIP BETWEEN BLOOD AND SEMINAL CONCENTRATIONS FOR 2-ETHOXYETHANOL (EE) AND ETHYLENE DIBROMIDE (EDB). D. Oudiz, M. Hurt, and H. Zenick Dept. of Envir. Hlth., Univ. of Cincinnati, Cincinnati, OH 45267.

Xenobiotics have been detected in the semen of both animals and man. The present study was designed to examine the distribution and time course of two environmentally relevant compounds, EE and EDB in the blood and semen and their predictive relationship. Male, dutch belted rabbits (4-6 months) were intubed with either 1000 mg/kg/EE or 22 mg/kg/EDB. Blood samples were obtained at 0.25, 0.5, 2, 4, 6, and 8 hrs. and semen samples were collected at 0.5, 2, 4, and 6 hrs. EDB treatment was continued for 10 days with blood and semen collected on the last day. Blood and semen concentrations of EE peaked within 30 min. The semen/blood ratios were greater than 1 at all time points (x=1.70±0.30). The curves for both the blood and semen levels were parallel, however, suggesting that the blood levels could be used to predict the semen concentrations for this compound. The data obtained from the males exposed to EDB was considerably more variable and a consistent relationship between blood and semen concentrations could not be determined. In general, levels of EDB in the semen were considerably higher than the blood levels. After 10 days of exposure, the blood levels decreased while the semen levels remained constant or increased. Therefore, blood does not appear to be a good predictor for semen levels with EDB (NIOSH 1-R03-DH01859).


The interaction of chlorine with natural organic material in drinking water results in the formation of halogenated acetonitriles (HAN). HAN are suspect as mutagens and carcinogens, and acetonitrile (AN) is teratogenic to the hamster. We have examined AN and 5 HAN for prenatal toxicity in an in vivo bioassay using the Long-Evans rat. Pregnant dams received 50-55 mg/kg/day of test compound by intubation on days 7-21 of gestation. Litters were evaluated on post partum days 1 and 4 within and across the variables of birth weight, viability, weight gain, sex ratio. On day 7 litters were culled to 4 and retained until puberty. Administration of all HAN resulted in reduced maternal weight gain and litter size. Birth weight, survival and growth of pups were also diminished, the severity of effects increasing with halide substitution. Both di- and trichloroacetonitrile (TCAN) caused early and late resorptions, and TCAN produced extensive neonatal death. Since the percentage of administered HAN excreted as thiocyanate is reduced with substitution at the α-carbon, these data suggest that cyanide release is not implicated in their developmental toxicity. (This abstract does not necessarily reflect U.S. EPA policy).


Methoxy-, ethoxy- and butoxyacetic acids (MAA, EAA and BAA) are metabolites of the corresponding ethylene glycol monomethyl, ethyl and butyl ethers (EGME, EGE, EGBE) respectively. EGME and EGBE produce testicular damage in the rat after oral or inhalation exposure. Further, MAA and not EGME is responsible for the testicular toxicity observed in vitro. The purpose of the present study was to compare the testicular effects of MAA, EAA and BAA in the rat at doses equimolar with 100, 250 and 500mg EGME/kg at varying times (up to 40) following a single oral administration. MAA produced damage specifically to spermatocytes at all dose levels within 24hr. Similar effects were seen with EAA but at the high dose level only. BAA was without testicular effects at all dose levels and time points studied. In testicular cell cultures, results indicated a specific toxicity to pachytene spermatocytes within 24hr with MAA and EAA treatment (5μM) only, with the former being more potent. The results suggest that the pachytene spermatocyte is the target cell, with these metabolites producing a marked structure-activity relationship in terms of testicular damage.


Ethylene glycol monomethyl ether (EGME) and its major metabolite 2-methoxyacetic acid (MAA) have been shown to produce a rapid, spermatogenic-stage specific degeneration of spermatocytes in rats. 2-Methoxyacetaldehyde (MALD) is a postulated intermediate in the metabolism of EGME to MAA. Mature male rats were administered either a single oral dose of MALD at levels of 195 and 974μg/kg or a single i.p. injection at 97 and 487μg/kg. Comparisons were made with a testicular toxic dose of MAA at 392μg/kg by both routes. Animals were killed 3 days after dosing and tissues removed for histological evaluation. MAA produced a characteristic testicular lesion by both routes of exposure. MALD did not significantly reduce testicular weight, but did cause a similar pattern of cellular damage to that seen with MAA. Sertoli-cell and germ cell cultures were exposed to both MAA and MALD over a range of doses (0-5μM) for 24hr. MALD and MAA produced significant increases in germ cell shedding, predominantly of pachytene spermatocytes (MALD > MAA). Hence, MALD produced the pattern of effects previously seen with MAA and EGME, and may play a critical role in the toxicity of EGME and an intermediate metabolite.

2-Ethoxyethanol (EE) has been shown to be a testicular toxin with its metabolite, ethoxyacetic acid (EAA), being the active agent. In a previous study, the pachytheine spermocyte was shown in vivo to be the germ cell stage most sensitive to EE. It has been reported that the pachytheine spermocyte has limited capabilities for energy metabolism. The current investigations have centered on the effects of EE or EAA on this function as measured by changes in rates of O2 consumption and ATP levels in pachytheine spermocytes. Pachytheine spermocytes were isolated by centrifugal elutriation. The cells were preincubated at 320°C for 30 min. with either 1 mM EAA, 10 mM EAA, 10 mM EE or vehicle. The cells were monitored for changes in O2 rates with the sequential addition of lactate and 2,4-dinitrophenol (DNP). There was a significant decrease in the DNP rate, an increase in lactate/endogenous ratios and an increase in DNP/lactate ratios when the cells were treated with 10 mM EAA.

Moreover, treatment of separate samples with 1 or 10 mM EAA produced a significant reduction in ATP levels when compared to lactate-only controls. These data indicate that EAA may, in effect, be producing an uncoupled respiratory state in the spermocyte which could contribute to the toxicity seen in these cells. (NIHES ES-07072).

LOCALIZATION OF RADIOACTIVITY FROM 2-METHOXYETHANOL (1.2-14C) IN MATERNAL AND CONCEPTUS COMPARTMENTS. R.B. Sleet, J.A. John-Greene and F. Welsch. C.I.T., Research Triangle Park, NC

2-Methoxyethanol (ME) given by gavage specifically induces paw malformations in CD-1 mice on gestation day (gd) 11 (vaginal plug + day = gd 0). Tissue localization of 14C from ME (13 μCi; 0.93 μmoles/mouse) was examined in dams and conceptus at times ranging from 5 min to 48 hrs after gavage on gd 11 by autoradiography and 14C quantitation. 14C incorporation into fractions soluble in organic solvents, hot acid or alkali was measured. 14C absorption and distribution were rapid and generalized in maternal and conceptus compartments as indicated by autoradiographic analysis of whole body and uterine sections. Blood 14C peaked by 30 min, remained stable for 1.5 hrs thereafter and later declined by 35% at 6 hrs and by 90% at 48 hrs. Plasma 14C accounted for 100% of blood 14C at 6 hrs and 50% at 48 hrs. Liver 14C at 6 hrs was twice blood 14C (one gram-one ml) and by 48 hrs was 4 times greater. On average, conceptus and embryonal 14C were greater than blood 14C at 6 and 48 hours, ranging from 1.1 to 1.4 times. Total embryonal 14C was 65% acid soluble and 35% acid insoluble at 6 hrs. 14C from ME or its metabolites freely distributed throughout both compartments with some embryonal accumulation. ME derived 14C was incorporated into macromolecules synthesized by the embryo.

2-METHOXYETHANOL (ME) DEPLETES TESTICULAR LACTATE IN RATS. P.J. Beattie and M.J. Brabec, Dept. of Env. & Ind. Health, Toxicology Program, The University of Michigan, Ann Arbor, MI 48109-2029.

Exposure to ME depletes primary spermocytes, resulting in aspermia and testicular atrophy in experimental animals. The major metabolite of ME, methoxyacetic acid (MA), produces similar testicular changes. Little is known about the mechanism of action. Therefore, several biochemical parameters in the testis were investigated. Adult male Sprague-Dawley rats were dosed by gavage with ME, 300 mg/kg/day for 1 to 10 days. Testis/body weight ratios, citrate and lactate concentrations, and sorbitol dehydrogenase (SDH) and gamma-glutamyl transpeptidase (GGT) activity were measured. No alterations were observed in either enzyme activity, or citrate concentration at any time point. Testis/body weight ratios were depleted 25% (p<0.01) by day 5, and 41% by day 10. Testicular lactate concentration was decreased from 212.8 ± 9.3, to 134.9 ± 6.6 μg/g (p<0.001) 24 hr following a single dose of 300 mg/kg ME. Lactate is reported to be an essential substrate for spermocyte viability in vitro. Therefore, this early lactate depletion may contribute to the spermocyte death observed following ME exposure.


While it is clear that DBP induces testicular atrophy, inhibits spermatogenesis and affects Sertoli cells within 3 h, its effects on steroid hormone production have been less well studied. In our studies that investigated the effects of DBP treatment on the morphological and behavioral development of pubertal rats and hamsters, we noted effects which suggested that DBP reduced serum testosterone (T) levels (Gray, 1983). The present study was designed to investigate the effects of DBP on pituitary and testicular hormone levels and testicular IF levels. Seventy five male rats were gavaged with 2 g/kg/day for 4 days. After the last dose the rats were necropsied, serum was taken, the testes were weighed and IF was collected (Sharpe, 1988). DBP treatment reduced testes weight (8%) and IF content (21%). T levels were reduced by 75 to 80% in both the serum and IF in the DBP treated rats while serum and IF progesterone levels were reduced by 50%. In contrast, serum FSH and LH levels were normal in the treated males. These results indicate DBP treatment alters Leydig cell steroidogenesis which in turn affects intratesticular T levels and IF production.

Research supported in part by US Army MRDC IAG # RM21931062-01-0
CORRELATION OF DOSE-DEPENDENT FUNCTIONAL PATHO- 
PHYSIOLOGICAL CHANGES INDUCED BY Di-(2-ETHYLHEXYL)- 
National Toxicology Program, NIEHS, Research Triangle Institute, Research Triangle Park, NC.

Dietary exposure of F344 male rats to 0, 320, 1250, 5000, or 20,000 ppm DEHP for 14 consecutive days resulted in a dose-dependent reduction in total body, testes, epididymis, and prostate weight at 5000 and 20,000 ppm. Degenerative changes were observed in testes along with decreased epididymal sperm density and motility, and increased occurrence of abnormal sperm at 20,000 ppm. There was a trend towards reduced testosterone and increased luteinizing hormone and follicle-stimulating hormone in serum at 5000 and 20,000 ppm. Reductions in fertility parameters were correlated with gonadal effects, although not marked in severity. Average litter size was reduced at 20,000 ppm, but initial pup weights and growth were unaffected, and there were no observed abnormalities in the offspring. Characteristic toxic manifestations of DEHP included increased liver weights and reduced serum triglycerides and cholesterol at all doses. Total protein, albumin and glucose in serum, urinary specific gravity, and urinary excretion of proteins and glucose were increased at 5000 and 20,000 ppm. These data suggest a lack of reproductive dysfunction in male rats at doses of DEHP below those which produce mild testicular degeneration.

THE TERATOGENIC EFFECT OF INHALED ACROLEIN VAPOR IN SPRAGUE-DAWLEY RATS. J.M. Gerhart, R.S. Hatoum and C.L. Leach. IIT Research Institute, Life Sciences Research, Chicago, IL.

Acrolein (AC) is a reactive aldehyde, irritant, cytotoxin, metabolite of the immunosuppressive drug cyclophosphamide, and a constituent of cigarette smoke (440 ppm/puff). AC is considered non-teratogenic by virtue of its in vitro and intraperitoneal test dose; however, in vivo segment-II test results are lacking. Therefore, an in vitro study was designed in which groups of 21 to 26 Sprague-Dawley dams were exposed to AC vapor at target concentrations of 0.1, 1.0 or 3.0 ppm. A sham control group of equal size received filtered air only. Dams were exposed for 6 hr/day on gestation days 7-16. Slight maternal toxicity, evidenced by a reduction in dam body weight at term, and a slight decrease in male, but not female, pup body weight were seen in the 3.0 ppm group only, which suggested potential fetal growth retardation. Skeletal examination revealed an increased incidence, relative to sham controls, of anomalies/malformations which primarily involved the sternum, rib cage and pelvic girdle of the 1.0 and 3.0 ppm dosage groups. Salient skeletal observations included missing ribs, sternum, and pelvic bones and gnailed rib cages. External and wet visceral examinations revealed no malformations of the soft tissue. These data suggest that AC vapor exposure induces teratogenic events in Sprague-Dawley rats and warrant further in vivo investigation of this compound.
The present study was designed to evaluate the effects ACR on male reproductive function. A pretreatment (baseline) assessment of copulatory behavior and ejaculated semen (sperm count, morphology, motility) was conducted on adult, male Long-Evans hooded rats. Males were then begun on ACR treatment (0.5, 100, 200 ppm) administered in the drinking water (15/group). Copulatory behavior was monitored biweekly. Semen evaluations were conducted on Week 9 of treatment and fertility tests in the controls and 100 ppm group at Week 10. Hindlimb spaying appeared in the 200 ppm group by Week 4 and progressed to the extent that these males were sacrificed at Week 6. In the absence of hindlimb spaying, males receiving 100 ppm ACR displayed incessant intromissions during copulation. As a result, ejaculation and sperm transport were impaired at Week 9, such that semen was recovered from the uterus of only one female sacrificed post-ejaculation. This impairment also was reflected in subsequent fertility tests. All females that mated to 100 ppm males were sperm positive; however 2/3 showed no evidence of impregnation. Yet, epididymal sperm counts and testicular histopathology were normal. An unanticipated finding was a significant increase in post-implantation loss in the impregnated dams of the 100 ppm males. This research, supported by USEPA CR808880, does not necessarily reflect policy or opinion of USEPA.

The response of primary testicular cell cultures to ethylene glycol monomethyl ether (EGM) and its major metabolite methoxyacetic acid (MAA) was studied. In vivo these testicular toxins specifically damaged the germinative and dividing spermatocytes. Mixed cultures of Sertoli and germ cells were prepared from testes of immature rats by a two-step enzymic digestion (FA Chem. Toxicol. 22 123, 1984). Cultures incubated with 50 mM EGM or EG monomethyl ether for 24 - 72 hr showed no morphological changes but MAA (2-10 mM) caused degeneration of the pachytene and dividing spermatocytes. As in vivo, earlier spermatocytes, spermatogonia and Sertoli cells were unaffected. Ethoxyacetic acid produced similar but less marked changes whereas propoxy- and butoxyacetic acid and methoxyacetylglucine, a further metabolite of MAA, had no such effects. The same differences in toxicity were observed in vivo. Decreases in carnitine acetyltransferase and lactate dehydrogenase-X in the cultures paralleled the morphological signs of toxicity. Cultures did not convert [14C] EGM to MAA or further metabolise [14C] MAA. The results show a close correspondence between the testicular toxicity of alkoxycarboxylic acids in vivo and in cell culture, suggesting a common mechanism of action in each case.

The role of metabolism in ethylene glycol monomethyl ether (EGM) induced testicular toxicity has been investigated using Sprague-Dawley rats. Following administration of [14C] EGM (1.5 ppm kg⁻¹ body wt.) to a control group of animals, there was evidence for testicular damage, identified as depletion of the spermatocyte population. Radioactivity detected in urine over 48 hours after dosing accounted for 55% of the dose. The major urinary metabolites were identified by HPLC and isotope dilution analysis, as methoxyacetic acid (MAA) and methoxyacetylglycine (50 - 60% and 18 - 25% of urine radioactivity respectively). Analysis of plasma revealed a rapid conversion of EGM to MAA (t½ for disappearance of EGM = 0.6 ± 0.03 hr) and gradual clearance of radioactivity (t½ = 19.6 ± 2.3 hr). Pretreatment of animals with pyrazole (i.p., 400 mg kg⁻¹ body wt.) one hour prior to [14C] EGM dosing gave complete protection against the testicular toxicity of EGM. Radioactivity detected in the urine over 48 hours (182) was significantly lower than in the control group. The major radioactive peak co-chromatographed with EGM, (30 - 36% of the total urinary radioactivity). MAA and methoxyacetylglycine were not major metabolites. Analysis of plasma revealed almost complete inhibition of the conversion of EGM to MAA. (t½ for disappearance of EGM = 42.6 ± 5.6, clearance of radioactivity t½ = 51.0 ± 7.8 hr)

Inhaled or ingested EGME is teratogenic in Segment II tests. The present study examined the developmental phase specificity of EGME's embryotoxicity. Pregnant mice [day of vaginal plug positive-gestation day (gd) 0] received by gavage single or multiple doses of EGME between gd 7 and 14. Fetuses were examined on gd 18 with standard methods. EGME induced no apparent maternal toxicity, either after multiple exposures to 250 mg/kg or after a single dose of 500 mg/kg. In conceptuses, embryotoxicity and embryolethality were manifested as reduced gd 18 fetal weights and increased resorptions. The induced anomalies were phase specific. EGME in multiple doses between gd 7 and 10 caused exencephaly, while paw anomalies (syndactyly, oligodactyly, stunted digit #1) prevailed during later stages of development. Maximal sensitivity of embryos to EGME-induced paw lesions was on gd 11, and forepaws appeared to be more susceptible than hindpaws. These results demonstrate the sensitivity of paw morphogenesis in mice to EGME.


Methyl-2-butyl ether (MTBE) is utilized as an octane enhancer in gasoline; an inhalation teratology study was conducted under the auspices of the American Petroleum Institute to determine potential hazards to developing fetuses. Mated female CD® Sprague-Dawley rats (25/group) and CD-1 mice (30/group) were exposed during gestation days 6-15 to target concentrations of 0, 250, 1,000, and 2,500 ppm MTBE; animals were sacrificed on day 20 (rats) or day 18 (mice). There were no maternal deaths during gestation, and no significant effects were noted on maternal parameters. In rats there were no MTBE related changes in uterine implantation rates, fetal size, or fetal sex distribution, and no significant effect on incidence of gross or soft tissue abnormalities, skeletal malformations, or ossification variations. A slight increase in fetal absorptions in the low and high-concentration groups of mice was attributed to two mice in each group. The incidence of mouse fetus gross abnormalities and soft tissue or skeletal abnormalities was not significantly increased. A slight increase in fused sternebrae, the only ossification variation, was attributed to fetotoxicity of MTBE.

LETHALITY AND TESTICULAR EFFECT IN MALE RATS FOLLOWING ACUTE INHALATION EXPOSURE TO ORDRA ME®. S.M. MacAskill, G.M. Zwicker and G.L. Sprague, Stauffer Chemical Company, Environmental Health Center, Farmington, CT

ORDRAM®-a synthetic anthraquinone derivative, a novel rodent-specific, testicular atrophy in rats and mice. This study was conducted to establish potential effects following a single, 4-hour inhalation exposure. Adult, Sprague-Dawley rats were exposed to 1.52, 1.84, 2.08 or 3.08 mg/l ORDRAM®. The 14-day LC50 for ORDRAM was 7.21 mg/l in male rats. The average MMAD was between 3-4 microns for each of the 4 exposures. Acute toxicity seen at 1.84 mg/l included labored breathing prior to death. Necropsy showed a high incidence of pulmonary congestion and edema. Pulmonary changes were considered the cause of mortality at high concentrations. The testes of surviving rats were small along with epididymides, seminal vesicles, prostates and coagulation glands. The no-effect level for testicular changes was determined to be 0.08 mg/l in a previous study. In conclusion, a single, inhalation exposure to ORDRAM® produced relatively low toxicity (LC50>2 mg/l) and reduced testes weights in rats.

STUDIES WITH AN INHIBITOR OF OVULATION IN THE RAT. C.M. Milne, R.L. Hassamll, and M.C. Middleton, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: E.A. Lock

The many different processes involved in female reproductive function are coordinated and controlled by circulating peptide and steroid hormones. The control systems are likely to be perturbed, primarily or secondarily, by reproductive toxicants. The mode of action of a substituted triazole (1,1-di-(4-fluorophenyl)-2-(1,2,4-triazol-1-y1)-ethanol; R151885) which delays ovulation in the rat, has been investigated by examining plasma concentrations of those hormones involved in the control of ovulation. Hormones were measured by RIA. A single oral administration of 5mg/kg at midday on day 3 of the 4-day oestrous cycle, delayed ovulation by 24h. The preovulatory peaks of progesterone, follicle stimulating hormone and luteinizing hormone (LH) were delayed by 24h in treated animals, consistent with the delay in ovulation. There was a normal preovulatory oestradiol (E2) peak but E2 levels were depressed late on day 3 and early on day 4. Exogenous E2, given during the first 12h after dosing, prevented the inhibitory effects of R151885 on ovulation, confirming the importance of depressed E2 levels. R151885 appears to delay ovulation by reducing E2 at a critical time. This may prevent adequate priming of the pituitary with consequent inhibition of the LH surge necessary for ovulation.
477 METABOLIC STUDIES WITH AN INHIBITOR OF OVULATION IN THE RAT. A.J. Gray, A.L. Worthington, and M.C. Middleton, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, UK. Sponsor: E.A. Lock.

1,1-Di-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl)-ethanol (R151885) caused a 24 hour delay in ovulation when administered orally at 5mg/kg on day 3 of the oestrous cycle. Under these conditions, triazole and carbonyl labelled R151885 were excreted slowly (approximately 20% over 24hrs), mainly in urine (65-70% over 7 days). Conjugates of R151885 eliminated in bile accounted for 50% of the dose over 72hrs. Approximately 40% of the triazole labelled R151885 was excreted in urine as a polar metabolite, tentatively identified as 1,2,4-triazole. The majority (44%) of the carbonyl labelled R151885 was excreted in urine as a very polar metabolite and a further 15% as 1,1-di-(4-fluorophenyl)-ethane-1,2-diol (identified by GC-MS). Both labels were distributed throughout the body with some accumulation and retention in adrenal cortex, nasoethmoid and liver. Since it has been demonstrated that R151885 itself inhibits oestradiol biosynthesis in granulosa cells in vitro. The in vivo persistence of R151885 could result in a sufficiently long inhibition of oestradiol synthesis to account for the observed delay in ovulation.

478 ALTERATION OF LACTATE METABOLISM IN SERTOLI CELLS AND SPERMATOCYTES BY 1,2-DIBROMO-3-CHLOROPROPANE. Miller, C.E., Welsh, M.J., and Erbsec, M.J. Program in Toxicology and the Dept. of Anatomy and Cell Biology, The University of Michigan, Ann Arbor, MI 48109.

The antispermatogenic properties of 1,2-dibromo-3-chloropropyl (DBCP) have been described in laboratory animals and in exposed human populations. During spermatogenesis, there occurs extensive biochemical interaction between developing germ cells and Sertoli cells. Recent evidence suggests that spermatocytes depend almost exclusively on lactate derived from Sertoli cells as a metabolic substrate. When DBCP (0.25-2.00mM) was added to primary Sertoli cell cultures derived from 20-day-old Sprague-Dawley rats, rates of lactate secretion into culture media were stimulated (0-70%) in a dose-dependent manner. Analysis of culture media for LDH leakage revealed little cytotoxicity under the conditions of DBCP exposure. DBCP also altered lactate metabolism by spermatocytes isolated from 34-day-old rats. Metabolism of [14C]-lactate to [14C]-carbon dioxide by spermatocyte preparations was reduced to 90% and 60% of control values when incubation mixtures contained 0.5mM and 1.0m DBCP, respectively. These observations are consistent with DBCP's disruptive effect on mitochondrial function as previously reported by this laboratory, and further suggest that DBCP's antispermatogenic effects may be, in part, to alterations in lactate homeostasis within the seminiferous tubule.

479 Reproductive Function of Adult Male Rats Following Neonatal Exposure to 1,2-Dibromo-3-Chloropropene. E.M.K. Lui, Dept. of Pharmacol. and Toxicol., University of Western Ontario, London, Ont. Canada.

1,2-Dibromo-3-Chloropropene (DBCP) is known to be a reproductive toxicant in adult mammals. In the present study, we have examined the effect of neonatal exposure to DBCP on the reproductive function of adult male rats. Male rats received s.c. injection of DBCP (1, 5, 10 and 20 mg/kg) once daily on alternate days during the first 20 days of age, and were examined in adulthood. Treatment with 1 mg/kg DBCP did not alter the reproductive parameter examined except a reduction in the seminal vesicle to body weight ratio. Treatment with 10 mg/kg DBCP, however, resulted in marked reduction in testes to body weight ratio as well as serum androgen levels. Analysis of androgen production in vitro from testicular tissues revealed that the basal rate of androgen production per gm of tissue was significantly elevated by neonatal DBCP exposure; however, the responsiveness of testicular tissues to HCG stimulation was obliterated by treatment with 10 and 20 mg/kg of DBCP. The testicular histology of these DBCP-exposed animals showed few numbers of seminiferous tubules and hyperplasia of the interstitium. These data suggest that the development of the male reproductive system could be adversely affected by neonatal DBCP exposure. (Supported by NSERC, Canada).


Timed-pregnant Fischer 344 rats and CD-1 mice were exposed to MIBK vapors by inhalation on gestational days (gd) 6 through 15 at 0, 300, 1000 or 3000 ppm. At sacrifice (gd 21, rats; gd 18, mice), live fetuses were examined for external (100%), visceral (50%) and skeletal (50%) malformations and variations. In both species, maternal toxicity was observed only at 3000 ppm: in rats, by decreased weight, weight gain, and food consumption, clinical signs and increased absolute and relative kidney weight; in mice, by increased mortality, clinical signs, and increased absolute and relative liver weight. Fetal toxicity was observed only at 3000 ppm in both species, rats exhibited reduced fetal weight and reduced skeletal ossification; mice exhibited increased fetal deaths, reduced fetal weight and reduced skeletal ossification. Exposure to 1000 or 300 ppm MIBK during organogenesis resulted in no treatment-related maternal, or fetal toxicity. No increase in embryonic toxicity or fetal malformations was seen at any exposure concentration employed. Sponsored by Chemical Manufacturers Association.
Mice (strain C57B/6, 10 per group per sex) were gavaged with 6 μg/kg TCDD (dioxin) in corn oil, clean soil, 6 μg/kg TCDD added to clean soil, and TCDD contaminated soil equivalent to 6 μg/kg from a 2,4,5-T manufacturing site. Male mice were dosed weekly for at least eight weeks before mating. Female mice were dosed at least two weeks before mating. Pregnancies, birth, litter size and survival, gross malformations, weights, and signs of toxicity were noted.

When TCDD treated males were mated to control females, reproductive outcomes were normal, except for a greater number of conceptions. These small males had a marked greying of their coats. When treated females were mated with control males, a marked reduction in successful reproduction occurred for females dosed with TCDD, and TCDD on control soil, but not in mice treated with contaminated site soil. Vaginal smears were normal for controls and females treated with contaminated soil, but they indicated the absence of estrus cycles in females treated with TCDD, and TCDD on clean soil. These data suggest that the TCDD in contaminated soil is not highly bioavailable.

Supported by contract no. C-29786 N. J. Dept. of Environmental Protection.

A dominant lethal study was conducted in male Sprague-Dawley rats to determine the mutagenicity of nitrobenzene (NB) following treatment by oral gavage for 5 consecutive days. Based on preliminary studies, doses selected for the study were 0 (corn oil vehicle), 35, 70, and 140 mg/kg/day. Several hours after the final treatment, 20 males per group were randomly selected and bred to two females each for 1 week. The females were replaced by two new females per male each week for a total of 8 weeks of breeding. Evaluation of the females during gestation showed that exposure of the males to 140 mg/kg of NB resulted in severe impairment of fertility during Weeks 3 through 6 of breeding. A dominant lethal effect was also suggested, but the effect was obscured by the marked reduction in fertility. A dose response was not evident in that the few effects seen in the 35- and 70-mg/kg groups were scattered and did not appear to be biologically significant. Responses to the final 2 weeks of breeding showed steady improvement in fertility, indicating that the spermatogonial stem cells were probably not damaged; in other words, the reproductive effects of acute NB treatment appear to be transient.

(Research supported by U.S.E.P.A. Contract No. 68-02-3703.)
TERATOGENICITY OF HEXABROMINATED NAPHTHALENE, A TOXIC CONTAMINANT OF POLYBROMINATED BI-PHENYLS, IN C57Bl/6N MICE. C.P. Miller, N.W. Harris, L.S. Birnbaum. NIEHS, Research Triangle Park, NC

Brominated naphthalenes have been identified as toxic contaminants of the polybrominated biphenyl mixture Firemaster which was found to be responsible for the 1973 Michigan contamination incident. Fetal wastage was associated with consumption of the contaminated feed. In order to characterize the embryotoxic and teratogenic properties of hexabrominated naphthalene (HBN), a component of Firemaster, pregnant C57Bl/6N mice were treated on gestation d. 6-15 with 0.5, 2.5, 5, 7.5, 10 mg HBN/kg body weight/day and sacrificed on d. 18. Maternal and fetal toxicity were examined and a complete teratological evaluation was performed. Dose related effects on maternal body and thymus weight, liver/body weight ratios and liver pathology were seen. Dose related increases were observed for subcutaneous edema, involution of lymphatic organs, delayed cranial ossification and fetal mortality. A steep dose response curve was shown for cleft palate, with 4.8% and 98.6% of the fetuses per litter having cleft palate at 1.0 and 2.5 mg/kg, respectively. Kidney lesions were an even more sensitive indicator of toxicity with 100% of the fetuses having bilateral dilated renal pelvis at 1 mg/kg. Thus, HBN is a potent teratogen, producing a spectrum of teratogenic and toxic lesions similar to TCDD and other toxic halogenated aromatic hydrocarbons.

TOXICOKINETICS OF 7,12-DIMETHYLBENZ[a]ANTHRACENE (DMBA) IN RATS FED HIGH LARD OR CONTROL DIET. C.T. Walsh, S. Lee and A.E. Rogers. Department of Nutrition and Food Science, M.T.T. and Department of Pharmacology and Pathology, Boston University Medical Center, Boston, MA.

High lard diets enhance mammary gland tumorigenesis induced by oral administration of DMBA in rats. The dietary effect is exhibited at least in part at the time of carcinogen exposure. Studies were conducted to determine whether a high lard diet alters the plasma or liver concentrations of DMBA or its metabolites. Female Sprague-Dawley rats were fed diets with 12% corn oil and 4% (control) or 23% lard from days 21 to 53 of age. On day 55, 23-DMBA (2.5mg, 50ug, in 0.2ml sesame oil) was administered by gastric intubation. During the subsequent 0.5-30 hr, blood samples were withdrawn from canulas implanted in the jugular vein and livers removed. Radioactivity was determined in plasma, liver homogenates, and their hexane extracts (which recovered 90% of DMBA in control studies). The plasma and liver concentration-time curves for hexane-extractable and total radioactivity did not differ in the animals fed control and high lard diets (ANOVA, P>0.05). Therefore, the high lard induced enhanced carcinogenesis cannot be explained by a change in the gastrointestinal absorption or plasma clearance of DMBA or its metabolites. (Supported in part by the American Institute for Cancer Research and by NIH Grant CA-25538.)

REPRODUCTIVE DYSFUNCTION IN MINK AND FERRETS EXPOSED TO HEXACHLOROBENZENE. M.R. Bleavins, R.J. Aulerich, and R.K. Ringer, Michigan State Univ., East Lansing, MI. Sponsor: M.T.S. Hsla

Carnivores, being at the apex of the food chain are particularly likely to be exposed to high concentrations of chemicals that biomagnify. In these experiments, the highly lipophilic chemical hexachlorobenzene (HCB) was fed to mink and ferrets. Chronic exposure to HCB resulted in reduced reproductive performance as indicated by litter size, stillbirths, kit mortality, and kit growth. Mink birth weights were reduced at 1, 5, and 25 ppm HCB, while ferrets showed this effect at 5 and 25 ppm HCB. Offspring survival exhibited a dose-dependent decrease in both species, with mink kit mortality being 2-3 times greater than was seen for ferrets at each concentration of HCB. To more accurately characterize the HCB toxicity, a cross-fostering study was conducted. HCB-treatment resulted in lengthened gestations without altering other litter parameters. Exposure to HCB via the milk caused an elevated kit mortality over control values (13.6 vs. 5.0%). Continuous exposure to HCB (gestation & lactation) resulted in an even higher rate of kit death (20.5%). However, in utero exposure alone was as serious a threat to offspring survival (22.5% mortality) as was continuous HCB insult. Comparisons to published work shows the mink to be 5-10 times more sensitive to HCB than the rat. The ferret, although less sensitive than the mink was found to be at least 4 times less resistant than rats.

THE ROLE OF HALOGENATION IN THE TERATOGENICITY OF DIPHENYL ETHERS. P.M. Francia and R.L. Metcalf, University of Illinois, Urbana IL 61801.

Eight chlorinated diphenyl ethers were administered percutaneously to outbred Swiss mice either in single doses on day 8 of gestation or in 10 doses between days 5-14 of gestation (day of plug = day 0). The most toxic compound was 2,4,5-trichlorophenyl 4'-nitrophenyl ether, causing 100% mortality when single doses of >16 mg/kg were given. The toxicity of its analog 2,4,6-trichlorophenyl 4'-nitrophenyl ether (CHP) was closer to that of 2,4-dichlorophenyl 4'-nitrophenyl ether or nitrofen, which induced significant neonatal mortality at single doses of >150 mg/kg. Of the monochlorinated phenyl 4'-nitrophenyl ethers, the 2-chloro analog was less toxic than the 4-chlorophenyl 4'-nitrophenyl ether. The latter, like the unchlorinated phenyl 4'-nitrophenyl ether, did not cause excess neonatal mortality even at 10 doses of >150 mg/kg/day. Although 4-chlorophenyl 4'-nitrophenyl ether was not as teratogenic as nitrofen, the analogous 4-chlorophenyl 4'-chlorophenyl ether was more teratogenic than nitrofen. These data suggest that the 4'-nitro substituted does not significantly contribute to the teratogenic potential of diphenyl ethers. Absence of Harderian glands was more readily induced by multiple than by single doses of nitrofen, and was not seen in pups exposed to other diphenyl ethers. Prenatal exposure to 4-chlorophenyl 4'-chlorophenyl ether caused compulsive circling
SENSITIVITY OF MATHEMATICAL MODEL OZONE (O₃) DOSIMETRY TO ANATOMICAL AND VENTILATORY PARAMETERS OF LABORATORY ANIMALS. John H. Overture, Jr., Frederick J. Miller, HERL U.S.E.P.A. Res. Tri. Pl. NC 27711. Sponsor: Judith A. Graham

An O₃ dosimetry model was used to simulate the local absorption of O₃ in the lower respiratory tract (LRT) of rats and guinea pigs. The model takes into account LRT anatomy, transport in the lumen and air spaces, transport and chemical reactions in the mucous and surfactant layers and in the underlying tissue and capillaries. Two anatomical models (based on literature data) for each species were used to investigate their influence in predicting absorption. Absorption along typical paths in different lobes was also studied as well as the effects of tidal volume and inhalation time. A nine zone anatomical model for rats resulted in a 91% uptake regardless of the different ventilatory parameters used; the peak O₃ dose occurred in the terminal bronchioles. However, the other anatomical model, with 23 generations, showed a much greater sensitivity of dose to ventilatory parameters as well as a lower percent uptake (50% to 75%, depending on parameters). The largest O₃ dose occurred in the first generation after the terminal bronchioles. Results for the guinea pig will be presented also. (This abstract does not necessarily reflect EPA policy.)

NASAL DEPOSITION OF ACETONE AND ETHANOL VAPORS - A PHYSIOLOGIC MODEL. J.B. Morris and D.G. Cavanagh, School of Pharmacy, Univ. of Connecticut, Storrs, CT.

Pulmonary ventilation-perfusion models are based on the assumption that inspired non-reactive gases equilibrate with the alveolar capillary blood. These models predict that deposition will remain constant with respect to time (quasi-steady state deposition under constant velocity flow conditions provided there is no recirculation of absorbed gas molecules in the bloodstream), with the ratio of deposition to penetration (D/P) linearly related to the inverse of the inspiratory flow rate. To determine if upper respiratory tract (URT) acetone and ethanol deposition could be described by these models, the deposition of these vapors was measured in the surgically isolated URT of male Sprague-Dawley rats at inspiratory flow rates of 70, 100, 150, 300 or 500 ml/min and exposure times ranging between 3.3 and 13.3 min. URT acetone deposition exhibited quasi-steady state conditions. A linear relationship was observed between the D/P ratio and the inverse of the inspiratory flow rate for both acetone (r=0.988, n=16, p<0.0001) and ethanol (r=0.963, n=20, p<0.0001). These results suggest that nasal capillary blood becomes saturated (i.e., equilibrates) with these gases and, therefore, URT deposition of these gases in the rat is dependent upon both the URT ventilation and the URT perfusion rates.

A QUANTITATIVE METHODOLOGY FOR ASSESSING HEALTH EFFECTS OF INHALED TOXICANTS. E.D. Swolko, R.L. Wolpert, D.J. McKeen, and B.B. Mangel. Depts. of Pharmacology and Medicine, Comprehensive Cancer Center, Duke Univ. Med. Center, Durham, NC and OAQPS, USEPA, Research Triangle Park, NC

This methodology was established to provide a quantitative understanding of the health effects of inhaled toxicants. An additional benefit is the identification of data gaps which limit our understanding of these effects. Our basic approach is to analyze available literature on health effects of an inhaled toxicant, to predict regional lung dose with a dosimetric biologic model, and to develop relationships between these two data sets. Our first application of this methodology was an analysis of pulmonary ozone dose predictions generated by the Miller Model. The mechanism for obtaining potentially comparable studies, subsequent comparison of these studies, their combination with Miller predictions, and gaps in available data which impede our efforts are presented. This approach could also be used in the study of other inhaled toxicants. Literature analyses would require few modifications; and assuming knowledge regarding uptake, transport, and chemical reactions, models could be developed. Thus, this methodology is generally applicable and provides a mechanism for reducing uncertainties inherent in qualitative interpretations of data. (Supported by EPA Contract 68-02-3869.)


The dual effects of age and sex on the metabolism of theophylline in New Zealand White rabbits was investigated following exposure to 0.3 ppm O₃ for 3.75 hrs. Young animals were 3-4 mo. old while the old were over 2 yrs. Animals were given an air sham 7 days before and after 5 consecutive days of ozone exposure. The elimination t½ of theophylline was significantly (p=.004) prolonged on Day 1 and Day 5 of O₃ compared with the sham days for the old rabbits with no effect being detected in the young. No change was seen in the volume of distribution to account for this effect. No difference was detected based on sex of the animal.

The results suggest an age related sensitivity to the O₃ effect of inhibition of microsomal xenobiotic metabolism.
OZONE STUDIES ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT RED CELLS. M. A. Amoroso, D. Easton, J. Ryer, C. Witz and B. D. Goldstein, Dept. of Environmental & Community Medicine, UMDNJ-Rutgers Medical School/Rutgers Univ. Joint Graduate Program in Toxicology, Piscataway, NJ

The A⁺ variant of glucose-6-phosphate dehydrogenase deficiency (G6PD, A⁺ variant) is a sex-linked inheritable enzyme deficiency which affects approximately 14% of black males and 2% of black females in the United States. It has been suggested that individuals with this disorder will be at risk for significant hematologic effects due to inhalation of atmospheric ozone or other oxidant species. In order to get a true estimate of the potential effects of ozone on G6PD deficient red cells, in vitro and in vivo studies were carried out. Exposure of G6PD deficient (C57 1/2) and non-deficient (C57 6/3) mice to ambient levels of ozone had no effect on red cell GSH. Exposure to higher ozone levels (3 ppm x 4 hrs and 5 ppm x 4 hrs) also had no significant effect on red cell GSH. In vitro exposure of human G6PD deficient red cells to 5 or 10 ppm ozone for 2 hrs flowing at 20 ml/min did not change the GSH concentration, while 5 ppm at 80 ml/min for 4 hrs caused only a 16% decrease in GSH levels. These data suggest that individuals with the A⁺ variant of G6PD deficiency will not be at risk to hematologic effects arising from lowered red cell GSH levels since the latter are not a consequence of exposure to ambient levels of ozone.

EFFECTS OF OZONE ON DENTARY VITAMIN E UPTAKE AND TURNOVER IN THE MOUSE LUNG. D.L. Morgan, T.L. Furlow, and D.B. Mengel, Departments of Pharmacology and Medicine, Comprehensive Cancer Center, Duke University Medical Center, Durham, N.C. 27710

The protective effects of vitamin E in mice to the toxic effects of O₃ suggest that vitamin E in lung tissue is destroyed or metabolized faster on exposure to this pollutant. The effects of dietary fat and O₃ exposure on the kinetics of vitamin E uptake and turnover in the lung were investigated. CD-1 mice were equilibrated to either a polysaturated fat diet (5% stripped corn oil) or a saturated fat diet (5% stripped lard), free of vitamin E or supplemented with 105 mg d, l-α-tocopheryl acetate/kg diet. Equilibration of vitamin E in lung, liver, and plasma was determined by HPLC after feeding the vitamin E-supplemented diet for various times. Exposure to O₃ during vitamin E equilibration of mice appeared to cause an initial increase in tissue and plasma levels. To determine the effects of inhaled O₃ on vitamin E turnover, mice were initially fed a vitamin E-supplemented diet. These same animals were then simultaneously fed a vitamin E-deficient diet and exposed to 1.0 ppm O₃, 8 hr/day for 1, 2, 4, or 8 days. O₃ exposure caused an initial increase in plasma Vitamin E and a decrease in lung vitamin E levels. No detectable changes were observed in liver vitamin E content. (Supported by EPA Grant RB80283.)


Although rodents are the most commonly studied species for ozone (O₃) research, no acute pulmonary function studies have been reported. We examined the pulmonary function of Fischer-344 rats (250 gm) before, during and after exposure to ambient concentrations of O₃. To enhance test sensitivity, rats were simultaneously exposed to O₃ and CO₂. CO₂ increased minute volume, thereby increasing pollutant uptake, much like exercise in controlled human exposure studies. Rats were surgically prepared with an intrapleural catheter one day prior to testing. On the day of testing, four awake rats were loaded into a head-out plethysmograph attached to a 0.3 m³ stainless steel exposure chamber. Measurements of ventilation, airway resistance and dynamic compliance were recorded. Exposure to O₃ (0, 0.12, 0.25 or 0.5 ppm) was for 2.25 hr with pulmonary function evaluated every fifteen min. During exposure to O₃, 15 min exposures to CO₂ (2, 4, 6, 8%) were added. Each CO₂ exposure was followed by a 15 min recovery period. After the 8% CO₂ recovery period, O₃ exposure was terminated. Preliminary results indicate that at 0.5 ppm O₃ breathing rate and expiratory resistance increased while tidal volume and dynamic compliance decreased. Exposure to 0.25 ppm showed similar effects but of decreased magnitude, while 0.12 ppm did not appear to effect pulmonary function.

NO₂ produces changes in lung morphology at ambient concentrations. However, the functional consequences of these alterations have not been determined. The present study examined newborn and young adult rats continuously exposed to NO₂ (0.5, 1.0 or 2.0 ppm) for up to 6 weeks with twice daily 1 hr spikes equal to 3X the baseline concentration. This spike to baseline ratio was chosen to simulate morning to evening urban rush hour conditions. Pulmonary function of newborn Fischer-344 rats and young adult rats was measured after 3 and 6 week exposures. Young adult rats were examined after 1, 3 and 6 week intervals. Lung volumes, pulmonary mechanics, and efficiency of ventilation were evaluated. Lung volumes increased in the neonatal rats after 3 and 6 week exposures to 1.0 and 2.0 ppm and were unchanged in young adult rats. Lung compliance was increased in neonatal rats exposed to 1.0 or 2.0 ppm for 3 weeks, was unchanged in neonatal rats exposed for 6 weeks, as well as in the young adult rats exposed for 1 and 3 weeks. Compliance decreased in young adult rats exposed to 2.0 ppm for 6 weeks, however this effect was not seen after a 3 week recovery. The observed changes in compliance were the result of differences in lung volume. All other pulmonary function measurements in neonate and young adult rats were unchanged by NO₂ exposure. (This abstract does not necessarily reflect EPA policy.)

EFFECTS OF ACUTE NITROGEN DIOXIDE(NO₂) EXPOSURES ON LUNG CLEARANCE PATHWAYS. T. A. Vollrath, R. Driscoll, and R. B. Schlesinger, Institute of Environmental Medicine, New York University Medical Center, New York, NY

Clearance of inhaled particles deposited in various regions of the respiratory tract is mediated through different mechanisms. Mucociliary transport removes particles from the ciliated airways, whereas clearance from the alveolar region is mediated by alveolar macrophages (AM) which are ultimately removed via the mucociliary escalator. These two clearance pathways were studied in rabbits in vivo following a 2 hour exposure to either 0.0(0) ppm, 0.3, 1.0, 3.0, or 10.0 ppm NO₂. Both mucociliary and early (14d) alveolar clearance were assessed simultaneously in the same animal by external retention measurements of radioactively tagged tracer aerosols. NO₂ produced no observable change in mucociliary clearance rate at any concentration. On the other hand, an apparent dose related response in alveolar clearance rate was evident, with the greatest effect observed at the 1.0 ppm level. These results suggest that the altered rate of clearance from the alveolar region was not due to dysfunction of mucociliary clearance. Rather a change in AM functional activity is likely responsible. In addition, although the change in alveolar clearance rates at the higher NO₂ levels was less than that at the lower ones, it is probable that a more adverse effect on the functional capabilities of the AM was produced. Assays are currently being performed to test this hypothesis.

LUNG CELL PROLIFERATION AFTER SHORT-TERM EXPOSURE TO DIESEL EXHAUST, CARBON BLACK OR NITROGEN DIOXIDE. E. S. Wright, Biomedical Science Dept., GM Research Labs, Warren, MI. Sponsor: E. W. Lee

Diesel exhaust (DE), a complex mixture of carbon particles and several gases, is one of many inhaled pollutants that has shown to cause changes in lung cell populations. The effect of exposure to DE on DNA synthesis in lung tissue and type II cells was investigated. Parallel experiments were performed with carbon black (CB) and nitrogen dioxide (NO₂), with exposure conditions designed to mimic exposure to individual components of DE. DNA synthesis was measured by the in vitro incorporation of 3H-thymidine into rat lung DNA. Labeling index in alveolar wall cells and type II cells was determined using light microscopy and autoradiography. Continuous exposure to 6 mg/m³ DE particulate or 7 ppm NO₂ elicited significant increases in DNA synthesis and type II cell labeling index. The effects of DE and 7 ppm NO₂ were similar in that the maximal response occurred after 2 days of exposure, and all measures returned to control levels by 1 week of continuous exposure. The peak response to 7 ppm NO₂ was significantly greater than the response to DE. Exposure to 6 mg/m³ CB or 2 ppm NO₂ did not cause similar changes. These findings suggest that the initial transient wave of cell proliferation in the lung after exposure to a high concentration of DE is attributable to the presence of NO₂ in the gas phase of the exhaust.

ASSESSMENT OF ELASTASE INSTILLATION, ELASTASE INSTILLATION AND CHRONIC NO₂ INHALATION, AND CHRONIC NO₂ INHALATION AS INDICATORS OF EMPHYSEMA. D. M. Stewart, D.C. Archuleta, L.M. Holland, and K.E. Lehnhrt. Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM. Sponsor: P. E. Morrow

A study was performed to assess emphysematous changes in the rat lung induced by: 1) intrabronchial instillation of elastase (E), 2) a 25-day exposure to 35 ppb nitrogen dioxide (NO₂), and 3) elastase instillation followed by a 25-day exposure to 35 ppb NO₂ (E+NO₂). The mean linear intercept values (LMI) of the E and E+NO₂ lungs were markedly higher than the LMI obtained with lungs from sham-air exposed rats (S). The LMI of the S and NO₂ lungs, however, were not different. Residual volumes of the E and E+NO₂ lungs [2.3 and 2.1 ml, respectively] were significantly greater than those of the S and NO₂ lungs [1.3 and 1.4 ml, respectively]. Directionally similar changes in functional residual capacities, and total lung capacities were found in the E and E+NO₂ groups. No differences in arterial blood gas and pH values, minute ventilation, or breathing frequencies were found among the experimental groups. This study demonstrates elastase instillation brings about architectural, and lung volume and capacity changes consistent with panlobular emphysema, but, these changes did not compromise the ventilatory and respiratory gas exchange status of unanesthetized rats. Also, exposure to 35 ppm NO₂ for 25 days does not result in changes in the size of the alveolar spaces, 2) alter lung volumes of capacities, or 3) impair ventilatory function and gas exchange. [This work was performed under the auspices of the DOE].
Male and female Sprague-Dawley rats were exposed to RP/BR aerosols, ranging from 0.40 to 1.20 mg/l or filtered air for 2.25 hr/day, four days/week for four weeks. During the exposure period, wheezing and labored breathing were observed in male rats exposed to the high dose. Decreased body weights and reduced food consumption were seen at all exposure levels in male rats only. Body weight gains returned to normal after a 14-day recovery period. Biological endpoints were examined within 1-hr after the last exposure and after a 14-day recovery period. Examination of the pulmonary free cells collected by lung lavage showed an increase in total numbers, increased cellular ATP levels and decreased ectoenzyme activity (5' nucleotidase) after most of the RP/BR exposures in both sexes. Protein levels in the lavage fluid were elevated after the high doses. Mild to moderate terminal bronchial fibrosis was found in rats of both sexes exposed to the medium and high doses. (No treatment related changes were found in tissues outside the respiratory tract.) Except for the fibrosis, most toxic changes were reversible.

(Supported by the U.S. Army Medical R&D Command, Contract No. DAMD17-82-C-2121)

The leading cause of residential fire deaths is inhalation of smoke from cigarette ignition of upholstered furniture. A literature review of the combustion toxicity of FPUR, the most widely used filling material in upholstered furniture, was conducted to compare results using different test methods. Five major methods were compared: University of San Francisco/National Aeronautical and Space Administration and Federal Aviation Administration/Civil Aeromedical Institute test methods which measure time to incapacitation (t1) and time to death (t2), and the National Bureau of Standards, Federal Republic of Germany (DNV) and University of Pittsburgh tests which measure mortality expressed as the LC50. Based on limited toxicological data, it was concluded that despite differences in composition, densities, etc. of the FPUR used, the data were comparable when the same toxicological indices were compared. Further, the differences between t1 and t2 were not considered sufficiently sensitive to provide a meaningful index for incapacitation.

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Sulfur dioxide deposits efficiently in the nasal cavity, yet little is known about its effects in these airways. To study the nasal toxicity of SO2, male Sprague-Dawley rats were exposed to filtered air or 80 ppm SO2 for 6 hr/day for 1 or 5 days. Following exposure upper respiratory tract (URT) uptake of acetone vapor was measured. Rats were then killed and nasal tissues were removed and pooled to form two homogenates: anterior tissues (nasomaxillary turbinates, septum) and posterior tissues (ethmoturbinate). The ability of the nose to scrub vapor from the airstream is an important nasal defense mechanism; SO2 had no effect on URT acetone uptake. Total protein, blood, and net protein content (net-total minus blood content) of anterior tissues were unaltered by 5 day SO2 exposure. While total protein and blood content of posterior homogenates were increased 3-fold by SO2 (p<0.025), net protein was unaltered, indicating the importance of correcting nasal tissue parameters for contaminating blood. No change in any of these parameters was observed following single 6 hr SO2 exposure. Thus, slight injury to posterior nasal tissues was observed. The lack of effect in anterior tissues suggests a resistance to the effects of this irritant. The mechanism(s) of this resistance remain obscure.

The leading cause of residential fire deaths is the inhalation of smoke resulting from cigarette ignition of upholstered furniture. As most upholstered furniture available contains PUF, evaluation of its combustion toxicity is one important factor to consider when assessing fire safety. Fire retardants have been added to many foams to improve fire performance without regard to their effects on combustion toxicity. Therefore, the toxicity of combustion products of two FR and the corresponding NFR PUF was assessed using the National Bureau of Standards Toxicity Test. Rats were exposed for 30 min to flaming or non-flaming combustion products of various concentrations of PUF. CO, CO2, O2 and HCN and blood carboxyhemoglobin (COHb) were measured during exposure. Animals were weighed daily during the 14 day post-exposure period. Only the flaming FR material caused within exposure deaths. All other deaths occurred post-exposure usually following extensive weight loss. Comparison of the 30 min + 14 day LC50 values of the FR and NFR foams indicated they were not toxicologically significantly different. Based on gas levels and COHb values, deaths were not due to CO or HCN alone. In one case, interaction of CO, CO2 and HCN could account for the observed toxicity within exposure.

In postcrash aircraft fires, only a few minutes are often available for escape. To assess the potential of combustion gases to impair human escape, a signalled avoidance task was developed for use with the juvenile African Savannah baboon. After a 5-minute exposure, the animal was required to select and depress the correct lever to open an escape door and exit into the adjacent compartment of a shuttlebox. With CO, the EC50 for escape failure was 6850 ppm. Acrolein (12 to 2780 ppm) neither prevented escape nor affected escape times, despite irritant effects at all concentrations. Similar results were obtained with HCl (190 to 17,200 ppm) in that, despite severe irritant effects, all animals successfully performed the escape task. With a comparable shuttlebox and escape paradigm for rats, the EC50 of CO was 6780 ppm. Five-minute exposures to HCl (11,800 to 76,730 ppm) did not prevent escape but severe post-exposure respiratory effects and lethality occurred at 18,000 ppm and higher. In both species, escape time was not affected by HCl but a concentration-related increase in intertrial responses was evident. The data suggest that humans may be able to tolerate much higher concentrations of irritant gases than anticipated without prevention of escape. (Supported by FAA Contract No. DTPA03-81-00065)


Inhalation toxicities of 0 quartz (Min-U-Sil) and fly ashes from a fluidized bed combustor (FBC) and a pulverized coal combustor (PCC) were compared. Fischer-344 rats were exposed 7 hr/day for 20 days to aerosols of these materials at concentrations of 36 to 38 mg/m3. Control rats were exposed to filtered air. Animals were sacrificed at 2, 4, 6, and 26 weeks after initiation of exposures. Functional, biochemical, and histological endpoints were evaluated on lungs and lung-associated lymph nodes. Respiratory function was not affected in quartz or fly ash-exposed animals. Clearance of particles was impaired following inhalation of both quartz and fly ash, but more severely following inhalation of quartz. At 4 weeks after exposure, significant increases in airway lactate dehydrogenase, airway protein content, and airway β-glucuronidase activity occurred in quartz-exposed rats which were 2 to 3 times greater than those caused by fly ash. The extent of influx of nucleated cells was similar after inhalation of all materials. Histopathological lesions of pulmonary fibrosis were markedly more severe in animals exposed to quartz. Compared to the known responses to quartz, fly ash has a lower order of pulmonary toxicity. (Research performed under U.S. DOE Contract DE-AC04-76EV01013.)

506 TOXICOLOGICAL EFFECTS OF THE INTERACTIONS OF FIRE GASES AND THEIR USE IN A TOXIC HAZARD ASSESSMENT COMPUTER MODEL.  B.C.  Levin, M.  Paabo, G.  Bailey, S.E.  Harris, and J.L.  Gorman.* National Bureau of Standards, Gaithersburg, MD.

The toxicity of single and multiple fire gases is being studied to determine whether the toxic effects of a material's combustion products can be explained by the interactions of the major fire gases or if minor, more obscure gases need to be considered. LC50 values for Fischer 344 rats have been determined for carbon monoxide (CO) in air for 10, 20, 30, and 60 minute exposures using the NBS Toxicity Test Method. Similar LC50 values have been calculated for hydrogen cyanide (HCN) in air. Combination experiments of CO and HCN indicated that they act in an additive manner. Non-lethal concentrations (1-5%) of carbon dioxide (CO2) in combination with sublethal concentrations of CO caused death of the rats. Decrease of the oxygen (O2) concentrations in the presence of various combinations of the major toxic fire gases increased the toxicity even further. Examination of the combustion products from some materials tested at their LC50 values indicated that the observed toxicity could be explained by the interaction of the main toxic fire gases. These results on the toxicological effects of multiple fire gases are being used in the development of a computer model for predicting the toxic hazard that people will experience under various fire scenarios.

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Rats were exposed to TiO2 by inhalation exposure at concentrations of 0, 10, 50, and 250 mg/m3 for 2 years. Inhaled particles were mostly engulfed by intraalveolar macrophages. A fraction of the inhaled particles was retained in the membranous pneumocytes and interstitial macrophages. A dense accumulation of dust cells was found in the perivascular or peribronchial lymphoid tissue. Some dust cells entered peribronchial lymphatics or pulmonary blood vessels and the general circulation. Dust cells in the hyperplastic peribronchial lymphoid tissue were exposed directly in the luminal surface of the airways and were subsequently eliminated via airways. Massive dust deposition was observed in the tracheobronchial lymph nodes. Dust transmigration was markedly reduced in the cervical lymph nodes, but only a trace amount of dust particles was found in the mesenteric lymph nodes. Some dust cells entered either blood or lymphatic vessels in the lymph nodes and then migrated into the general circulation. The incidence of extrapulmonary dust deposition in the liver or spleen was increased in a dose-related fashion similar to the lung dust burden. Since there was no tissue response to translocated particles in the lymph nodes, spleen, or liver, potential health effects appear to be negligible.

Exposure of F-344 rats to selected concentrations of FA for 6 hr resulted in the formation of DNA-protein cross-links (DPX) in the nasal respiratory mucosa (TAP 76, 26 (1984)). The concentration of DPX was observed to increase non-linearly with increases in the FA concentration. To investigate the role of FA oxidation by FA dehydrogenase (FDH) in defense against the formation of DPX, the concentration of DPX in the respiratory mucosa of normal rats and of rats depleted of GSH (by administration of phorone, 300 mg/kg, i.p.) was determined at 0.9, 2, and 6 ppm. Exposures to [3H]- and [14C]FA were carried out for 3 hr, one day after a single 3-hr exposure to the same concentration of unlabeled FA. The yield of DPX increased non-linearly with exposure concentration, both in normal rats and in GSH-depleted rats. Concentrations of DPX in the DNA (pmoles/mg) of normal rats at 0.9, 2, and 6 ppm were 0.0 (-0.5 ± 0.8), 11.5 ± 2.7, and 82.7 ± 13.0, respectively. Corresponding concentrations in the DNA of GSH-depleted rats were 4.9 ± 1.2, 37.1 ± 11.6, and 154.3 ± 16.0, respectively. GSH depletion had larger effects on the yield of DPX at lower FA concentrations, indicating that oxidation of FA by FDH is more effective at low than at high exposure concentrations in preventing DPX.


FA and AA are nasal carcinogens in rats. In rats exposed to FA at 6 or 15 ppm for 3 hr, there was no significant change in the concentration of non-protein sulfhydryl (NPSH) in the nasal mucosa. In contrast, 3-hr exposures to AA at concentrations of 0, 100, 500, 1500 or 3000 ppm resulted in a dose-dependent decrease in the NPSH concentration in the respiratory and olfactory nasal mucosa. Respiratory NPSH was depleted to a greater extent than olfactory NPSH. The depletions of NPSH by AA were not expected, since AA does not bind irreversibly to GSH. Further studies showed that organic peroxides (OP) are present in both distilled and undistilled AA. OP form after AA is exposed to air, and they react with GSH rapidly and irreversibly. AA free of OP was prepared by distillation under nitrogen; AA containing a high concentration of OP (0.35%) was also prepared by distillation in air. Subsequent exposures of rats to equal concentrations of AA vapor (1500 ppm) containing either no detectable OP or OP (1 ppm) resulted in similar depletions (20%) of NPSH in the nasal respiratory mucosa. These results suggest that NPSH depletion in the nasal mucosa was not due to inhaled OP. Although the mechanism of nasal mucosal NPSH depletion by AA is unknown, these data indicate that AA and FA exert different toxic effects in this tissue.

BIOLOGICAL RATE OF DIFFERENT 1-NITROPYRENE AEROSOLS AFTER REPEATED INHALATION EXPOSURES IN RATS. J.D. Sun, R.K. Wolff, and R.O. McClellan. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

1-Nitropyrene (NP) is potentially carcinogenic and found in many air pollutants. Fisher-344 rats were exposed by nose-only inhalation to aerosols of pure 3H-NP (7 mg/m3), pure 3H-NP (8 mg/m3) + 5 ppm SO2, 3H-NP (8 mg/m3) coated on Gac203 particles (27 mg/m3), or 3H-NP (8 mg/m3) on Gac203 particles (28 mg/m3) + 5 ppm SO2 for 2 hr/day for 1, 1 wk, 2 wks, or 4 wks. Particle sizes were 0.1-0.2 μm, and exposure times were 1/2 < 1 hr. However, the clearance rate of radiolabel from kidneys was increased with longer exposure times (1 d exposure, 1/2 1 d vs. 4 wk exposure, 1/2 8 d.), but unaffected by the different exposure atmospheres. Also, 3H levels in lungs after 4 wk exposures were 2-3 fold higher using 3H-NP aerosols with Gac203 as compared to 3H-NP without Gac203. These data suggest that kidneys may be a target organ for NP toxicity, and repeated exposure may intensify this toxicity. Also, particle association may increase the lung retention of this compound. (Research supported by the Health Effects Institute's Interagency Agreement No. BS-16 under U.S. Department of Energy Contract No. DE-AC04-76EV01013.)

DISTRIBUTION OF BENZO(A)PYRENE IN THE MOUSE FOLLOWING INTRATHRACHEAL OR INTRANASAL INSTILLATION. C. T. Schmittlin and A. E. Monson. Dept. of Pharmacol. and Toxicol. Medical College of Virginia, VCU, Richmond, VA 23298.

As part of a study to assess the effects of a subchronic benzo(a)pyrene (BaP) exposure on local and systemic immunity, the deposition patterns of a 3H-BaP suspension (2.5 mg/kg) following intrathoracal (IT) or intranasal (IN) instillation in B6C3F1 mice were compared. The vehicle contained micelles of phosphatidyl choline, the major phospholipid of surfactant. The affinity of BaP for the phospholipid resulted in a homogeneous suspension of BaP in saline. Approximately 50 times more 3H-BaP reached the lung by the IT route. The clearance of 3H-BaP in the lung was followed by measuring radiolabel in lavage fluid and cells, lung interstitial fluid and cells, and in lung strata. Radioactivity was immediately detected in the blood, liver, spleen and intestine after both routes. Two to 3 times more 3H-BaP was recovered in the blood and intestine after IT instillation. The liver, spleen and lung-associated lymph nodes appeared to preferentially retain BaP, containing a higher ratio of radiolabel than that found in the blood and intestine. Although some BaP was deposited in the lung by the IN route, the low efficiency of deposition makes this route unacceptable compared to the IT route. Supported by PHS Grant ES05319-01.
513 EXCRETION RATES OF 1-NITROPYRENE IN RATS FOLLOWING ACUTE AND EXTENDED INHALATION EXPOSURES. R.K. Wolff, J.D. Bond, and R.O. McIlveen. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Fischer-344 rats were exposed nose-only to $^{3}$H-1-nitropyrene (NP) aerosols for 2 hr/day for 1, 2, 4, or 4 wks. There were 4 exposure atmospheres: pure NP (7 mg/m³), pure NP (8 mg/m³) plus 5 ppm SO₂, NP (8 mg/m³) coated on Ga₃O₅ particles (27 mg/m³), and NP (8 mg/m³) on Ga₃O₅ (28 mg/m³) plus 5 ppm SO₂. Rats were also exposed for 1 hr to pure $^{14}$C-NP aerosols. Particle size was 0.1-0.2 µm activity median diffusion diameter for all aerosols. Excretion patterns were similar for all exposure lengths and modes with 80 ± 6% of the radioactivity excreted in the feces. Clearance half-times of 21 ± 3 hr for both urine and feces for the extended exposures were similar to values of 16-20 hr measured after 1-hr exposures at lower concentrations (0.07-1.0 mg/m³). Exposed exposure produced little retention of NP, at least in lung, and excretion was not prolonged or pathways altered. Thus, results from these acute exposures can be used to model dose relationships at high exposure concentrations for long periods of time. (Research supported by the Health Effects Institute's Interagency Agreement No. 83-16 and U.S. DOE Contract No. DE-ACO4-76EY01013.)

514 NITROSAMINE FORMATION IN THE ISOLATED VENTILATED PERFUSED LUNG. C.R. Shoaf, J.R. Stokes, and D.R. Helfant. Departments of Pharmacology and Medicine, Comprehensive Cancer Center, Duke University Medical Center, Durham, N.C. 27710

This study seeks to determine the uptake of hydroxyproline (HP) into the lung from the vascular system using the IVPL system and further to determine whether this amine is trapped sufficiently to act as a reactant with NO₂ in the production of nitromonomo- hydroxproline (NHP). NHP was synthesized and analyzed: C, 37.65%; H, 5.12%; O, 39.90%; N, 17.30%. Separation of HP and 3H-HP was effected by HPLC. Detection of 3H-HP was by scintillation counting, and detection of NHP was done spectrophotometrically. Using 14C-dextran as an extracellular marker, the absorption of HP by the lung from the vascular system at supply rates of 2.52 X 10⁻⁴, 1.19, and 12.0 umol/min was 8.99 X 10⁻³, 4.15 X 10⁻¹, and 1.08 umol/min, respectively. HP had a greater volume of distribution than the vascular marker and appears to be removed from the vasculature by a carrier mediated process rather than a diffusional process since at high concentrations the net rate of uptake is not proportional to the concentration infused. Using HP as a trapped amine in the IVPL system ventilated with NO₂ and the NHP detection system developed, the ability to quantitate nitrosamine formation in the lung is now available. (Supported by EPA Cooperative Agreement CR809715.)


This study was designed to characterize the fibrogenic action of asbestos in a variety of animal species. Wistar, Sprague-Dawley, F-344, and Long-Evans rats; B6C3F1 mice; Golden Syrian hamsters; and Hartley guinea pigs, were intratracheally instilled with 0.7 mg crocidolite/g lung once/week for 3 weeks. At 3, 6, and 12 months post-institution, 4 animals from each group were evaluated for pulmonary function, histopathology, and biochemistry. Histopathologic examination of 1.0 micron sections of glycol methacrylate-embedded lung tissue demonstrated giant-cell granuloma in peribroncholar, endobroncholar, and periductal regions at 3 months post-institution. A transition to fibrocellular granuloma with radiating bands of interstitial fibrosis was seen at 6 months. Measurements of CO diffusing capacity, quasistatic compliance, and forced vital capacity, indicated that the injury was obstructive in nature. Biochemical measurements showed significantly increased levels of hydroxyproline, elastin, DNA, and protein at 3 and 6 months when compared to controls. The F-344 rat and B6C3F1 mouse showed significantly increased levels of hydroxyproline at 6 months in comparison to 3 months. This is in contrast to morphological data indicating that these two groups were the least responsive. Supported by U.S.D.O.E. No. DE-AC02-76CH00016.

516 RECOVERY AND CHARACTERIZATION OF LUNG-DEPOSITED KEVLAR® ARAMID FIBERS IN RATS. D. P. Kelly, S. J. Williams, G. L. Kennedy, Jr., and K. F. Lee, E. I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P. O. Box 50, Newark, DE.

Kevlar® is an aramid fiber which is being used as an asbestos replacement in gaskets and friction materials. A method has been developed to recover Kevlar® fibers from rats exposed to inhaled or intratracheally instilled fibers. Tracheobronchial and pulmonary tissues as a unit are saponified with 11% KOH in ethanol at 70°C for 4 hours. The fibers are isolated on cellulose acetate filters, then counted and measured using a phase contrast microscope. Quantitative recovery of fibers was demonstrated in Sprague-Dawley rats instilled with 50 µg (6 x 10⁶ fibers) Kevlar® suspended in 1.0 ml saline/Tween® 20. In inhalation studies using this technique, fibers were recovered from rats at periods up to 28 days after a 6-hour exposure to 400 Kevlar® fibers/cc. Airborne fibers had 12 µm median lengths and <0.5 µm diameters. Immediately after exposure 6.9 x 10⁶ fibers were recovered with a size distribution similar to that found in the chamber atmosphere. Although similar numbers of fibers were recovered from rats 4 and 21 days after exposure (approx. 4 x 10⁶ fibers), median fiber lengths dropped to 9.7 µm at 4 days and to 5 µm at 21 days after exposure. These results demonstrate the feasibility of conducting deposition and clearance studies with inhaled aramid fibers using this recovery technique.
The primary biomolecular targets of oxygen toxicity must be investigated without interference from biochemical changes resulting from extensive cell edema and inflammation. We have evaluated various lung injury parameters in order to establish the probable time of such damage-induced, secondary biochemical changes. Adult rats were exposed to 95% O₂ for 6, 12, 18, 24 and 48 hrs following a lung injury using semen, bronchoalveolar lavage and lung homogenate. At 48 hrs edema was indicated by an increased wet weight to dry weight ratio. Lavage content of protein, lactic acid dehydrogenase, and angiotensin converting enzyme (ACE) all more than doubled, probably as a result of edulation. Inflammation was indicated by an increase in lavageable neutrophils. None of these inflammation-exudation indicators were changed at 6, 12, 18 or 48 hrs. The earliest indication of lung endothelial cell injury was a decrease in total lung ACE of 10% and 16% at 18 and 24 hrs, respectively. These results suggest that mechanism-related biochemical changes can be investigated up to at least 24 hrs of O₂ exposure without complications resulting from biochemical and cellular alterations which occur secondary to damage. (Supported by US DOE contract No. DE-AC02-76EV03490 and NIH Training Grant No. 5 T32-HL07026).

Several sulfite-binding proteins were separated from rat lung and plasma, and a human lung carcinoma cell line (A549 cells) after exposure to Na₂S₃SO₃ in vitro for 2 hours. Proteins were separated by gel filtration on Sephadex G-200, and analyzed by isoelectric focusing (IEF) and SDS polyacrylamide gel electrophoresis. Rat lung cytosol had a sulfite-binding protein with an aggregate molecular weight > 200,000 on non-reducing SDS gels, and a pI of approximately 7.0. This species is not present in samples reduced with 2-mercaptoethanol. A protein of similar characteristics was found in A549 cells. Rat plasma has three sulfite-binding proteins ranging in molecular weight from 56,000 to 70,000 on SDS gels. These proteins separate into at least 5 bands on an IEF gel, all with pIs between 4 and 5. No sulfite-binding proteins were found in rat liver cytosol. These proteins may represent specific sites of interaction of sulfite or inhaled SO₂ with cellular biopolymers. Their role in the toxicity of SO₂ is being investigated. (Supported by NIHES Grants ES07031 and ES02916.)

METHYL BROMIDE TOXICITY: A TARGET ORGAN? S.B. Haber,¹ R.T. Drew,¹ S. Eustis,² and R.S.H. Yang.³

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B6C3F1 mice exposed to 200 ppm methyl bromide, 6 hrs/day, for 10 days exhibited a high mortality rate (60% for females and 90% for males), while mice exposed to 120 ppm, 6 hrs/day for 63 days (12 week study), showed minimal exposure-related effects. Rats exposed to 0, 30, 60, or 120 ppm methyl bromide, 6 hrs/day, 5 days/week, for 13 weeks showed a reduction in body weight in the 120 ppm males and females in the latter half of the study. To further investigate the target organ toxicity of methyl bromide, a 6 week study was conducted in which male and female B6C3F1 mice and Fischer 344 rats were exposed to either 160 ppm methyl bromide or air 6 hrs/day, 5 days/week. The animals were scheduled for sacrifice either after 3, 10, or 30 exposures. When 50% mortality was observed in any group, the surviving animals in that group were sacrificed. Mice were evaluated for body weight, mortality, organ weights, hematology and histopathology. In addition to these endpoints, urine chemistry and plasma enzymes were assessed in the rats. Significantly different mortality rates were observed between the two species, with the mice demonstrating a higher sensitivity to 160 ppm methyl bromide than rats. Body weight differences were exposure-related and similar to those reported earlier.

ACUTE AND SUBCHRONIC INHALATION TOXICITY OF 1,4-BUTANEDIOL IN RATS. L. A. Kinney, B. A. Burgess, E. P. Stula and G. L. Kennedy, Jr., R. I. du Pont de Nemours and Co., Inc., Haskell Laboratory, P. O. Box 50, Newark, DE 19714

1,4-Butanediol is a clear, slightly viscous liquid with a low vapor pressure used in manufacturing polyester glycols and polyurethanes. The material has a low order of acute inhalation toxicity with a 4-hour AIC of 15 mg/L in male rats. In the subchronic study, groups of 10 male Crl:CD® rats were restrained and exposed nononly, 6 hours/day, 5 days/week for 2 weeks to aerosol concentrations of 0, 0.29, 1.1 or 5.2 mg/L. Rats were given pathological, urinalysis, and clinical chemical examinations after the 10th exposure and after 2 weeks recovery. No adverse effects were observed in rats exposed to either 0.29 or 1.1 mg/L. Rats exposed to 5.2 mg/L had depressed body weights from the third exposure to 4 days post exposure. After 10 exposures, these rats had increased erythrocyte counts and hematocrits and decreased serum cholesterol concentrations. Pathological examination showed slight atrophy of lymphoid cells in the thymus and depressed mean heart weights. No adverse effects were observed after 2 weeks of recovery. Both 0.29 and 1.1 mg/L were "no effect" concentrations under the conditions of this test. At 5.2 mg/L, rats showed non-specific systemic effects which were reversible after 2 weeks.
Whereas butadiene (BD) was weakly active in an inhalation bioassay in rats (IISRP, 1982), a recent long-term inhalation study (NTP bioassay program) in mice (B6C3F1) revealed a considerable carcinogenic activity. In rats, metabolism of BD proceeded via oxidation to 1,2-epoxybutene (EB) and followed saturation kinetics. Saturation of BD metabolism occurred at an atmospheric concentration of about 1000 ppm. Inhalation pharmacokinetics of BD in mice also revealed a saturation pattern, but metabolic saturation was not reached until 1800 ppm. Metabolic clearance (first order metabolism) was 6.500 (rat=4.500) ml * h^-1; maximal metabolic elimination rate was 4500 (rat=2200) nmol * h^-1. Covalent binding of reactive BD metabolites to nucleic acids served as a dose monitor for the actual concentrations of EB in both species. The results show that BD is metabolized by mice at a higher rate compared to rats. This may be one determinant for the higher susceptibility of mice to BD induced carcinogenesis.

In a study on the hemotoxicity of ethylene oxide in mice we previously reported (Toxicologist 4: 161, 1984) that C57BL/6J mice survive for at least 10 weeks when exposed to 255 ppm of ethylene oxide for 6 hours per day and 5 days per week. We have recently observed that SEC/R1 and SEC/18e mice succumbed when similarly exposed. All 31 of the SEC mice were dead after 19 exposures (22 after 10 exposures). Histological sections revealed extensive internal hemorrhage, particularly in the lungs. In contrast, only 4 of 85 C57BL/6J mice died after at least 20 exposures to ethylene oxide. (SEC/R1 X C57BL/6J)F1 hybrids, like their C57BL/6J parents, were resistant to ethylene oxide; thus sensitivity of strain SEC/R1 mice to ethylene oxide appears to be a recessive trait. A population of backcross animals, progeny of SEC/R1 females mated with (SEC/R1 X C57BL/6J)F1 males, are being classified for visible and biochemical markers. These mice will be exposed to ethylene oxide to determine whether sensitivity versus resistance is controlled by one or more genes that can be mapped to specific chromosomes of mice.

In a study designed to investigate their response to heavy metals, a group of American alligators were administered a single intracardiac injection of cadmium chloride, at a dose of 1 mg Cd/kg body weight. At sacrifice, the highest concentration of Cd was found in the liver (22.5 μg/g), bound to a soluble protein with characteristics similar to mammalian metallothionein (MT). Gel filtration of cytosol, using Sephadex G-75, revealed a peak containing Cd and to a lesser extent Zn, having the same relative elution volume (Ve/Vo) as rat hepatic Cd,Zn-MT. Anion-exchange chromatography (using DEAE-Sephacel) of material having a Ve/Vo of 1.7-1.9 revealed a major Cd-peak corresponding to rat Cd,Zn-MT(I) and a minor peak corresponding to Cd,Zn-MT(II). Neither of the peaks contained significant amounts of Zn. Heat denaturation. of alligator hepatic cytosol, followed by selective acetone precipitation (at 0-40, 40-60 and 60-80 percent acetone (v/v)) yielded material in the 80 percent pellet which had a molar Cd:Zn ratio of 9.15:1, compared to 0.67:1 in the corresponding material isolated from Cd-pretreated rat liver. Ultraviolet spectral analysis of the material purified from alligator liver displayed an absorbance peak between 250 and 260 nm with no peak at 280 nm, characteristic of mammalian MT.
525 INDUCTION OF METAL-BINDING PROTEINS AS AN ADAPTIVE MECHANISM TO ENVIRONMENTAL METAL EXPOSURE. W.H. Benson and W.J. Birge. Division of Pharmacology, Toxicology and Nuclear Pharmacy, School of Pharmacy, Northeast Louisiana University, Monroe, LA and the Graduate Center for Toxicology, University of Kentucky, Lexington, KY. Sponsor: T.H. Eickholt.

The induction of tolerance to heavy metals in natural populations of fish has been previously demonstrated. Fish taken from a metal-contaminated flyash pond were significantly (p<0.05) more tolerant to cadmium and copper than fish collected from relatively uncontaminated hatchery ponds. However, after ash pond fish were transferred to uncontaminated water, tolerance to cadmium and copper decreased significantly. These results indicated that in natural populations of fish an adaptive mechanism functions in response to prolonged metal exposure. To examine the induction of metallothionein (MT) in response to environmental metal exposure, MT content of gill cytosol was determined by examining protein fractions eluted in the 10,000-molecular weight range from a Sephadex G-75 column. While no protein or cadmium was detected in the MT fraction of the hatchery fish, the respective mean values of protein and cadmium were 90 μg and 20 mg for flyash pond fish. The cadmium-binding capacity of gill cytosol samples also was determined. The biochemical mechanism responsible for the adaptation of the flyash pond fish was, in part, attributed to increased MT levels.


Translocation of inhaled particulates from the nasopharynx and upper tracheobronchial area to the gastrointestinal tract is a major route of exposure for particles with a mass median diameter of greater than 1 micron. Previous studies in this lab with particulate Mn304 show that preweaning rats have substantially higher tissue Mn concentrations than similarly treated adults indicating possible differences in uptake or elimination or both. This study was conducted to evaluate changes in gastrointestinal movement and retention of particulate matter in the preweaning and weaned rat. 95Sr labeled microspheres were used to evaluate gastrointestinal transit rate (TR) while particulate Mn304 was used to evaluate particulate retention at selected ages. Results show stomach retention time in the preweaning is at least twice that of the postweaning (90 vs 42 min). In general intestinal TR was not different in any of the ages evaluated while transit time increased as intestinal length increased. Analysis of the Mn data demonstrated that the preweaning rat had a 2 component retention curve with halftime between 2 and 6 h for the short component and between 24 and 26 h for the long component. In the postweaning rat only 1 component was identified and had half-time between 2 and 5 h.


The effect of GSH depletors and enzyme inducers on hepatic GST activity and the biliary excretion of GSH, methymercury, cadmium and zinc were studied in rats. Methyl iodide and diethyl maleate, depletors of GSH, did not influence hepatic GST activity but benz[a]pyrene, phenobarbital, pregnenolone-16α-carbonitrile (PCN) and trans-stilbene oxide (TSO) increased the conjugation of various substrates by 16-33, 44-89, 53-97 and 208-279%, respectively. The biliary excretion of GSH was decreased by the depletors of GSH (-88%) whereas two of the transferase inducers (benz[a]pyrene and TSO) had no effect and two (phenobarbital +113% and PCN +149%) increased it. The biliary excretion of methylmercury, cadmium and zinc was reduced by the GSH depletors (-97, -74 and -93%) and enhanced by phenobarbital (+96, +280 and +220) and PCN (+150, +121 and +160%). Treatment with benz[a]pyrene and TSO did not affect the excretion of methylmercury and zinc into bile and decreased that of cadmium. It is assumed that phenobarbital and PCN enhance the biliary excretion of these metals by increasing the transport of GSH, the carrier molecule, from liver to bile. These results suggest that the hepatic activity of GST is not important in determining the rate of biliary excretion of these metals but strongly support the importance of biliary excretion of GSH.

528 METHIONINE AS A SULPHYDRYL SOURCE FOR ZN-INDUCED METALLOTHIONEIN IN CULTURED RAT HEPATOCYTES. A.F. Stein, W.M. Bracken and C.D. Klaassen. Dept. Pharmacol., Toxicol., & Therap., Univ. Kansas Medical Center, Kansas City, KS.

The utilization of methionine (Met) as compared to cysteine (Cys) for the synthesis of metallothionein (MT) is not known; therefore, studies were designed to determine if both these amino acids serve as sulphydryl (SH) sources for Zn-induc ed (300 μM) biosynthesis of MT in cultured rat hepatocytes. Varying concentrations of the amino acids between 0 and 0.5 mM demonstrated that the hepatocytes were able to synthesize only low levels of MT when the concentration of both amino acids was extremely low; however, when either amino acid was present at a high concentration, production of MT was independent of the other amino acid concentration. Induction of MT was compared in four media: complete (0.5 mM Met, 0.5 mM Cys), Met (0.5 mM), Cys (0.5 mM), and SH free. Higher concentrations of MT were produced in the Met than in the Cys media, and no differences were observed between the Met and complete media. The inhibition of the cystathionine pathway, propargylglycine, was added (1 mM) to each of the four media. Reductions in MT levels were not observed in the cells cultured in the complete and Cys media; however, 95 and 92% reductions were observed in the Met and SH free media, respectively. These results suggest that Met can serve as a SH source for the in vitro biosynthesis of MT via the cystathionine pathway. (Supported by ES-01142 and ES-07079).
INDUCTION OF HEPATIC METALLOTHIONEIN BY CHELATING AGENTS IN MICE. P.J. Goering, S.K. Tandon and C.D. Klaassen. Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

Induction of metallothionein (MT) synthesis in liver occurs following exposure to a variety of chemical and environmental insults and, in some cases, has been attributed to enhanced hepatic uptake of zinc. Chelating agents, commonly used in therapy of heavy metal intoxication, alters the levels of essential metals in liver, kidney and serum. Therefore, the purpose of this study was to determine the effects of acute exposure to seven common chelators on the concentration of MT in liver. Adult male Swiss Webster mice were injected with the chelators and hepatic MT was quantitated by a Cd-radioassay. Ethylene-diaminetetraacetic acid (EDTA) increased hepatic MT 5 to 6 fold between 12 and 48 hrs. Cadmium, a known inducer of hepatic MT, produced a 14-fold increase in the hepatic concentration of MT. Other chelators produced the following increases: diethylidithiocarbamate (10-fold), 2,3-dimercaptopropanol (5-fold), 2,3-dimercaptopropanol (5-fold), diethylaminedithiocarbamate (5-fold), diethylthiourea (5-fold), and nitrotriacontic acid (2-fold). Therefore, chelating agents can increase the concentration of MT in liver of mice. This may have implications for their efficacy in the treatment of cadmium intoxication because Cd bound to MT is less available for elimination. (Supported by USPHS Grants ES-011142 and ES-07079.


Cultured rat hepatocytes could provide a model system for evaluating whether various metals can increase metallothionein (MT) synthesis. Thus, the objective of this study was to measure MT synthesis in cultured hepatocytes exposed to either zinc (Zn), cadmium (Cd), mercury (Hg), copper (Cu), manganese (Mn), lead (Pb), cobalt (Co), nickel (Ni) or vanadium (V). Following a 48 hr exposure period, MT was quantitated by a Cd/hemoglobin binding assay. Cell viability was monitored by protein synthesis activity and cellular K+ concentration. Increases in MT concentration were noted for Zn (2100%), Cd (640%), Cu (240%), and Mn (220%). However, even at maximum tolerated concentrations Cu, Mn, Pb and V did not increase MT. Of the non-inducing metals, only Cu competitively interfered with the Cd/hemoglobin assay. MT synthesis was also monitored by S-cysteine incorporation into MT. Cd and Zn enhanced S-cysteine incorporation 2-4 fold following a 4 hr exposure; Cu and Mn failed to increase S-cysteine incorporation into MT above control levels. These data establish that Cd, Zn, Hg, Cu and Mn exposure increases MT concentrations in primary cultures of adult rat hepatocytes but Cu, Mn, Pb and V do not. (Supported by USPHS Grants ES-011142 and ES-07079.)


The intracellular distribution of Cd has classically been explored by cell disruption and isolation of Cd-metallothionein (MT). This study was designed to quantify intracellular kinetic pools of Cd in intact cells and to identify the Cd-MT containing pool with conditions known to modify MT synthesis. Hepatocytes were obtained by collagenase perfusion of liver biopsies and plated as monolayer cultures. Cells were loaded with 0.5 μCi 109Cd/ml and CdCl2 to give 0.2 to 1 μM Cd in Williams' Medium E. Cells were treated with Cd alone or combined with 0.2 μM dexamethasone, 5 μM cycloheximide, or both. After 20 hrs 109Cd efflux was measured. In 0.2 μM Cd, cellular Cd was distributed in three kinetic pools: Total Cd (S3) was 0.42 nmol Cd/mg protein with distribution in S1, S2, and S3 of 3, 3, and 94%, respectively. S1 and S3 exchanged with T1/2 of 1 and 8 min, while the S3 T1/2 was 3d. Dexamethasone treatment increased S3 by 23%. This increase was prevented by concomitant treatment with cycloheximide which indicates that Cd-MT is contained in the S3 pool. Increasing Cd concentration increased S3 while S1 and S2 remained low capacity pools. These results are consistent with the physiological T1/2 of Cd and offers a useful model for studying Cd metabolism.
PHARMACOKINETICS OF SOLUBLE NICKEL AEROSOLS IN RATS. D.L. Deal, M.I. Tayyeb, and B.R. Henkel. Departments of Pharmacology and Medicine, Comprehensive Cancer Center, Duke University Medical Center, Durham, NC 27710.

Nickel is inhaled as both atmospheric particles and cigarette smoke. We have reported that nickel is absorbed from lung of rats exposed to NiCl₂ aerosols by a carrier-mediated process, with an apparent Km of 1292 ng/lung and an apparent Vmax of 39 ng/hr. We now report the pharmacokinetics of Ni(NO₃)₂ aerosol. Ni(NO₃)₂ is also absorbed from the lung by a concentration-dependent process, with rates which parallel those found with NiCl₂. Male Sprague-Dawley rats were exposed to aerosol concentrations of 100 and 1000 μg Ni⁺⁺/m³ as Ni(NO₃)₂ (MMAD 0.8 μm, σg 1.1) for 2 hours and their lungs were removed at various times after exposure and analyzed for Ni⁺⁺ by AA spectrophotometry. The t½ for absorption of Ni⁺⁺ was 48 hours for 1000 μg/m³ and 25 hours for 100 μg/m³. The removal of NiCl₂ from the nasopharyngeal region of rats exposed to 400 or 1000 μg/m³ was also investigated, by analyzing nasopharyngeal lavage samples taken at various times after aerosol exposure. We found that Ni⁺⁺ is absorbed from the nasopharyngeal region by a concentration-dependent process, with a t½ of 0.4 hours for the 400 μg/m³ exposure and a t½ of 1.4 hours for the 1000 μg/m³ exposure. We conclude that lung burdens are dependent on nickel chemical speciation and not the anion. (Supported by NIH Grant ES01859.)

538 COMPARATIVE TOXICITY OF FIVE NICKEL COMPOUNDS TO RAT LUNG. J.M. Benson, R.F. Henderson, R.D. McClellan, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; and A.H. Rebar, Purdue University, W. Lafayette, IN.

We have investigated early biochemical, cytological, and morphological changes in rat lung exposed to four nickel (Ni) compounds of widely differing solubility in aqueous media: nickel oxide (NiO, highly insoluble), nickel sulfide (NiS₂, intermediate solubility), nickel sulfate (NiSO₄, soluble), and nickel chloride (NiCl₂, soluble). Groups of 48 (24 male, 24 female) Fischer 344/Lov rats were administered a single Ni compound by intratracheal instillation. Twelve rats within each group were administered 0.01, 0.10, or 1.0 μmol Ni, and 12 served as vehicle controls. Rats were sacrificed at 1 or 7 days after Ni administration. The right lung was lavaged and the fluid obtained was analyzed for lactate dehydrogenase (LDH), β-glucuronidase (BG), total protein (TP), and nucleated cells (NC). The left lobe was examined for morphological changes. Minimal changes in LDH, BG, TP, or NC were observed at one day. No significant changes in any parameter occurred in animals exposed to NiO. NiS₂, NiSO₄, and NiCl₂ caused dose-dependent increases in LDH, BG, TP, and NC at 7 days. Alveolitis was seen in NiS₂-, NiSO₄-, and NiCl₂-exposed animals. The results indicate the following toxicity ranking: NiS₂ > NiSO₄ > NiCl₂ > NiO. (Research performed under U.S. Department of Energy Contract No. DE-AC04-76EVO1013.)

539 DIFFERENTIAL EFFECT OF LEAD UPON REGULATORY ENZYMES ACTIVATED BY CALCIUM. Gary W. Goldstein and Diane Ar, Depts. of Pediatrics and Neurology, University of Michigan, Ann Arbor, MI 48109. Sponsor: J. C. Pounds.

Some toxic effects of lead appear mediated by an interaction with calcium. Phosphodiesterase (PDE) is activated by calcium, but only in the presence of calmodulin. The activation of phospholipase A₂ (PLA) by calcium is independent of calmodulin. The basal activities of PDE and PLA purified from bovine heart and Naja moscambique venom were determined in calcium-free media with 10 μM EGTA. PLA produced a 4.7-fold increase in PDE activity with 2 μg/ml of calmodulin. Calcium did not increase PDE activity in the absence of calmodulin. 20 μM lead without Ca⁺⁺ produced a 3.7-fold increase in the activity of PDE. Activation of PDE by lead also required calmodulin and was inhibited 50% by 20 μM trifluoperazine, an inhibitor of calmodulin binding to enzyme activation sites. In contrast, calmodulin did not influence the 4.2-fold stimulation of PLA by 10 μM Ca⁺⁺. Further, 1 to 100 μM lead did not activate PLA in the absence of Ca⁺⁺ or alter the ability of calcium to stimulate PLA. Lead appears to act as a calcium agonist in the calmodulin-mediated activation of PDE but is without effect in the calmodulin independent activation of PDE by calcium. These differences may in part explain the selective cellular toxicity of lead.

540 MODIFICATION OF LEAD TOXICITY AND DISTRIBUTION BY DIETARY SULFUR AMINO ACIDS IN GROWING CHICKS. D.M. Latta and W.E. Donaldson. Toxicology Program. N.C. State University. Raleigh, N.C.

The effects of inadequate vs. adequate levels and sources of total sulfur amino acids (TSAAs) on lead (Pb) toxicity in growing chicks were studied. The TSAAs inadequate, basal diet contained .3% methionine (MET) and .3% cystine (CYS). The basal diet was supplemented with either CYS, MET, or MET and CYS to give total TSAA contents of 0.9%. Pb was fed at 0 or 1000 mg/kg with each diet. Pb depressed body weight gain with all diets. Addition of MET or MET and CYS improved growth compared with the basal diet regardless of Pb level fed. Pb addition improved growth only in the presence of Pb. Pb increased hepatic non-protein sulphydryl concentration (NPSH) in chicks fed all diets. All CYS and MET additions increased NPSH over basal levels regardless of Pb level. With the diets containing Pb, CYS addition resulted in the highest NPSH concentrations. Pb accumulation in muscle, bone and blood was reduced with all TSAA adequate diets. Additionally, the MET diet resulted in lower Pb levels in kidney and liver as compared to basal. The data suggest that addition of CYS and MET facilitates increased excretion of Pb in growing chicks fed diets deficient in sulfur. However, for maximum alleviation of the growth depression by Pb, dietary MET level must be adequate.
Pathologic changes in the brain microvasculature and brain edema accompany the development of acute Pb encephalopathy. Investigation of Pb metabolism and toxicity in BBE cells may provide information necessary to understand the pathogenesis of Pb encephalopathy in vivo. Experiments were performed to examine the cellular homeostasis of 210Pb and concentration dependent metabolism of Pb in BBE cells. BBE cells were isolated by enzymatic digestion of brain tissue. The cellular homeostasis of 210Pb was investigated by labeling cultures for 20 hr in 210Pb (3 μM) and kinetically analyzing 210Pb washout curves. Cellular Pb metabolism was defined by 3 intracellular pools, S1, S2, and S3. Nearly half of S1 was chelatable and identified as an extracellular fraction of S1. Doubling of Pb concentration resulted in higher Pb concentrations in all pools, indicating lead homeostasis in BBE cells differs from other cell types in which lead accumulates primarily in the S3 (mitochondria) pool. At 50 μM Pb, BBE Pb concentration was 5 times greater than at 3 μM. The relative pattern of lead distribution did not change with concentration, indicating that Pb accumulation is concentration dependent and that Pb homeostasis is similar at high and low concentrations.

Although high hepatic levels of Zn/Cu-Metallothionein (MT) have been reported in newborn rat liver, little is known about the maternal nutritional status on the synthesis of MT. Tied pregnant rats were fed either with a Zn-D, Cu-D, Fe-D or control diet from day 12 of gestation until delivery. The dams and pups were sacrificed on day one of birth. The livers from pups were analyzed for Zn, Cu and Fe levels by atomic absorption spectroscopy and MT by Cd-hem method. The low plasma levels of Zn, Cu or Fe in dams confirmed the respective maternal deficiencies. Maternal ZnB resulted in about 50% decrease in hepatic MT levels in newborn rats, whereas CuD resulted in a 25% reduction and FeD had no effect on the MT levels. The nutritional deficiencies of newborn rats were demonstrated by reduced levels of the respective metals in the hepatic tissues. Fractionation of hepatic cytosols from the pups by Sephadex G-75 gel filtration showed that in FeD pups, the Fe was depleted from the high mol. wt. fraction, whereas in both ZnB and CuD pups, the respective metals were depleted mainly from the low mol. wt. MT fraction. These results demonstrate a specific effect of maternal Zn or Cu deficiencies on the storage of Zn and Cu as MT in newborn rats.

(Supported by MRC, Canada and NIH.)

Hepatocyte nodules (HN) generated by different models exhibited a decrease in phase I and an increase in phase II drug metabolizing enzymes. This unique pattern was considered responsible for the resistance of the HN towards several xenobiotics. The present study was designed to determine whether this biochemical pattern is unique for HN or a general property of the liver cell but is expressed when perturbed by other conditions as well. In this study, a biochemical pattern very similar to that seen in HN was observed in lead nitrate treated rat liver. Male rats were given i.v. 0.9% NaCl or 25, 50 and 100 μmol/kg of lead nitrate. Rats were sacrificed after 36 hrs. Lead nitrate treatment resulted in a significant increase in liver weight compared to control. An increase in DNA content and protein concentration in all fractions occurred when the results were expressed per liver. Administration of lead nitrate resulted in a decrease of phase I components such as cytochromes P-450 and aminopyrine N-demethylase activity and an increase in phase II components such as glutathione, glutathione S-transferase and DT-diaphorase. The various biochemical parameters studied were correlated and were comparable to HN. This study demonstrates that the biochemical pattern in lead nitrate treated rat liver is very similar to that seen in HN.

Safetv evaluation studies of trace metal additives Zn, Cr, Cu and Mn in rats, rabbits and dogs. L.G.K. Wong and B.S. Brar, Department of Drug Safety Evaluation, Revlon Health Care Group, Tuckahoe, NY.

Trace metal additives Zn, Cr, Cu and Mn were formulated to be used in total parenteral nutrition (TPN) via iv drip. In acute studies 5 rats/sexdose were administered iv via tail vein Zn, Cr, Cu and Mn solution at dose levels of 1815, 6.8, 680 or 362 μg/kg, respectively. Similarly, rabbits received half of these dose levels on a body weight basis. Body weight, clinical observations and gross necropsy at the end of 7 days revealed no adverse effects. In order to conduct subchronic safety studies rats were permanently implanted with a PE10 catheter threaded approximately 5 cm into tail vein. The test article was administered iv with an infusion pump at the rate of 4 ml/kg/hr for 7 to 10 hours daily for 14 days. The dosage levels were 363 μg/kg/day of Zn, Cr, Cu or Mn solution added to 5% dextrose for 5 animals/sex/group. Similarly, 3 dogs/sex/group received 108, 404, 403 or 21.5 μg/kg/day of Zn, Cr, Cu or Mn, respectively by iv drip for approximately 8 hours daily for 14 days. Control animals received 5% dextrose solution. During the course of these studies iv infusion of heavy metals produced no adverse effects on growth, daily observations, clinical pathology and gross and histopathological evaluation of tissues.

The bioavailability of Pb in kidney is mediated in part by binding to high affinity cytotoxic lead-binding proteins (PbBPs), which are not found in liver. Addition of semipurified 11,500 dalton PbBP to liver ALAD reaction mixtures reverses the inhibition of this enzyme by Pb, which may explain the relative insensitivity of renal ALAD to Pb inhibition in vivo and in vitro. This study was undertaken to determine the mechanism by which this PbBP attenuates Pb inhibition of ALAD. Sephadex G-75 chromatography disclosed that addition of renal PbBP to hepatic ALAD reaction mixtures resulted in a marked increase in binding of $^{203}$Pb to the PbBP. Zinc (Zn) is known to activate ALAD and is also present in the PbBP fraction (6 $\mu$M in reaction mixtures). Zn activates hepatic and renal ALAD over a range of 1.5 to 25 $\mu$M and also reversed the IC$_{50}$ Pb-Inhibited activity. The possible displacement of Zn from PbBP under ALAD assay conditions (37°, + GSH) was assessed by Sephadex G-25 chromatography. Approximately 25% of the Zn in the PbBP fraction was released, and this value was not affected by addition of IC$_{50}$ Pb or altered assay conditions (4°, - GSH). Thus, the PbBP fraction attenuates Pb inhibition of ALAD in vitro by chelating Pb and apparently serving as a potential Zn donor for this enzyme.

Supported by NRSA 5732 ES 07126.

BINDING OF MANGANESE IN HUMAN AND RAT PLASMA. A. M. Scheuhammer and M.G. Cherian, Dept. of Pathol., Univ. of Western Ontario, London, Ontario, CANADA.

Albumin (AB), transferrin (TR) and transmanganese (TMn) have all been proposed as the major Mn-binding ligand in plasma. The present investigations were initiated in order to resolve these discrepancies. Compared to other metals (Cd, Zn, Fe) $^{55}$Mn was found to bind very poorly to purified AB in aqueous solution at neutral (7.4) or basic (8.6) pH. The addition of 6 mg oxogenous AB to a 100 $\mu$M aliquot of plasma did not result in an increased level of Mn radioactivity associated with the high mol. wt. proteins after subsequent incubation and fractionation on a G-75 column. Also, incubation of $^{55}$Zn-AB in the presence of excess Mn$^{2+}$ (1mM) did not result in the displacement of Zn from AB. In contrast, $^{55}$Mn was bound to purified TR, not as readily as Fe$^{3+}$ but better than Zn or Cd$^{2+}$. Saturation of TR by pre-incubation with Fe (1.6 mg Fe/mg TR) prevented the binding of subsequently added Mn indicating that Mn probably binds to Fe sites on TR if it is not saturated with Fe. Polycrystalline gel electrophoresis further demonstrated the selective association of $^{55}$Mn with TR rather than with AB in both human and rat plasma. The amount of $^{55}$Mn recovered with TR increased as incubation time was increased due to the requirements that Mn$^{2+}$ be oxidized to Mn$^{3+}$ before incorporation into the protein.

(Supported by Ont. Grad. Scholarship).

THE INFLUENCE OF LACTOSE AND MILK REPLACER ON LEAD ABSORPTION AND LEAD TOXICITY IN CALVES FED GRAIN-HAY DIET. J. Zmudzki, G.R. Bratton, Dept. of Veterinary Anatomy, Texas A&M University, College Station, Texas. Sponsor: S. Safe.

Fifteen Holstein bull calves (5-6 wks old) were divided into 5 dietary groups. Group 1 - grain-hay diet; Group 2 - 10% lactose; Group 3 - 40% lactose; Group 4 - 40% milk replacer, all in grain-hay diet; and Group 5 - liquid milk replacer diet. All calves were dosed orally with 5 mg Pb/kg b.w./day for 7 days via nursing bottle. Within 7 days, all calves on liquid milk diet showed signs of Pb intoxication and one died. No calves from the other groups showed any clinical signs of poisoning. All calves from the groups which had grain-hay in the diet accumulated significantly less lead than the calves on liquid milk replacer diet. However, calves fed 40% lactose or 40% milk replacer accumulated approximately 2 times more Pb in the tissues than calves on grain-hay only or 10% lactose. The highest Pb levels were found in kidney of calves on liquid milk replacer diet (104.87 mg/kg) and liver of grain-hay diet groups (20.02, 19.93, 31.13, and 38.92 mg/kg, respectively). Blood Pb reached a maximum level within 6 h after the first dose, while ALAD activity dropped to about 20% of pre-treatment value within 12 h. These data show that lead-hay diet significantly decreases Pb toxicity and Pb accumulation in young calves. Lactose, on the other hand, is a major factor in milk responsible for an increased lead absorption in calves. (USDA Grant 879-CRS2-0485)

BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES ON THALLIUM-MEDIATED ALTERATIONS IN HEPATIC MIXED FUNCTION OXIDASE (MFO) ACTIVITY. J.S. Woods, Univ. of Washington, Seattle, WA and B.A. Fowler, N.I.H., Research Triangle Park, NC

Previous studies demonstrated impaired hepatic MFO activities following acute thallium (Tl) treatment in rats. These studies investigated the ultrastructural and biochemical basis of that effect. Tl as TlCl$_3$·4H$_2$O was given IP to male rats at 0,50,100 or 200 mg/kg 16 hrs prior to sacrifice. Ultrastructural examination of hepatocytes from Tl-treated rats showed a dose-related loss of ribosomes from the endoplasmic reticulum (ER) and diffuse ER disaggregation with relatively little mitochondrial alteration. Biochemical studies showed a dose-related decrease in microsomal (mic) anionpyrene demethylase and NADPH cytochrome P-450 reductase to 50 and 54% of control, respectively. Concomitantly, a 2-fold increase in mic hemoglobin oxygenase and a 50% reduction in 5-aminolevulinic acid (ALA) synthetase were observed. However, no changes in mic heme or cytochrome P-450 content were seen. Additionally, no changes occurred in the soluble or mitochondrial-bound enzymes ALA dehydratase, MAO or ferrochelatase. In contrast, Tl (0,50,100,200 mg/ml) directly inhibited all SH-dependent enzymes tested in vitro. These results indicate that thallium MFO activity following Tl treatment is principally mediated via physical disruption of the ER and concomitant inhibition of associated mic enzymes, rather than as a result of altered heme biosynthesis or degradation.
HEPATIC AND EXTRAHEPATIC EFFECTS OF ARSENIC ON HEME METABOLISM. M.E. Cobrian, J.C. Connelly and J.W. Bridges. The Robens Institute, University of Surrey, Guildford, Surrey. U.K.

Arsenic exposure is known to alter the activities of some enzymes of heme metabolism, resulting in porphyrimuria in rats. We examined (1) The time course and dose relationships of As effects on the hepatic free heme pool; (2) The target organ selectivity of sodium arsenite (AsIII) and arsenate (AsV). Male Wistar rats (200 g) were dosed subcutaneously 16 h before sacrifice with AsIII (12.5-75 μM/kg) or AsV (25-200 μM/kg). AsIII produced a dose-related decrease in the degree of heme saturation of hepatic tryptophan pyrrolase. The effects of AsV were similar, but less pronounced. AsIII administration resulted in a dose-related induction of hem oxygenase (HO) activity in liver and kidney. Despite the unexpectedly high control activity, large doses of AsIII also increased testicular HO activity by about 50%. AsV induced HO in kidney at doses ineffective in liver or testis. These results in liver suggest that as has two apparently opposing properties i.e. it depletes the regulatory heme pool but induces HO, which contrasts with the effects of many other agents that induce porphyria. Differences in organ susceptibility appear to be related to the oxidation state of As, which has been reported to influence its tissue distribution in rodents (Lindgren et al., Acta Pharmacol. et Toxicol. 51, 253).


Gallium Arsenide (GaAs) has replaced silicon in some semiconductor applications, and electronics industry workers may be exposed via inhalation. Absorption, distribution, metabolism, and excretion, following intracranial instillation of GaAs (mean volume diameter 5.8 μm.) Arsenite (As) and Arsenate (AA) at a dose of 5 mg As/kg were examined in male Syrian Golden hamsters (n=4) 1, 2, and 4 days after administration. Blood, kidney, liver and lung samples were collected. Urinary metabolite profiles were determined on a mixed anion-cation exchange column. Total As content was analyzed by direct hydride flame atomic absorption spectrophotometry after digestion. Blood levels of GaAs, As, and AA were 0.185 ± 0.041, 0.596 ± 0.117 and 0.310 ± 0.056 ppm, respectively, after one day, with GaAs showing a continued absorption phase at two days while the arsenic oxides blood levels decreased. At day one, the liver contained 0.56 ± 0.036, 2.62 ± 0.26, and 0.579 ± 0.14% percent of the dose of GaAs, As, and AA, respectively. The arsenic was rapidly excreted in the urine with 5.4 ± 0.3 (GaAs), 48.5 ± 2.6 (As), and 48.5 ± 2.9 (AA) percent appearing after 4 days. Total recoveries were GaAs: 75.8 ± 4.6%, As: 81.0 ± 5.0%, and AA: 78.5 ± 7.9%. Ratios of the two major urinary metabolites (dimethylarsenic acid/inorganic As species) were 1.41, 1.71, and 0.983 for GaAs, As, and AA, respectively. GaAs metabolism appears to be similar to As.


The acute response of mice and rats exposed to an aerosol of BeSO4 was characterized by lung cell kinetics. Male (n=344) rats and male BALB/c mice were exposed to a nose-only inhalation chamber for 1 hour to a concentration of 13 μg Be/L. Animals were killed at intervals over 21 days. Labeling index (alveolar parenchymal cells labeled with 3H thymidine/total cells counted) peaked at day 8 for rats (3.4% vs 0.4% for controls) and day 5 for mice (1.4% vs 0.2% for controls). Morphologically, greatest tissue reaction was observed in rats at day 10-15 with increased interstitial macrophages, prominent Type II pneumocytes, alveolar fibroin, hemorrhage, and alveolar macrophages with ragged cell membranes and microvesicular cytoplasm. In a separate study, bronchoalveolar lavage analysis showed maximum levels of lactate dehydrogenase on day 8 in rats (30 X control) and day 5 in mice (3 X control). Acid phosphatase peaked at day 3 in rats (4 X control) while alkaline phosphatase was highest on day 3 for both rats (31 X control) and mice (3 X control). Research sponsored by the Office of Health and Environ. Res., USDOE, under contract DE-AC05-84021400 with the Martin Marietta Energy Systems, Inc.
INORGANIC AND ORGANIC MERCURY TOXICITY TO GROWING QUAIL: PO VS. IM EXPOSURE. E.F. HILL. U.S. FISH AND WILDLIFE SERVICE, PATUXENT WILDLIFE RESEARCH CENTER, LAUREL, MD.

The relative toxicity of HgCl₂ and CH₃HgCl may vary markedly, even reverse, among mammalian models depending on the route of exposure, age, and physiologic status of the subjects. This study compared 1) the toxicity of HgCl₂ and CH₃HgCl to coturnix at various ages by single-dose po and in injections and by ad libitum feeding for 5 days, and 2) the inherent toxicity (1m) of the mercurials and toxicity when affected by gastrointestinal deterrents to absorption (po and dietary). Acute po and im LD50 values increased consistently from hatching through 4 weeks of age (prepubescent) for both mercurials, and then remained constant to adulthood (8 weeks) for CH₃HgCl and decreased to about the 2-week level for HgCl₂. Subacute dietary LC50 values also increased through subadulthood. Toxicity ratios, i.e., HgCl₂/CH₃HgCl, for im and po exposures averaged about 1.5 and 2.0 at the various test ages compared to 100 for the 5-day feeding trials, e.g., at 2 weeks the po LD50s for CH₃HgCl and HgCl₂, were 18 and 42 mg/kg, and the dietary LC50s were 47 and 5,086 ppm. Rate of excretion seemed more important than absorption to the differential toxicity of the mercurials. The transient nature of the toxicity was monitored by brain and plasma cholinesterase activity.

IN VITRO EMBRYOTOXICITY OF TWO DIRECT ACTING ALKYLATING AGENTS, METHYLNITROSUREA (MNU) AND ETHYLNITROSUREA (ENU). E. Faustman-Watts, M. Varnum, D. Gage and J. Lottsfeldt. Dept. of Env. Health, Univ. of Washington, Seattle, WA.

These studies were designed to examine the embryotoxicity of two direct acting alkylating agents on rodent embryos during organogenesis. A modification of the whole embryo culture system of New et al (1971) was used for these studies. Concentrations of up to 420 µM ENU were added to a balanced salt buffer containing day 10 Sprague-Dawley rat embryos. After two hours of exposure, the rat embryos were transferred to supplemented Waymouth's medium for an additional 23 hours of culture. After only two hours of exposure to 200 µM ENU, this ethylating agent caused a 50% drop in embryo viability and 100% incidence of malformed embryos on day 11. In a similar series of studies, MNU, a methylating agent caused a 50% decrease in viability at concentrations of approximately 100 µM and resulted in a 100% incidence of malformations. These effects were also observed after only two hours of exposure to the agent. The spectrum of malformations observed in the day 11 embryos was similar after exposure to either agent except that MNU appeared to be approximately two times more potent than ENU. Dramatic cephalic malformations were noted. Future studies will examine the embryotoxicity of other methylating and ethylating agents. These studies have been supported by NIH grant ES-03157 and Univ. of Washington Graduate School Research Fund.


As part of a large study of the teratogenic effects of industrial alcohols, three concentrations of n- and iso-propanol were administered by inhalation to groups of 15 pregnant Sprague-Dawley rats for 7 hrs/day throughout gestation. Dams were sacrificed on day 20; one half of the fetuses were evaluated for skeletal malformations, and the other half for visceral malformations. The highest concentration of n-propanol (10000 ppm) produced little maternal toxicity, but the same concentration of isopropanol produced narcosis in the dams and retarded weight gain and feed intake. This concentration of both propanol isomers produced an increase in resorptions, a reduction in fetal weights, and an increase in skeletal and visceral malformations. At 7000 ppm, exposure to isopropanol retarded dam weight, and both chemicals reduced fetal weights and increased skeletal malformations. At 3500 ppm, no detectable maternal or embryofetotoxic effects were observed. In summary, extremely high levels of n- and iso-propanol were teratogenic in rats, but neither solvent was embryofetotoxic at lower levels.

A TERATOLOGY STUDY IN RATS WITH DIAZINON TECHNICAL, AN ORGANOPHOSPHATE INSECTICIDE. W.R. Campbell, CIBA-GEIGY Corp., Agricultural Division, Greensboro, NC, R. Infruma and A. Arthur, CIBA-GEIGY Corp., Pharmaceuticals Division, Summit, NJ. Sponsor: J.T. Stevens.

Diazinon Technical was studied in rats to evaluate its potential embryotoxic, fetotoxic, and/or teratogenic effects. The compound was administered once daily by gavage at doses of 0, 10, 20, and 100 mg/kg/day during gestational days 6 through 15, the period of organogenesis. Maternal toxicity, as evidenced by negative weight gain, was observed in the high dose group. Fetal toxicity, associated with the maternal toxicity, was observed in the high dose group. Diazinon Technical was concluded not to be embryotoxic or teratogenic in this test system.
TWO DEUTERATED PROCARBAZINE ANALOGS DIFFER IN DEPRESSION OF SPERMATOGENESIS IN MICE.
G.S. Yost and M.G. Horstman, College of Pharmacy, Washington State University, Pullman, WA

Although the antineoplastic agent procarbazine, \((\text{CH}_3)_2\text{C\text{H}}=\text{NHCOC}_2\text{H}_2\text{NHCH}_2\text{CH}_3\) (1) and \((\text{CH}_3)_2\text{C\text{H}}=\text{NHCOC}_2\text{H}_2\text{NHCH}_2\text{CD}_2\text{NHCH}_3\) (2), were administered to male BDF, mice, with depression of spermatogenesis, measured by epididymal sperm count, as an index of toxicity. At a dose of procarbazine (200 mg/kg) which produced a 34% reduction in sperm count compared to controls, the equivalent doses of 1 and 2 caused depressions of 34% and 1%, respectively. This marked difference between 1 and 2 in their abilities to depress spermatogenesis is most likely due to a deuterium isotope effect. Further, this implicates oxidation at the benzylic carbon as an event necessary to the toxic activation of procarbazine.

Supported by PHS grant number CA35763.

ENVIRONMENTAL TESTICULAR TOXICITY: TUBULIN DYSFUNCTION? K. Boekelheide, Division of Biology and Medicine, Brown University, Providence, RI. Sponsor: D.G. Graham.

Preliminary studies have identified an alteration in tubulin assembly in tissue extracts from 2,5-hexanediol intoxicated rats. Six Sprague-Dawley rats (200 gm) were divided into two groups. Control animals were given food and water ad libitum and the experimental group received 1% 2,5-hexanediol in the drinking water for three weeks. The treated rats showed a significant relative loss in body and testicular weight. Microtubule polymerization of brain homogenate supernatants was monitored by observing changes in absorbance at 350 nm. Assembly of tubulin from the 2,5-hexanediol intoxicated animals occurred both earlier (time to 1/2 maximum O.D., 2.42 ± 0.51 versus 6.13 ± 1.10 minutes) and more rapidly (maximum rate as change in absorbance/minute, 0.104 ± 0.012 versus 0.059 ± 0.013). In vitro incubation of twice cycled porcine brain tubulin with 2,5-hexanediol simulated the in vivo alteration; longer periods of 2,5-hexanediol exposure produced earlier and more rapid tubulin assembly. The following hypothesis of n-hexane induced testicular atrophy is proposed: n-hexane is metabolized in vivo to the ultimate toxin 2,5-hexanediol, 2,5-hexanediol reacts covalently with tubulin lysyl residues to form pyroles; these pyroles undergo spontaneous oxidation; the oxidized pyroles react further to form covalent cross links between tubulin monomers; the covalently linked tubulin multimers change the in vitro and in vivo kinetics of tubulin assembly; testicular injury occurs in the whole animal because of the unique dependence of the testis on microtubule integrity for maintenance of homeostasis and spermatogenesis.


DNB is an aromatic nitro compound that is used widely in the manufacture of dyestuffs, explosives, plastics, and the production of other chemicals. The most remarkable clinical effect of exposure is hypoxia due to a methemoglobinemia. In the rat, testicular atrophy and hypoperfusion have also been reported following long-term exposures. In the present study male rats (72 d), treated orally with a single dose of either corn oil or DNB (48 mg/kg), were killed 1,2,4,8 and 16 days postdosing. In the testis, the primary spermatocyte was selectively affected by 24 h. By d 4, these cells appeared to be phagocytized by Sertoli cells or selectively sloughed into the lumen, causing other cells and cytoplasmic debris to be released. The mature spermatids appeared to be more resistant to sloughing. This pathology was followed by observing the progressive movement of large round cells and cytoplasmic bodies through the epididymis, reaching the vas deferens by d 16. The types of cells and debris in the lumen of the excurrent ducts were differentiated, defining this sequence of cellular release. Residual bodies were the first components to appear, followed by spermatids, spermatocytes and multinucleated giant cells.


Cimetidine has weak anti-androgenic activity in rats but 950mg/kg orally daily by gavage did not affect male rat fertility. Anand et al (Science, 1962: 215: 493-4) and Parker et al (Gastroenterology, 1984: 86: 55-50) claimed that giving cimetidine to pregnant rats in the drinking water caused feminisation of male pups, small sex organs, low 11bido and low serum testosterone. Anand et al gave approx 170mg/kg and Parker et al 11.1mg/kg daily.

We tested these claims using large groups of pregnant Wistar rats given at least 1800mg/kg cimetidine daily in the drinking water. Estimations included: anogenital distance exactly 24h and 120h after birth; serum testosterone at 55 and 110 days of age; mating performance at 110 days and (after castration and testosterone implantation) at 134 days; testis, prostate and seminal vesicle weights at 55 and 135 days.

Maternally-administered cimetidine was completely without effect on all the parameters measured in the male offspring.

We conclude that giving cimetidine to pregnant rats does not feminise their male offspring.
TOXIC EFFECT OF COBALT ON MALE REPRODUCTION
N.G. Pedigo, M.B. Anderson, and W.J. George,
Depts. of Pharmacology and Anatomy, Tulane University School of Medicine, New Orleans, LA 70112
Sponsor: R.E. Billings

The dominant lethal assay was employed to evaluate
the effects of acutely administered cobaltous chloride (CoCl₂) on male reproduction. Male CD-1 mice were injected daily with 200 µmoles/kg of CoCl₂, i.p. for 3 days. Treated males were then
mated with untreated females for 8 weeks in order to
determine cobalt's effects on the complete
spermatogenic cycle. Pregnant females were sac-
rificed and autopsied on days 16 to 18 of gesta-
tion. Fetuses were evaluated for viability and
abnormalities, including reabsorptions, premat-
uration losses and undersized fetuses. There
was a 91% increase in the incidence of fetal ab-
normalities in fetuses from the cobalt treated
cohort as compared with controls in the first week of
matting after treatment. There was an increase
in all the above types of abnormalities in the
cobalt treated group, except for non-viable
fetuses. There was no change in sperm concentra-
tion, motility and viability, or morphology.
There was no change in the number of litters pro-
duced per male. Therefore, it is unlikely that the
fertilizing ability of sperm or male libido was
affected by cobalt. The effect seen at one week corresponded with the stage of spermatogenes-
esis at which time mature sperm are stored in the
epididymis.

TESTICULAR EFFECTS OF BIS(2-METHOXYETHYL)ETHER IN THE ADULT MALE RAT: EQUIMOLAR DOSE COMPARISON WITH 2-METHOXYETHANOL AND 2-ETHOXYETHANOL.
TMS, DBBS, NIOSH, Cincinnati, OH.
Sponsor: T.H. Lewis.

The onset of testicular pathology in the rat and subsequent recovery over an 8-week period were
evaluated after the administration of up to 20
daily oral doses of bis(2-methoxyethyl)ether at
684 mg/kg bw. Primary and secondary spermatocyte degeneration and spermatid giant cells
were observed after seven treatments. The
testes-to-body weight ratio was significantly
reduced by day 12. Testicular pathology for
bis(2-methoxyethyl)ether appeared to be reversible
even though the testes-to-body weight ratio
continued to be depressed after 8 weeks. Equi-
molar doses of the related glycol ethers,
2-methoxyethanol (388 mg/kg bw) and 2-ethoxy-
ethanol (460 mg/kg bw), administered under the same
regimen showed similar pathology. Spermatocytes were
affected after only 1 treatment with
2-methoxyethanol, whereas eleven 2-ethoxyethanol
treatments were required. The animals treated
for 11 to 13 days with 2-methoxyethanol showed
spermatogonia and spermatid cell degeneration.
Testicular LDH-X enzyme activity was significant-
antly decreased in animals after 5 treatments with
2-methoxyethanol or 18 treatments with bis(2-
methoxyethyl)ether, but not after as many as
20 treatments with 2-ethoxyethanol. Serum
LDH-X activity in these animals was not affected.

THE POTENTIAL ROLE OF THE CARNITINE ACETYLTRANS-
FERASE SYSTEM IN CADMIUM-INDUCED STERILITY IN MALE FISCHER 344 RATS. P. Adates, R.G. James, and R.D. Harbison.
Div. of Toxicol., Univ. Ark. for Medical Sciences, Little Rock, AR 72205.

Cadmium is a well known reproductive toxicant that
can produce sterility, testicular necrosis and
oligospermasia. Cadmium inhibition of sulfhydryl
containing enzymes is established, therefore it
should have adverse effects on the carnitinergetic
system which is thought to be essential to sper-
matogenic function. The purpose of this study was
to examine the effects of cadmium on the carniti-
ergetic system of the epididymis and epididymal
spermatosors to determine whether or not cadmi-
ium induced changes in this system and sperm motility
occur at lower doses than those needed to pro-
duce testicular necrosis. Sexually mature male Fischer
344 rats were treated with cadmium chloride daily
for 4 days. Cadmium significantly reduced carni-
tine acetyltransferase activity in the caput por-
tion of the epididymis. The effect was dose depen-
dent with 89, 44 and 14% inhibition of enzyme
activity observed at dosages of .5, .35 and .25
mg/kg respectively. A dose dependent decrease in
the carnitine levels of the seminal plasma fluid
was also observed. These changes in the carni-
tinergetic system correspond to parallel decreases in
sperm motility of 82, 40 and 30%, respectively.
These results suggest that the cadmium-induced
inhibition of carnitine acetyltransferase may be
the mechanism by which it alters sperm motility
and possibly fertility at non necrotizing doses.
(Supported in part by USPHS Grant ES02824).

ETHYLENE DIBROMIDE (EDB) AND BUTHIONINE SULFOSKIMINE (BSO) POTENTIATION OF ETHYL METHANESULFONATE (EMS)
INDUCED DOMINANT LETHAL MUTATIONS. C.H. Teaf, J.B.
Bishop and R.D. Harbison.
Ctr. for Toxicological Research, Jefferson, AR.

EDB and BSO are both capable of lowering tissue
 glutathione (GSH) in vivo. GSH and a high specific
GSH-S-transferase activity exist in the testis and
epididymis of male F344 rats. EMS is a reactive
electrophile known to induce GSH-dependent metab-
olism. The purpose of this study was to determine
the effect of pretreatment with EDB or BSO on EMS-
induced dominant lethal mutations. BSO adminis-
tered as 3 subcutaneous injections of 3 mmol/kg at 12
hr intervals resulted in a lowering of GSH to 70,
41 and 40% of control in testis, caput, and cauda
epididymis respectively. Similarly, EDB (75 mg/kg
ip) significantly lowered GSH in the same tissues
and inhibited GSH-S-transferase. Pretreatment of
male F344 rats with BSO or EDB significantly in-
creased the incidence of EMS-induced dominant le-
thal mutations. Neither BSO nor EDB administered
alone produced a significant increase in dominant
lethal mutations. The decidualoma/implant, live
fetuses/pregnant females and Dominant Lethal Index
were all significantly altered by BSO and EDB at 2
and 3 weeks following EMS treatment (50 mg/kg). Pre-
treatments with EDB or BSO potentiate the germ cell
mutagenicity of EMS. The mechanism by which these
pretreatments exert their effects appears to be a
specific lowering of GSH levels in the male repro-
ductive tract and perhaps specifically the epi-
didymis. (Supported in part by USPHS Grant ES02084).

In a study designed to assess the embryotoxic and teratogenic potential of EDS hydrotreated naphtha (HN), pregnant female Sprague-Dawley rats were exposed 6 hours/day to HN vapor in concentrations of 0.2 to 5.0 mg/L. Animals were exposed from day 6 to 19 of gestation (a). Dams were sacrificed on day 20 G, and the uterine contents were examined. The experimental and control groups did not differ significantly in mean implantation frequency, fetal viability, weight, crown-rump length, or incidence of malformation. In a second study, to assess the effect of HN exposure on reproduction, male and female Sprague-Dawley rats were exposed to HN vapor, 6 hours/day, 5 days/week for 13 weeks. Exposure levels ranged from 0.2 to 5.0 mg/L. Following the exposure period, treated animals were mated, and the females and offspring were then maintained to postnatal day 21. No significant differences were found in fertility, number of live births, or fraction of litter surviving through the lactational period. A low incidence of malformed offspring, probably the expression of inbred recessive mutations, were observed in the exposed groups. The results of these studies suggest that HN exposure is unlikely to interfere in the reproductive or developmental processes.


We are evaluating a protocol which uses alterations of puberty as a model system to screen chemicals for reproductive toxicity. The present study is a report on the effects of methoxychlor (M) on easily measurable behavioral and morphological changes associated with puberty in the rat and hamster. Animals were dosed from weaning, through puberty, young adulthood and gestation with oil, 100 or 200 mg/kg/d. In the rat we measure the ages of vaginal opening and first estrus, cyclicality, sperm in the smear, age at parturition, litter size and viability until 5d after birth. At 75 males are necropsied, the reproductive organs are weighed and motility and counts of caudal sperm are determined. The serum, pituitary and testis are saved for hormonal determinations if warranted. The other testis is used for histopathology. Females are necropsied on d5 postnatal or d95, the numbers of implantations and resorptions are determined and corpora lutea are counted if warranted. Observations using hamsters also include sex behavior and flank gland development. M, a weak estrogen, accelerated puberty in the female, an effect noted within the first week of dosing, but delayed development in the male. Supported in part by US Army MRDC IAG #W21931662-01-0.

653 EFFECTS OF ETHANOL ON REPRODUCTION IN SHR Rats. R. Manke, R. Lefevre, R. Lyon, J. Fieshe, and A. Santiago, Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY, 12208.

Ethanol and hypertension are fetal and maternal risk factors. Groups of A-a strain SHR (groups 1 and 2) and Wistar-Kyoto (groups 3 and 4) rats were given: 20ml/kg H2O (controls - groups 1 and 3); or 4.0ml/kg ethanol (groups 2 and 4) from day 6 to 15 of gestation. Dams were monitored for arterial pressures, body weights and signs of toxicity. At necropsy (day 20), parameters of fecundity were recorded; offspring examined; and maternal hearts and kidneys examined. During acclimation, hypertension developed (MABP-WKY of 105 to 114mmHg and MABP-SHR of 137 to 148mmHg). From day 6 to 15 of gestation, groups 2 and 4 had higher arterial pressures. SHR rats given alcohol did not experience a "prebirth" hypotension. Most (84% - group 2, 86% - group 4) offspring of alcoholic rats were dead or malformed. Low birth weight occurred in group 4. Hydrocephalus, microphthalmia and hydrenephrosis/hydrourereter were common in group 2. Hydrenephrosis/ hydrourereter was increased in group 4 (WKY) pups. A low incidence of focal myocardial hynalinosis and renal calcifications was seen in alcoholic SHR rats. These data confirm the effects of alcohol in two strains of rats and suggest altered hypertensive response during pregnancy.


We have previously reported that toxaphene (T) is an inhibitor of ATPases involved in ion transport and oxidative phosphorylation in mice. Since T is a mixture of 177 different components, studies were extended further to understand the effective component of the mixture. For the present study, T fractions were divided into three categories based on the polarity of the component i.e., non-polar (N), intermediate polar (I) and polar (P). Rat brain synaptosomes were prepared by Ficoll-sucrose gradient centrifugation method. ATPase activities were determined by measuring the Pi liberated during ATP hydrolysis. These fractions inhibited Na+-K+-ATPase significantly with IC50 values of 25, 25, 15, and 13 uM for T, N, I and P respectively. K+-ATPase was inhibited significantly to a maximum inhibition of 26% by 75 uM of T, with an IC50 of 25 uM for P and no significant effect was observed with N and I fractions. O.S. Mg++-ATPase was also inhibited significantly with IC50 values of 12, 25 and 10 uM for T, I and P fractions respectively. N did not effect the enzyme activity significantly. These results support our earlier observations that T affects ATPases involved in active ion transport and oxidative phosphorylation. Besides, it is clearly evident that P fractions seem to be more effective in vitro than other fractions of T. (Supported by NIH/NIEHS grant SO6 ES090047).
In vitro activation of tyrosine hydroxylase by organophosphates. J.K. Marquis and Y. Tsuzuki. Boston University School of Medicine, Boston, MA 02118.

Tyrosine hydroxylase (TH) activity of bovine caudate nucleus was studied in 0.2M phosphate-acetate buffer, pH 6.2, in the presence of ATP, Mg,K, protein kinase, catalase and Triton X-100. These buffer conditions were previously shown to optimize TH activity in tissue homogenates and a 30,000xg supernatant. The organophosphates paraoxon, diisopropylfluorophosphate and ecolithosphate activated TH at concentrations (µM) in the range of acetylcholinesterase inhibition by these compounds. Double-reciprocal analysis demonstrated that TH activation was produced by a noncompetitive or allosteric increase in the Vmax of hydroxylation with no change in the Km for tyrosine. These findings are interpreted with reference to other studies of catecholaminergic actions of organophosphates, and it is proposed that in vivo alterations of brain levels of dopamine and norepinephrine may be produced by phosphorylation of TH.

(This work was supported by the U.S. Army Research Office, DAAG29-82-K-0042.)


Earlier studies from this lab demonstrated that toxaphene (T) altered the activity levels of Na,K-ATPase and Mg⁺-ATPase in fish, mice and rat. The present studies were initiated to study the effect of T in vitro and in vivo on calcium transport and brain subcellular calmodulin (CaM). Rat brain synaptosomes were prepared by ficoll-sucrose gradient centrifugation method and Ca⁺⁺-ATPase activity was determined. Ca⁺⁺-uptake was measured by incubating synaptosomes with radioactive Ca⁺⁺. CaM levels were determined by radiolmmunnoassay method. T inhibited Ca⁺⁺-ATPase in vitro to a maximum of about 50% at 150 µM. Substrate activation kinetics of Ca⁺⁺-ATPase in synaptosomes revealed that T inhibited the enzyme activity non-competitively by decreasing Vmax without affecting the enzyme substrate affinity. Synaptosomal Ca⁺⁺-uptake was inhibited by T with an IC50 of 4 µM. T also inhibited the CaM activated Ca⁺⁺-ATPase in a concentration dependent manner with an IC50 of 10 µM, a concentration at which no significant effect was observed on basal enzyme. Nuclear and P fraction CaM levels were reduced significantly in the rats treated with T. The synaptosomal Ca⁺⁺-ATPase was reduced by 45% in T treated rats and the activity was restored to normal level by the exogenously added CaM. These results suggest that T may cause synaptic dysfunction by interfering with CaM regulated events in brain.

(Distributed by NIH/DBS 8309028107.)


The molecular forms of human brain AChE were characterized by sucrose density gradient centrifugation and separated by gel chromatography. 3 major forms are evident in tissues extracted in 1M NaCl, 0.5% Triton X-100, 10mM Tris buffer, pH 7.4: an asymmetric 16S (A12: mw 560,000) and globular 10.3S (G4: mw 435,000) and 4.6S (G1: mw 115,000) forms. Regional variations in the ratio of the major globular forms (G4/G1) were defined in 11 dissected brain areas and correlated with the cholinergic activity of the distinct brain regions. It is proposed that a major portion of the G4 enzyme is presynaptic in origin. In addition, pharmacological differences in the sensitivity of isolated G4 and G1 to inhibition by carbamates, organophosphates and noncompetitive enzyme inhibitors are defined and discussed with reference to the selective central neurotoxicity of these compounds.

(This work was supported by the U.S. Army Research Office, DAAG29-82-K-0042.)

Human serum A-esterases: 0,0-dimethyl-0,2,2-dichlorovinyl phosphate hydrolase. N. Pierini, A. Moretto, and R. Lotti. Istituto di Medicina del Lavoro, Università di Padova, Italy.

Two groups of esterases interact with organophosphate compounds (OP) (A and B-esterases). OPs are either substrates or inhibitors. The nature of the interaction is the same. Substrates and inhibitors are differentiated on the basis of the velocity of reappearance of the free enzymes: rapid for A-esterases (OPs as substrates), very slow for B-esterases (OPs as inhibitors). Many A-esterases hydrolyzing OPs have been described in the serum of various animal species, including man. We report the characterization of an A-esterase in human serum which hydrolyses dichlorvos (0,0-dimethyl-0,2,2-dichlorovinylphosphate hydrolase, DH). DH activity is calculated from the loss of inhibitory power of dichlorvos on human nucleus caudatus acetylcholinesterase (AChE), caused by the incubation of the inhibitor with serum. AChE activity was measured with Ellman's method, slightly modified. The loss of inhibitory power of dichlorvos (24M) is linear according to the time of incubation with serum [0-60 min] and proportional to protein content. The incubation of dichlorvos (24M at 37°C, pH 7.4) with different concentration of serum (72 mg of protein/ml 36 mg/ml 18 mg/ml) causes 50% loss of inhibitory power on human after 12, 24, 65 minutes respectively. Apparent Michaelis constants were calculated according to Lineweaver-Burk. Km was 52.5M and Vmax was 62.4. Measurements of serum DH activity might be useful to monitor dichlorvos hydrolysis in man.
569 DELAYED NEUROTOXICITY OF
TECHNICAL AND PURIFIED EPN IN CHICKEN

Salah A. Soliman and G. Wayne Sovocool
Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, and Environmental Monitoring Systems Laboratory, USEPA, Las Vegas, NV 89114, USA.

Eight groups of chicken (10 hens each) were orally treated with either technical or purified EPN at 0.5, 1.0, 2.0 and 3.0 mg/kg.d respectively for 90 days. Results indicated that, non of the technical EPN-treated hens developed any sign of organophosphorus-induced delayed neurotoxicity and by the end of the experiment they were as normally active as the vehicle control chickens. On the other hand, 4 and 8 hens of the 2.0 and 3.0 mg/kg.d groups of purified EPN-treated chickens, respectively developed clear signs of neurotoxicity. These results suggested that the neurotoxic symptoms induced in hens following exposure to small doses of technical EPN could be attributed to impurities present in the technical samples per se or to a synergistic effect due to such impurities. Analysis of the technical EPN sample by gas chromatography combined with mass-spectroscopy indicated that this sample contains impurities. Some of these impurities are known to be delayed neurotoxicants.

570 EFFECT OF METHYL AND ETHYL DERIVATION ON THE
DELAYED NEUROTOXICITY OF 0-ALKYL O-(4-BROMO-2,5-
DICHLOORO PHENYL) PHENYLPHOSPHONOTHIOATES

Salah A. Soliman, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University, Alexandria, EGYPT.

The delayed neurotoxic effect of lepto phosphonothioates, [0-methyl O-(4-bromo-2,5-dichloro phenyl) phenyl phosphonothioate] and its ethyl analogue was studied in chicken. Results demonstrated that, lepto phosphonothioates induced clinical signs of delayed neurotoxicity and inhibited more than 80 and 70% of the brain and spinal cord neurotoxic esterase (NTE) activity, respectively in treated hens at dose levels as low as 250 mg/kg. Treatments with the ethyl analogue at dose levels ranged from 250 to 1000 mg/kg inhibited 80 and 70% of the brain and spinal cord NTE of the treated hens, respectively. However, no clinical signs of organophosphorus-induced delayed neurotoxicity (OPIDN) were demonstrated in hens treated with the ethyl analogue at this range of dosing. This diminish from the NTE inhibition/OPIDN relationship could be attributed to differences in rate of transferring of the methyl and ethyl moieties from the phosphorylated enzyme (aging), a process suggested to be a necessary step in the genesis of OPIDN. This is supported by the finding that, higher doses (1250 mg/kg) of the ethyl analogue induced symptoms of OPIDN in treated hens.

571 ACUTE AND DELAYED NEUROTOXICITY OF METHYL AND
ETHYL PHOSPHONOTHIOATES. R.L. Metcalf, J.C.
Francis, R.A. Metcalf, University of Illinois, Urbana IL 61801.

A series of methyl and ethyl O-halogenated-phenyl O-alkyl phosphonothioates were evaluated for acute toxicity in house flies and mice and for delayed neurotoxicity (OPIDN) in hens. Comparisons were made with the corresponding phenylphosphonothioates. The methylphosphonothioates were 3-5 times more acutely toxic to fly and mouse than the phenylphosphonothioates. Both series of compounds were very effective in producing OPIDN, but the magnitude of this effect was highly dependent on the number and position of the halogen substituents and on the O-methyl and O-ethyl esterification. Production of OPIDN by the phosphonothioates is clearly dependent upon the overall effects of the three substituents of the P-atom (i.e., P-C, P-O-aryl and P-O-alkyl). The considerably more bulky phenylphosphonothioates are more sensitive to the protective effects against OPIDN induced conferred by changes from O-methyl to O-ethyl, and by specific patterns of halogenation of the O-phenyl group.

572 PHENYL MethylSULFONYL FLUORIDE (PMSF) PRETREATMENT
PROTECTS RATS FROM ORGANOPHOSPHATE-INDUCED
DELAYED NEUROPATHY (OPIDN). B. Veronesi, S.

Initiation of OPIDN is thought to consist of the phosphorylation of the putative target enzyme, neurotoxic esterase (NTE), and a subsequent "aging" reaction which transforms the inhibited NTE into a charged moiety critical to the neuropathic process. Compounds that inhibit NTE but can not "age" theoretically abort the initial process and protect against the neuropathy (Murphy, 1974). In support of this we report that prior exposure to a non-"aging" NTE inhibitor PMSF, protects rats from OPIDN after subsequent exposure to the neurotoxic organophosphate Mipafox. Adult, male, Long Evans rats were exposed to either PMSF (250mg/kg, SC) or to Mipafox (15 mg/kg, IP). A course of brain NTE inhibition and recovery was defined. A separate group of PMSF-treated rats was exposed to the neuropathic dose of Mipafox when NTE inhibition was 87.7%. Conversely, rats pretreated with Mipafox were dosed with PMSF when NTE inhibition was 90.2%. Histopathological survey (444 post-exposure) showed severe cervical cord damage in the following frequency: PMSF (n=10) 0%; Mipafox (n=12) 82%, PMSF + Mipafox (n=5) 0%; Mipafox + PMSF (n=8) 100%. These data indicate that PMSF protects against neurological damage and underscore the relevance of NTE's "aging" in the development of OPIDN in rats.

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The potent convulsant \( ^{35} \text{S} \)-butylcyclophosphorothionate (TBPS) acts at a site associated with the GABA-activated chloride channels. GABA lowers the rate of association and increases the rate of dissociation of \( ^{35} \text{S} \)TBPS from its brain-specific receptor. The Scatchard plot for binding of \( ^{35} \text{S} \)TBPS to EDTA/water-dialyzed rat brain membranes becomes nonlinear at concentrations of GABA 1 \( \mu \text{M} \) with increasing \( K_d \) for an apparent low-affinity site. \( ^{35} \text{S} \)TBPS binding is inhibited by GABA-antagonists (e.g., picrotoxinin and picrotoxinin and photobacterium epoxide), the o-methylopyrophosphoryl omocyanopeptidase (e.g., [1R,2S]-cis-cypermethrin), and dimethylbutylbarbituric acid (DMBB). These GABA/GABA-A antagonist/pyrophosphate-DMBB binding sites of the allosteric GABA-receptor ionophore complex are also differentiated on solubilization with the surfactant detergent CHAPS. GABA strongly enhances cypermethrin and DMBB and for displacing \( ^{35} \text{S} \)TBPS from these membranes, supporting earlier evidence that the binding domains of cypermethrin and DMBB, although apparently different, are both regulated by GABA.

Effects of exposure to organophosphate acetylcholinesterase inhibitors upon cyclic nucleotide synthetic and degradative enzymes were studied in rat striatal slices 15 min after subcutaneous administration of Somar or Sarin (120 \( \mu \text{g/kg} \)) or Tabun (240 \( \mu \text{g/kg} \)). Age-matched control rats were administered saline, s.c. At the onset of severe symptoms of neurotoxicity (15 min after administration), such as tremors and convulsions, Somar reduced both adenylate cyclase and guanine cyclase activities to 74 and 62 percent of control activity, respectively. On the other hand, sarin had no effect on cyclase activity, and a significant increase (124\% of control) in guanine cyclase activity was observed 6 hr after injection of Tabun. Sarin was the only agent that significantly affected phosphodiesterase activity at 15 min. This was a 20\% increase in cyclic GMP phosphodiesterase activity. Six hr after Tabun administration, these were small, but significant, increases in both cyclic AMP and cyclic GMP phosphodiesterase activities (113 and 116\% of control levels, respectively).

These results show not only that these irreversible inhibitors of acetylcholinesterase affect cyclic nucleotide metabolism, but also that the three compounds differ in the biochemical effects they produce. (Supported by USAMRDC-DAMD17-81-C-1236.)

An \textit{in vitro} procedure was developed for assessing potential efficacy of oximes as nerve agent antidotes using purified eel acetylcholinesterase (AChE). Preliminary data are presented testing MB-4, 2-PAM, and toxogonin against sarin (GB), paraoxon, and methylphosphonic acid analogs (MPP), and pyridostigmine. After 95\% inhibition of AChE by 1.4 \( \mu \text{M} \) GB, 1.4 \( \mu \text{M} \) MPP, or 1.4 \( \mu \text{M} \) pyridostigmine, the inhibited enzyme was separated from excess inhibitor by HPLC within 2 minutes. When GB was the inhibitor, the rank order of oxime potency was 2-PAM (68\%) > TM-4 (54\%) = toxogonin (56\%). For MPP the oxime rank order was TM-4 (79\%) > toxogonin (67\%) > 2-PAM (40\%). For pyridostigmine there was no significant oxime effect on spontaneous reactivation. Differences among oxime accelerated and spontaneous rates of enzyme recovery were statistically significant at \( p < 0.005 \). The data suggest that not only is reactivation of AChE dependent on oxime potency, but also that oxime potency rank order is relative to the inhibiting agent. Work is in progress to determine oxime potency rank orders in the presence of other G-agents, and after pretreatment with either MPP or pyridostigmine. This approach, when coupled with tissue and in vivo studies, may assist in the selection of a new oxime for treating chemical warfare casualties.

One of the preseizure events following methionine-sulfoximine (MSO) is an increase in the cerebral methylation of membrane proteins and phospholipids. Phenyltoin and phenobarbital prevent MSO seizures and many of the MSO induced changes in cerebral methylation. In this study we report the effect of diazepam (DZ) on MSO seizures and cerebral methylation. Mice were injected ip. with from 5-50 mg/kg DZ alone or with 170 mg/kg MSO. MSO seizures were completely blocked by 5 mg/kg or greater of DZ. DZ (5 mg/kg) increased brain levels of the methyl donor, S-adenosylmethionine (AdoMet) 32\%, and the endogenous methyltransferase inhibitor S-adenosylhomocysteine (AdoHcy) 36\%, while at 10 mg/kg only AdoHcy was increased (30\%). Conversely, at the highest dose of DZ, AdoMet and AdoHcy were decreased 30 and 40\%, respectively. The \textit{in vitro} activity of phospholipid methyltransferases (PMT I and II) were determined after ip. injection of 5 or 50 mg/kg DZ. DZ (5 mg/kg) had no effect on PMT I but increased PMT II activity (40\%), while at 30 mg/kg DZ decreased PMT I activity (38\%) and had no effect on PMT II. The different effects of low and high doses of DZ on cerebral methylation may be an indication of therapeutic and toxic responses, respectively. Perturbations in amino acid and phospholipid metabolism associated with these responses are currently under investigation. Supported by a grant from the Epilepsy Foundation and NU BRSG 89462-21.
ALTERATIONS IN THE LEVELS OF TISSUE TRYPTOPHAN IN THE RAT FOLLOWING TCDD EXPOSURE. J.A. Sweatlock and T.A. Gastiewitz, Env. Hlth. Sci. Ctr. Univ. of Rochester, Rochester NY 14642

TCDD causes a variety of responses in experimental animals. However, all animals lose or gain body weight at a reduced rate following exposure. The naturally occurring and essential amino acid, L-tryptophan (TRP), and its metabolite, serotonin (5HT), play a role in the regulation of food intake. Therefore, we determined the levels of TRP in serum (total and free), liver and whole brain of male Sprague-Dawley rats treated with a single 200 ug/kg dose of TCDD. This dose was below the LD50. Animals were sacrificed 8 days later and data analyzed by paired T-test. Total serum TRP was increased compared to all control groups, pair-fed (FFC), ad libitum fed (ALC) and 48-hour fasted (FC), 22.93 vs 13.88, 14.77 and 13.72, respectively. No significant differences were observed in the serum free or liver concentrations. Whole brain levels in TCDD treated rats were significantly increased versus FFC and ALC both on a weight basis (3.83 vs 2.58 and 2.80 ug and 6.90 vs 4.86 and 5.18 ug, respectively). Thus, TCDD treatment causes an increase in serum total and whole brain levels of TRP which directly or indirectly through its metabolism may contribute to the toxic response observed. (NIH Grant ES02859, NIH Training Grant ST32ES07026 and EHS Center Grant ES01247)

IN VITRO GROWTH OF CANINE BONE MARROW PROGENITOR CELLS AND ITS POTENTIAL APPLICATIONS IN INVESTIGATIVE TOXICOLOGY. A. Deldar, L. Weiss, A. Apostoli, H. Lewis, and J. Bloom, School of Veterinary Medicine, University of Pennsylvania and Smith Kline & French Laboratories, Philadelphia, PA. Sponsor: W. R. Hewitt.

In vitro systems for studying hematopoiesis in mouse and man are widely employed, but few such systems have been applied to the dog, a species used extensively for testing the effects of drugs and chemicals on hematopoiesis. We have adapted assays for canine erythroid (CFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells using modified micro-plate clot and soft mouse systems. CFU-GM colonies stimulated with stem III sheep plasma are counted after 2 days of incubation in 5% CO2. CFU-GM colonies stimulated with pooled serum from dogs treated with endotoxin are examined after 7-8 days incubation in 10% CO2 at 37°C in 52 CO2. The presence of 10% autologous sera in CFU-GM cultures increases yield by 30-60%, but markedly reduces the size and number of CFU-GM colonies. A linear relationship exists between the number of cells plated and colonies counted. These cloning systems are reproducible and reliable permitting the routine determination of the effects of drugs, chemicals or other variables, including drug interactions and the reversibility of drug effects on hematopoietic stem cell growth and differentiation. This approach is a useful adjunct and offers an alternative to in vivo hematotoxicity testing.

LEAD- AND CADMIUM-INDUCED ALTERATIONS IN MARINE BONE MARROW DETECTED USING MULTIPARAMETER FLOW CYTOMETRY. S. W. Burchiel, W. M. Hadley, C. L. Cameron, R. H. Fincher, T. W. Lim and C. C. Stewart, Immunopharmacology Laboratory, University of New Mexico College of Pharmacy, Albuquerque, NM and the Los Alamos National Laboratory, Los Alamos, NM Sponsor: A. R. Dahl

Bone marrow and spleen cells obtained from young adult (12 weeks old) and aged (greater than 70 weeks old) female Balb/c ByJ mice given a single i.p. injection of sodium acetate (12 mg/kg), cadmium acetate (0.9 mg/kg), or lead acetate (12 mg/kg) were analyzed by multiparameter flow cytometry and conventional immunologic assays. Flow cytometry analyses included Coulter volume, light scatter, and FITC fluorescence using computer-based multiparameter single cell analysis at the Los Alamos National Laboratory. Several rat monoclonal antibodies were used to examine cell surface markers present on subsets of bone marrow and spleen cells using indirect immunofluorescence. A time-dependent (24 and 72 hour) increase in the relative number of myeloid/macrophage cells was detected by Coulter volume analysis of the bone marrow in response to both cadmium and lead, but not in the control mice. An increase in the relative number of Lyt-7 expressing cells (T cell precursors) was also detected in the bone marrow at 72 hours. A modest decrease was noted in the LPS, Con A, and PHA proliferative response of spleen cells taken at 72 hours from both the cadmium and lead treated mice.

ASSAY OF HUMANERYTHROCYTE PYRIMIDINE AND DEOXY-
PYRIMIDINE 5'-NUCLEOTIDASE BY REVERSED-PHASE ISOCRATIC HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). L.R. Cook, M.A. Mitchell, C.R. Angle, and S.J. Stoba, University of Nebraska Medical Center, Omaha, NE.

Human erythrocyte pyrimidine 5'-nucleotidase (PS5') dephosphorylates the 5'-pyrimidine and 5'-deoxypyrimidine nucleotides through the action of two or more isoenzymes. PS5' activity is affected by several hematological disorders and in vivo or in vitro by dervalent cations. Lead, mercury and cadmium markedly decrease the activity of PS5'. This report describes a rapid and reproducible assay for the activity of pyrimidine 5'-nucleotidase (PS5') and deoxypyrimidine 5'-nucleotidase (dPS5'). The nucleotides CMP, dCMP, UMP, dUMP or dTMP are incubated 30 m at 37°C with erythrocyte hemolyate and 4 mM MgCl2 in Tris, pH 7.4. The nucleoside products are assayed (280 nm) in less than 10 min by reverse phase high performance liquid chromatography (HPLC) with 2-16% methanol in 0.01 M KH2PO4. Each nucleotide-nucleoside pair is cleanly separated using the specified conditions. Nucleoside production in mmol/h/g hemoglobin or international units is calculated from the chromatographic response of appropriate standards. The linear range of this assay should facilitate biomedical applications in screening for decreases in PS5' as a correlate of blood lead.
Benzene is an erythropoietic toxin in the rainbow trout. *Salmo gairdneri* (Cooper, et al., *Dev. Metab. Res.* 13(4):573, 1984). The characteristics of benzene metabolism by the liver and the kidney, which is the major site of erythropoiesis, were determined. The major benzene metabolites produced by liver and kidney were phenol and phenyl glucuronide. Liver microsomal metabolism of benzene was dependent upon NADPH, inhibited by 0.1 mM SKF 525A (61.3% of control), and induced by 8-naphthoflavone. Thus, benzene metabolism by trout liver preparations had characteristics consistent with P-450 mediated metabolism. Preliminary findings indicated that benzene metabolism by head kidney preparations, in contrast to liver preparations, was not NADPH dependent. Most products formed by head kidney preparations were covalently bound to endogenous proteins. Following one hour incubations of 4 mM benzene with head kidney 10,000xg supernatants, the level of covalent binding was 8% moles per mg protein (70% of total metabolites) whereas in the liver the level of covalent binding was 75% moles per mg protein (14% of total metabolites). We have yet to determine the mechanism of benzene metabolism by the head kidney.

PHENOL, the major metabolite of benzene, is metabolized by BQ and EQ (Snyder et al., *Rev. Biochem. Tox. 3, 123, 1981*). In bone marrow preparations EQ and BQ inhibited RNA synthesis in nuclei (Post et al., *Chem. Biol. Interact.* 48, 203, 1984) and mitochondria (Kalf et al., *Chem. Biol. Interact.* 42, 333, 1982) and covalently bound to mitochondrial DNA (Rushmore et al., *Chem. Biol. Interact.* 49, 133, 1984). To study the nature of DNA adducts calf thymus DNA was incubated with [14C]BQ in the presence of Fe++ at pH 7.4, 37°C overnight and then dialyzed exhaustively. Density gradient centrifugation of the DNA in a CsCl gradient revealed a single peak of [14C] labeled DNA. DNAse treatment rendered the peak acid soluble. These data support the concept that BQ was covalently bound to DNA.

Hydroquinone, a metabolite of benzene, and acetaldehyde, a metabolite of ethanol, induce SCEs at relatively high concentrations. Because both compounds are reported to form glutathione (GSH) conjugates, experiments were done to see if a synergism occurred when low concentrations were added to human lymphocyte cultures. 4x10^-5M hydroquinone slightly increased SCEs in some individuals, but not in others. The rate of SCEs was more than doubled if cells were pretreated with diethyl maleate (DEM), which transiently depletes cellular GSH. Acetaldehyde increased SCEs at 10^-6M, but, in the presence of DEM, it increased SCEs at 10^-6M. When acetaldehyde was added to cultures containing 4x10^-5M hydroquinone, synergism occurred. Furthermore, the lowest effective concentration of acetaldehyde varied among individuals from 10^-6M to 10^-8M. These observations suggest that GSH is involved in the detoxification of hydroquinone and acetaldehyde in lymphocytes and that the simultaneous presence of both chemicals may saturate this mechanism and thus increase their genotoxic potency.

Genetic differences in GSH metabolism may govern the concentration of acetaldehyde at which synergism occurs in different individuals. Supported by NIHES 5 T32 ES 07106.

Previously, we reported that benzene (B) can inhibit DNA synthesis of bone marrow cells (BM cells) in B exposed mice (Toxicologist, 4:108, 1984). In this study, the inhibitory nature was investigated in the in vivo system. BM cells obtained from female ICR mice were incubated at 37°C in an in vitro medium with 5x10^-5M fraction of rat liver homogenate. Effect of B (0.55 to 4.42 mM) was measured 10 or 30 min after B incubation with BM cells by a 30 min incorporation of [3H]dTdr or [3H]dTTP into DNA. DNA polymerase activities were measured using activated calf thymus DNA as template 30 min after B interaction with the enzyme by a 30 min [3H]dTTP incorporation. B concentrations of 2.21 and 4.42 mM inhibited [3H]dTdr incorporation by 31 and 67%, respectively, in the presence of B. However, after removal of the reacted B from the cell culture system, no inhibition was observed with 2.21 mM, while 18% inhibition was produced with 4.42 mM, indicating that B alone is capable of inhibiting DNA synthesis. Similar inhibitory effect was also observed when [3H]dTTP was used as a DNA precursor in the cell system. Furthermore, B inhibited calf thymus DNA polymerase activity in a cell free system. BM cells reacted with 4.42 mM B in the presence of S-9 inhibited 34% of the control activity as opposed to 14% inhibition without S-9. These results indicate that B alone can inhibit DNA synthesis by inhibiting DNA polymerase in the mouse BM cells and that enhanced B metabolism is also responsible for the inhibition of DNA synthesis.

**DNA OR NUCLEOSIDE ADDUCTS WITH HYDROQUINONE (BQ) AND BENZOQUINONE (BQ)**. L. Jowa, G.F. Kalf, G. Witz and R. Snyder, Joint Graduate Program in Toxicology, Rutgers University and UMDNJ/Rutgers Medical School, Piscataway, NJ 08854 and Thomas Jefferson University, Philadelphia, PA. 19107.

Phenol, the major metabolite of benzene, is metabolized by BQ and EQ (Snyder et al., *Rev. Biochem. Tox. 3, 123, 1981*). In bone marrow preparations EQ and BQ inhibited RNA synthesis in nuclei (Post et al., *Chem. Biol. Interact.* 48, 203, 1984) and mitochondria (Kalf et al., *Chem. Biol. Interact.* 42, 333, 1982) and covalently bound to mitochondrial DNA (Rushmore et al., *Chem. Biol. Interact.* 49, 133, 1984). To study the nature of DNA adducts calf thymus DNA was incubated with [14C]BQ in the presence of Fe++ at pH 7.4, 37°C overnight and then dialyzed exhaustively. Density gradient centrifugation of the DNA in a CsCl gradient revealed a single peak of [14C] labeled DNA. DNAse treatment rendered the peak acid soluble. These data support the concept that BQ was covalently bound to DNA.

**DNA or Nucleoside Adducts with Hydroquinone (BQ)** and Benzquinone (BQ). L. Jowa, G.F. Kalf, G. Witz and R. Snyder, Joint Graduate Program in Toxicology, Rutgers University and UMDNJ/Rutgers Medical School, Piscataway, NJ 08854 and Thomas Jefferson University, Philadelphia, PA. 19107.
Previous studies have not consistently distinguished smokers from non-smokers by measuring the SCE frequency in human lymphocytes. In order to detect cytogenetic damage in smokers we have developed an assay using in vitro addition of AFN to human lymphocytes. In these experiments human peripheral lymphocytes were cultured for 72h in the presence of 2% PHA, 20 μg 5-bromo-2-deoxyuridine and AFN and assessed for SCEs. SCE induction in lymphocytes from smokers was linearly dependent on concentrations of AFN from 0.5-5 μg/ml. The maximum effect on the SCE/metaphase was 50% over baseline. In cultures incubated without AFN no difference in the SCE frequency between 4 smokers (8.35) and 8 non-smokers (8.07) was detected. The AFN (11 μg/ml) induced SCE levels were 12.79 (smokers) and 9.09 (non-smokers) (p<0.003), with both of the values also being different from their respective controls (p=0.01 and p=0.003). A SCE (AFN-controls) ranged from 3.12 to 5.72 in smokers vs 0 to 1.96 in non-smokers. Two mechanisms for the enhanced sensitivity of lymphocytes to cytogenetic damage are possible. Smoking might alter rates of critical metabolic activation/deactivation pathways for AFN or it might produce genetic instability. In conclusion, our results suggest that in vitro challenge to AFN results in a more sensitive SCE assay, which distinguishes smokers from non-smokers.

**In Vitro Challenge to α-Naphthoflavone (AFN) Increases the Sensitivity of the Sister Chromatid Exchange (SCE) Assay in Detecting Cigarette Smoking. K. Lundgren and G. Lucier. NIEMS, RTP, NC 27709**

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Tissue-type plasminogen activator (t-PA) is currently undergoing clinical trials for thrombolysis in the treatment of acute myocardial infarction. Low dose t-PA given to animals or patients does not substantially deplete the systemic circulation of compounds important for hemostasis (fibrinogen, Fg; plasminogen, Pg; α2 antiplasmin, α2AP), therefore avoiding post treatment hemorrhage, a frequent occurrence after conventional thrombolytic drugs. However t-PA might cause systemic fibrinogenolysis at high doses after high affinity binding sites on participating enzymes become saturated. Therefore, we used known rate constants for the major enzyme reactions of the fibrinolytic system with selected plasma concentrations of t-PA to model reaction kinetics on a VAX 11/780 computer. At plasma t-PA = 1 nM for 1 h the kinetic model predicted no depletion of Fg, whereas at 10 nM and 50 nM it predicted 5% and 30% depletion. Corresponding values for Fg and α2AP were 4% and 32%, and 85% depletion. Our simulations support the concept that it is possible to obviate the clot selectivity of t-PA with sufficiently high doses. Data from patients (N = 10) given i.v. t-PA support the implications of these computer simulations. Thus, although t-PA is the safest thrombolytic agent tested to date, high pharmacological doses can upset the hemostatic balance.

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The subchronic inhalation toxicity of a raw shale oil was evaluated in Flock 344 rats. Colorado oil shale was retorted using the Lurgi-Hubas technology producing an oil with an API gravity of 17.9° and a distillation range of 117-557°C. Groups of 15 male and 15 female rats were exposed 6 hrs/day, 5 days/wk for 13 wks to aerosol concentrations of 0, 56, 120, or 492 mg/m3. Ten of 15 males and 7 of 15 females exposed to 492 mg/m3 died during the study. Body weight gain was suppressed at all exposure levels in a dose-related pattern. Pathological changes clearly associated with repeated doses of the test compound included: (1) inflammatory and hyperplastic lesions in the lungs and the upper respiratory tract; (2) atrophy of the thymus and thymic-dependent lymphocytic portions of the peripheral lymph nodes and spleen, and (3) severe pancytopenia of the bone marrow. These changes were seen in all high dose animals and were present with decreasing severity in the lower dose groups. Other lesions present more frequently in the 492 mg/m3 group included focal necrosis of renal tubular epithelium, atrophy of thyroid follicular epithelium, centrolobular and focal hepatic necrosis, and patchy atrophy of the testicular epithelium. These findings were associated with organ weight changes and pronounced clinical pathological abnormalities.

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**Effects of Chlordcone on Isolated Ovine Erythrocytes. S. D. Sibleau and D. E. Moreland, Toxicol. Prog., Dept. of Crop Science, N.C. State Univ., Raleigh, NC**

Chlordcone (4 - 30 μM) altered the permeability of isolated ovine erythrocyte membranes as evidenced by a concentration-dependent induction of K+ efflux and hemolysis. The addition of chlordcone induced a short burst of hemolysis (< 1 min). The rate of hemolysis slowed after this initial rapid rate, followed by a time-dependent increase in the rate of hemolysis. Hemolysis was markedly delayed when the erythrocytes were suspended in isotonic sucrose. Chlordcone-induced hemolysis and K+ efflux were dependent upon the pH of the external medium. Raising the pH from 7.4 to 9.4 prevented both hemolysis and K+ efflux. Low concentrations of chlordcone (0.2-3 μM) protected erythrocytes against hypotonic hemolysis. Chlordcone (30 μM) did not induce the release of trapped K+ from KCN-loaded, ovine erythrocyte-lipid, bilamellar liposomes. It is suggested that chlordcone interacts with membrane proteins and increases the permeability of the membrane to cations. This interaction leads to colloid-osmotic hemolysis.

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The subchronic inhalation toxicity of a raw shale oil was evaluated in Flock 344 rats. Colorado oil shale was retorted using the Lurgi-Hubas technology producing an oil with an API gravity of 17.9° and a distillation range of 117-557°C. Groups of 15 male and 15 female rats were exposed 6 hrs/day, 5 days/wk for 13 wks to aerosol concentrations of 0, 56, 120, or 492 mg/m3. Ten of 15 males and 7 of 15 females exposed to 492 mg/m3 died during the study. Body weight gain was suppressed at all exposure levels in a dose-related pattern. Pathological changes clearly associated with repeated doses of the test compound included: (1) inflammatory and hyperplastic lesions in the lungs and the upper respiratory tract; (2) atrophy of the thymus and thymic-dependent lymphocytic portions of the peripheral lymph nodes and spleen, and (3) severe pancytopenia of the bone marrow. These changes were seen in all high dose animals and were present with decreasing severity in the lower dose groups. Other lesions present more frequently in the 492 mg/m3 group included focal necrosis of renal tubular epithelium, atrophy of thyroid follicular epithelium, centrolobular and focal hepatic necrosis, and patchy atrophy of the testicular epithelium. These findings were associated with organ weight changes and pronounced clinical pathological abnormalities.
Anguidine (diacetoxydicaprinol or DAS) is a trichothecene mycotoxin that elicits a number of responses from biological systems, some of which resemble radiation injury. There is rapid and severe injury to cell populations with a rapid turnover rate, including the gonads, thymus, spleen, lymph nodes, and bone marrow. Comparative sensitivity varies among species and with different routes of exposure. The LD₅₀ for young Fischer rats is about 1.0 mg/kg body weight by i.p. or s.c. administration. For CD-1 mice, it is about 20, 15, or 11 mg/kg body weight by i.p., s.c. and respiratory routes respectively. We have examined comparative absorption of DAS by skin exposure using a newly designed exposure chamber. The rat absorbs DAS quickly and metabolites disappear from urine in about 24 hours. The mouse absorbs DAS more slowly with about 85% of an i.p. dose excreted in the urine over a 4-day period, the skin acting as a reservoir in this species. The skin and other lesions of both species are the same but time course of development and healing are markedly different. Toxicokinetic and morphologic data will be presented. (Supported in part by USAMRIID, US Army R&D, Walter Reed Institute for Research).

Incubation of isolated rat hepatocytes in the absence of extracellular calcium leads to an accelerated depletion of cytoplasmic and mitochondrial glutathione (GSH), formation of GSH disulfide (GSSG), and efflux of GSH as compared with hepatocytes incubated in calcium-containing medium. These alterations in GSH status enhance the susceptibility of hepatocytes to cell injury (Parisa, et al., Biochem. Biophys. Res. Commun. 121:102, 1984). The addition of vitamin E succinate (25 μM) to the incubation media markedly attenuated the depletion of cytoplasmic GSH during incubation in calcium-free medium, without affecting mitochondrial GSH, GSH and GSSG efflux, or formation of protein-mixed disulfides (pr-SG). This treatment resulted in a net increase in total cellular glutathione (GSH + GSSG + pr-SG) content after 5 hours. An elevation in intracellular α-tocopherol content preceded this increase in glutathione content, with a resultant increase in protection against cell injury. Vitamin E succinate did not prevent the enhanced depletion of GSH by buthionine sulfoximine (BSH). These data indicate that vitamin E protects isolated rat hepatocytes against GSH depletion and cell injury by potentiating GSH synthesis.

Supported by grants ES07060 and ES01978.

Anguidine (DAS) and CFU-S System. V. Supakarn and P.M. Newberne. Massachusetts Institute of Technology, Cambridge, MA.

Anguidine [Diacetoxydicaprinol (DAS)] is highly toxic with a rapid turnover time. Lethal and sublethal doses of DAS cause extensive necrosis of bone marrow and lymphatic system, an effect seen as early as one hour after exposure. This study investigated the effect of anguidine on the nature and cellular basis of variation in the proliferative capacity of marrow cell populations. A semi-quantitative measure of this capacity of the marrow was obtained by determining the ability of the marrow to form colonies in the spleen of irradiated recipient mice (CFU-S) 10⁵ marrow cells in 0.5 ml volume from mouse or rat treated with 25 mg/kg DAS on skin for 3 days, were injected into 30 days old, CD-1, male mice, previously irradiated with 850 rads 137 Cesium. Ten days after injection, the mice were sacrificed and the spleens removed, fixed in Bouin's solution and counted. There was a significant difference in CFU/femur between treatment with DAS, DMSO, or control groups (p<0.001). DAS destroyed most of the hematopoietic stem cell population whereas DMSO had only a minimal effect. Skin exposure to DAS in Fisher rats was significantly more traumatic than a similar exposure in mice. The CFU-S system is a simple technique useful in identifying effects of chemicals on hematopoietic stem cells. (Supported in part by US Army R&D Command).


High concentrations of halothane (H) have been reported to be toxic to rat hepatocytes in suspension (RHS), while enflurane (E) is nontoxic (Anes 48:17, 1978). However, no biotransformation of either E or H was reported. This study examines the toxicity and defluorination (def) of E and H in RHS with regards to O₂ tension, time, and concentration. RHS from phenobarbital-treated rats (5 x 10⁹/3 ml media) were exposed to volatileized E and H (1-20 μl) in 25 ml sealed flasks. At various times (15 min-6 hr), aliquots were examined for the leakage of cellular lactic dehydrogenase (LDH) and K⁺ and the common metabolite, F⁻. In 95% O₂, 5 μl H caused decreased cellular K⁺ and increased leakage of LDH while 20 μl E had no effect. However, at this O₂ tension, H is not reductively def while E is def. At 21% O₂, 10 - 20 μl E is toxic to RHS, but not as toxic as H. At 21% O₂, H is def, but high levels decrease def. Def of E at 21% O₂ is less than in 95% O₂. At lower levels (1-3 μl), both E and H are mildly toxic to RHS (15-21% O₂) while their def is both time and dose dependent. Below 15% O₂ the toxicity of E and H is difficult to determine due to hypoxic effects. Toxicity of H and E at high dose levels appears to be unrelated to def and due solely to a solvent effect. At low dose levels, both H and E are slightly toxic, but primarily in hypoxia-stressed RHS. (NIH AM16715)
4-Bromophenol (4-OH) and 4-bromocatechol (CAT) are metabolites of bromobenzene (BB) found in vivo and in isolated hepatocytes. Both of these metabolites may potentially contribute to the toxicity of BB. BB metabolism in hepatocytes isolated from phenobarbital treated rats forms 0.12 - 0.17 mM 4-OH and CAT in two hours, with 1 - 3 mM BB. The roles of 4-OH and CAT in BB covalent binding and toxicity were investigated using isolated hepatocytes, under conditions identical to those used for the metabolism studies. Significant covalent binding of 0.25 mM 4-OH and CAT was observed only with the addition of 1mM unlabelled BB. Under these conditions, incubations of 0.25 mM 4-OH and CAT with isolated hepatocytes resulted in no significant toxicity, and approximately 4% and 2%, respectively, of the covalent binding associated with BB itself. Two and six hour incubations with higher BB concentrations demonstrated that 1 - 3 mM substrate concentrations were required for cytotoxicity. These results show that metabolically produced 4-OH and CAT do not play significant roles in BB cytotoxicity in isolated hepatocytes, and that they contribute only modestly to BB covalent binding.

Supported by NIH grant ES02868.


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594 ABSENCE OF THIOBARBITURIC ACID-REACTIVE SUBSTANCES (TBARS) IN ISOLATED RAT HEPATOCYTES DURING A TOXIC CHEMICAL INSULT. M.W. Paris, G.A. Passoe, and D.J. Reed, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR. 97331

Using a novel in vitro method for assessing the mechanism of chemical-induced toxicity (Reed and Paris, Pharmacological Rev. 36: 258, 1984), we examined the role of lipid peroxidation in chemically-mediated cell death. Hepatocyte suspensions were exposed to toxic levels of carbon tetrachloride, bromobenzene, ethyl methanesulfonate or adriamycin in combination with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and the concentration of TBARS in viable cells, medium and the cell suspension was monitored hourly for 6 hours. Prior to chemical-induced cell death (LDH leakage), a 5-15 fold increase in the cell suspension TBARS concentration was observed. The concentration of TBARS in chemically damaged viable hepatocytes, however, did not increase during the toxic insults. The amount of TBARS in the medium accounted for the increase observed in the cell suspension. These results suggest that (1) TBARS are rapidly transported or diffuse from isolated hepatocytes into the extracellular space and (2) chemical-induced cell death is not caused by cellular accumulation of TBARS.

Supported by grants ES07900 and ES03978. (*Present address: Department of Pathology, Medical College of VA., Richmond, VA. 23298).

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596 AN IN VITRO MODEL FOR TOXICITY TESTING USING HEPATOCYTES FRESHLY ISOLATED FROM ADULT MAMMALS. G. Krack, P. Vossen, and M. Roberfroid, Unit of Biochemical Toxicology. Catholic University of Louvain, 1200 Brussels, Belgium. Sponsor: L Glenn Sipes.

The present report demonstrates the validity of isolated hepatocytes surviving in suspension as a model to study the cytotoxic effects of drug mixtures. Many pharmaceutical preparations contain more than one component but it is, according to the classical toxicological procedure, difficult and even impossible to test for the toxicity of such associations. Critical biochemical functions of the hepatocytes (protein, glycogen metabolism) were chosen as the evaluation of the cytotoxic effects of the tested compound. Those endpoints were preferred to the usual cell viability criterion, LDH release, because of their sensitivity and their reliability which have been previously demonstrated. By testing for the effects of each individual component (cyclovalone, retinoic acid, triliodothyroacetic acid) of the drug preparation and by comparing these effects with those of the agents taken as binary and ternary mixtures, it has been possible to identify cyclovalone as the most cytotoxic component and to suggest a new formulation which could be less hepatotoxic. Such a demonstration opens new ways to reinforce the importance of in vitro toxicity testing, especially using freshly isolated hepatocytes from adult mammals.
TOXICITY OF 2-AMINOFLUORENE (2AF) AND 2-ACETYLAMINOFLUORENE (2AAF) IN RABBIT HEPATOCYTES.
C.A. McQueen, M.J. Miller, B.M. Merrill and G.M. Williams. American Health Foundation, Valhalla, NY.

Conjugation generally is a step in xenobiotic detoxification; however, one type of conjugation, N-acetylation, has been implicated in the activation of aromatic amines. The cytotoxicity, genotoxicity and metabolism of 2AF and 2AAF were investigated to compare the effect of acetylation on toxicity. In monolayer cultures of hepatocytes from male rabbits, 2AF was more cytotoxic than 2AAF. Cultures exposed to $10^{-3}$ M 2AF showed a significant decrease in cell number and viability while the same concentration of 2AAF had little effect. Genotoxicity was monitored as autoradiographic DNA repair synthesis. Maximum repair was induced at $10^{-7}$ M for 2AF and $10^{-3}$ to $10^{-4}$ M for 2AAF. Metabolites were separated by HPLC. The rate of disappearance of 2AAF was log linear; a half-life of 2.5 hours was observed for $10^{-5}$ M. Although a biphasic curve was seen with 2AF, percent of unmetabolized parent at 20 hr. was the same as seen with 2AAF. Over 75% of an initial $10^{-5}$ M dose was converted to sulphate and glucuronide conjugates. The greater toxicity of 2AF, then does not seem to be due to less detoxification. These results suggest that reactive metabolites lacking the acetyl group may be produced in larger quantities or cause greater damage than acetylated products.

OXYGEN-MEDIATED CELL INJURY IN THE KILLING OF CULTURED HEPATOCYTES BY BROMOBENZENE.

The sensitivity of cultured rat hepatocytes to bromobenzene was manipulated in ways that suggest the involvement of activated oxygen species in the cell killing produced by this hepatotoxin. S9 CNONU inhibited GSTC-reductase activity by 86% without affecting GSH-peroxidase, catalase, or cellular viability and potentiated by almost an order of magnitude the toxicity of bromobenzene. At the same time, there were no changes in the metabolism or covalent binding of [14C]-bromobenzene in hepatocytes treated with CNONU. Pretreating hepatocytes with 20mM deferoxamine reduced the toxicity of bromobenzene. Sensitivity was restored by the addition of 2mM iron to the culture medium. Again bromobenzene metabolism and covalent binding were unaffected by pretreatment with deferoxamine or by the addition of iron. These data implicate a role for hydrogen peroxide and iron in the cell killing produced by bromobenzene. It is suspected that an ultimate oxidizing species is formed from the reaction of free or chelated iron with hydrogen peroxide in a Fenton-like reaction. (Supported by NTH Grant AM 31114 and ES 5380)

THE MECHANISM OF SKF 92994 (OMERIDINE) CYTOTOXICITY IN ISOLATED RAT HEPATOCYTES.
Sponsor: W. R. Hewitt

SKF 92994 is an H2-receptor antagonist that has been associated with abnormal hepatic function in patients on maintenance therapy. Isolated rat hepatocytes exposed to SKF 92994 (500 μM) rapidly lost viability as estimated by increased enzyme leakage, decreased intracellular [K+] and increased formation of surface blebs in the plasma membrane. SKF 92994 (500 μM) also rapidly decreased hepatocyte oxygen consumption (QO2) and ATP content prior to changes in hepatocyte viability. SKF 92994 (50 μM) blocked pyruvate/malate-supported, state 3 (ADP-stimulated) respiration, caused a decrease in the ADP/O ratio and a loss of respiratory control in isolated rat liver mitochondria (IRLM). In contrast, SKF 92994 did not block succinate-supported, ADP-stimulated QO2 in IRLM. The mechanism of SKF 92994-induced hepatocyte injury therefore appears to be due to a sustained inhibition of mitochondrial oxidative phosphorylation leading to decreased ATP content and eventual cell death. Although the exact site of action of SKF 92994 within mitochondria has not been completely elucidated, it appears to occur prior to ubiquinone-oxidoreductase in the inner mitochondrial membrane electron transport chain.

EFFECTS OF HEPATOTOXIN ON MOUSE AND RAT HEPATOCYTES FROM PRENEOPLASTIC/NEOPLASTIC LESIONS AND FROM PARTIAL HEPATECTOMIZED ANIMALS. L.W. Chang, M.A. Pereira, J.E. Klaunig and R.J. Ruch. HERL, U.S. EPA, Cincinnati, OH 45268 and Dept. of Path. Medical College of Ohio, Toledo, OH 43699

Preneoplastic/neoplastic lesions (P/N) in rat liver have been shown to be resistant to hepatotoxins (HT) that are normally selectively stimulated to proliferate in the presence of HT. We investigated whether mouse and rat hepatocytes (HC) in primary culture from P/N or from partial hepatectomized (PH) animals were resistant to HT. In rats, P/N were induced by either 1) diethylnitrosamine (DENA) followed by phenobarbital or 2) DENA followed by 2-acetamidoanilene and CCL4. In mice, P/N were induced by DENA. HC were exposed to the test substance for 24 hrs. and the toxicity determined by Trypan blue exclusion. In rats, HC from P/N and from PH animals were resistant to HT except in the case of haloalkanes (HA) where HC from PH but not P/N were resistant. In mice, HC from PH animals were resistant to HT including Adriamycin, DCPA, mitomycin (MX) and lindane while P/N were resistant only to lindane and MX. In conclusion 1) P/N in mice unlike rats, are less resistant to HA and 2) P/N are not resistant to HA. Therefore, enhancement of liver cancer by HT in mice unlike rats, might not result from the selective proliferation of P/N. (This abstract does not necessarily reflect EPA policy.)

Little is known of the possible toxic interactions of halogenated hydrocarbons. The hepatotoxicity of monochlorobenzene (MCB) and o-, m-, and p-dichlorobenzene (DCB) was evaluated for each compound alone and in combination with CCl₄. Male F344 rats were given a single i.p. injection of 1.0 mmol CCl₄/kg and 0.9 mmol/kg of MCB or one of the DCBs in corn oil. Plasma GPT activity was evaluated 24 hr later as an index of liver injury and found to be significantly elevated in animals treated with both MCB and CCl₄ (321 units/ml) relative to MCB or CCl₄ alone (42 and 198, respectively). No such potentiation of toxicity was seen with the isomers of DCB. When given alone, m- and p-DCB were essentially nontoxic at doses up to 2.7 mmol/kg while o-DCB alone produced a dose related increase in GPT activity to 1740 units/ml at 2.7 mmol/kg. Interestingly, concomitant administration of 1.0 mmol CCl₄/kg along with o-DCB (2.7 mmol/kg) resulted in a decline in GPT activity to 209 units/ml. The data presented herein demonstrate a potentiation of hepatotoxicity when MCB and CCl₄ are administered simultaneously, while CCl₄ apparently decreases the hepatotoxicity of o-DCB. Studies to delineate the mechanisms of the differential toxicities of the DCB isomers and the apparent antagonism of o-DCB toxicity by CCl₄ are ongoing. (Supported by NIH #T32 ES 07091 and N01-ES-3-5031.)

Cimetidine (CIM) is known to inhibit liver microsomal drug metabolism. Trichloroethylene (TRI) is metabolized by the hepatic mono-oxynogenase system to a reactive intermediate responsible for its toxicity. So we have studied the effects of CIM on the hepatotoxicity (HT) and the metabolism of TRI in adult male Sprague-Dawley rats. Groups of rats received a single i.p. dose of TRI (0.05, 0.65 ml/kg) with or without a CIM treatment (3 doses of 120 mg/kg: 1 and 7 h before and 4 h after). Urines were collected for 24 h and the rats sacrificed. The HT of TRI at 0.65 ml/kg as measured by SGPT response and liver histopathology, was found to be increased by CIM. The urinary excretion of trichloroacetic acid at 0.65 ml/kg of TRI was reduced by CIM. In in-vitro studies, CIM inhibited the activities of aminopyrine N-deethylase (ADM) and aniline hydroxylase (AH). The epoxide hydratase activity was reduced due to combined CIM treatment compared to CIM treated group, without modifying ADM and AH. The lack of reduction of these liver enzymes after 24 h in liver microsomes of treated rats suggest a competitive inhibition which disappeared after 24 h. Thus potentialization of HT of TRI due to CIM seems to be somewhat related to the metabolism of TRI (Supported by CAFIR, Univ. de Montréal).

606. PROTECTIVE ROLE OF GLUTATHIONE IN HEPATOTOXICITY DUE TO ALLYL ALCOHOL. Mostafa Z. Badr and Ronald G. Thurman, Dept. of Pharmacology, Univ. of N.C., Chapel Hill, NC.

 Allyl alcohol (AA) is a hepatotoxin which specifically damages perportal (PF) yet spares pericentral (PC) regions of the liver lobule; however, the toxic intermediate acrolein is also generated in PC areas. Therefore, studies were designed to identify factors involved in vulnerability of PF and/or protection of PC regions. AA (350 µM) was infused for 60 min into perfused livers from fed, fasted (24 h), and diethylmaleate (DEM; 0.7 g/kg)-treated rats. Half-maximal lactate dehydrogenase (LDH) release was attained at 45, 35, 22 or 17 min for livers of fed; fasted; fed, DEM-treated; or fasted, DEM-treated, respectively. The damage in PC areas, as measured by trypan blue uptake, was nearly 100% in all groups; however, in PC areas it was 16, 77, 97 and 100% in fed; fasted; fed, DEM-treated; and fasted, DEM-treated rats, respectively. Methionine infusion for 20 min prior to AA reversed the effects of DEM treatment and increased the half-time for maximal LDH release to 43 min. Damage to PF and PC regions was 79 and 46%, respectively, under these conditions. Thus, factors that deplete glutathione (fasting and DEM treatment) accelerate AA-induced LDH release from PC areas and allow AA to damage PC regions. Conversely, methionine, which stimulates glutamine-synthesis, provided partial protection. These data indicate that glutathione is involved in protection of PC regions of the liver lobule against AA-induced toxicity. (ES-07126 and ES-02759)


The extent of hepatotoxicity caused by cis- and trans-1,2-dichloroethylene (DCE) was examined in male rats. Dose response studies indicated that 25 mmole/kg trans-DCE, i.e., elevated plasma sorbitol dehydrogenase (SDH), and plasma aspartate aminotransferase (AST) activities and slightly depressed hepatic glutathione (GSH). When administered orally in a dose of 51 mmole/kg, trans-DCE did not affect plasma enzyme activities or hepatic GSH content. A time course study using 25 mmole/kg trans-DCE, i.e., showed that hepatic GSH content varied between treatment groups and plasma alanine aminotransferase (ALT), AST, and SDH activities were significantly elevated above control levels. Dose response studies with cis-DCE showed that 26 mmole/kg i.p. significantly elevated plasma AST and SDH activities. When administered orally, 51 mmole/kg cis-DCE significantly elevated plasma AST and SDH activities, but did not depress hepatic hepatic GSH. Although variable responses were observed, these studies indicate that the cis and trans isomers of 1,2-DCE are equipotent hepatotoxins that are more toxic when given by the intraperitoneal than by the oral route, and that the hepatotoxicity of each is not mediated through changes in hepatic GSH content. (Supported by Burroughs-Wellcome Toxiology Scholar Award)


The hepatic portal necrosis induced by allyl alcohol (AA) is inhibited following acute or chronic α-naphthylthioacetate (ANIT) administration. Bile duct ligation (BDL) was utilized to determine if the cholestatic property of ANIT is responsible for this effect in male F-344 rats. The common bile duct was ligated using a tube suture. Ligation was performed after 2 days. Sham operated rats were used as controls. Rats were killed 1 or 2 days after ligation. Additional rats were killed 1–9 days after ligature release. AA (32 mg/kg i.p.) was administered 4 hr prior to ligature release or 1 or 8 days after release and rats killed 24 hr later. Alanine aminotransferase (ALT) was 60X control at 24 hrs and 25X at 48 hrs after BDL. ALT activity was 3X control at 24 hrs after release. In the sham operated rats AA produced a 218% increase in ALT. This response was suppressed by 93% when AA was administered 4 hr prior to, or 24 hrs or 48 hrs after ligature release. In conclusion, transient cholestasis (chemically or mechanically induced) cause a prolonged suppression of AA toxicity.
The objective of the investigation was to characterize the acute, subacute, and subchronic toxic potential of ingested carbon tetrachloride (CCL). Male Sprague-Dawley rats of 300-350g were gavaged with 0, 20, 40, or 80 mg CCL/kg bw in corn oil once daily for 5 days, rested for 2 days, and dosed once daily for 4 more days. Rats of 200-250g were similarly dosed with 0, 20, 80, or 160 mg CCL/kg. Single 20 and 40 mg/kg doses had no apparent toxic effect at 24 hr, though 80 mg/kg caused mild hepatic centrilobular vacuolization and increases in some serum enzyme levels. In general, there was progressively severe hepatic injury at each dosage level over the 11-day study periods. CCL was more hepatotoxic to the 200-250g rats than to the 300-350g rats. In the subchronic study, rats initially 200-250g were gavaged 5 times weekly for 12 weeks with 0, 1, 10, or 33 mg CCL/kg and monitored for signs of toxicity at 2-week intervals. One mg/kg had no apparent adverse effect. Ten mg/kg produced slight elevations in sorbitol dehydrogenase activity and mild hepatic centrilobular vacuolization. Thirty-three mg/kg caused marked hepatotoxicity. Serum enzyme levels in the 33 mg/kg animals returned towards normal during a 2-week recovery period, but hyperplastic nodules and cirrhosis were still evident. (U.S. EPA CR811215)

The objective of these studies was to gain a clearer understanding of the phenomenon of temporal sensitivity to CCL hepatotoxicity. Male S-2A rats of 250-300g were maintained on a 12-hr light dark cycle with light from 0600 to 1800 hr. Fasted and nonfasted rats were given a single oral dose of 0.5 ml CCL/kg in corn oil at intervals over a 24-hr period. Assays of serum enzymes 24 hr post dosing revealed a pronounced circadian rhythm in susceptibility to CCL hepatotoxicity. Susceptibility was maximum at 1800 hr in both nonfasted and fasted rats, with the latter exhibiting much more severe injury. Liver samples were also taken over a 24-hr period from fasted and nonfasted rats with no CCL, exposure, in order to assess the role of several cellular processes in temporal susceptibility to CCL. Glutathione levels were: a) inversely related to CCL susceptibility over the 24-hr period in both groups; b) lower at each time-point in the fasted group. CCL was incubated in vivo with hepatic microsomes and protein binding measured as an index of CCL metabolism. There was a circadian rhythm in CCL metabolism, maximum metabolism coinciding with maximum susceptibility to injury in both groups. Metabolism was greater in the fasted group at each time-point. P-450 levels were also higher in the fasted rats, but circadian rhythm was seen in either group. (U.S. EPA CR811215)
PLASMA MEMBRANE ALTERATIONS AND COVALENT BINDING TO ORGANELLES AFTER AN HEPATOTOXIC DOSE OF ACETAMINOPHEN (APAP). G.L. Ginsberg and S.D. Cohen, Toxicology Program, Sch. of Pharm., Univ. of Conn., Storrs, CT 06268.

APAP covalent binding is associated with hepatotoxicity, although its role in the toxic process remains unclear. We studied the subcellular distribution of covalently bound APAP, and effects on plasma membrane (PLM) enzyme activities at 2,4 and 6 hours after 600mg APAP/kg, p.o., in fasted mice. Covalent binding per mg organelle protein was greatest in PLM, followed by cytosol (Cyt), microsomes (MCS) and mitochondria (Mito) through 6 hours. Total binding per fraction was greatest in Cyt followed by MCS, Mito and PLM. This binding decreased between 2-6 hours by 36, 32, and 26% in homogenate (HOM), Mito, and Cyt, respectively, but was unchanged in MCS and PLM. Protein content of fractions at 6 hours was 68, 61, 75, and 79% of control in HOM, Mito, MCS and Cyt, respectively, but was unchanged in PLM. NαH4+ ATPase and 5‘nucleotidase total activities in PLM were 60 and 60% of control at 6 hours, while the activities declined to 77 and 66% of control, respectively. High specific binding in PLM and decreased PLM enzyme activities suggest that damage to PLM may play a critical role in hepatocellular necrosis after APAP. (Supported by NIGMS-31460 and Stafker Chm. Co. Fellowship in Tox. to GLC)


Alterations in APAP metabolism may explain the potentiation of APAP induced hepatotoxicity by RA in F344 rats. These experiments were designed to quantify the effect of RA on plasma APAP concentrations and urinary metabolites, and covalent binding. Rats were administered RA (50 mg/kg, p.o.) or vehicle (VE) 30 min prior to (3H)-APAP (750 mg/kg, p.o.) and killed 2, 4, 6, 8, 12 or 24 hr later; blood was collected and liver and muscle excised. Urine was collected for 24 hours. Plasma APAP concentrations and urinary metabolites were quantified by HPLC. Plasma APAP concentrations in RA rats were 193% and 277% of those in VE rats 2 and 4 hours after APAP, respectively. Similarly, covalent binding of APAP to hepatic macromolecules (24 hrs after APAP) in RA rats was approximately twice that observed in VE rats. Urinary excretion of glucuronide (APAP-GLUC) and sulfate (APAP-SO4) conjugates of APAP were affected by RA. APAP-GLUC excretion was decreased 34% while APAP-SO4 excretion was increased by 28% in RA pretreated animals. Therefore, these studies indicated that APAP metabolism, following a hepatotoxic dose, was altered by RA pretreatment. These alterations in metabolism may explain the potentiation of APAP-induced hepatotoxicity by RA.

CHARACTERIZATION OF RANITIDINE-ACETAMINOPHEN (APAP) INTERACTIONS IN F344 RATS: EFFECTS ON APAP HEPATOTOXICITY. T.B. Leonard, D.G. Morgan, and J.G. Dent, Deps. of Drug Metabol. and Path., Smith Kline and French Labs., Phila, PA 19101

In contrast to cimetidine which protects against acetaminophen hepatotoxicity, ranitidine (RA), another H2 receptor antagonist, has been demonstrated to potentiate APAP induced hepatotoxicity in F344 rats. These studies were designed to characterize RA effects on APAP hepatotoxicity and to evaluate possible mechanisms. Toxicity was evaluated using serum ALT activity and histopathology. RA administered (50 mg/kg, p.o.) 30 min prior to APAP (750 mg/kg, p.o.) potentiated APAP hepatotoxicity but did not alter the time course of toxicity. At APAP doses ranging from 500 to 1000 mg/kg, pre-treatment with RA enhanced APAP hepatotoxicity (5 to 12X). Potentiation of APAP hepatotoxicity by RA was dose-dependent at RA doses from 10 to 50 mg/kg, while RA doses 100 mg/kg protected against APAP hepatotoxicity. RA was not hepatotoxic when administered at up to 500 mg/kg or following induction of mixed function oxidase activity or depletion of glutathione (GSH). Hepatic GSH was not markedly reduced by RA and RA mediated increases in APAP oxidation are improbable. In conclusion, these results have demonstrated a RA-APAP interaction in rats which increases APAP hepatotoxicity and support the suggestion that RA mediated inhibition of APAP conjugation may be the underlying mechanism.


Experiments were conducted to examine the ability of zinc to protect against acetaminophen (AAP) hepatotoxicity in male rats. Animals were pretreated twice with zinc acetate (6 mg Zn/kg, ip) at 48 and 24 hr prior to AAP (1 g/kg, ip). At 4 and 24 hr following AAP, aspartate aminotransferase (ALT) activities and hepatic glutathione (GSH) levels were measured. Four hours after AAP, both serum SDH and ALT activities were elevated in AAP treated rats while hepatic GSH levels were reduced. Pretreatment with zinc prevented the AAP increase in ALT but not SDH activity. Zn did not alter the AAP decrease in hepatic GSH. At 24 hr, serum SDH and ALT activities were markedly increased and hepatic GSH decreased in rats receiving AAP. Zinc prevented the AAP increases in ALT and SDH but did not prevent the decrease in hepatic GSH. The binding of 3H-AAP to hepatic macromolecules was decreased at 24 hr by zinc pre-treatment. Subcellular distribution of AAP showed only increased in the cytosolic fraction in Zn treated rats. Zn treatment also significantly decreased cytochrome P-450 levels in hepatic microsomes. Thus, these data suggest that Zn protection of AAP hepatotoxicity is partially mediated through inhibition of drug metabolism but not in changes in hepatic glutathione. (Supported by a Burroughs-Wellcome Toxicology Scholar Award).
Morphological and Biochemical Characteristics of Perfluorooctanoate-Induced Liver Enlargement. T.P. Pastoor, K.P. Lee, and F.J. Gillies, E.I. du Pont de Nemours & Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Rd., P.O. Box 30, Newark, DE 19714

Perfluorooctanoic acid (PFOA) causes liver enlargement (hepatomegaly) in rats. These studies were undertaken to examine the morphological and biochemical basis of PFOA-induced hepatomegaly. Male rats were dosed orally with 50 mg PFOA/kg body weight for three successive days. Hepatocytes were enlarged, occluded sinusoidal spaces, and contained more mitochondria and microbodies (peroxisomes). Biochemically, the concentration of cytochrome P-450 was increased more than 3-fold together with a 2-fold increase in benzphetamine-N-demethylase activity. Carnitine acetyltransferase (CAT), a marker enzyme for both mitochondria and peroxisomes, was elevated 12-fold and carnitine palmitoyltransferase (CPT), a marker enzyme for mitochondria, was elevated 2-fold. The incorporation of [14C]-acetate into hepatic phospholipids, triacylglycerols, and cholesteryl esters was also increased. Plasma total cholesterol and triacylglycerols were unaffected. Thus, the factors underlying PFOA-induced hepatomegaly are proliferation of the smooth endoplasmic reticulum, mitochondria, and, to the greatest extent, peroxisomes. However, unlike peroxisomal proliferating compounds reported thus far, PFOA is not hypolipidemic. Thus, PFOA may be considered as a novel peroxisome proliferating agent.

EFFECT OF THIOBENZAMIDE (TB) ON HEPATOCELLULAR MEMBRANE-BOUND ENZYMES. J.A. Zempel and G.J. Traiger, Department of Pharmacology & Toxicology, University of Kansas, Lawrence, KS.

Chemically-induced cholestasis has been associated with inhibition of hepatocellular membrane-bound enzymes. Acute cholestatic doses of TB increase plasma bilirubin and GPT activity in the rat. This study was designed to assess the effect of TB on bile canalicular and sinusoidal membrane (BCM and SM, respectively) associated Na+/K+-ATPase, Mg++-ATPase and S'-nucleotidase (5'-Nase). TB (1.82 mmole/kg, ip) inhibited BCM and SM Na+/K+-ATPase, Mg++-ATPase and 5'-Nase at 24 hrs. At 48 hrs BCM Na+/K+-ATPase and 5'-Nase recovered while BCM Mg++-ATPase and SM enzymes remained inhibited. Plasma bilirubin, GPT activity and SM protein were maximally increased at 24 hrs.

N-octylimidazole (NOI; 2 X 0.06 mmole/kg, ip) or methylphenylthiourea (MPTU, 0.91 mmole/kg, ip), inhibitors of TB metabolism, were administered with TB (1.82 mmole/kg, ip). TB-induced BCM Na+/K+-ATPase inhibition and hyperbilirubinemia were reversed by NOI and MPTU, while TB-induced SM Na+/K+-ATPase inhibition was partially reversed by NOI. BCM and SM Mg++-ATPase and 5'-Nase inhibition was not reversed by NOI or MPTU. TB-induced increases in GPT activity and SM protein were antagonized by both inhibitors. In vivo administration of TB inhibited hepatocellular membrane-bound Na+/K+-ATPase, Mg++-ATPase and 5'-Nase. Na+/K+-ATPase inhibition may be involved in TB-induced hyperbilirubinemia. Supported by NIH Grant No. ES-02335.

RELATIONSHIP BETWEEN HEPATIC GLUTATHIONE DEPLETION AND INHIBITION OF PROTEIN SYNTHESIS INDUCED BY ADMINISTRATION OF HEPATOTOXIC CHEMICALS. L.J. Fischer, M.D. Green and C.J. Nichols, Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA.

The ability of acetaminophen (APAP) and some other hepatotoxins to inhibit in vivo hepatic protein synthesis (HPS), measured by [3H]-leucine incorporation after a pool expanding dose of the amino acid, was examined in mice. Because many, but not all, hepatotoxins produce a depletion of hepatic glutathione (GSH), treatments were selected so that a possible relationship between HPS and GSH could be detected. A dose-dependent inhibition of HPS, measured at 2 hr. after APAP (100-500 mg/kg), correlated well with the dose-related depletion of GSH. The time course of HPS inhibition produced by 500 mg/kg APAP was the same as the time course of GSH depletion, both parameters reaching a nadir at 2 hr. after the drug. Prevention of APAP-induced GSH depletion with N-acetylcysteine or piperonyl butoxide pretreatments prevented the drug-induced inhibition of HPS. Two other chemicals known to deplete GSH, diethylmaleate and phorone, also produced an inhibition of HPS. Allyl alcohol did not lower GSH and was not inhibitory to HPS measured 2 hr. after a hepatotoxic dose. It appears that a link between HPS and GSH exists and details of this relationship remain to be investigated. (NIH GM-26811).

ENHANCEMENT OF IRON-INDUCED IN VIVO-LIPID PEROXIDATION BY COMPOUNDS UNDERGOING REDOX CYCLING. M. Younes, S. Cornelius, and C.-P. Siegers, Institut für Toxikologie, Medizinische Hochschule Lübeck, D-2400 Lübeck, FRG.

Treatment of male mice with the redox cycling compounds nitrofurantoin, parquat, diguet or menadione failed to elicit in vivo-lipid peroxidation as evidenced by ethane exhalation. The first three led to an enhanced ethane production, however, when the animals were pretreated with a low dose of Fe²⁺. While GSH-depletion by phorone pretreatment alone had no influence on the in vivo lipid peroxidation in the presence of either compound, the combined treatment with phorone, Fe²⁺ and nitrofurantoin, parquat or diguet led to a further enhancement of ethane exhalation. These results indicate that redox cycling compounds do not initiate lipid peroxidation by themselves, but are well capable of stimulating the iron-induced LPO.

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THE ROLE OF IRON IN THE ACETAMINOPHEN- AND CCl₄-INDUCED LIPID PEROXIDATION AND HEPATOTOXICITY. M. Younes, and C.-P. Siegers. Institut für Toxikologie, Medizinische Hochschule Lübeck, D-2400 Lübeck, FRG

Treatment of non-induced or phenobarbital-induced, glutathione-depleted mice with 400 mg/kg acetaminophen led to a marked ethane exhalation as an index of in vivo lipid peroxidation and to a significant elevation of liver-specific serum enzyme activities. Similar effects were seen with rats treated with 0.5 ml/kg CCl₄. Pretreatment with the iron-chelating agent deferroxamine clearly suppressed lipid peroxidation in all cases, but inhibited only the CCl₄-induced hepatotoxicity. These findings indicate that lipid peroxidation requires the presence of Fe²⁺-ions, regardless of the initiating agent, and that LPO is involved in CCl₄-toxicity, but most probably not in acetaminophen-induced liver damage. Furthermore, Fe²⁺-ions might play a role as mediators of CCl₄-hepatotoxicity.


DIT compounds, olitipraz (OTP) and anethol dithiolthione (ADT), have been reported to have antimutagenic and radioprotective activity. These studies were undertaken to characterize the biochemical effects of these compounds. OTP and ADT (4.3 mmole/kg) or vehicle (25% glycerol in 1% CMC, 10 ml/kg) were administered po to male CDF1 mice at 96 and 48 hr prior to sacrifice. GSH content, but not GSSG, was significantly increased by both agents. GSSG reductase and γ-glutamylcysteine synthetase activities were also increased compared to controls. Cytochrome P450 content was not influenced by DIT administration. Both OTP and ADT decreased GSH peroxidase mediated catalysis of cumene hydroperoxide and H₂O₂. GSH-S-transferase (GSH-Tx) was measured using five electrophilic substrates (1:1-chloro-2,4-dinitrobenzene; 1:1,2-dichloro-4-nitrobenzene; 1:1;1;1-p-nitrobenzyl chloride; 1-bromosulfophthalein; V1;2-epoxy-3-(p-nitrobenzoyl) propane). Only conjugation of V was not increased by DIT administration. These DIT-induced biochemical changes may be related to their protective effects. Furthermore these agents may be useful in studying the role of GSH-5-Tx in hepatotoxicity. (Supported by NIHES ES-02425 and Burroughs Wellcome Toxicology Scholar Award).

LIPOPEROXIDATIVE PROPERTIES OF PHENOBARBITAL IN RAT LIVER. M.-M. Iba. Graduate Program in Toxicology, Rutgers University, Piscataway, NJ

Phenobarbital (PB) has been reported to stimulate lipid peroxidation in rat liver. Suggested mechanisms include: the induction of (i) prooxidative enzymes - e.g., NADPH-cytochrome c reductase and (ii) peroxidizable substrates - e.g., polymethylated fatty acids. In the present study, the potential of the drug PB to stimulate lipid peroxidation was assessed. Increase in the 393 nm/417 nm peak ratio of the absorption spectrum of oxidized microsomes, abolition of the type I spectral change by added PB, and inhibition of cytochrome P-450 peroxidase activity were characteristic of hepatic microsomes from PB-pre-treated rats. Similar effects were observed in hepatic microsomes from control rats in the presence of added PB. In vivo correlates of these in vitro effects included increased "lipofuscin-like" fluorophores and cytochrome P-450 in the 9,000 g precipitate of liver homogenates. These two parameters increased with duration of PB pretreatment and may be better markers of in vivo lipid peroxidation than thiobarbituric acid reactive substances. In the presence of NADPH and added PB, no products of PB were detected in microsomes from control or PB-pre-treated rats but H₂O₂ formation was stimulated. The results suggest that redox cycling by cytochrome P-450 PB complex in the rat liver may be important in the enhancement of lipid peroxidation by the barbiturate.


Phenylenebis(oxy)bis[alkanoic acids] and their derivatives increase HDL cholesterol in rats treated with high lipid diets. Quantitative electron microscopic evaluations were conducted to establish peroxisome proliferating potential of the most pharmacologically active compound in this series compared to gemfibrozil (LOFID). Cl-924 and gemfibrozil were administered orally to male CDF-1 albino rats for 4 weeks at dose levels of 5 and 25 mg/kg. Both treated and untreated control rats received a diet consisting of 93% ground chow, 1.5% cholesterol and 5.5% peanut oil. Comparably, statistically significant increases in liver weights and peroxisome populations occurred with both compounds, in a dose-related manner. Peroxisome volume fraction per cell and per gram organ confirmed this proliferating potential. However, changes in individual peroxisome volume and diameter determinations suggest significantly increased organelle size in Cl-924 treated groups. The significance of the increased peroxisome size has not been established, but may be related to lipid regulation potency. Comparative studies in rats indicated that Cl-924 produced an equal elevation of HDL cholesterol at one-third the dose required for gemfibrozil.

Studies of the oral and inhalation toxicity of methyl isoamyl ketone (MIK) indicate that the most consistent biological effect of MIK in liver enlargement but large oral doses (2000 mg/kg) may result in hepatocyte degeneration, hypertrophy and foci of altered cellular morphology. Metabolically, MIK is extensively converted to branch-chain alcohols which may have biological properties similar to that of 2-ethylhexanol, an inducer of hepatic peroxisomes. To explore the possibility that MIK might mediate its effects through induction or proliferation of hepatic peroxisomes, MIK was given to rats by gavage at a dose of 2000 mg/kg/day, five days per week for approximately three weeks. MIK increased liver weight and serum cholesterol levels but had no effect on peroxisome size or number. 2-Ethylhexanol given orally at a dose of 1500 mg/kg clearly induced hepatic peroxisomes by promoting an increase in peroxisome size and peroxisome number or density per hepatocyte.

MODIFICATION OF BILIARY TREE PERMEABILITY IN RATS TREATED WITH A MANGANESE-BILIRUBIN COMBINATION. P. Ayotte and C.L. Plaa, Dépt. de pharmacologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7

Previous studies in this laboratory demonstrated incorporation of manganese (Mn) and bilirubin (BR) in rat liver bile canalicular membrane (BCM) following a cholestatic regimen composed sequentially of Mn plus BR. The present study investigates biliary tree permeability using segmental retrograde intrabiliary injection (SRII) with Mn-mannitol and Mn-inulin as markers. Male Sprague-Dawley rats were given the following (iv): a) Mn (high and low dose), b) BR, c) Mn-BR, d) BSP, e) Mn-BSP-BR (a non-cholestatic regimen). Results obtained with mannitol showed a ~24% decrease (p < 0.05) in marker recovery following administration of Mn or BR alone. Mn-BR in combination resulted in a 63% decrease (p < 0.05). BSP alone had no effect on mannitol recovery. However, when administered in the Mn-BSP-BR sequence, BSP abolished the Mn-BR response. With inulin, Mn (high dose), Mn-BR and Mn-BSP-BR all produced a ~65% decrease (p < 0.05) in recovery, while BSP and BR alone caused a ~25% decrease (p < 0.05). Mn (low dose) was without effect. These results suggest: 1) Mn-BR treatment increases biliary tree permeability by altering both BCM and the junctional complex; 2) BCM alteration is probably the critical event, since BSP only protects against the Mn-BR induced change in mannitol recovery. (Supported by the MRC.

EFFECT OF CHLORDEcone AND MIReX ON THE ACUTE HEPATOTOXICITY OF ACETAMINOPHEN IN MICE. B.L. Fouse and E. Hodgson, Interdepartmental Toxicology Program, N.C. State University, Raleigh, NC

Acetaminophen (APAP), in large doses, is known to cause hepatotoxicity due to tissue necrosis caused by the binding of its reactive intermediate to tissue macromolecules. APAP is converted to its reactive intermediate by the hepatic cytochrome P-450 dependent monoxygenase system. The chlorinated insecticides, choridecone and mirex have been shown to induce the enzymes of the hepatic microsomal monoxygenase system, although the patterns of induction differ from one another. Neither compound follows the classical pattern of phenobarbital or 3-methylcholantherene. C57BL/6 mice (20-25 grams) were administered mirex (30 mg/kg), chlordecone (30 mg/kg), or trileon vehicle (10 ml/kg) p.o. once each day for 2 days. 24 hrs. after the last dose, APAP (150 mg/kg) was administered i.p. in sterile saline. Hepatotoxicity was measured by the activities of the serum enzymes ALT, AST, LDH, and alkaline phosphatase. Neither the trileon or chlordecone treated mice showed any signs of hepatotoxicity without APAP treatment. Mirex dosed mice showed some signs of hepatotoxicity without APAP administration. Both compounds caused increases in the APAP hepatotoxicity with the mirex having the greater effect. These initial studies suggest that both chlordecone and mirex can potentiate the hepatotoxicity of APAP. Histological studies are now in progress.

HEPATOTOXICITY OF 1,1-DICHLOROACETONE: COMPARISON TO AND INTERACTION WITH CARBON TETRACHLORIDE. L.M. Condie and R.D. Laurie, Toxicology and Microbiology Division, USEPA, Cincinnati, OH

This study was designed to investigate toxicological properties and interactions of drinking water contaminants. 1,1-Dichloroacetone (DCA, 0.25 ml/kg) was shown to deplete hepatic homogenate and hepatic mitochondrial glutathione (GSH) levels by 60% and 80%, respectively, in fasted male mice following oral administration. Hepatic thiolobarbituric acid reactive material (TRM) levels were not changed by DCA treatment. Serum alkaline phosphatase, lactic acid dehydrogenase, aspartate and alanine aminotransferases were greatly elevated by DCA exposure (0.25 ml/kg). For specific durations of exposure subthreshold doses of both DCA (0.01 and 0.05 ml/kg) and carbon tetrachloride (CC14, 0.1 and 0.2 ml/kg) were administered. At dose levels that did not deplete hepatic GSH levels, DCA pretreatment potentiated the effect of CC14 on increasing hepatic TRM levels. Serum enzyme levels were elevated in mice given both DCA and CC14. It appears that subthreshold doses of DCA and CC14 are hepatotoxic when given together and that DCA is a more potent hepatotoxin when administered acutely than CC14. (This abstract does not necessarily reflect EPA policy.)
HEPATIC LESION ASSOCIATED WITH ADMINISTRATION OF CHLOROWAX 40\(^{\circ}\) TO F344/N RATS. J.R. Bucher and C. A. Montgomery, National Toxicology Program, RTP, NC, R. Thompson and J.D. Prejean, Southern Res. Inst., Birmingham, AL Sponsor: W.M. Kluiwe.

Chlorowax 40\(^{\circ}\), a mixture of paraffins of C20-30, chlorinated to 43% by weight, was administered to male and female F344/N rats, five days/week, by gavage in corn oil for periods of 14 days, 90 days, and 2 years. In 14-day studies no toxicity was observed from doses up to 3750 mg/kg, the highest dose feasible considering chemical viscosity. Therefore, doses of 0, 238, 469, 938, 1875 and 3750 mg/kg were given to groups of 10 male and female rats for 90 days. This treatment did not influence animal mortality, weight gain or clinical signs, but examination of the liver revealed a dose related lesion in the females characterized by a multifocal, randomly disseminated granulomatous and lymphohistiocytic inflammation. Intrauterine sacrifices of 10 rats/group at 6 and 12 months into an ongoing 2 year chronic toxicity and carcinogenicity study revealed similar lesions with a dose related frequency and intensity in female rats given 100, 300 and 900 mg/kg, and in males receiving 1875 and 3750 mg/kg. Although no effects were observed on mortality, weight gain or clinical signs, dose-related increases were noted in serum AST, ALT and SDH of up to 4 fold at 6 and 12 mo. in both males and females. The hepatic lesion was the primary toxicity resulting from the administration of Chlorowax 40\(^{\circ}\) to F344/N rats.


Compound LY171883, an alkoxitetrazoylactophenone, is a leukotriene D\(_4\) antagonist under development for treatment of asthma. Toxicity testing in rats revealed reduced food consumption and body weight at high doses and minor changes in serum glucose, bilirubin, alkaline phosphatase and alanine transaminase that were unrelated to dose. The major finding was dose-related and reversible hepatomegaly. Light microscopic analysis revealed hepatocellular hypertrophy with no other evidence of liver disease. There were increases in microsomal monoxygenases and cytochrome P-450, but mitochondrial cytochrome oxidase activity was not changed. Peroxisomal catale activity and 8-oxidation increased in a dose-related manner to a maximum of 2.5 and 9-fold. Morphological confirmation of peroxisome proliferation was obtained by electron microscopy. Hepatomegaly and induction of peroxisommal enzymes occurred in mice and hamsters, but not in guinea pigs, beagle dogs or rhesus monkeys given maximum tolerated doses. The doses required to elicit the effects in sensitive species were an order of magnitude higher than expected clinical doses. Both the species specificity and the dose considerations make it unlikely that rodent data can be meaningfully extrapolated to humans undergoing therapy with LY171883.


Models were developed to study zone-specific damage in portal (PP) and pericentral (PC) regions of the liver lobule due to hypoxia produced in the perfused liver either by nitrogen or by perfusion with low-flow followed by reflow. Damage was assessed by LDH release and trypan blue uptake in specific regions. Perfusion for up to 2 hours under the conditions employed in both models failed to damage liver from well-fed rats. In contrast, perfusion of livers from fasted rats for 30 min with N\(_2\)-saturated buffer produced dye uptake of 37% and 66% in PP and PC regions, respectively. Damage tended to be greater in this model when calcium was omitted from the perfusate (69% and 88% staining of PP and PC regions, respectively). Release of LDH correlated well with the percentage of cells stained with dye. In livers from fasted but not fed rats, ninety min of low-flow (ca. 1 ml/g/min) followed by 30 min reflow at normal flow rates (ca. 4 ml/g/min) produced damage exclusively to PC regions of the liver lobule. On average, about 40% of hepatocytes were stained with the dye under these conditions. When perfusion was in the retrograde direction, PP areas were damaged while PC regions were spared. Thus, a model has been developed to study zone-specific damage due to hypoxia under nearly physiological conditions in the perfused liver. The data indicate that nutritional status is an important determinant of hepatotoxicity. (ES-02739 and AA-03624).

PARADOXICAL EFFECT OF THE GLUTATHIONE (GSH) BIOSYNTHESIS INHIBITOR, BUTHIONINE SULFOXIMINE (BSO), ON HEPATOTOXICITY OF THE KETOBIOLOGICALLY-ACTIVATED ALKYLATING AGENT, 2-METHYLPURAN (2-MP) V. Navindranath and N.E. Boyd. M.CI, Bethesda, MD

2-MP, a commonly occurring constituent of cigarette smoke and coffee is hepatotoxic in the rat. 2-MP is metabolically activated by mixed function oxidases to acetylcrocin, which binds covalently to microsomal proteins in vitro. Intraperitoneal administration of 145 2-MP to male Sprague-Dawley rats resulted in covalent binding of the label to tissue macromolecules. Maximal covalent binding was observed to liver proteins and DNA; damage to liver was also observed by an increase in SGPT levels. Pretreatment of rats with BSO, an inhibitor of γ-glutamyl cysteine synthetase, decreased both the covalent binding to liver macromolecules and SGPT levels as compared to rats treated with 2-MP alone. Diethylmaleate (DEM) pretreatment, however increased both the covalent binding and SGPT levels. Administration of oxythiasolidine and BSO prior to 2-MP also caused a decrease in covalent binding and SGPT levels. Thus, depletion of GSH by DEM potentiates hepatotoxicity while BSO which depletes GSH by inhibiting GSH synthesis protects against 2-MP toxicity. BSO is known to produce a transient increase in cysteine levels. The protective effect of BSO may be due to increased cysteine levels in the liver. Cysteine is a better trapping agent of acetylcrocin than GSH in rat liver microsomal incubation in vitro.
633 CORRELATION BETWEEN ACETONE-POTENTIATED CCl₄-INDUCED LIVER INJURY AND PLASMA CONCENTRATIONS AFTER INHALATION OR ORAL ADMINISTRATION.
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In experiments of acetone-potentiated liver injury, acetone is usually given by gavage. Industrial exposure to acetone occurs mostly by inhalation. It is of interest to verify if the route of administration influences the potentiation. Male Sprague-Dawley rats were exposed for 4 hr to acetone vapors or treated orally with acetone; minimal effective doses (MED) to potentiate CCl₄-induced liver injury were 2500 ppm and 0.25 ml/kg, respectively. Groups were treated with acetone using 0.4, 1, 2, 4 or 6 times the MED. Half of each group was killed at various times after the treatment, and acetone plasma concentrations were measured by GLC; the others were challenged 18 hr after acetone with CCl₄ (0.1 ml/kg, ip), and were killed 24 hr later. Plasma ALT activity and bilirubin concentrations were measured. Inhalation and oral administration of acetone both potentiated CCl₄ toxicity. Correlations between ALT activities and maximal plasma concentrations were highly significant for both routes. The acetone potentiating effect was predictable on the basis of acetone plasma concentration, irrespective of the route of administration. Thus, the route of administration does not influence the potentiation. (Supported by NSRSTQ and NSERC)

635 MECHANISM FOR INHIBITION OF HEPATIC KETOGENESIS BY VALPROATE. M.J. Olson and R.G. Thurman. Dept. of Pharmacology, U. of North Carolina, Chapel Hill, NC.

Valproate (V), a clinically useful anticonvulsant, produces fatty liver in man and experimental animals. Infusion of oleate (250 μM) to perfused livers of fasted rats increased O₂ uptake and ketogenesis and reduced flavin and pyridine nucleotides rapidly. Pre-infusion of V blocked reduction of flavin and pyridine nucleotides by oleate and prevented 50% of the oleate-stimulated increase in ketogenesis in the presence or absence of L-carnitine (500 μM). In contrast, V did not affect ketone body production from octanoate (250 μM). Oleate is activated by an ATP- and CoASH-dependent cytoplasmic process and transported into mitochondria as oleoylcarnitine. Octanoate is activated within the mitochondrial matrix. Since V inhibits ketogenesis from long-chain fatty acids preferentially, mechanisms other than ATP or CoASH depletion are most likely involved. This conclusion is supported by the observation that V did not diminish the extra O₂ uptake due to oleate. V does not affect the hydroxymethylglutaryl CoA pathway directly since the ability to form ketones from octanoate is unimpaired by V. Oleate and octanoate both failed to stimulate ketogenesis in the presence of 4-pentenoate, an inhibitor of β-ketoacyl-CoA thiolase, indicating that V probably does not inhibit that enzyme. Thus, V or a metabolite diminishes ketogenesis by inhibiting transport of long-chain CoA compounds into the mitochondria and/or by inactivating the mitochondrial long-chain acyl CoA dehydrogenase specifically. (ES-02759, ES-05292)

634 LIVER AND LIPIID EFFECTS OF DIETHYHEXYL PHTHALATE (DEHP) AND DIETHYHEXYL ADIPATE (DEHA) IN FISCHER 344 RATS. A. Linton¹, B. Schneider¹, E. Moran¹, M. Stoltz², J. Windels² and M. El-hawari².
Chemical Manufacturers Association, Washington, D.C.¹ and Midwest Research Institute, Kansas City, MO.²

In rats, DEHP, but not DEHA was shown to be a weak liver carcinogen. However, both compounds were nongenotoxic in a battery of assays. The purpose of this study was to assess the effects of these esters on liver and lipid metabolism at comparable doses used in their chronic studies. F-344 rats were fed DEHP (0.1, 0.6, 1.2x) or DEHA (0.1, 1.2, 2.5x) and sacrificed after 1 or 3 weeks or after 3 weeks feeding followed by a 2-week recovery period. Both esters depressed serum triglycerides and cholesterol levels and increased liver weight and hepatic peroxisomal enzymes (catalase, carnitine acetyl transferase and fatty acyl CoA oxidase). Liver function tests were normal consistent with negligible effects seen by light microscopy. An increase in hepatic peroxisomes was observed by electron microscopy in the high dose groups. DEHP was more potent than DEHA for all biological endpoints. The lipid and liver effects of DEHP and DEHA were more prominent in males than females and were reversible in both sexes.

636 MODIFICATIONS IN RAT HEPATOBILIARY FUNCTION FOLLOWING TREATMENT WITH KETONES AND CHLOROFORM. L.A. Hewitt, P. Aytote and G.L. Plaa. Dépt. de pharmacologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Potentiation of CHCl₃-induced hepatonecrosis by acetone (A), 2-butanone (MEK), 2-hexanone (MBK) and chlorodecone (C) is well known. This study investigates the hepatobiliary effects of these treatments. Liver necrosis, evaluated by ALT and GST, was modulated by varying the time interval between pretreatment with A, MEK, MBK (15 mmol/Kg, ip) or C (50 mg/Kg, po) and a challenge dose of CHCl₃ (0.5 ml/Kg, ip). The data were compared to bile flow rate and plasma bilirubin (BR) as indicators of hepatobiliary function. Results show: 1) ketones alone had no effect; 2) CHCl₃ alone did not affect bile flow rate but increased plasma BR; 3) ketones potentiated the effects of CHCl₃ on plasma BR, which correlated with necrosis; 4) the combination resulted in cholestasis, but this did not correlate temporally with necrosis. A preliminary study of biliary tree permeability by the segmented retrograde intrabiliary injection technique (mannitol as the marker) indicated that CHCl₃ alone decreased mannitol recovery (50%) and that pretreatment with MBK or C enhanced this effect (80%). Thus, ketones can augment CHCl₃-induced hyperbilirubinemia and biliary tree permeability alterations. The combination is also capable of inducing cholestasis, an effect not characteristic of the individual agents. (Supported by NRC and NSERC).
Conversion of one differentiated cell type to another is referred to as transdifferentiation.

Pancreatic hepatocytes have been induced in hamsters and rats. In this experiment we describe the development of hepatocytes in pancreas in another experimental model system. Male, Wistar rats were given a single iv injection of 4-HAQO, and 22 weeks later they were placed on a copper deficient diet for 10 weeks, followed by normal diet. Rats were sacrificed at 3-week intervals after return to normal diet for an 18-week period. The pancreas showed marked loss of acinar tissue and severe fatty infiltration. In addition, pancreata of all rats sacrificed at 6 weeks or later contained foci of hepatocytes. At 6 weeks only a few foci were present; however, the number and size of hepatic foci increased with time. Hepatocytes were randomly distributed throughout the pancreas, and were positive for albumin and catalase, and negative for amylase, insulin, and glucagon. No hepatocytes were present in rats treated with 4-HAQO alone. Further studies are required to delineate the role of copper deficiency in the development of pancreatic hepatocytes. The rapid development of hepatocytes in 100% of animals in a highly reproducible manner should facilitate studies on the control and modulation of gene expression during transdifferentiation in this system. Supported by NIH Grants CA16954 and GM23750.

Intestinal absorption of nutrients and xenobiotics has been suggested to change during aging. Transport processes were examined using non-metabolizable glucose analogs as markers in an in situ single pass intestinal perfusion preparation. At 27 months of age were used in this study. At least 6 rats were used per age group. Net active and passive transport was determined using 0.85% NaCl solutions containing [14C] 3-O-methylglucose (3OMG) and [3H] 2-deoxy-D-glucose (2DG) and phenol red as a nonabsorbable volume marker. Rats were anesthetized and approximately 20 cm of jejunum was perfused 30 minutes. Final glucose analog concentrations were corrected for volume changes, and compared with initial concentrations. Changes in marker concentrations were normalized to the dry weight of the perfused intestinal segment. There was no significant change in the net transport of 2DG, a compound thought to be passively absorbed (range 2.7-5.1 mg/g/h). In the same animals, the rate of net 3OMG absorption, an actively transported compound, was generally an order of magnitude greater than 2DG. In addition, 3OMG was significantly (P<.05) decreased in the 16 and 27 mo. rats compared with the 2.4 and 10 mo. old animals (39.4 vs 68.7 mg/g/h). There appears to be a gradual decrease in net active transport with age. The mechanism responsible for this age-related change remains to be explained.


We examined dietary selenium (Se) influence on the liver biochemical response to inhaled ozone (O3). We fed weaning female strain A/STc mice a diet containing either 0 or 1 ppm Se for 11 weeks, then exposed half of the mice to 0.8 ppm O3 and the other half to room air, for 5 days. After exposure we analyzed the livers for Se content and a series of biochemical indices of toxicity including DNA and protein contents, oxygen utilization, sulfhydryl metabolism and related NADPH production. In air-exposed mice, Se deficiency resulted in lower liver Se content and glutathione peroxidase activity with no apparent effect on the other parameters. After O3 exposure all parameters decreased relative to air-exposed mice, but the decreases were significant only with Se deficiency. The decrease in protein content and oxygen uptake reflects a depression in the overall liver metabolism. In addition, decreased sulfhydryl metabolism and NADPH production reflect a depressed detoxifying mechanism. This depression in liver biochemical parameters is in contrast to the elevated response known to occur in the lung. The results suggest that O3 inhalation results in secondary toxic effects extending beyond the lung to other organs such as the liver.

Proponitride (Ethyl Cyanide, PCN) is a component of cigarette smoke and an industrial solvent and grain fumigant. Studies have shown PCN to be a potent duodenal ulcerogen (Szabo and Selye, 1972). The objective of this study is to elucidate the ulcerogenic mechanisms by examining PCN toxicokinetics. Female Sprague-Dawley rats were injected IV with a tracer dose of 2-14C PCN. At various time intervals, animals were sacrificed and used either for whole-body autoradiography or for dissection and subcellular fractionation of selected organs. The levels of 14C excreted in urine, feces and expired air over the 24-hour period after administration were measured. Biliary levels of radioactive PCN metabolites were assessed by bile duct cannulation. Less than 10% of the initial dose was excreted over the first 24 hours, indicating long retention of PCN in the body. Our whole body autoradiography studies indicate that PCN is sequestered in the submesenteric wall of the duodenum, the site of its primary ulcerogenesis. Biliary studies indicate extensive hepatic elimination of PCN metabolites. Biliary delivery of toxic PCN metabolites into the duodenum could play a role in the ulcerogenic activity. (Partly supported by NIH Grant ES01871).
641 HISTOPATHOLOGIC EFFECTS OF DIETARY SOYBEAN ISOLATE ON THE MOUSE PANCREAS. C.G. Willhite, F.D. Wilson, P.V. Allen and J.C. Smith. Toxicology Research Unit, WERC, USDA, Albany, CA

Female Swiss-Webster mice [Sim:SMFBR] were fed a semipurified diet (American Institute of Nutrition #76) or diets containing 7.2% (0.2% trypsin inhibitor, TI) or 35.9% (1.0% TI) precipitated soy protein ad libitum for at least 14 days prior to breeding. Males were fed Purina #5001 stock diet. The day following the evening of breeding was day 1 of gestation. The mice were killed on day 18 of pregnancy and when uteri were found without implantation sites, conception was deemed not to have occurred. The maternal pancreas was weighed and routinely processed. There was no significant (p > 0.05) difference in food consumption, net maternal weight gain or mean fetal body weight between any of the groups. Dietary TI failed to induce a significant increase in the number of litters containing abnormal offspring. Pancreatic weights were elevated significantly (p < 0.05) in pregnant mice fed 1.0% TI compared to mice fed 0.2% TI or the control diet. The numbers of acini/1000x field were reduced significantly in the high soy-fed group. The pancreatic acinar cells of mice fed 1.0% TI contained more zymogen granules. The mean numbers of pancreatic mitotic figures in soy-fed mice were not significantly different from control. Pancreatic hypertrophy associated with high soy diets may be related to their trypsin inhibitory activity. Supported by USDA CRIS Work Unit #5102-20340-010.

642 DRUG-INDUCED INHIBITION OF HEPATIC EXCRETION IN RATS. D.G. Hoyt and R.E. Larson. College of Pharmacy, Oregon State University, Corvallis, OR

The carcinostatic drug 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) causes intrahepatic cholestasis in rats by inhibiting bile salt independent bile production. Prior to the onset of cholestasis which occurs between 36 and 96 h after administration of 20 mg/kg BCNU i.p., biliary excretion of a single i.v. injection of sulfobromophthalein (BSP 50 mg/kg) is inhibited. Liver cytosol isolated from rats, pretreated with BCNU retains BSP and the hepatic content of reduced glutathione is elevated with respect to pair-fed controls. This implies that the metabolism of BSP is unimpaired. To help decide whether BSP excretion is inhibited by an action at the sinusoidal or canicular membrane of the hepatocyte, the transport maximum for BSP (BSP Tm) was measured 48 h after male Sprague-Dawley rats were given 20 mg/kg BCNU or corn oil i.p. This was accomplished by constant i.v. infusion of BSP (2.5 mg/min/kg). BCNU reduced the BSP Tm from 24.7±2.2 µg/min/g of liver in controls to 13.5±2.2 µg/min/g in treated rats (p<0.01). The biliary concentration of BSP at the BSP Tm was decreased from 11.15±3.4 µg/µl in controls to 5.86±1.84 µg/µl in treated rats (p<0.005). These results are similar to those obtained after a single i.v. injection of BSP and suggest that canicular excretion is inhibited although hepatic uptake of BSP may be decreased.


Male and female F-344 rats and B6C3F1 mice were exposed to 50, 150, or 1000 ppm methyl isobutyl ketone (MIBK) vapor 6 hrs/d, 5 d/wk, for 14 wks. Exposure levels were based on results of a 2-wk probe study at 2000, 500, 100 or 0 ppm. At 2000 and 500 ppm there was evidence of increased liver and kidney wt (absolute and relative). Histologically, the only changes observed were an increase in regenerative tubular epithelia and hyaline droplets in kidneys of male rats exposed to 2000 or 500 ppm. During the 14-week MIBK study, no adverse effect on the clinical health or growth of rats or mice were observed. Male rats and mice exposed to 1000 ppm MIBK had a slight (p<0.05) increase in liver wt and liver wt/body wt ratio. Liver wt was also increased slightly in male mice exposed to 250 ppm; however, no gross or microscopic hepatic lesions were observed. The only microscopic change observed was an increase in the number of hyaline droplets within proximal tubular cells of the kidneys of male rats exposed to 250 and 1000 ppm of MIBK. Hyalin droplet change appears to be peculiar to male rats and therefore has no relevance in other species. In conclusion, rats and mice exposed to MIBK at levels up to 1000 ppm for 14 weeks was without significant toxicity.


In a prior oral study, liver sections from rats exposed to 2000 mg/kg/day methyl isomyl ketone (MIK) 5 days/week for 13 weeks, revealed areas of hepatocyte degeneration, hypertrophy, and hyperplasia. Since MIK exposure is likely to occur by the inhalation route, a two-week (2000, 1000, or 0 ppm) and 90-day (2000, 1000, 200 or 0 ppm) inhalation study of MIK in rats was conducted. The highest exposure concentration was selected to produce similar peak blood MIK concentrations to that of the prior oral study. Rats were exposed 6 hrs/day, 5 days/week. Body weights, hematology and serum clinical chemistry determinations were comparable to controls in both inhalation studies. Clinical signs of toxicity were lethargy and decreased aural response (2000 ppm, two-week study; 2000 and 1000 ppm, 90-day study) and nasal and eye irritation (2000 and 1000 ppm, 90-day study). Absolute and relative liver and kidney weights were increased in both sexes following exposure to 2000 and 1000 ppm in the two-week and 90-day studies. Hepatocyte hypertrophy was observed on microscopic examination. Kidney changes seen in both studies at 2000 and 1000 ppm were minor. The toxicity of MIK following inhalation exposure was not as extensive or severe as that resulting from the prior oral study. The inhalation no-observed-effect level of toxicity was 200 ppm MIK.
THE EFFECTS OF ETHYLENE OXIDE (EO) EXPOSURE ON TISSUE GLUTATHIONE (GSH) LEVELS IN RATS AND MICE. J. A. McKelvey and M. A. Zemaitis. Bushy Run Research Center, Export, PA and University of Pittsburgh School of Pharmacy, Pittsburgh, PA.

Male Fischer 344 rats and male Swiss-Webster mice were exposed to different atmospheric concentrations of EO for 4 hours. The EO concentration of bone marrow (rats only) and other selected tissues was determined at various times after termination of exposure. In mice sacrificed immediately after exposure to 100, 450 or 900 ppm EO, there was a concentration-related decrease in the GSH levels of all tissues examined. Similar findings were obtained in rats immediately after exposure to 100, 600 or 1200 ppm EO except that blood GSH levels were not affected at any exposure concentration. In both species, lung and liver GSH were depressed at all exposure concentrations. Liver GSH was depleted 38% in rats and mice at their respective highest EO exposure levels. Twenty-four hours after exposure to 1200 ppm EO, the GSH concentrations of rat bone marrow and testis had not returned to control levels. Only blood GSH levels remained depressed in mice 48 hours after exposure to 900 ppm EO. These results indicate a marked species difference between rats and mice regarding the effects of EO exposure on blood GSH levels. This may have important toxicologic implications with respect to animal models that relate EO alkylation of histidine in hemoglobin with its alkylation of DNA in tissues.

LOCALIZATIONS OF XENOBIOTIC-METABOLIZING ENZYMES IN NASAL TISSUES OF UNTREATED AND 3-METHYLCOLANTHRENE (MC) AND AROCLOR 1254 (A) PRETREATED RATS. J.M. Voigt, F.P. Guengerich, and J. Baron. Toxicology Center, Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242 and Dept. of Biochemistry, Vanderbilt Univ., Nashville, TN 37232

Xenobiotics such as benzpyrene that must be bio-activated in order to exert their toxic effects can be metabolized in nasal tissues. To determine the sites for xenobiotic metabolism within nasal tissues of untreated rats and if and where nasal xenobiotic-metabolizing enzymes can be altered by MC and A, the localizations of NADPH-cytochrome P-450 reductase, cytochrome P-450 MC-B, and epoxide hydrolase were investigated at the light microscopic level in fixed, paraffin-embedded tissues using antibodies raised against the rat hepatic enzymes in immunohistochemical staining techniques. In untreated rats, staining for each enzyme was detected in respiratory and olfactory epithelia, Bowman's glands, and ducts. Treatments of rats with MC (40 mg/kg/day for 4 days, i.p., in corn oil) or A (a single 500 mg/kg dose, i.p., in corn oil) did not alter staining for either the reductase or epoxide hydrolase in nasal tissues. Although MC did not affect nasal P-450 MC-B, treatment of rats with A resulted in enhanced staining for P-450 MC-B in respiratory and olfactory epithelia and Bowman's glands, suggesting that A may render these cells more susceptible to toxins that are activated by cytochrome P-450 MC-B. (Supported by USPHS Grants GM33253, ES01590, and ES02205.)

PURIFICATION AND RECONSTITUTION OF THE CYTOCHROME P-450 MONOOXYGENASE SYSTEM FROM HUMAN PLACENTA. M.M. Lewandowski and E. Hodgson. Interdepartmental Toxicology Program, North Carolina State University, Box 7613, Raleigh, NC 27695.

Two forms of cytochrome P-450, with molecular weights of 52,000 and 55,000 daltons, were partially purified from human placental microsomes of nonsmokers using octylsepharose, hydroxylapatite, DEAE-cellulose and CM-cellulose chromatography. Both forms appear to be predominantly low spin and the minor form (55,000 daltons) is less stable than the major form (52,000 daltons). NADPH cytochrome P-450 reductase was purified from human placenta. The cytochrome P-450 monooxygenase system was reconstituted by combining purified NADPH cytochrome P-450 reductase from either human placenta or mouse liver, cytochrome P-450 and phosphatidylycholine. Both forms showed aromatase activity; net form showed benzo(a)pyrene activity; and the minor form showed 7-ethoxycoumarin deethylase activity.

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CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO RAT MICROSOMAL CYTOCHROME P-450(c), PURIFIED AFTER AROCLOR 1254 ADMINISTRATION. Z. Amelizad and P. Deemch, Institute of Toxicology, University of Mainz, D-6500 Mainz, FRG, Sponsor: L.W. Robertson

Cytochrome P-450(c) was purified and characterized after Aroclor 1254 administration in male Sprague Dawley rats. Seven monoclonal antibodies (Mabs) (named (676-D7), (676-B9), (670-E), (676-D7), (676-E), (658-C9), (670-B)) were developed towards this microsomal cytochrome P-450. Each was tested for cross-reactivity using Ouchterlony double-diffusion analysis and Elisa test. None of them showed cross-reaction in Ouchterlony plates, but all of them showed very clear single bands in blotting on nitrocellulose sheets, and high titration values, using the Elisa test for cytochrome P-450(c). Rat microsomal fractions after 3-methylcholanthrene, Aroclor 1254, BNF, PCN administration gave positive reactions with all of the 7 Mabs in Elisa tests and a single band on immunoblots (i.e. no reaction with cytochrome P-4500). However using untreated rat and rabbit or PB-treated rat microsomes, we could not obtain any reaction. A loss of activity towards 7-ethoxycoumarin and 7-ethoxyresorufin was observed corresponding with a high degree of cross-reactivity with the Mabs, however, using benzphetamine as substrate (selective for the PB-induced cytochrome P-450) no loss of activity was measured.
DBBD has been shown to antagonize MC induction of cytochrome P-450 in Dub:ICR mice yet has no effect on phenobarbital (PB) induction (Biochem. Pharmacol., in press). CS78L/6 mice, an Ah responsive strain, also produced a similar response when treated with PB + DBBD and MC + DBBD. The hypothesis that DBBD, although not a cytochrome P-450 inducer itself, competes with MC for binding to the Ah receptor was tested. Using sucrose density gradients, the Ah receptor was measured in Dub:ICR and CS78L/6 male mice. DBBD was unable to displace TCDD or MC from the Ah receptor, in vitro, in either strain. These in vitro data suggest that DBBD does not compete with MC for binding to the Ah receptor. In vivo experiments are in progress to confirm this finding. Presumably the site of inhibition is at some other level of the induction process.

Effects of pretreatment with pyrazole (PYR) and inducers of mixed function oxidases on DNA repair elicited by dimethylnitrosamine (DMN) in rat hepatocytes in vitro and in vivo. D.J. Kornbrust and T.D. Dietz. NIEHS, and Research Triangle Institute, Research Triangle Park, NC.

Experiments were performed to investigate the relationship between the rate of oxidative metabolism of DMN by liver microsomes (i.e., DMN demethylase activity, DMNA) and its genotoxicity in liver, as assessed by the in vivo/in vitro and in vitro rat hepatocyte/DNA repair (HPC/DR) assays. Pretreatment of rats with PYR resulted in a 4-fold increase in DMNA and a 3-fold greater DNA repair response to in vivo administration of 5 mg DMN/kg body weight. Pretreatment with phenobarbital (PB), DDT, 3-methylcholanthrene (3MC), 3-naphthoflavone (BNF) or Aroclor 1254 produced variable inhibition of DMNA and had no significant effects on the response to DMN in the in vivo/in vitro HPC/DR assay. DNA repair elicited by DMN in vitro was significantly decreased in hepatocytes from rats pretreated with PB and 3MC, while DDT, BNF and Aroclor had no effect. In contrast, PYR pretreatment increased the in vitro DNA repair response to DMN 4.5- to 6.7-fold, and extended the concentration range over which a positive response was detected. None of the inducers had any effect on DNA repair elicited by direct acting genotoxic agents (e.g., methylmethanesulfonate), suggesting that the pretreatment-related changes in DMN-induced repair were due to alterations in DMN rather than to effects on the DNA repair capacity of the hepatocytes.

Oxidation of phorate by purified FAD-containing monooxygenase and purified cytochrome P-450 isozymes. P.E. Levy, P.J. Sabourin and E. Hodgson. Interdepartmental Toxicology Program, North Carolina State University, Raleigh, NC.

Oxidation of [14C-ethyl] phorate, (C2H5O2)P(S)SC2H5SC2H5, was studied using five cytochrome P-450 isozymes and the FAD-containing monooxygenase, all purified from uninduced mouse liver microsomes. Phorate was rapidly oxidized by the FAD-containing monooxygenase to form an optically active phorate sulfoxide as the only metabolite. No metabolites were produced when [14C-ethylene] phorate sulfoxide was used as a substrate.

All cytochrome P-450 isozymes, when incubated with phorate in a reconstituted system, yielded phorate sulfoxide as the major product, although the rate differed with the individual isozymes. Only trace amounts of other metabolites, including phorate oxon, were detected. Cytochrome P-450, in contrast to the FAD-containing monooxygenase, further metabolized phorate sulfoxide to form phorate oxon sulfoxide, phorate sulfone, and phorate oxon sulfone.
**653** A RAPID AND SENSITIVE ASSAY OF 7-ETHOXYCOUMARIN O-DEETHYLASE. W.H. Lee, C. DiColio, P.A.G. Malys, Stuart Pharmaceuticals, A Division of ICI Americas Inc., Wilmington, DE. Sponsor: L.K. Gonsler

Ethoxyzoumarin O-deethylase (EOD) assay is widely used to assess the inducibility of hepatic mixed function oxidases. Assays available require multiple extraction steps followed by measurement of 7-hydroxycoumarin (7-OHC), a reaction product of ethoxyzoumarin which is unstable at pH above 10. A simple and sensitive method for EOD has been developed eliminating tedious sample preparation steps. The formation of 7-OHC by EOD is assayed by measuring the fluorescence of 7-OHC in the supernatant, after precipitating protein with methanol at pH 7.8. EOD activities in rat liver microsomes prepared from 100 mg/kg/day of phenobarbital treatment for 4 days and control were 1.93 and 0.870 n moles of 7-OHC/min/mg protein, respectively, which are approximately 2 to 4 fold higher than that obtained by existing methods. The intra run and day-to-day variations of standard curve (0.04 µg to 4.00 µg/ml) were c.v. of 5.2% (N=4) and 7.9% (N=14), respectively. With the present method, the fluorescence of 7-OHC in the methanolic solution is stable over 4 hours, while with the existing methods it is unstable at the pH above 10 losing 50% fluorescence during the same period of time. The new method permits rapid and sensitive assay for microsomal EOD.


Biochemical studies were conducted to investigate the potential for hepatic microsomal enzyme induction by the insect growth regulator, MV-678 [1-[8-methoxy-4,8-dimethyl)nonyl]-4-[1-methylethyl]benzene], when administered to male and female dogs. Three dogs of each sex received either 0, 10, 200, or 800 mg/kg/day MV-678 Technical by gavage for 4 weeks. Significant increases in absolute and relative liver weight, microsomal protein content, cytochrome P-450 content, NADPH-cytochrome P-450 reductase activity, ethylmorphine N-demethylase activity, and aniline hydroxylase activity were observed in the male and female dogs at the 800 mg/kg/day dose level at 4 weeks. Significant increases in cytochrome P-450 content, ethylmorphine N-demethylase activity, and aniline hydroxylase activity were also observed in males and females at the 200 mg/kg/day dose level at 4 weeks. A significant increase in aniline hydroxylase activity was observed in both male and female dogs at the 10 mg/kg/day dose level, and a significant increase in cytochrome P-450 content was noted in females at this dose level at 4 weeks. The results of this study suggest that MV-678 Technical acts as a hepatic microsomal enzyme inducer when administered to dogs by gavage for 4 weeks at dose levels of 10, 200, or 800 mg/kg/day.


PAH are converted to active metabolites such as arene oxides and diol epoxides, which can interact with nucleophiles in macromolecules. The balance between metabolic activation of PAH and detoxification of their active metabolites may play a vital role in controlling mutagenic and carcinogenic processes. We studied the effects of P. ginseng C.A. Meyer on several parameters of monooxygenase system, including aryl hydrocarbon hydroxylase (AHH) and epoxide hydratase (EH), as well as the effects of ginseng on the conjugation system. Sprague-Dawley rats (200 g) receiving ginseng extract (75% EtOH) at a single dose of 20 mg/kg b.w. induced EH activity in the 10,000 x g supernatant fraction of liver homogenates up to 150% of the control, whereas no significant changes were observed with AHH activity even at 40 mg/kg b.w. Aniline hydroxylase activity also did not exhibit any changes even after 4 hrs. of treatment at a daily dose of 10 mg/kg b.w. Glutathione transferase activity was increased by 40% at a single dose of 20 mg/kg b.w. The selective induction of EH activity combined with a marked increase in conjugation capacity, without a concurrent induction of AHH suggests that ginseng could be used as a valuable tool in the study of the role of EH in the overall biotransformation of PAH leading to mutagenic and carcinogenic processes.


Neonatal administration of PB during the first five days after birth results in increased P-450 dependent xenobiotic metabolism at adulthood. PB is generally thought to act as a transitory inducer of the P-450 system, resulting in increased which quickly dissipate. PB's inductive effects is due to increased synthesis of specific P-450s which are electrophoretically and immunochromically different from the major constitutive forms and those induced by other inducers. A technique for quantitating specific forms of P-450 is the Western Blot, employing antibodies specific to various P-450s. It was employed to determine if the mechanism responsible for PB imprinting is similar to induction by surveying for P-type P-450s in neonatally imprinted adults. Eight to 40 mg of microsomal protein resulted in no detectable levels of P-type P-450s in PB-imprinted rats, although 8 mg from PB-induced rats produced a band of reaction. PB imprinted microsomes also did not react with antibody to alloisoform of (SF) induced P-450, which cross reacts with 3-methylcholanthrene induced P-450. If PB imprinting acts via increased synthesis of P-type cytochrome P-450s during adulthood, the form(s) synthesized must be antigenically different from those induced by PB, 3-MC or ISF. Supported by NIEHS R01 ES03403.
657 INDUCTION OF THE MICROosomal METABOLISM of PHENOxOZINE AND ITS ALKYL AND ARYL ETHERS IN RAT  
S. Thompson, C. R. Elcombe, R. T. Mayer, and M. D. Burke  
Dept. of Pharmacology, Aberdeen University, Central Toxicology Labs, I.C.L., p.l.c., Alderley Park, U.K., USDA, PO Drawer G.E., College Station, U.S.A.  
Sponsor: M. T. Smith

The aim of this study was to characterize the induction of different isozymes of rat hepatic cytochrome P450 by their metabolism of a homologous series of phenoxzone ethers related to ethoxyresorufin. The substrates used were phenoxzone, benzyloxophenoxzone, and a homologous series of alkyl ethers of phenoxzone with increasing alkyl ether side chain length (IC-8C). Male Sprague Dawley rats were pretreated with one of a number of inducers (phenobarbital (PB), 3-methylcholanthrene (3MC), Aroclor-1254, isosafrole, SKF-525A). The induction of different isozymes of cytochrome P450 could be "fingerprinted" by distinctive changes that occurred in the metabolism of the phenoxzone ethers.

658 EFFECTS OF PERFLUORO-N-DECANOIC ACID (FFDA) ON MICROsomal ENZYMES in RAT LIVER. M. J. Van Ralghem and M. E. Andersen, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB OH

FFDA causes toxic effects in rats similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin and an increase in the ratio of total oleic to total stearic acid in rat livers. We studied the effects of a single ip dose of FFDA (30 mg/kg) on several hepatic microsomal enzymes and microsomal electron transport chain components in Fischer 344 rats 14 days after treatment. Stearoyl-CoA desaturase activities, which convert stearic to oleic acid, were not detectable in either FFDA-dosed or pair-fed control rats. FFDA-treated rats did have a marked reduction in the rate of electron transfer in the stearoyl-CoA desaturase assay system, as compared to either ad lib fed or pair-fed controls. Cytochrome b5, a constituent of this electron transport chain, was significantly reduced in FFDA rats (49% control). Cytochrome P-450 content was slightly elevated in FFDA-dosed rats (116% control) but pentobarbital sleep times were significantly increased (393% control). Aminopyrine demethylase (AD), a microsomal monooxygenase enzyme, was significantly reduced (7% control). Although pair-fed control rats also had prolonged pentobarbital sleep times and reduced AD activity, these changes were not as dramatic as in FFDA-dosed rats. FFDA clearly impairs function of certain membrane bound microsomal enzyme systems.

659 SELECTED CHANGES in HEPATIC MICROsomal ENZYME ACTIVITY FOLLOWING FOOD RESTRICTION OR STREPTOZOTOCIN in the GUINEA PIG. J.C. Kaepghan, M-J. Schlosser, and A-R. Verlangieri. Atherosclerosis Research Laboratories, Dept. Pharmacol., Sch. Pharmacy, Univ. of Mississippi, University, MS 38677. Sponsor: W.L. Guess

Alterations in the activity of selected hepatic microsomal enzymes occur with an accompanying decrease in body weight (BW) in experimental diabetes. Presently, it is not clear how these BW changes affect the observed changes in microsomal parameters. Male Dunkin-Hartley guinea pigs were divided into the following treatment groups: 1) STZ (streptozotocin) treatment (150 mg/kg, i.e. fed ad libitum), 2) food restricted (FR) animals to match BW changes in STZ group for 20 d, and 3) controls fed ad libitum. Hepatic microsomal enzyme assays were performed on the 12,500 g supernatant fraction. Total microsomal protein was depressed in STZ and FR animals; however this was significant in the FR group only. Total aminopyrine N-demethylase (per liver) was significantly lower in STZ and FR groups compared to controls, while there was no change in total aniline hydroxylase. Specific activity (per mg microsomal protein) was not affected for aminopyrine N-demethylase; however, aniline hydroxylase was elevated in the FR group only. Food restriction alone, therefore, produced a pattern of changes in liver microsomal enzyme activity similar to, but quantitatively different from diabetic animals with the same BW loss. (Supported by the Coronary Heart Disease Research Project of the American Health Assistance Foundation, Washington, D.C.).


Choline-deficient diets fed to rats have been shown to affect hepatic carcinogenesis, possibly by fat accumulation resulting in liver cell necrosis and subsequent regeneration (Lombardi et al. 1971, Glimmbarresi et al. 1982). The present study addresses whether choline deficiency affects the ability of hepatic microsomes to metabolize xenobiotics. As the woodchuck (Marmota monax) has been developed as a research model for viral hepatitis and for liver cancer, dietary effects on its hepatic biotransformation have been studied. Male woodchucks and Sprague Dawley rats were assigned to 1 of 4 diet groups (high or low fat, with or without choline) for 12 weeks. As a measure of functional damage to their biotransformation pathways, liver microsomal cytochrome P-450 was quantitated spectrophotometrically, as was p-nitrophenol glucuronid transferase (p-NP-GT), p-nitrophenol hydroxylase (ANH) activity was assayed fluorometrically. Analysis showed no statistically significant effect of choline or fat on rat microsomal activities. In woodchuck, presence or absence of choline had no effect; however, the high fat diets caused 38% higher P-450, 38% higher p-NP-GT, and 56% higher ANH than the low fat diets.
PURIFICATION AND CHARACTERIZATION OF THE DOG HEPATIC CYTOCHROME P-450 ISOZYME(S) RESPONSIBLE FOR THE METABOLISM OF CERTAIN POLYCHLORINATED BIPHENYLS. D.B.A. Duignan, J.R. Halpert, and J.G. Sipes, Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.

Previous studies have shown that among the dog, monkey, rat and human, only the dog is capable of metabolizing 2,2',4',5,5'-hexachlorobiphenyl (245-HCB) to a significant degree. In addition, pretreatment of dogs with phenobarbital (PB) enhances the in vitro metabolism of 245-HCB on both a per nanomole cytochrome P-450 and a per mg protein basis. In dog hepatic microsomes, we have detected at least two PB induced isozymes of cytochrome P-450, as evidenced by SDS-polyacrylamide gel electrophoresis and column chromatography. Our chromatographic protocol employed Octylamino-Sepharose followed by DEAE-Sephalcel, which led to the partial purification of the two isozymes. In vitro incubation with a reconstituted system has shown a substantial rate of metabolism of 245-HCB by one of the isozyme fractions, which is comparable to that found in the PB-induced dog microsomes. By further purification of the dog hepatic cytochrome P-450 isozymes, we hope to determine why the dog is unique among the species tested in its ability to metabolize 245-HCB. Additional studies using 4,4'-dichlorobiphenyl and 2,2',3,3',5,5'-hexachlorobiphenyl are in progress. (Supported by NIH Grant #T32 ES 07901.)

STUDIES ON THE REGULATION OF GLUTATHIONE TRANSFERENCE AND PEROXIDASE ACTIVITIES IN THE RAT FOLLOWING TREATMENT WITH INDUCERS. L.W. Robertson, P. Oesch, and H. Schramm, Institute of Toxicologie, University of Mainz, D-6500 Mainz, FRG.

We have previously reported (Toxicologist 3 #398) that following the administration of Aroclor 1254, a powerful inducer of glutathione transferase (GST), the activities of glutathione peroxidase (GPx) were diminished. This was particularly surprising since some of the inducible GST isozymes possess GPx activity (towards organic hydroperoxides as cumene and t-butyl hydroperoxides). We also observed a decrease in the GPx activity with H2O2, a substrate for the discrimination of Se-dependent and non-Se-dependent GPx activity (of the GST isozymes). The reported effects of Aroclor 1254 have been generalized to include other potent inducers of GST, e.g. 3-methylcholanthrene, trans-stilbene oxide and 3,3',4,4'-tetrachlorobiphenyl, which either had no effect on or slightly diminished the GPx activities. Assays specific for the inducible GST subunits, which possess GPx activity (Ta: 4', androstenedione, Ye: echacinol) indicate that these GST activities are increased following treatment with inducers without the concomitant increase of GPx activities. In separate in vitro experiments Aroclor 1254 neither activated nor inhibited GPx activities. (Supported by the DFG. L.W.R. is the recipient of an A. v. Humboldt Research Fellowship)

EFFECTS OF 3-METHYLCOLANTHRENE (MC) AND AROCLOR 1254 PRETREATMENTS ON THE DISTRIBUTION OF CARCINOGEN-METABOLIZING ENZYMES WITHIN THE RAT PANCREAS. T.I. Kawabata, F.F. Guengerich, and J. Baron, Dept. of Pharmacology, The Univ. of Iowa, Iowa City, IA and Dept. of Biochemistry, Vanderbilt Univ., Nashville, Th.

Although the pancreas is known to possess carcinogen-metabolizing enzymes, the effects of xenobiotics on the distribution of these enzymes have not been established. Immunohistochemical staining methods were used to localize the following enzymes within pancreases of rats pretreated with either MC or Aroclor: cytochrome P-450 (P-450), PB-B and BNF-B (major isozymes of P-450 present within livers of rats pretreated with phenobarbital and P-naphthoflavone, respectively); NADPH-P-450 reductase; epoxide hydrolase; and glutathione S-transferases B, C and E. As biochemical correlates, benz(a)pyrene (BaP) hydroxylase activity was localized within pancreases by employing a fluorescence histochemical technique. BaP hydroxylase activity and staining for P-450 BNF-B within acinar and duct epithelial cells were increased after either MC or Aroclor pretreatment, whereas staining for the other enzymes was not altered. The distribution of P-450 BNF-B and BaP hydroxylase activity within pancreases of rats pretreated intraperitoneally with either MC or Aroclor was different than that observed after intragastric administration. (Supported by USPHS Grants CA30140, ES01590, and ES02205.)

DISTRIBUTION OF EPOXIDE HYDROLASE ACTIVITY IN MOUSE LIVER MITOCHONDRIAL FRACTIONS. S. Kaur and S. S. Gill, Division of Toxicology and Physiology, University of California, Riverside, CA 92521

Mouse liver light and heavy mitochondrial fractions contain significant epoxide hydrolase activity in addition to that present in the cytosol and microsomes. As mitochondrial fractions contain a number of organelles, experiments were designed to determine the localization of the epoxide hydrolase activity in these fractions. Subcellular fractions were prepared using livers from 6-8 week old Swiss-Webster male mice. Using trans-stilbene oxide (TSO) as substrate, the highest activity was localized in the cytosolic fraction, followed by the light mitochondrial fraction. Subfractionation of the light mitochondrial fraction by isopycnic sucrose density gradient resulted in the separation of mitochondria from peroxisomes as monitored by marker enzymes. Distribution of TSO hydrolase activity in the sucrose gradient fractions closely resembled the activity distribution of peroxisomal markers catalase and urate oxidase. Treatment of mice with clofibrate selectively induced TSO hydrolase in the cytosol without affecting this enzyme activity in the mitochondrial fraction. There was no difference in the distribution pattern of TSO hydrolase and marker enzymes in sucrose density gradients of mitochondrial fractions from clofibrate-treated and control mice.
Nicotine undergoes metabolism dependent covalent binding to rabbit liver microsomal macromolecules. Metabolism of nicotine to its major metabolite cotinine involves an initial 2e oxidation catalyzed by cytochrome P-450, resulting in the generation of a reactive electrophile, nicotine $\Delta^1(5')$iminium ion. Incubation of $3H$-nicotine in the presence of NADPH, air, and liver microsomes led to a time and concentration dependent irreversible binding to microsomal macromolecules, directly paralleling its metabolism. No covalent binding was observed in the absence of NADPH. An increase in covalent binding (217%), which correlated with an elevation of cytochrome P-450 content (267%), was observed when comparisons were made between phenobarbital induced and control microsomes. The cytochrome P-450 inhibitor, SKF-525A, decreased covalent binding by 50%. In each case, formation of the iminium ion, monitored by detection of the enamine, correlated with the extent of covalent binding. Thus covalent binding of nicotine to rabbit liver microsomes appears to be mediated by cytochrome P-450 and to involve the formation of the iminium intermediate. Such covalent binding may have relevance to toxic mechanisms underlying tobacco related diseases.

**METABOLISM OF ACETAMINOPHEN IN RATS EXPOSED TO CIGARETTE SMOKE.** M.J. Gagliano and H.W. Dorough, Dept. of Entomology and Graduate Center for Tok., University of Kentucky, Lexington, KY 40546

Adult male rats exposed nose-only to cigarette smoke 20 min/day for 6 mo showed a 15% lower level of hepatic GSH than did the sham control animals. To determine if this reduction, or smoking in general, altered GSH conjugation and other processes important in xenobiotic metabolism, the fate of $^{14}$C-acetaminophen (APAP, 300 mg/ kg i.p.) in the smoke-exposed rats was compared with that of the controls. Urinary excretion of radioisotope after 24 hr was greater in the smoke-exposed rats (81.1 ± 3.4 % of dose, x ± SE) than in the controls (74.3 ± 5.5 %). Excretion via the feces was low (2-4%) in all animals. Radioactive components of the urine consisted of the parent compound (26% of urinary radioactivity), APAP-sulfate (33%), APAP-glucuronide (34%) and APAP-mercapturic acid (3,4%) with no significant difference (p<0.05) between the smoked and control rats. Pretreatment of the rats with the GSH depletor methyl iodide (30 mg/kg i.p. 24 hr before injection of APAP) did not alter the excretion rate of APAP but the percentages of the urinary radioisotope as the parent compound (16%) and APAP-mercapturic acid (2%) were reduced, while the APAP-sulfate (42%) and glucuronide (38%) metabolites were increased. As with APAP alone, however, there was no difference between smoke-exposed and control rats. (Supported in part by THRI Grant No. 4E014)


The metabolism and distribution of lindane in the developing Fisher 344 rat was investigated at 2, 9, 16, and 23 days of age following subcutaneous administration of 20 mg/kg of lindane containing 0.5 μCi [U$^{14}$C]-lindane in peanut oil. Groups of ten pups (5 males and 5 females) were sacrificed at 4 hr intervals during a 24 hr period following dosing. Adrenals, brain, heart, lung, liver and kidney were analyzed for radioactivity. Blood and urine samples were analyzed for radioactivity and metabolites of lindane. The effect of age on the distribution and excretion of radioactivity were examined. The high levels of radioactivity in the blood, brain, and lung and the low level of radioactivity excreted in the urine suggest that lindane metabolism is significantly lower in the 2 and 9 day old pups. By day 16 the levels of radioactivity in tissue and urine suggest that lindane metabolism is increasing and by day 23 the radioactivity excreted in the urine suggest that metabolism of lindane has reached the levels seen in young adult rats.
EFFECT OF ALTERATION OF CYTOCHROME P-450 ACTIVITY ON ETHYLENE DICHLORIDE (EDC) TOXICITY IN MICE FOLLOWING INHALATION EXPOSURE. R. J. Francovitch, N.A. Schor and W.J. George. Depts. of Pharmacology and Pathology, Tulane Medical School, New Orleans, LA 70121; Sponsor: R.E. Billings

Ethylene dichloride (EDC) has been shown to produce toxic effects in various organ systems in both man and animals. An intermediate formed during the metabolism of EDC has been suggested to be at least partially responsible for its toxicity. Studies were performed to investigate the role of cytochrome p-450 metabolism in mediating the toxic responses associated with EDC. Mice were exposed by inhalation to selected concentrations of EDC (1000, 1250, 1500 ppm). Prior to exposure, mice were pretreated with phenobarbital (PB) or 3-methylcholanthrene (3-MC) to induce cytochrome P-450. SKF 525A was administered to inhibit this form of biotransformation. Ethylene dichloride produced a dose-dependent increase in mortality at 24 and 48 hr post exposure. This response was enhanced by PB and 3-MC pretreatment and attenuated by administration of SKF 525A. EDC exposure was also associated with pathological changes in the kidney. Renal toxicity was not significantly altered by the various pretreatments. These results support the hypothesis that a product of cytochrome P-450 metabolism of EDC is involved in the mortality of mice following EDC exposure but may not be responsible for toxic changes in the kidney.

ALTERATION OF BENZ(a)PYRENE METABOLISM IN RAT LUNG BY THE ORGANIC SOLVENT, p-XYLENE. A. Roberts, M. Bogdannify, D. Brown and R.A. Schatz. Tox. Prgm., Northeastern U., Boston, MA.

Studies in our laboratory have shown that acute inhalation of p-xylene is capable of inhibiting Benzo(a)pyrene B(a)P metabolism in rats. This inhibition was shown to be due in part to corticosterone. Rat lung cytochrome P450 is partially destroyed by treatment with the solvent, p-xylene. We therefore examined the effects of P450 destruction on the ability of rat lung to metabolize B(a)P. In lung microsomes prepared from rats treated with p-xylene (i.e., 1 mg/kg), it was found that the ability to hydroxylate B(a)P measured by aryl hydrocarbon hydroxylase (AHH) activity was decreased by 41% (p < 0.05). Further analysis by HPLC showed changes in formation of individual B(a)P metabolites. The 3-OH, 9-OH, and 4, 5, 9, 10 and 9, 10 diol B(a)P were inhibited by 32%, 27% and 50% respectively. Only the B(a)P 4, 5 diol was significantly reduced by p-xylene treatment (p < 0.05). Levels of the 1, 6 and 3, 6 and 6, 12 B(a)P quinones were unchanged as were the 7, 8 and 9, 10 diol metabolites. In order to assess changes in the balance between activation and detoxication pathways, ratios were calculated between the 9, 10 diol and the 3-OH B(a)P metabolites. Interestingly, a significant increase was noted for this ratio in rats treated with p-xylene (p < 0.05). The results suggest a p-xylene mediated inhibition of X region metabolites and shifts in the balance between toxicication and detoxication toward toxicication.
Hepatic cytosolic aldehyde dehydrogenase (ALDH) has previously been implicated in the oxidation of acrolein. In contrast, our results indicate that acrolein is a potent inhibitor of ALDH present in mitochondrial and cytosolic fractions of rat liver. Livers were obtained from male Sprague-Dawley rats for preparation of cytosolic (C) and mitochondrial (M) fractions. Incubations of ALDH from C and M fractions with NAD and acrolein did not result in production of NADH detectable by standard spectrophotometric assays. The inability of NADH to oxidize acrolein in the presence of NAD was verified by the absence of acrylic acid formation quantitatively by HPLC analysis. Only small amounts of acrylic acid (0.36 mmol/min/mg protein) were produced when C-ALDH was incubated with NAD. Incubation (5 min) of C-ALDH with 10, 30, or 60 µM acrolein prior to addition of propional resulted in 3, 38, and 56% inhibition of the low-Km enzyme and 3, 13, and 20% of the high-Km C-ALDH. These concentrations of acrolein inhibited the low-Km M-ALDH 56, 88, and 100%, respectively. Total high + low-Km M-ALDH activity was inhibited 100% by preincubation with 100 µM acrolein. The time course of inhibition demonstrated that incubation of acrolein (100 µM) for 120 sec with C-ALDH resulted in 41% decrease in total activity and 76% inhibition of the low-Km C-ALDH. The low-Km M-ALDH was inhibited 80% after a 120-sec incubation with 10 µM acrolein. (Supported by NTAA Grant AA03527.)

Phencyclidine (PCP) undergoes metabolism-dependent covalent binding to liver microsomal proteins (Law, Toxicol. Appl. Pharmacol. 57: 263, 1981) and is a mechanism-based inhibitor of cytochrome P-450 (Hogg, et al. Drug Metab. Dispos. 12: 371, 1984). Oxidative metabolism of PCP by cytochrome P-450 involves formation of an electrophilic iminium intermediate which may interact with microsomal macromolecules (Ward, et al. Drug Metab. Dispos. 10: 690, 1982). The incubation of PCP iminium perchlorate with rabbit liver microsomes resulted in an irreversible loss of N-demethylase activity and a loss of cytochrome P-450 content. Both effects required NADPH and exhibited time and concentration relationships similar to those of PCP itself. Covalent binding of 3H-PCP iminium ions to microsomal proteins also required NADPH. Thus, both enzyme inactivation and protein binding appear to depend on the further metabolism of the iminium intermediate which does not itself exhibit characteristic properties of the ultimate reactive species. A partition ratio of 1:216 was calculated from comparison of the rate of suicide inactivation of cytochrome P-450 by the PCP iminium species with the rate of its metabolism by liver microsomal enzymes. (This work was supported by NIDA Grant #CA 34050-01. The work was supported by NIH Training Grant #5T32GM07175-08.)

The antibiotic chloramphenicol (1-p-nitrophenyl-2-dichloroacetamido-1,3-propanediol=CAP) inactivates cytochrome P-450 by virtue of the covalent modification of the protein moiety of the enzyme. The major reactive metabolite responsible for the enzyme inactivation is chloramphenicol oxamyl chloride, which is formed during the oxidative dechlorination by cytochrome P-450 of the dichloromethyl moiety of CAP. We are determining the effect of structural alterations of the remainder of the CAP molecule on its effectiveness and isozyme specificity as a suicide substrate of rat liver cytochrome P-450. For P-450. We have focused on the role of the propenoid side chain and the p-N2 group. 1-p-Nitrophenyl-2-dichloroacetamidothane (p-N2-DCAE) and 1-phenyl-2-dichloroacetamidothane (DCAE) have been synthesized. Both compounds exhibit time and concentration-dependent inactivation of cytochrome P-450 in a reconstituted system and in intact rat liver microsomes. The p-N2 group and the hydroxyl groups thus do not seem to be required for inactivation of cytochrome P-450. In fact, DCAE and P-N2-DCAE appear to be at least ten-fold more potent inactivators of P-450, both in vivo and in vitro. Preliminary studies indicate that p-N2-DCAE is metabolized to a reactive oxamyl chloride intermediate which covalently binds to P-450 in a manner similar to CAP. (Supported by NIH Grant #T32 ES 07091.)

Glucuronidation of 7-hydroxycoumarin (HC) in perportal (PP) and pericentral (PC) regions of the lobule in livers from untreated and 3-methylcholanthrene (MC)-treated rats. J.G. Conway, F.C. Kaufman, T. Tsukada and R.G. Thurman, Deps. of Pharmacology, U. of N.C., Chapel Hill, NC and U. of Maryland, Baltimore, MD. Under normoxic conditions, the fluorescence of free HC was monitored from the surface of perfused livers using micro-light guides placed on PP and PC regions of the liver lobule. Formation of nonfluorescent HC glucuronide was then inhibited completely by perfusion with N2-saturated perfusate containing ethanol. Maximal rates of glucuronidation calculated from fluorescence quenching in PP and PC regions were 11.9 and 12.8 µmol/g/h, respectively, in livers from untreated rats. In livers from MC-treated rats, maximal rates were 15.3 and 17.5 µmol/g/h in PP and PC regions, respectively. Glucuronosyltransferase (GT) activity in microdissected tissue samples assayed in vitro with 9 mM UDPGA was 5- to 8-fold greater in livers from MC than untreated rats and 2-fold greater in native microsomes incubated with 0.4 mM UDPGA. GT activity was 2.4-8-fold greater in PP than PC regions and was equal in both regions in livers from MC-treated rats. Thus, maximal rates of glucuronidation did not correlate with GT activity. Factors other than GT activity (i.e., UDPGA supply or β-glucuronidase activity) are most likely important in the regulation of glucuronidation in PP and PC regions of the liver lobule under these conditions (supported by CA-23080 and CA-20807).
Fungi have the enzymatic capacity to oxidize polycyclic aromatic hydrocarbons (PAHs) such as benz[a]pyrene, benz[a]anthracene, 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene and 1-nitropyrene to nontoxic metabolites as well as compounds that have been implicated as biologically active forms of PAHs in higher organisms. Fungi metabolize PAHs to trans-dihydridols, phenols, quinones, dihydridol-epoxides and sulfate, glucuronide and glucoside conjugates of these primary metabolites. Fungal extracts of PAHs incubations were assayed for mutagenicity toward S. typhimurium TA98 and TA 100. The mutagenic activity of the biodegradation products of the PAHs tested was significantly decreased. The results suggest that the microbial degradation of PAHs may detoxify these potential environmental pollutants.

3-Methylindole (3MI) is an anaerobic ruminal bacterial metabolite of L-tryptophan that causes acute pulmonary edema and emphysema in cattle, sheep and goats. 3MI when infused into horses and ponies selectively damages bronchiolar epithelia. Experiments with fresh lung tissue were placed in buffer with 50 mM H-3MI for 30 min, fixed, washed, plastic embedded and autoradiographed. Labeling was 8 times greater per area to bronchiolar epithelium than to interalveolar septa. Washed horse lung microsomes were incubated with or without glutathione, an NADPH-generating system and 14C-3MI. Protein precipitates were collected on filter paper, extracted, oxidized to 14C-CO2 and counted. Metabolites in incubation supernatants were separated by HPLC and detected by radioactivity and 280 nm. In the presence of glutathione, an apparent glutathione adduct was formed and binding to microsomal proteins was reduced. Inhibitors of cytochrome P450, SKF-525A and piperonyl butoxide reduced the covalent binding to 75% and 12% of the controls, respectively. These results suggest that the target cells of 3MI-induced injury in the horse, the bronchiolar epithelial cells, are alkylated by an electrophilic 3MI intermediate. (NIA HL-13645)
Male Fischer 344 rats were given a single po dose of approximately 1 or 8.7 mmole/kg of [14C] PGME-α-isomer (1-methoxy-2-propanol) or [14C] PGME-β-isomer (2-methoxy-1-propanol). After dosing, expired air, excreta and tissues were analyzed for C activity, and metabolites in urine were isolated and identified. There were pronounced differences in the metabolism and disposition of the two PGME isomers. About 10 to 20% of the administered 14C was excreted in urine while 50 to 60% was eliminated as 14CO2 within 48 hr after giving the alpha isomer. In contrast, 70 to 80% of the 14C was excreted in urine while 10 to 20% was eliminated as 14CO2 within 48 hr after giving the beta isomer. The urinary metabolites indicated that the two isomers were metabolized via different routes to different types of metabolites. While metabolism of the two isomers is different, there is a substantial toxicological data base which clearly shows that the commercial grade PGME mixture (2-5% β-isomer) has a low degree of biological activity.

1 Co-sponsored in part by ARCO Chemical Company

Experimental models for halothane hepatotoxicity require microsomal enzyme induction by phenobarbital or trilodothyronine pretreatment and hypoxic conditions. In phenobarbital-pretreated male rats exposed to 1% halothane for 2 h combined with hypoxia (10% O2) significant increases in serum enzyme activities of alanine aminotransferase (GPT) and sorbitol dehydrogenase (SDH) were observed 24 and 48 h later indicating liver damage. Pretreatment with phenone to deplete hepatic reduced glutathione further aggravated the halothane-induced hepatotoxicity. In both models cianidanol (200 mg/kg po before exposure to halothane) provided protection against halothane-induced liver injury as evidenced by reduced serum enzyme elevations and histomorphological examinations. It is now generally excepted that halothane can mediate a direct hepatotoxicity reaction by reductive metabolism, whereas the oxidative route of metabolism seems to be responsible for the immune mediated toxic reaction. Our experimental data indicate that from cianidanol a protection against the direct toxic reaction of halothane might be expected, which needs to be verified by clinical studies.

TRICHLOROETHYLENE POTENTIATES CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY.

Both 1,1,2-trichloroethylene (TCE) and carbon tetrachloride (CCl4) are hepatotoxins in the rat. In this study the effect of TCE on CCl4-induced hepatotoxicity was examined. In one experiment F344 rats were given TCE (0.05-1.0 ml/kg, i.p.) and CCl4 (0.016-0.10 ml/kg, i.p.) simultaneously. No dose of TCE alone and only the highest CCl4 dose resulted in elevated plasma glutamic-pyruvic transaminase (GPT) activity 24 hours after administration. When TCE (0.05 ml/kg) and CCl4 (0.10 ml/kg) were given together plasma GPT was elevated 18-fold over that due to CCl4 alone. TCE was also administered in the rats' drinking water (0.1-5.0 mg TCE/ml 1% EmulphorR 620) for 3 days and followed by a challenge of CCl4 (0.10ml/kg, i.p.). Plasma GPT was not increased by any concentration of CCl4. However, when challenged with CCl4, plasma GPT values rose from 146 to 37 units/ml in controls receiving CCl4 alone to 1600 to 600 units/ml in animals also exposed to 3 mg TCE/ml drinking water. Parallel results were observed in liver histology. This potentiation was not observed 24 hrs after cessation of TCE exposure. Liver cytosolic N1-spermidine acetylase activity also increased 6 hours after CCl4 challenge from 11 ± 3 to 25 ± 3 pmol N-acetylspermidine formed/min/mg protein in rats exposed to 0 and 3 mg TCE/ml drinking water, respectively. Thus, TCE causes a reversible potentiation of CCl4 hepatotoxicity. (Supported by NIH Grant #T32 ES 07091.)

EFFECT OF GAMMA-IRRADIATED SEWAGE SOLIDS (GISS)
AS A DIETARY SUPPLEMENT ON XENOBIOITIC BIOTRANSFORMATION IN SHEEP. J.B. Watkins, G.S. Smith and D.M. Hallford. Indiana University School of Medicine, Bloomington, IN and New Mexico State University, Las Cruces, NM.

Municipal sewage represents a resource of nutrients especially usable by ruminants. Data were formulated to meet nutrition requirements for breeding ewes with 3.5% cotton-seed meal for controls and 7% GISS for experimental ewes. After 4 yr of continuous feeding, tissues were removed and the activities of phase I and II biotransformational enzymes were assayed under standard conditions. Total cytochrome P-450 content in liver and benzphetamine N-demethylase activity in rumen were decreased 40 and 70%, respectively, whereas renal and ileal values were not affected by GISS feeding. Glutathione S-transferase activity toward chlorodinitrobenzene was depressed by 20% in rumen wall and that toward ethacrynic acid was increased by 25% in ileum. UDP-Gluconuronyltransferase activity toward 1-naphthol and testosterone was increased 20 to 60% in rumen. No other differences were noted in any tissue for other substrates for the above enzymes or for epoxide hydrolase or sulfotransferase. Long-term consumption of 7% GISS caused slight alterations in enzyme activity, however there were no major differences in the capacity of hepatic, renal or ileal tissues to execute these reactions. (Supported by Dept of Energy Contracts DE-AC04-76ET30926 and DE-AC04-83AL21776 with NMSU.)

Trimetrexate (TMQ; 2,4-Diamino-5-methyl-6-[3,4,5-trimethoxyanilino]methyl[3]quinazoline) is a "nonclassical" antifolate which is advocated as an alternative to methotrexate. In mice TMQ is rapidly and extensively demethylated to a metabolite which is equipotent with the parent drug with respect to in vitro dihydrofolate reductase inhibition, but is only weakly cytotoxic. The effects of clinical imidazole drugs (ketocanazole [K], miconazole [M], clotrimazole [C], clotrimidine [Et], clindamycin and clindamine) on rat hepatic microsomal demethylation of TMQ, in vitro, was investigated. The nitrogen-substituted imidazole drugs (K, M, and C) were potent noncompetitive inhibitors of TMQ metabolism with IC50 values obtainable at therapeutic doses (<2μM). Ketocanazole and clotrimazole were comparatively weak, competitive inhibitors of TMQ metabolism (IC50 > 300μM) while clindamycin and clindamine were even less inhibitory (IC50 > 1mM). In vivo, the administration of K to male rats (40 mg/kg i.p. daily for 3 days) prolonged the elimination half-life of a 20 mg/kg i.v. dose of TMQ when TMQ was given 1 hour after the last K administration. TMQ pharmacokinetics may thus be subject to modification by clinical imidazole drugs.

METABOLISM OF DINITROBENZENES BY ISOLATED RAT HEPATOCYTES. P.A. Cossum and D.E. Bickert, CIIT, Research Triangle Park, NC 27709.

The metabolism of the isomeric dinitrobenzenes (DNB) was compared in hepatocytes freshly isolated from male Fischer-344 rats. Hepatocytes (8×10^6 cells) were incubated for 30 min under air with 100 μM [U-14C] o, o', m, or p-DNB. Metabolites were separated by reverse phase HPLC and were quantitated by liquid scintillation spectrophotometry. Metabolite identification was by coelution with standards on HPLC, GC/MS, specific enzyme hydrolysis and, where appropriate, deasulfuration with Raney nickel. The half-lives for disappearance of o-, m-, and p-DNB were 2.6, 12.8 and 3.5 min respectively. The major metabolites of o-DNB were o-nitroaniline (30.8%), S-(2-nitrophenyl)glutathione (39.8%) and a glucuronide conjugate (3.2%). Non-enzymatic conjugation of o-DNB with glutathione occurred very slowly in aqueous solution. These findings are in accord with previous work which showed that glutathione transferase is necessary for the conjugation of o-DNB with glutathione (Asaoka and Takahashi, J. Biochem 90:1237, 1981). The major metabolites of m- and p-DNB were the corresponding nitroanilines (70.8 and 80.2%, respectively). Thus, each isomer is metabolized to nitroaniline under aerobic conditions, and o-DNB is the only isomer from which a detectable glutathione conjugate is formed.

EFFECTS OF HEPATIC GLUTATHIONE DEPLETION ON THE DNA BINDING AND HEPATOBILIRARY DISPOSITION OF AFLATOXIN B1 IN VIVO. C.J. Holeski and D.L. Eaton. Dept. of Pathology and Environmental Health, University of Washington, Seattle, WA.

Conjugation with glutathione (GSH) is a major metabolic pathway for the inactivation of aflatoxin B1 (AFB). Previous studies have shown that depletion of hepatic GSH increases the severity of AFB-induced hepatic lesions. This study examined the effects of GSH depletion on the hepatic disposition of AFB. Hepatic GSH content of rats was depleted by i.p. injection of 0.7 ml/kg diethylmaleate (DEM) two hrs before i.p. administration of 0.25 mg/kg, 25 μCl/kg ^3H-AFB. After collection of bile for 2 hrs, the liver was removed and DNA was isolated. DEM treatment reduced the amount of AFB metabolites excreted in bile and significantly increased the amount of AFB remaining in the liver. However, DEM-treatment did not significantly alter the covalent binding of AFB to hepatic DNA, nor did it alter the distribution of AFB metabolites among polar, non-polar and covalently-bound fractions. Urinary excretion of AFB-related metabolites accounted for less than 2% of the administered dose in both control and DEM-treated rats. These data suggest that the intracellular concentration of GSH is not rate-limiting in the inactivation of AFB, even when GSH is reduced by as much as 95%.

(Supported by Am. Cancer Soc. Grant IN-26M).

EFFECT OF BILE ACIDS ON PROGESTERONE BINDING IN RAT LIVER MICROSOMES. A. Ghoshal, S. Gracon, L. Stuhne-Saklaer, M.W. Roomi, R.G. Cameron, F.A. de la Iglesia and G. Feuer. Department of Clinical Biochemistry and Pathology, University of Toronto, Canada, and Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan, USA.

Since progesterone was shown to cause an increased biliary cholesterol output involved in the onset of bile acid-induced cholestasis the effect of bile acid accumulation was studied on H-progesterone binding in hepatic microsomes in vivo and in vitro. Applying lithocholate, glycolithocholate, taurolithocholate, deoxycholate, glyco-deoxycholate, chenodeoxycholate, hyodeoxycholate, cholate, glycocholate and taurocholate, the in vitro competition experiments indicated that none of the bile acids could displace H-progesterone from binding sites, but the monohydroxy derivatives, particularly taurolithocholate increased progesterone binding. Microsomes isolated from the liver of female rats treated with cholesterol doses of taurolithocholate, lithocholate, chenodeoxycholate or cholate in vivo, have shown that only taurolithocholate treatment increased progesterone binding. This was dose dependent. Taurolithocholate also raised microsomal cholesterol content suggesting that this bile acid may induce a conformational change resulting in a greater capacity for progesterone and cholesterol binding. This membrane effect may be a component in the development of certain forms of cholestasis.
PHARMACOKINETICS AND METABOLISM OF A DERMAL DOSE OF \(^{14}\text{C}\)2,5-Hexanediol in Hens. E. Sumita, A.A. Nofel, and M.B. Abou-Donia. Duke University Medical Center, Durham, NC.

A dermal dose of 50 mg/kg (7.5 Ci/kg) of \(^{14}\text{C}\)2,5-hexanediol (2,5-HD) was applied on a protected area on the back of hens. Five groups of three hens were killed after 4, 8, 24, 36, and 48 hrs. The results were released rapidly from the application site with an initial half-life of 0.4 hr. After 45 hrs most of the radioactivity was collected as volatile materials (35%), mostly as 2,5-HD, followed by \(^{14}\text{C}\)CO\(_2\) (11.9%) while the combined urinary-fecal excreta accounted for 11.8% of the dose. Radioactivity level in tissues reached a peak of 7.6% of the dose in 8 hrs. The highest concentration of \(^{14}\text{C}\) was detected in the bile followed by liver, kidneys, and bone marrow whereas brain, spinal cord, and peripheral nerves showed smaller concentrations. The half-lives for the elimination of \(^{14}\text{C}\) were longest for muscle (71 hr) and bone marrow (61 hr) and shortest for adipose tissue (12 hr). Half-lives of \(^{14}\text{C}\) in neural tissues ranged from 23 to 30 hr while plasma had a half-life of 12 hr. Most of the radioactivity in the plasma at 2 hrs was identified as the parent compound followed by 2,5-dimethylfuran and 5-hydroxy-2-hexanone. As time passed, 2,5-dimethylfuran concentration increased at the expense of both 2,5-hexanediol and 5-hydroxy-2-hexanone. (Supported in part by NIOSH Grant OH00823).

KINETICS OF \(^{14}\text{C}\)-TRIMETHYLVIN IN THE RAT. M.G. Paule, J.C. Lipscomb, and W. Skilker, Jr., Division of Teratology Research, National Center for Toxicological Research, Jefferson, AR.

The mechanisms responsible for trimethyltin (TMT)-induced behavioral and neuropathological consequences remain unknown but likely relate to its time-course of exposure. To determine TMT's pharmacokinetic profile, we injected rats with 2.0 mg/kg TMT-chloride plus 12.5 microcurie \(^{14}\text{C}\)-TMT intravenously (IV) via a femoral vein cannula or intraperitoneally (IP). Serial whole blood samples (20 ul) were collected for 6 hours after IV and IP administration and at 24 hour intervals for 2 weeks after IP administration. Total \(^{14}\text{C}\)-radioactivity was determined at selected times in blood and selected tissues. Peak blood levels were similar by both routes (IV=34.4(n=9) and IP=38.4(n=4)), occurring 1 minute after IV and 30 minutes after IP dosing. Substantial radioactivity (40% of peak IP values) remained in blood 2 weeks after treatment. Peak tissue levels (within 4 hours) were highest in liver and kidney. In brain, levels in putatory > brainstem > hippocampus > thalamus. \(^{14}\text{C}\) disappearance from tissue, was similar to that noted for blood. Since the half-life of TMT is approximately 2 weeks, single 'acute' treatments actually represent prolonged exposures.

JOINT NEUROTOXIC ACTION OF METHYL 1,4-BUTYL KETONE (MBK), METHYL 1,4-BUTYL KETONE (MBK) AND \(\eta\)-HEXANE: INDUCTION OF CYTOCHROME P-450. Daniel M. Lapadula, Gerald A. Campbell, and Mohamed B. Abou-Donia. Duke University Medical Center, Durham, NC 27710.

Previous investigations from this laboratory demonstrated a joint neurotoxic action of dermal EPN (O-ethyl-O-4-nitrophenyl phenylphosphonothioate) and inhaled MBK, as well as a synergistic effect of \(\eta\)-hexane-induced neurotoxicity by the non-neurotoxic MBK. We have studied a possible mechanism of this neurotoxic action. Hens were administered i.p. injections of 5 mmole/kg of \(\eta\)-hexane, MBK, MBK, MBK (MBK:MBK:MBK, 7:3), or 2,5-HDHO (2,5-hexanediol) for 3 consecutive days. In another experiment, hens were given continuous inhalation of 1000 ppm \(\eta\)-hexane, 1000 ppm MBK, or 1000 ppm \(\eta\)-hexane + 1000 ppm MBK for 30 to 50 days. Treatments of MBK, MBK, MBK, MBK, 2,5-HDHO, and phenobarbital (PB), as well as inhalation of MBK or \(\eta\)-hexane + MBK significantly induced cytochrome P-450 content. No induction was observed in \(\eta\)-hexane treated animals. Analysis of microsomes by SDS-Polyacrylamide gel electrophoresis demonstrated that a PB type of cytochrome P-450 was induced. The joint neurotoxic action of these chemicals is at least partially due to the activation of liver microsomal cytochrome P-450 — enhancing the metabolism of \(\eta\)-hexane or EPN to more neurotoxic chemicals. (Supported by NIOSH Grant No. OH00823).

UPTAKE, DISPOSITION AND ELIMINATION OF ACRYLAMIDE IN RAINBOW TROUT. D.W. Petersen, K.M. Kleiman, R.S. Knebel, and J.J. Lech. Medical College of Wisconsin, Milwaukee, WI and American Cyanamid Co., Wayne, NJ.

The uptake, disposition and elimination of 2,3-\(^{14}\text{C}\)-AC was studied in fingerling rainbow trout exposed to 0.388 mg/L and 0.710 mg/L 2,3-\(^{14}\text{C}\)-AC at 12°C (static water) for 72 hours (h). \(^{14}\text{C}\) levels in carcass (C) and viscera (V) were determined from 4 h to 72 h after the beginning of AC exposure and 4 h to 96 h after transfer of the fish to flowing water. Uptake of \(^{14}\text{C}\) was initially rapid and plateaued after 72 h of AC exposure. No appreciable bioaccumulation occurred in C or V at either exposure level and \(^{14}\text{C}\) distributed approximately equally to all tissues studied. Elimination of \(^{14}\text{C}\) from C and V was biphasic with a terminal half-life of approximately 7 days. \(^{14}\text{C}\) elimination was the most rapid from blood and gill and the slowest from muscle and intestine. In addition, 10-15% of the initial total \(^{14}\text{C}\) in C or V was associated with the protein fraction at all time points in the depuration period. Approximately 20% of an i.p. administered dose of 14C-AC was eliminated via the gills, 7% via the urine, and 11% via the bile in 2 h. Four biliary metabolites were isolated using high pressure liquid chromatography. Supported by NIHES Grant No. 01080 and by American Cyanamid Co.
The kidney of the male Syrian golden hamster is a target for estrogen-induced carcinogenesis. The mechanism is unknown, but believed to involve hormonal factors and covalent binding of reactive metabolite(s) to critical macromolecules. Covalent binding of the synthetic estrogen DES to kidney macromolecules was observed after incubation of male hamster kidney slices with \(^{3}H\)-DES. Covalent binding increased with increased incubation time and concentrations of DES. Hamster kidney slices incubated with 2 mM KCN under a nitrogen atmosphere neither covalently bound nor metabolized \(^{3}H\)-DES. Conjugated and oxidative metabolites were observed in control incubation media. Kidney slices from male hamsters had a two-fold greater covalent binding of \(^{3}H\)-DES as compared with kidney slices from female hamsters. These studies provide evidence that DES covalently binds to macromolecules of the hamster kidney and that covalent binding of \(^{3}H\)-DES depends upon oxidative metabolism. The sex difference in covalent binding of \(^{3}H\)-DES may be relevant to the sex difference in tumor susceptibility.

Relatively little is known of the role of the lymphatic system in the gastrointestinal absorption of lipid-soluble drugs. \(^{14}C\)-LY154356 (0-methoxy labeled) in the above vehicle. During the next 24 hours (food and water provided), 43.9 ± 3.3% (n = 5) of the radiocarbon dose was recovered in the lymph. Lymphatic absorption was highly variable (range 28-72%) and appeared to be unrelated to lymph flow. The T1/2 for appearance of radioactivity in lymph was 3.4 hr.; the peak absorption rate (7.4%/hr.) occurred at 3 hr. Analysis of lymph samples by GLC showed that the drug was absorbed unmetabolized. About 70% of the lymphatic radioactivity was recovered in the "cholemicron" fraction.

Orpanoxin demonstrated nonsteroidal anti-inflammatory activity in animals. This study assessed its toxic potential upon repeated peroral dosing for 6 months. Four Beagle dogs/male received 0(T1), 40(T2), 50(T3), 80(T4), 120(T5), and 150(T6) mg/kg/day. 40 and 60 mg/kg/day were no-effect drug levels, which were relatively high dosages in dogs as compared to other NSAIDS. 80 mg/kg/day or more caused varying degrees of toxicity associated with salivation, emesis, lab weakness, decreased activity, body weight loss, and pale mucosa. There was one death in T6 by day 28, and 7/8 of this group, 7/8 T5, and 1/8 T4 were terminated prematurely in moribund condition. Hemograms ranged from mildly decreased to frank anemia with depression of marrow erythroid cells. Depressed neutrophil counts correlated with marrow myeloid immaturity. T5-T6 induced renal papillary tip neomycin and gastric mucosal erosions, which did not penetrate the lamina propria. T2-T6 caused a persistent hypocholesterolemia, commencing within 10 days of initiation of dosing, which was considered a physiological response rather than a toxic manifestation.
This study was conducted to evaluate the chronic toxicity and carcinogenic potential of TNT in F344 rats. Rats received 0, 0.4, 2, 10 or 50 mg/kg/day in their diet for 24 months. Clinically, rats had reduced food intake and decreases in body weight gain at 10 and 50 mg/kg/day. A lethal dose was not seen. At the 2 highest doses, rats were anemic with compensatory reticulocytosis apparent. Splenic lesions, including hemosiderosis and sinusoidal congestion, were secondary to the anemic state and suggestive of hemolysis. This mechanism of anemia was further supported by methemoglobinemia and Howell-Holly and Hinz bodies, all of which reflect the oxidizing nature of TNT and/or its metabolite(s). Liver injury at doses of 10 mg/kg/day or greater was evidenced by altered lipid and protein metabolism and by hepatomegaly with hepatocellular hyperplasia. At the highest dose given, renal injury occurred which included elevated BUN and serum potassium levels. Kidney weights were increased and microscopic lesions including hyperplasia of the renal pelvis were observed. Hyperplasia and carcinoma of the urinary bladder were also seen for high dose females. (Supported by the U.S. Army Medical Bioengineering Research Laboratory under Contract No. DAMD17-79-C-9120).
THE TOXICOLOGY OF DENTAL PRODUCTS CONTAINING

Laboratory and clinical studies suggest that the retention of sanguinarine in dental plaque coupled with its antimicrobial profile against oral bacteria are responsible for the antiplaque activity of products containing sanguinaria. Dentifrices and oral rinses containing sanguinaria or sanguinaria extract were evaluated according to Food and Drug Administration guidelines for animal and human acute toxicity. Preparations containing sanguinaria extract or pure sanguinarine did not induce animal and human mucosal membrane irritancy, human irritation or sensitization when used at exaggerated doses and usage patterns. In rats the products were found to have an acute oral toxicity greater than 5 g/kg. Similar preparations containing pure sanguinarine presented similar toxicological profiles. Acute toxicity was absent in sanguinaria preparations as high as 25 g/kg in rats. Sanguinaria extract did not induce anaphylaxis when tested in guinea pigs. From these profiles presented it is concluded that the products with sanguinaria extract or pure sanguinarine present a more than acceptable safety profile in the use of the products for the purposes indicated.

SUBCHRONIC TOXICITY OF AN ALPHA 1-ADRENERGIC BLOCKER IN RATS AND DOGS. T. Terrell, D. Bidwell, J. Averre, M. Wilson, R. Hull and F. Andrew. Syntex Research, Institute of Toxicologic Sciences, Palo Alto, CA

A new proposed α1-adrenergic antagonist antihypertensive agent (9-[(2-[indol-3-yl]ethyl]-1-oxa-3-oxo-4,9-diazaspiro[5.5]undecane hydrochloride) was tested orally in rats and dogs. Rats were dosed with 0, 10, 30, 60 or 100 mg/kg/day, and dogs with 0, 3, 10 or 30 mg/kg/day for 4 months. Some animals in each group were allowed a 1 month recovery period. The drug was well tolerated in rats. High-dose dogs developed generalized alopecia. Cataracts were observed in mid and high-dose rats and in high-dose dogs. Pathologic lesions in high-dose rats were lenticular opacities, testicular atrophy, adrenal cortical hyperplasia, and hyperkeratosis of footpads. Changes in high-dose dogs included lenticular opacities, leukocytosis with a degenerative shift, anemia, decreased A/G ratio, decreased serum lipids and elevated liver enzymes in some animals. Dermatologic changes in treated dogs were associated with histopathological lesions of cutaneous candidiasis. After 1 month without dosing some recovery was seen for most changes. The toxicologic profile for this agent was different than that reported for other α1-adrenergic antagonists.


Recently sanguinarine has found use as an antiplaque agent in dentistry and may have use in the treatment of plaque-associated oral diseases such as dental caries, gingivitis and periodontal disease. However, the toxicology of purified sanguinarine has not been adequately studied or reported in the literature. The oral LD 50 of sanguinarine was 1.66 g/kg rats while the i.v. LD 50 was 25.7 mg/kg rats. Sanguinaria extract had a similar oral LD 50 of 1.44 g/kg. Acute ocular and oral mucous membrane irritancy of sanguinaria in various species were sufficient to classify sanguinaria as a mild irritant but not an oral mucosal irritant. Sanguinaria demonstrated an LC 50 of approximately 2.2 mg/L. In the Ames mutagenic assay, sanguinaria did not demonstrate mutagenic activity. In human subjects no dermal sensitization or irritation was observed. The toxicological profile presented by sanguinaria and sanguinaria extract suggest that the material is safe for oral care products. While sanguinaria is not volatile, it is suggested that reasonable precaution be used during processing to protect against excessive inhalation exposure.


Aldehydes are common environmental pollutants and irritants of the respiratory tract. Aldehyde dehydrogenase (ALDH) plays an important role in their detoxication. Biochemical studies using tissue homogenates have demonstrated that ALDH is present in different quantities in every organ. These studies, however, have not localized ALDH in individual cell types in a heterogeneous tissue. Thus, a cold GMA embedding procedure which preserves ALDH activity was developed for histochecmical localization of the enzyme. ALDH was identified in Fischer-344 rats by modifying the method of Gabler (Acta Histochem. 33:323-330,1969) to include 1.5 mM pyrazole and a 24 h incubation at 37°C. The appearance of formazan granules, indicative of ALDH, was inhibited by preincubation with 10 μM dehydroacetaldehyde or 100 μM chloral hydrate. ALDH activity was identified in numerous sites including A) the luminal aspect of the ciliated epithelium of the respiratory tract, not olfactory nasal epithelium, B) Clara and other epithelial cells of bronchioles, C) basal cells of the esophagus, D) external limiting plate of the liver, and E) proximal tubules of the kidney. These studies demonstrated the superior morphology provided by GMA embedding versus frozen section histochemistry and identified potentially important sites of enzymatic detoxication of acetaldehyde.

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FOURTEEN DAY INTRATHecal SAFETY EVALUATION STUDY OF SALMON CALCITONIN IN RATS. J.W. Dietrich, B.S. Brar, R. Chau, L.C.K. Wong, T.P. Prues, and E.S. Neiss; Department of Drug Safety Evaluation, Revlon Health Care Group, Tuckahoe, NY.

In order to allow clinical evaluation of the analgesic activity of salmon calcitonin (sCT) after subarachnoid administration, the peptide was administered daily to rats via a catheter which was surgically implanted through the cisterna magna into the lumbar region. The doses used were 0, 5, 15 & 45 µg/kg/day. Rats were weighed and observed daily for 14 days.

One female (mid-dose) became moribund after 13 days and was sacrificed. Clinical signs associated with sCT included increased sensitivity to touch and involuntary movement of the hindquarters. Treatment produced a 2-9% loss in body weight. Clinical chemistry changes included reductions in LDH & SGOT. Absolute heart and thymus weights were decreased, while relative kidney weight was increased, in high-dose females. Absolute liver, kidney and heart weights were decreased, and relative brain weight was increased, in males (mid- and high-dose groups). Histologic examination revealed no difference between control and treated rats.

We conclude that intrathecal administration of sCT has significant pharmacologic effects in rats.


Because of reported analgesic activity of salmon calcitonin (sCT) after subarachnoid administration, the peptide was administered daily to dogs via a catheter which was surgically implanted from the cisterna magna into the lumbar region. The doses utilized were 0, 5, 10 & 15 µg/kg/day. Animals were weighed & observed daily for 14 days.

1 female (mid-dose) died on the 2nd day of dosing. Clinical signs associated with sCT included dehydration, excess salivation, lethargy, reduced appetite, diarrhea, emesis & involuntary muscle movements. Body weight loss ranged between 5% & 34%. Possible drug-related clinical chemistry findings were reduced BUN, triglycerides, cholesterol, total protein, phosphorus & alkaline phosphatase. Absolute & relative spleen & thymus weights were reduced in males (all 3 doses) while in females there was a trend towards a decrease in thymus weight with treatment. Thymus involution was more severe in test animals.

We conclude that intrathecal administration of sCT has significant pharmacologic effects in dogs.
A SIMPLE BUT EFFICIENT STATISTICAL METHOD FOR TREND AND HETEROGENEITY 
ANALYSIS OF SUBJECTIVELY GRADED INCIDENCES 
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Sponsored by: Frederick E. Reno

In a toxicological study many of the important histopathological findings are recorded as subjectively graded responses on a qualitative scale such as none, minimal, slight, moderate, moderately severe, severe, etc. Examples of such grades include chronic nephropathy, myocardial degeneration, splenic pigmentation, etc. Because of subjectivity and overlapping of this type of gradation, ordinary X^2 tests for r x c tables of this sort often are not efficient to detect treatment related effect. The RIDIT (Relative to an Identified Distribution) method originally proposed by Bross for two samples and extended by Selvin for k samples as a test for heterogeneity among these categories, performs well under these conditions. In this work the classical method is extended to provide a test for linear trend. The mathematical algorithm is demonstrated by a toxicological example. A simple computer program is described to perform the analysis. The method naturally lends itself to incorporation of "historical" control data.

EARLY LUNG FREE CELL RESPONSE TO CADMIUM: DIFFERENTIAL 
LAVAGE CHARACTERISTICS OF ALVEOLAR MACROPHAGES AND 
POLYMORPHONUCLEATED LEUKOCYTES. 
B.E. Lehner, Y.E. Valdez, J.E. London, and D.M. Smith, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM. 
Sponsor: P.E. Morrow

Piscator-344 rats were intratracheally instilled with either 7 μg cadmium (Cd) in normal saline (NS) or with NS alone. A third group of uninstilled rats served as controls. One day after instillation the rats’ lungs were washed by three consecutive lavage series. Common to each series, the lungs were washed 6 times with 6 ml NS per wash. There was a two-fold increase in cell yields over the course of lavage with the Cd-instilled lungs compared to either the untreated or NS-instilled lungs; 98% of the lavaged cells from untreated or NS-instilled lungs were alveolar macrophages (AM). During the first six lavages of the Cd-instilled lungs, approximately 50% of the cells were polymorphonucleated leukocytes (PMN). The relative numbers of PMN in the two subsequent lavage series decreased to 36% and 32%, respectively. Histologically, PMN and AM in the alveoli were present in the Cd-instilled, but un Lavaged, lungs. The relative appearance of PMN decreased as the number of lung washings increased, with those more proximal to the airways being removed first. Overall, there was a 72% error in estimating PMN when data obtained from six lung washings were compared to those from 18 lung washings. The alveolar adherence characteristic of PMN may play an important role in their clearance from the lungs. (This work was performed under the auspices of the DOE.)

KINETICS OF INTRATRACHEALLY INSTILLED CdCl2 AND 
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Although metallothionein (MT) induction in the lung after Cd inhalation has been demonstrated, the fate of Cd-MT in the lung has not been studied. The purpose of our study was to examine both lung retention and distribution into other organs of CdMT1 and CdMT2 and compare this to CdCl2. Long Evans male rats consisting of 15 rats per group were instilled intratracheally with 40 μg Cd in 0.2 ml H2O as 105CdCl2, 105CdMT1 and 105CdMT2. Five rats per group were killed on day 1, 4 and 7 after the instillation and Cd content of the organs was measured. Rats instilled with CdCl2 showed distinct clinical symptoms of general and lung toxicity whereas rats instilled with the same amount of Cd as CdMT1 and CdMT2 did not. Although total body retention of Cd in rats 24 hrs after the instillation was similar in all groups, estimated to be 80% of Cd instilled, a distinct difference in body distribution between CdCl2 and CdMT groups was observed. Lung retention on day 70 was 6% and 4% for the MT’s and 10% for CdCl2. Liver was the major site of Cd deposition after CdCl2 instillation whereas kidney was the primary organ of Cd accumulation in case of CdMT instillation. This difference in organ distribution between the free cation and Cd bound to MT is similar to the results of studies with orally or iv administered CdCl2 and CdMT.

LUNG TOXICITY OF DIFFERENT Cd COMPOUNDS. G. 
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We compared the inflammatory response in the lung of different Cd compounds including Cd-thionein (CdMT). Lung epithelial permeability was determined using 99mTc-DTPA, and biochemical parameters in lung lavage were measured. Male Long Evans rats were intratracheally instilled with 10, 30 and 100 μg of Cd as CdCl2, CdO, CdO fired and unfired, CdMT1 and CdMT2, suspended or dissolved in 0.2 ml of saline. Control groups received TiO2 or 0.2 ml of saline. A dose dependent influx of inflammatory cells into the lung was observed for CdCl2, CdO and CdMT, and a toxicity ranking based on this and on cell viability is as follows: CdMT1 > CdMT2 > CdCl2 > CdO > CdO. However, lung epithelial permeability which was highly increased after CdCl2 and CdO, was not altered after CdMT and CdO. LDH and MDH in lung lavage fluid were significantly increased after CdO and CdCl2, whereas after CdMT and CdO only a very small increase was seen. Protein and stalic acid in lung lavage confirmed the high permeability changes after CdO and CdCl2, which did not occur after CdO and CdMT. This demonstrates that inflammatory cells do not necessarily produce an increase in epithelial permeability. The role of MT in lung toxicity of Cd needs further investigation. Cd particles showed little toxicity except for a decreased cell viability at the highest dose administered.
713 ANALYSIS OF THE LUNG'S FREE CELL RESPONSE TO A TOXIC INSULT BY MULTIPARAMETER FLOW CYTOMETRY. R.L. Lehnert, Y.E. Valdez, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM. Sponsor: F. E. Morrow

Mice were intratracheally instilled with 7 µg of cadmium (Cd) in normal saline, or normal saline alone, and their lungs were lavaged 24 hrs later. Harvested free cells (PC) were counted and the phenotypes of the PC were characterized by flow cytometry. Parameters evaluated included: electronic volume (EV), area light loss (ALL), light scatter, 2nd light scatter, and blue green-yellow auto-fluorescence. Based on their EV and ALL characteristics, two minor and one major subpopulation of alveolar macrophages (AM) were found with cells lavaged from untreated animals. The major population (PC-MH) could be further subdivided into PC-MHI and PC-MHII subpopulations by their unique ALL feature. These subpopulations clearly differed morphologically and light scatter, the PC-MHI and II phenotypes were each found to be composed of a minimum of 4 subpopulations. Following Cd insult, polymorphonuclear leukocytes (PMN) were readily distinguished from AM by their EV, ALL, and auto-fluorescence characteristics; PMN were sorted in ~95% purity. Cd-induced changes in the AM were denoted by alterations in the frequency of retrieval of some of the AM phenotypes. This study demonstrates the utility of flow cytometry for evaluating the lung's free cell response to a toxic insult, and the usefulness of this technology for selectively harvesting inflammatory cells for subsequent in vitro study. This work was performed under the auspices of the DOE and was supported, in part, by National Flow Cytometry Resource Grant No. RR01-01395-02.


Pulmonary alveolar macrophages (PAM) are the first line of defense against inhaled foreign material, including pathogenic bacteria. The purpose of this study was to assess the effect of the inhalation of brass particulates on several morphological and functional parameters of rat PAM. Changes in PAM function may be reflected in altered resistance to bacterial infection. Lung leukocytes were collected by bronchopulmonary lavage from control rats and from rats exposed to brass particles by inhalation. More than 90% of cells from normal lungs were PAM, but for the first 3d after exposure 80-95% of the cells were neutrophils associated with inflammation. Normal PAM were about 1.5% binucleated and rarely multinucleated; 3d post-exposure ~13% were binucleated and ~3% were multinucleated. This pattern of increased nucleation persisted for 2 wks after exposure. Increased PAM phagocytic activity was seen at 1d and remained elevated for about 3d. PAM chemotaxis was initially inhibited, but was considerably enhanced 3-6d post-exposure. The chemotactic alterations were interpreted as manifestations of lymphokine release. Changes in nucleation and phagocytic indices probably relate to particulate-induced macrophage activation.


Occupational exposure to numerous inorganic particles has been associated with interstitial lung disease. Pulmonary macrophages have been proposed as mediators of lung fibrogenesis. Recently we demonstrated that inhaled chrysotile asbestos fibers activate complement-derived chemotactic factors for macrophages at sites of deposition. These macrophages form a component of a lesion at alveolar duct bifurcations within 48 hrs. after exposure. In addition, we have shown that a variety of inorganic dusts activate complement in rat serum and lavage. Using pulmonary macrophage chemotaxis as a bioassay for complement activation, we have demonstrated that chrysotile and crocidolite asbestos fibers (p<0.05), fiberglass (p<0.05) and iron coated (Fe) chrysotile fibers (p<0.05) activate complement in comparison to Mt. St. Helens ash particles (NS). We have postulated that particles which activate serum complement in vitro and are deposited initially at alveolar duct bifurcations when inhaled will stimulate pulmonary macrophage migration to sites of particulate deposition. Our results suggest that a correlation exists between complement activation in vitro and macrophage accumulation at sites of particle deposition in vivo.


To investigate pulmonary retention and clearance of the particles, rats inhaled diesel exhaust diluted to 250 µg diesel particulate (DP)/m³ for 6 months and were serially sacrificed during the following 17 months. The lungs were examined by light and electron microscopy (EM). On conclusion of the exposure, most DP was in alveolar macrophages (AM) scattered among the alveoli with some diesel-filled AM clumped in the alveoli. After exposure, diesel-filled AM found in the alveoli were replaced by diesel-free AM. AM obtained by bronchopulmonary lavage had half-times of 6 weeks for the clearance of "diesel-laden" AM and of 17 weeks for the appearance of "diesel-free" AM. During 77 weeks postexposure, the pulmonary particulate burden declined from 0.66 to 0.25 mg. The remaining DP was observed in clusters of AM in alveoli at the pleural surface, at the terminal bronchiolar area and against the alveolar walls of the bronchi. The aggregates formed by interdigitation of AM filopodia. Polymorphonuclear leukocytes and lymphocytes were observed on the clustered AM suggesting that lymphokines influence the AM aggregation. The results indicate three clearance pathways for particle-laden AM: 1) mucoiliary clearance out of the lungs, 2) clearance to the regional lymph nodes and 3) clearance to dust macrophages, i.e. the interstitium and the bronchiolar wall. Consequently, pulmonary defenses are replenished with particle-free AM while particle-laden cells are sequestered in clusters.
PULMONARY CLEARANCE OF INTRATRAECHALEALLY INSTILLED NiS₂ AND TiO₂ IN MICE. G.L. Finch, G.L. Fisher*, and T.I. Haye$. Donner Laboratory, Lawrence Berkeley Laboratory, Berkeley, CA; *Toxicology Section, Battelle Laboratory, Columbus, OH.

To examine the short-term effects and clearance of toxic versus relatively nontoxic particles, male BALB/c mice were exposed by intratracheal instillation of low doses (0.5 mg/kg) of respirably sized NiS₂ and TiO₂ particles, then sacrificed at 15 minutes; 5, 20, 72, and 168 hours post-exposure. Compared with TiO₂ and saline-instilled controls, instilled NiS₂ exerted toxic effects including pulmonary hemorrhaging, increased numbers of polymorphonuclear leucocytes recovered by bronchopulmonary lavage, and decreased body weights at later timepoints. The lung Ti burden (by ICP spectroscopy) and tissue Ni levels (by liquid scintillation counting) were determined and clearance was modelled using biexponential regression analyses. Both Ni and Ti were rapidly cleared to the gastrointestinal tract (25 and 34% of dose) within 15 minutes; this rapid phase may in part be an artifact of the exposure route. Significantly less pulmonary Ni was present at 3 and 7 days post-exposure compared with Ti, with a factor of three difference in clearance halflives. Longer term Ni clearance rate constants were similar for lung, kidney, and blood, and were consistent with the hypothesis that Ni was solubilized in the lung and then transported through the blood. Supported by Battelle Laboratory, EPRI, and the U.S.DOE.

ASSÉSSMENT OF EARLY ALVEOLAR CLEARANCE AND MACROPHAGE FUNCTION FOLLOWING ACUTE INHALATION OF SULFURIC ACID MIST. B.D. Naumann and R.B. Schlegl-inger, Institute of Environmental Medicine, New York University Medical Center, NY, NY.

Rabbits were exposed to submicrometer sulfuric acid mist at 1 mg/m³ for 1 h to assess effects on alveolar clearance of a polystyrene latex (PSL) tracer aerosol. To examine underlying mechanism(s), bronchopulmonary lavage was performed at selected times after exposure for functional characterization of macrophages (AM) and neutrophils (PMN). In vivo PSL clearance was accelerated in acid exposed animals relative to sham control during the 14th observation period. Acid exposure produced no change in the viability or numbers of AM recovered. Although an increase in the number of PMN was observed by 1h in both acid and sham groups, compared to nonexposed controls, levels were normal by 12h in shams but continued elevated in the acid group through 24h. Reduced in vitro AM attachment and adherence were also observed after acid exposure. In vivo uptake of PSL by AM was enhanced during the first 5h after acid exposure and in vitro phagocytosis by PMN increased through 48h post exposure. These results indicate that functional alterations in free cells obtained after in vivo exposure to H₂SO₄ are consistent with and can be related to observed changes in clearance of tracer particles, and relatively low level H₂SO₄ exposure produces a mild inflammatory response, which may have implications in the pathogenesis of chronic lung disease.

TRANSLATION OF PARTICLES FROM THE LUNGS TO THE PLEURAL SPACE AND MEDIASTINAL LYMPH NODES. S. Hyler, Y.E. Valdez, B.E. Lehment, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM. Sponsor: P.F. Morrow

Groups of Sprague-Dawley rats were intratracheally instilled with 4 x 10⁵ fluorescent, polystyrene microspheres (1.9 μm diam.) in normal saline, or normal saline alone. On days 1, 14, and 30 after instillation, free cells were lavaged from the lungs and pleural space, and cells were harvested from the mediastinal lymph nodes. Day 1 after particle deposition, the numbers of lung free cells increased ~3-fold, with the increase being due primarily to an influx of polymorphonuclear leukocytes. Lung free cell numbers remained elevated on days 14 and 30 after instillation, with alveolar macrophages (AM) representing essentially all of the retrieved cells. On day 1, ~1 x 10⁶ particles were found in the lymph nodes and ~35% of these were associated with macrophages. The frequency distribution of particles in these macrophages did not parallel the distributions of particles in the AM. Similar results were obtained on days 14 and 30 post-instillation. After 24 hrs after particle deposition, ~1.5 x 10⁶ microspheres were found in the pleural space and ~60% of these were cell associated. Particles were also in the day 14 and 30 samples. This study shows particle translocation from the alveolar compartment to the mediastinal lymph nodes occurs within 24 hrs, and suggests these macrophages with particles in the lymph nodes were not migrating AM. Moreover, this study demonstrates that particles deposited in the lung can reach the pleural space within 24 hrs, and suggests such particle translocation may be an important alveolar clearance pathway. This work was performed under the auspices of the DOE and was supported, in part, by National Flow Cytometry Resource Grant No. BRAG-0315-02.

TWO-YEAR INHALATION TOXICITY STUDY OF PETROLEUM COKE (DELAYED PROCESS) IN RATS AND MONKEYS. D. Klonne, J. Burns, C. Halder, C. Holdsworth, and C. Ulrich, BRR, Export, PA, Hazleton, Vienna, VA, Standard Oil (TN), Chicago, IL, API, Washington, DC, IRGC, Mattawan, MI.

The purpose of this study was to investigate the chronic effects of petroleum coke dust inhalation in rats and monkeys. Sprague-Dawley rats (150/sex/group) and cynomolgus monkeys (4/sex/group) were exposed to concentrations of 0, 10.2 or 30.7 mg/m³, 6 hr/day, 5 d/wk for two years. Body weight, physical appearance, ophthalmologic, hematologic, serologic, and histopathologic changes were monitored on both species at various intervals during the study (histology on monkeys only at termination). Significant exposure-related effects were limited to the following parameters: increased lung weights, microscopic observations (deposition and phagocytosis of dust in the lung) for both sexes, species and exposure groups. Microscopic pulmonary alterations in rats also included chronic inflammation, sclerotic and metaplastic changes, and the presence of keratin cysts. These microscopic alterations were more frequent in the 30.7 mg/m³ group than in the 10.2 mg/m³ group. Thus, inhalation of petroleum coke dust in this study produced neither oncogenicity in rats nor significant toxicity in monkeys.
THIRTY DAY REPEATED DOSE TOXICITY OF REV 2871 INHALED BY RATS AND MICE. L.C.K. Wong, Litton Bionetics, Inc., Rockville, MD, and Revlon Health Care Group, Tuckahoe, NY.

4 groups each of rats & mice were exposed nose-only for 30 days to aerosolized REV 2871. Mean estimated exposure doses were 0, 10, 33 & 78 mg/kg/day for rats & 0, 8, 26 & 65 mg/kg/day for mice. 6 rats died during the course of these exposures, 4 from the high dose group while 14 mid & high dose mice died during the first 2 weeks before reducing exposure duration. There were significant dose related, body weight depressions in both species that were more persistent in males than in females, more severe in mice than in rats. Clearly dose-related effects including rough hair coat, respiratory abnormalities, tremors and decreased activity in both species disappeared within 2 hours of exposure. There was a dose-related decrease in triglycerides in rats. There were more clinical chemical changes in male than in female mice, but these changes were minor and inconsistent. There were no histopathologic changes in treated rats that could directly or indirectly be attributed to the test compound, while the dose-related atrophy of the thymus and spleen & reduction in mature lymphocytes in mice could have been due to stress. The nose-only exposure procedure appears to be stressful to both the rat & mouse species. The no significant effect level of REV 2871 following 30 day repeated nose-only inhalation exposure is greater than 10 mg/kg/day in male & 33 mg/kg/day in female rats & between 8 & 28 mg/kg/day in mice.

CHANGES IN THE CHEMICAL COMPOSITION OF THE RAT LUNG FOLLOWING SUBCHRONIC INHALATION EXPOSURE TO COBALT AND TUNGSTEN OXIDE. R.S. Kutner, B.N. Shiotsuka, and B.T. Drew. Medical Department, Brookhaven National Laboratory, Upton, NY.

Male Fischer-344 rats were exposed to cobalt (Co), tungsten carbide (WC), or both Co and WC to examine the fibrogenic potential of these dusts. Separate groups of rats were exposed to either filtered air, 1,0 mg Co/m³, 15 mg WC/m³, or 1.0 mg Co/m³ plus 15 mg WC/m³ for 6 hrs/day, 5 days/week for 62 exposures. Lungs of animals exposed to only Co weighed significantly (p < 0.05) more (1.48 g) than those of the control group (1.32 g). Relative to control lungs, those of the Co and WC-Co groups contained 11.1 and 9.9%, respectively, more elastin, and the Co group contained 14.8% more collagen. However, these tissue components were expressed in terms of dry lung weight the significant changes were negated. All of the lungs from exposed animals had increased numbers of macrophages. These cells had foamy cytoplasm in the animals exposed to only Co while pulmonary macrophages from the WC-Co and WC groups were pigmented. Alveolar wall thickening was more prominent among the WC-Co and WC animals than in those exposed only to Co. Type II cell hyperplasia was observed only in the WC-Co and WC groups. Supported by U.S.D.O.E. No. DE-AC02-76CH00016 and the National Toxicology Program under Inter-agency Agreement Number 222-T01-ES-9-0043.


Four groups of CD-1 mice and 5 groups of rats were exposed nose only to aerosolized REV 2871. Estimated exposure doses were 0, 49, 117 and 216 mg/kg in mice, and 0, 95, 149 or 170 mg/kg in single exposures, and 41 mg/kg/day for 5 days in rats. Mortality during exposure was dose related in male but not in female mice, while in rats, mortality occurred only at the two highest doses. The occurrence of tremors was dose, sex and species dependent, being most frequent in the male mice. The LD₅₀ was greater than 0.30 mg/L and 49 mg/kg appeared to be a no-effect exposure dose in mice. The LD₅₀ in rats was greater than 0.53 mg/L, and there were no cumulative effects from 5 days at 41 mg/kg/day. Beagle dogs of both sexes were exposed nose-only to REV 2871 for up to 4 hours a day for 10 days at estimated mean exposure doses of 0, 2.2, 6 and 12 mg/kg/day. One control dog died during the first day of exposure. Two male high dose group dogs died during the seventh exposure. Dose related observations during exposure included trembling and respiratory abnormalities. No effect was seen in body weight, hematology, serum chemistry, organ weight or weight ratios, gross necropsy or histopathology. The nose-only test procedure was found to be stressful to the dogs in the present study. There was no evidence of any persistent effect at any tested dose level.

PLACENTAL AND MILK TRANSFER OF PHOSFOLAN AND MEPHOSFOLAN IN MOUSE. Nabilah M. Basyry, A.K. Salama and H.A. Aly. Plant Protection Department, Faculty of Agriculture, University of Alexandria, Egypt.

A single oral dose of 8 mg/kg of technical phospholane (55% (0,0-diethyl-1,3-dithiolan-2-yldene phosphoramide) of Technical mephosphan (85%,3%) (0,0-diethyl-4-methyl)-1,3-dithiolan-2-yldene phosphoramide) was administered on day 17 of gestation to female mouse. Animals were killed at various times of gestation and the total recovered amount of phospholane or mephosphan from the fetuses was determined using FPD-DLC. The total recovered amount of phospholane from the fetuses reached high values at the two time intervals 10-min and 6-hr, while in the case of mephosphan the high values were reached at 0.5 and 6-hr. The values of phosphan were 25.07 and 27 µg which did account for 9.35 and 5.38% of the applied dose, while those of mephosphan were 3.03 and 5.21 µg which did account for 1.08 and 2.29% of the applied dose.

Milk transfer of both compounds was studied by giving a single oral dose of 2 mg/kg to nursing female mouse, directly after delivery. The total recovered amount of phospholane and mephosphan from suckling reached high value at the two time intervals 11 and 72-hr. 12 and 72-hr, respectively. The values were similar at the two time intervals in the case of phosphan (4.57 µg) which did account for 7.61% of applied dose and 5.97% of the applied dose. The values of mephosphan were 3.06 and 2.84 µg which did account for 5.12 and 7.10% of the applied dose.
LACK OF DOMINANT LEthal EFFECTS OF 0.0-DIMETHYLPHOSPHOROCHLORIDETHIOATE IN MALE RATS, J.M. Kronenberg, T.M. Fuhrmann, F.R. Johannsen, C.J. Levinakas, Monsanto Company, St. Louis, MO, and J.L. Schardein, International Research and Development Corporation, Mattawan, MI.

0.0-Dimethylphosphorochloridethioate (DMPTC), a chemical intermediate used primarily in the production of organophosphate pesticides, was reported to cause dominant lethal mutations in male rats. In that unpublished study (cited in draft Chemical Hazard Information Profile, U.S. EPA, 1982), males were gavaged for 5 days at dose levels up to 75 mg/kg/day and then mated for 7 weeks to untreated virgin females. In an attempt to confirm the reported effect, another dominant lethal study was conducted. The approach described by Green (Toxicol. Appl. Pharmacol. 39:549–552, 1977) was chosen, however, because of its reported advantages over the previously used protocol. Male rats were gavaged daily for 10 weeks and then mated to untreated virgin females for 2 weeks. Mid- and high-dose rats exhibited decreased body weight gains, excessive salivation and/or hair loss. No treatment-related effects were noted in mean testicular weights, sperm motility, sperm counts, male and female fertility indices, total implantations, implantation losses, viable embryos or number of dams with early resorptions. Thus, in contrast to the earlier studies, no male dominant lethal or fertility effects were noted, even at dose levels of DMPTC which induced obvious signs of toxicity.

TESTICULAR SORBITOL DEHYDROGENASE ACTIVITY INCREASES AFTER EXPOSURE TO DIFFERENT TOXICANTS. R.E. Chapin, R.R. Swaisgood, and J.C. Lamb, IV, National Toxicology Program, NIEHS, Research Triangle Park, NC 27709.

Previous studies with 2,5-hexanedione (Toxicol. Appl. Pharmacol. 62:262) found a slight but consistent elevation in testicular sorbitol dehydrogenase (SDH) activity prior to detectable cell loss, while hepatic SDH activity was unchanged. Further experiments were done to determine if SDH, localized to spermatocytes and spermatids, responds similarly after other toxicants. After 100 mg/kg acrylamide sc, testicular SDH activity rose significantly, from 0.0394 μmol per min/mg protein (control) to 0.0392 μmol/min/mg at 2 hrs, and returned to control levels at 4 and 6 hrs. Hepatic SDH was elevated only at 4 hrs, from 0.2188 to 0.2408 μmol/min/mg, when testicular activity was not different from control. Dose dependent increases in testicular SDH were also seen 2 hr after a single dose of ethylene glycol monomethyl ether (200 mg/kg po, up 12%), triethanol-cesyl phosphate (1000 mg/kg ip, up 10%) and dipentyl phosphate (2 g/kg po, up 17%). In each case, hepatic SDH was unchanged, and no changes in testicular histology were detected in perfusion-fixed, GNA-embedded tissue. Testicular aldose reductase was positively correlated with SDH in most treatment groups. These early changes may represent a common response to toxicant exposure.

TAMOXIFEN EFFECTS ON RAT UTERINE LUMINAL EPITHELIUM. W.S. Branham, D.R. Zehr, C.J. Nelson, and D.K. Sheehan, Div. of Teratogenesis Research and Div. of Biometry, National Center for Toxicological Research, Jefferson, AR and Dept. of Biology, University of Central Arkansas, Conway, AR.

Tamoxifen is a developmental toxicant in the rat and inhibits uterine gland genesis. Uterine glands appear in untreated rats on postnatal (PN) days 9-15, during a period of declining luminal epithelium (LE) hypertrophy. Tamoxifen, given on PN days 1-5 (sc in sesame oil; 10 μg/rat), increases LE hypertrophy between PN days 5 and 20 as compared to controls and inhibits uterine gland genesis. Cellular degeneration (vacuolated and/or granulated cytoplasm with various degrees of karyorrhexis) corresponds to the period of LE hypertrophy. Tamoxifen given on PN days 10-14 induces immediate LE hypertrophy and uterine weight gain, which is maintained to day 19, and inhibits gland genesis. By contrast, 17β-estradiol and diethyilstilbestrol maintain LE hypertrophy for shorter periods and allow gland genesis to proceed. Removal of endogenous estrogen by ovarietomy or ovarioectomy plus adrenalectomy decreases uterine weight but does not affect LE hypertrophy or gland genesis. We conclude that endogenous estrogen is not required for induction of uterine LE hypertrophy or gland genesis in the untreated rat. Additionally, tamoxifen insult is revealed by LE morphological changes and inhibition of uterine gland genesis.


A number of procedures are available for assessing the effect of toxic agents on reproductive function. However, few are designed to test directly their effect on the pituitary (pit.). We measured several indices of pit function including pit weight (wt), LH, FSH & PRL concentration (conc.). LH release was examined using a perifusion system. Pits from male Long-Evans rats were removed rapidly after decapitation & put in a 500 μl chamber through which a medium 199 buffer containing 10 mm Hepes & 0.3% BSA was passed (200 μl/min) for 360 min, at 37°C the effluent collected at 9 min intervals. After a 120 min basal period, the pits were challenged with a similar medium containing 100 ng/ml gonadotropin releasing hormone for 240 min. Diethyliestibestrol (DES) & estradiol benzoate (EB) treatment for 14 or 21 days caused a time- & dose-dependent increase in pit wt & a dose-dependent increase in pit LH & FSH conc & a rise in pit PRL conc. Methoxychlor caused a decrease in pit wt with no effect on pit LH conc. Within the perifusion system, basal & stimulated LH release showed a dose-dependent decline after DES- & EB-treatment. These studies show that changes in pit wt, LH, FSH & PRL conc & release are sensitive indicators of pit function & important measures of the reproductive system following exposure to various compounds.
DELAYED DEVELOPMENT OF REPRODUCTIVE FUNCTIONS AND ALTERATION OF Dopamine RECEPTOR BINDING IN HYPTHALAMUS OF RATS EXPOSED PRENATALLY TO PHENOTYINOX AND PHENOBARBITAL.  S. Takagi, P.K. Seth, F.R. Alleva, and T. Balas, Daiichi Seiyaku Co., Ltd, Tokyo and Food & Drug Administration, Washington, DC.

A recent epidemiologic study in a European country (unpublished) implicated maternal phentyoind (P) and/or phenobarbital (PB) treatments in the various reproductive abnormalities of their daughters. While previous work in experimental animals treated with PB revealed effects of this nature, little has been reported on the effect of P or a combination of both drugs (CB). Seventy-five pregnant Sprague–Dawley rats were administered either P (Na salt) at 20, 40 or 80 mg/kg po, PB (Na salt) at 20, 40 or 80 mg/kg sc or CB at the same doses daily from 5 to 20 days of pregnancy (4-12 rats/dose level). Twenty-four control rats received 12 methocel po and distilled water sc. Vaginal opening (puberty) was delayed only in the CB group. Regularity of estrous cycles was decreased in all treated groups with the P group being most affected. The binding of 3H-spiropenidol, known to label dopamine receptors, was decreased in the hypothalamus of all drug-treated groups, indicating that the alteration in neurotranscense function may be responsible for the above effects. Data suggest that prenatal exposure of rats to anticonvulsant drugs could lead to reproductive disorders.

PRENATAL EXPOSURE TO NITROFEN CAUSES ANOMALOUS DEVELOPMENT OF PARA- AND MESONEPHRIC DUCT DERIVATIVES IN THE HAMSTER.  L.E. Gray, Jr., J. Ferrell and J. Ostby. USEPA, NEIL, DBD, RTF, NC Sponsor:  M. Chernoff

The herbicide nitrofen is extremely teratogenic but relatively nontoxic to adults, having an LD50 greater than 1 g/kg. Studies using rats and mice exposed to low doses of nitrofen during gestation have shown that most of the pups are abnormal or die after birth even though few abnormalities are seen when treated fetuses are examined. The present study was designed to determine the effects of prenatal nitrofen administration on the postnatal development of the golden hamster. Dams were exposed to nitrofen at 400 mg/kg/d on days 8 and 9, 11 and 12 or 14 and 15 of gestation. Postnatal growth, viability, and reproductive development were measured. Early gestational treatment caused abnormal development of the para- and mesonephric ducts and the uretic bud as indicated by uterus unicorns, occasionally accompanied with ipsilateral renal agenesis in the female and unilateral agenesis of the vas and/or epididymis and seminal vesicle in males. Males treated later in gestation developed spermatogenic granulomas in the epididymides. The great majority of the agenesis was on the left side (50%). Sex hormone levels were also reduced in the treated males, teratogenic effects that are not apparent until adulthood.


This study was designed to evaluate the ability of diuretic agents to probe the function of diuretic segments in the neonatal rat. Acetazolamide (Z), furosemide (F), chlorothiazide (C) and amiloride (A) are diuretic agents which act specifically in the proximal tubule, loop of Henle, early and late distal tubule, respectively. Six day old rats were treated sc with the proximal toxin mercuric chloride (HC), 3.16 mg/kg, or the distal toxin Amphotericin B (AB), 20 mg/kg. One day later the glomerular filtration rate and the fractional excretion (FE) of water and various components of the filtrate were determined during a 2 hr clearance period immediately following injection of the diuretic. Both HG and AB treatments produced expected clinical symptoms of renal damage. The diuretic response to Z was markedly attenuated in the HG treated pups (as indicated by a decreased FE of water, total osmotic solutes and potassium) whereas the diuretic responses to F, C and A were unaffected, consistent with the proximal sites of action of Z and HG. In the AB treated pups, the responses to F, C and A, but not Z, were altered. These results indicate that there is an interaction in response to the diuretics when the location of pharmacologic and nephrotoxic action overlap.


Groups of 22 pregnant CD-1 mice and F-344 rats were exposed to isophorone vapor at 0, 25, 50 or 115 ppm on D 6 through 15 of gestation (G) for 6 hrs/d, to assess its teratogenic potential. On D 0, 3, 6, 9, 12, 15, 18 and 20, rats were weighed and examined externally; mice on D 0, 3, 6, 9, 12, 15 and 18G. Rats were sacrificed and examined by gross necropsy on 20G; mice on 18G. Live and dead fetuses were weighed, sexed, examined for gross abnormalities and crown rump distance. The heads of one-half of the fetuses from each litter were examined by the Wilson technique and the viscera by the Staples technique. The remaining fetuses were examined for skeletal malformations and ossification variations. Rat dams exposed to 115 ppm had reduced food consumption and dose-related neonatal increases in alopecia and cervical or anogenital staining. Further, the corrected 18G (mice) and 12G and 15G (rats) body wt's of the high dose dams were significantly lower than controls. Uterine implantation and fetal evaluations showed no significant differences between treated and control groups for any of the parameters evaluated. Isophorone did not cause teratogenic effects under the conditions of this study. The no-effect level was 50 ppm for maternal toxicity.
THE DEVELOPMENT OF ESTRUS CYCLE DEPENDANT RUNNING WHEEL ACTIVITY (RWA) IN THE FEMALE SD, LE AND F344 RAT. L.E. Gray, Jr., J. Gatty, and J. Ferrell. USEPA, HERL, DBR, Reproductive Toxicology Branch, RTP, NC Sponsors: N. Chernoff

The ability of toxic chemicals to alter the reproductive integrity of the female is usually limited to the observation of fertility rates and litter sizes over 2 or 3 generations. An evaluation of additional parameters is needed because fertility is an insensitive measure; even acyclic, constant estrus female rats and mice will breed because they become induced rather than spontaneous ovulators. The present study is a description of the ontogeny of RWA in the SD, LE and F344 female rat. In a second experiment SD rats were dosed sc with 50 μg of Estrogen (E), Testosterone (T), 1 mg of Chlordione (C) or the vehicle on d4 and RWA, vaginal opening (VO), age at first estrus and fertility were assessed. In the first experiment LE and SD rats had RWA peaks of 4 or 5 days and continued to cycle until ovariectomy (OVX). Only 2 of 10 F344 rats displayed RWA cycles. Hyperphysiological doses of E induced RWA in OVX SD and LE but not F344 females. In the second experiment neonatal treatment affected reproductive development, RWA and fertility. The age of the first RWA peak was delayed 12d by all treatments. C and E accelerated VO and E drastically lowered RWA and fertility in young females. Fertility and RWA peaks were reduced by all treatments in older females.


Male germ cells (MGC) are an important target in the study of genotoxicity. The response of MGC to a known genotoxin, bleomycin (BLM), was evaluated using the method of alkaline elution (AE) to measure single-strand DNA damage (SSD). Spermatogenic cells (>90%) were isolated from testes of propubertal, Sprague-Dawley rats using a two-step enzymatic digestion. Dose-response relationships were demonstrated following exposure of cells to varying concentrations of BLM for 1 hr at 0°C and 37°C. Compared to 0°C, SSD at 37°C was either reduced or abolished following exposure of cells to 0.5 or 0.05 μg/mL BLM, respectively. To evaluate the role of iron in BLM-induced genotoxicity, MGC were pretreated with deferoxamine mesylate (DM), a highly selective iron chelator. Preincubation of cells with 10 μM DM at 37°C for 1 hr abolished the SSD produced at varying concentrations of BLM. Cell viability was >90% in all experiments, as determined by trypan blue exclusion. Supported by a grant from the Pfeiffer Research Foundation.

Parenteral cadmium (Cd) administration can cause a high incidence of interstitial cell tumors. However, the interactions of Cd with interstitial cells (ICs) of the testes have not been well defined. Thus, this study was designed to assess the uptake of Cd into this potential target cell of Cd carcinogenesis. ICs, prepared by collagenase dispersion of rat testes and separated from seminiferous tubules by gravity sedimentation, were incubated at 33°C with CdCl₂ (1.0 to 100 μM) for 0.5 to 60 min. After incubation, cellular Cd was separated from Cd in the media by centrifugation through a silicone oil layer. Results showed 3 distinct phases of Cd influx into ICs: a primary rapid velocity phase (V₁; 0 to 1.5 min), a second intermediate velocity phase (V₂; 3 to 12 min), and a third low velocity phase (V₃; 15 to 50 min). V₃ appeared to have both influx and efflux components as efflux experiments indicated a 22% loss of Cd from 15 to 60 min. V₁ was found to be nonsaturable and was not decreased by addition of potassium cyanide (KCN, 1.0 mM), N-ethylmaleimide (NEM, 1.0 mM), or zinc acetate (Zn, 100 μM). However, V₂ was found to be saturable between 50 and 100 μM Cd and was markedly decreased by the inclusion of KCN, NEM or Zn. These data suggest that Cd is taken up into ICs, in part, by a transport system that may normally function in the uptake and may constitute a carrier-mediated or active transport.


Aqueous solutions of chlorine (0,1,0,2,0, and 5.0 mg/kg) were administered to male and female Long-Evans rats for approximately 66 days. Males were dosed for 56 days prior to breeding and throughout the 10-day breeding period. Females received chlorine for 14 days prior to breeding, and throughout breeding, gestation, and lactation. Selected pups were dosed following weaning until day 40 or the day of vaginal opening. No clinical signs of toxicity or body weight depression were observed. Fertility, fecundity, and litter weight were unaffected. The day of parturition was not influenced by chlorine exposure. No alterations in estrous cyclicity or day of vaginal opening were observed among F₁ females. F₂ males showed no adverse effects of chlorine exposure when sperm count, sperm morphology, motility, or velocity were evaluated. No histopathologic lesions of the reproductive tract were observed in males or females. (Supported by EPA Project No. CB810862-01. This abstract does not represent the policy or opinion of the USEPA).


Vehicle control subjects dosed with DW or CO in a series of teratology studies during 4 consecutive years were retrospectively compared. CD rats (164 DW; 149 CO) and CD-1 mice (130 DW; 87 CO) were dosed by gavage on gestational days (gd) 6–15. At sacrifice (gd 20, rats; gd 17 mice), each live fetus was weighed and examined for external, visceral, and skeletal malformations. Maternal body wt. on gd 0 did not differ across vehicles for either species. In CO-treated rats, but not mice, maternal body wt. at sacrifice, maternal gestational wt. gain and avg. fetal body wt./litter were significantly reduced in comparison to DW-treated subjects. The % fetuses malformed/litter and avg. number of defects (malformations and variations)/fetus/litter were significantly higher in CO- than in DW-treated litters for both species. A consistent pattern of increased malformation incidence in CO-treated litters was observed across the 4 year period in both species. These results suggest the importance of vehicle type as a relevant experimental variable in historical summaries of fetal morphological development. [Supported by NIEHS Contract No. 1-ES-6-2127; NCTR/NTP Contracts 222-80-2031(C) and 222-83-2010(C)].

740 PRENATAL INDUCTION OF SUPERNUMERARY RIBS IN THE RODENT: ROLE OF MATERNAL STRESS. N. Chernoff, P. Parsons, R.J. Kavlock, EPA, DDB, RTP, NC.

Litters of mice exhibit significant increases of supernumerary ribs (SNR) after the dams are exposed to chemicals at maternally toxic dose levels on day 8 of gestation. The present study describes the effect of non-specific stress on the induction of SNR. The embryonic period of greatest susceptibility to SNR formation was determined by treatment with sodium salicylate (a known SNR inducer). CD-1 mice and CD rats were given a dose of 1500 and 300 mg/kg respectively on a single day during 7-11 of gestation. In the mouse, day 9 was found to encompass the critical period (71% SNR < 29% on other days). Day 10 was the most sensitive in the rat (32% SNR vs 10% on other days). In the final experiments gravid females were stressed by restraint in a supine position for 12-hour periods during the predetermined sensitive days. Two concurrent control groups were used, one food and water deprived for a 12 hour period, and the other untreated. An increase in SNR was noted in mice (29% stressed; 15% food and water deprived; 9% untreated), but not in rats. Mice stressed during the 9am-9pm period (as compared to those stressed from 9pm-9am) showed the greatest increase in SNR (41%) and also exhibited significant incidences of fused ribs and exencephaly. These studies indicate that non-specific maternal stress can induce significant SNR and other terata in the CD-1 mouse.
A variety of xenobiotics enter female reproductive tract fluids and thus are potentially toxic to sperm residing in the tract prior to fertilization. We have initiated studies to examine this potential avenue of insult using ethanol as the model compound. Ethanol has been shown to have direct adverse effects on mature sperm cells. To begin these studies, the disposition of ethanol in uterine fluids was characterized. Fasted, female Long-Evans hooded rats were mated with vasectomized males. Five minutes after ejaculation females were administered 4 gr/kg (p.o.) of ethanol. Uterine and blood ethanol levels were determined at various times post-treatment. The time course for distribution into blood and uterine fluid was parallel, with a blood to uterine ratio of approximately one. Peak levels were attained within 1 hr. after treatment. Although the interindividual variation was quite large, for a given animal blood levels were a predictor for concurrent uterine levels. Thus tail bleeding provides a means of accurately monitoring uterine ethanol levels over time. By incorporating this type of monitoring into current studies one can assess 1) spermatozoal sensitivity to ethanol as a function of in vivo residence time; 2) susceptibility as a function of total ethanol exposure and 3) the interaction between these variables. Supported by March of Dimes 59-105.

The inhibitory of ovarian oestradiol production by a substituted triazole and its relationship to delayed ovulation in the rat. N.C. Middleton, S.C. Watson, R.L. Hasmall, and C.M. Milne, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: E.A. Lock

Ovulation in the rat is delayed by a single oral administration of the substituted triazole (1-1-di-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl)-ethanol; RS15885). The mechanism of this delay involves a reduction of plasma oestradiol (E2) levels at a critical time in the oestrous cycle. Reductions in plasma E2 may reflect an inhibition of ovarian E2 synthesis. Granulosa cells, within the ovary are the major site of E2 synthesis. Undifferentiated granulosa cells were prepared from ovaries of diethylstilbestrol primed immature rats. In the presence of both FSH and testosterone the cells synthesise E2 and progesterone (11.2 ± 1.3 and 85 ± 0.6 [±6] ng/105 live cells/48 h respectively). Addition of RS15885 resulted in a dose-dependent, specific inhibition of E2 production (no reduction by 10-6M). Progesterone production was unaffected. Thus, the triazole inhibits the induction or activity of granulosa cell aromatase in vitro. This inhibition appears to be competitive with testosterone and is reversible. The reduced circulating E2 levels detected in vivo are probably a consequence of a similar action with granulosa cell aromatase in vivo.


The reproductive toxicity of metronidazole was studied in rats. Male rats (10/group) were treated with metronidazole as a dietary admixture at dosages of 0 (control), 25, 100 and 400 mg/kg/day for 8 weeks. The reversibility of effects was determined in separate groups of 10 control and 400 mg/kg/day treated rats after a three and one-half month recovery period. After 2 and 4 weeks of metronidazole treatment, mating performance and fertility of treated rats were comparable to controls; however, all high dose rats were infertile after 6 weeks of treatment. Fertility was comparable to controls in low and mid-dose rats. After 8 weeks of treatment, testicular and epididymal weights, testicular spermatozoa counts and epididymal sperm counts were markedly decreased in high dose rats. Most of the few epididymal sperm present in high dose rats were morphologically abnormal. Histologically, severe degeneration of the seminiferous epithelium was observed; the tubules were generally devoid of primary or secondary spermatocytes and spermatids. Rats treated at the low and mid-dose exhibited normal testicular weights, testicular spermatozoa counts and epididymal sperm counts; epididymal sperm viability and morphology were comparable to controls. Minimal histologic changes that included degenerative fragmentation of spermatozoa and spermatids were observed in mid-dose rats. In high dose recovery rats, fertility was restored in most rats by 8 weeks after the cessation of treatment; however, at sacrifice after three and one-half months of recovery, the testicular and epididymal weights and sperm counts were still significantly decreased in treated rats. Histologically, spermatogenesis was observed in most tubules; however, a portion of atrophic tubules persisted. It is concluded that high dosages of metronidazole produce infertility in the male rat by an inhibition of spermatogenesis at the stage of the primary spermatocyte. This effect is partially reversible.

A number of pregnancies occur during the use of vaginal contraceptives (use effectiveness 1.5 to 29 pregnancies per 100 woman years) resulting in the exposure of the developing fetus to vaginal contraceptives. This study investigates the effects of vaginally applied octoxynol (a spermicidal agent used in vaginal contraceptives) on pregnant rats. Octoxynol solutions (1.5, 15 and 60% were administered vaginally three times daily to female rats from day one (presence of sperm in vaginal smear) to day fifteen of gestation. On day twenty of gestation the rats were sacrificed and the number of corpora lutea of pregnancy, implantation sites, normal fetuses and abnormal fetuses recorded. Fetal weights and crown rump lengths were also determined and each fetus examined for any gross anomalies. Fetal crown rump lengths were greater than control in both the 15% and 60% treated groups. Increased fetal weights were also observed in the 60% treated group when compared to controls. These effects paralleled the presence and severity of vaginal irritation. Other parameters observed did not differ from control. It appears that excessive vaginal irritation may lead to changes in the developing fetus.

746 TERATOGENICITY IN VITRO OF AFLATOXIN B1, [AFB1]; ROLE OF BIOTRANSFORMATION. P.T. Geissler and E.M. Faustman-Watts. Dept. of Env. Health, Univ. of Washington, Seattle, WA.

Using modifications of the whole embryo culture system of New et al. (1971), we have determined that AFB1 is a teratogenic agent in the rat embryo in vitro. Day-ten Sprague-Dawley rat embryos were explanted and cultured for 24 hours. When AFB1 was added to the cultures at a concentration of 50 µM, it caused decreased embryonic size and macromolecular content as well as embryonic neural tube malformations. Specific necrotic bands were observed in tissues dorsal to the mandibular arches. This malformation was highly reproducible and AFB1 concentration dependent. Upon addition of a complete monooxygenase system (hepatic S9 fraction and cofactors) to these embryonic cultures, the minimum embryopathic concentration of AFB1 was reduced. This reduction in the effective embryopathic concentration of AFB1 was more dramatic when hepatic S9 fractions from phenobarbital or 3-methylcholanthrene induced rats were used. These preliminary studies are consistent with previous studies which have shown that AFB1 requires biotransformation to elicit other toxic effects, i.e., carcinogenesis. Our studies suggest that AFB1 may require biotransformation for its embryopathic effects and we are currently investigating the possibility that embryonic tissues may have the capacity to biotransform AFB1. Supported by NIH grants ES-03157 and ES-07032.


Fungal secondary metabolites are commonly found in foodstuffs and are important because of their association with disease in humans and animals. One such metabolite, dicetoxycirsepelin (DAS) [3-hydroxy-4,15-dicetoro-12,13-epoxytrichothece-9-ene] produced by several species of Fusarium has been reported to be toxic to the rabbit, rat, mouse and guinea pig. The teratogenic potential of DAS was determined in in-utero Sprague-Dawley mice. DAS, dissolved in a 1:9 mixture of propylene glycol-saline, was administered intraperitoneally at levels of 2, 3, and 6 mg/kg in a single dose on one of the gestation days 7-11. Term fetuses were examined for anomalies by routine teratologic procedures. None of the DAS-treated dams died. Resorptions occurred in frequencies of 100% at 6 mg/kg on all gestation days; 30-99% at 3 mg/kg on days 7-9 and 100% on days 10 and 11; 10-33% at 2 mg/kg on days 7-9, 80% on day 10 and 100% on day 11. A reduction in mean fetal body weight and a variety of fetal malformations (i.e. exencephaly, hydrocephaly, protruded tongue and short jaws) were observed following exposure to DAS at 2 and 3 mg/kg. This is the first report to implicate DAS as a teratogen. (Supported by TAES 6215, COM 18920-4, DOD DAAG29-83-G0088 and USDA 84-CR-3-2-2401).

748 EFFECT OF SECALONIC ACID D ON THE DEVELOPING FETAL MOUSE PALATE. C.S. Reddy, Univ. of Missouri, Columbia, MO 65211. Sponsor: M.F. Raisbeck.

Nystatin secalonic acid D (SAD) is capable of inducing cleft palate in CDI mouse pups following maternal ip exposure during days 7 thru 15 of pregnancy. In order to determine the critical period of teratogenicity, pregnant mice were given, ip, 25 mg/kg of SAD on days 10, 11, 12, 13, 14 or 15 of pregnancy (day of vaginal plug is day 1). Incidence of cleft palate averaged 16.9, 28.4, 45.3, 39.9, 27.0 and 0.0 percent, respectively, indicating that day 12 is the most sensitive to the effects of SAD. A dose response on day 12 to 15, 20, 25 and 30 mg/kg of SAD indicated that 25 mg/kg of SAD is the optimal teratogenic dose at which 45.3% of pups in each litter had cleft palate with no increase in fetal resorptions of decrease in fetal weights. Palatal uptake of 3H-thymidine as well as mitotic activity in SAD-treated mouse fetal palate were not decreased by 25 mg/kg SAD compared to those in controls. Light microscopic evaluation of developing palatal sections gave no evidence of abnormal rate of cell death or a reduction in cell density in SAD treated palates compared to controls. These studies indicate that SAD can cause cleft palate at doses that have no obvious cytotoxic effects. Supported by USPHS grant DE06196.
EMBRYOTOXIC EFFECTS OF TWO ALKYLATED AGENTS ON JAPANESE MEDAKA FISH (Oryzias Latipes) EMBRYOS.
F.P. Solomon and E.M. Faustman-Watts. Dept. of Env. Health, Univ. of Washington, Seattle, WA.

The carcinogenic and mutagenic properties of N-nitroso compounds have been extensively investigated. Less attention has been given to their embryolethal and teratogenic effects. This study examined the embryotoxic properties of two direct acting N-nitroso compounds, methyl nitrosourea (MNU) and ethylnitrosourea (ENU), using Japanese medaka embryos in culture. Medaka embryos in early organogenesis were exposed for 2 hours to MNU or ENU at concentrations ranging from 0.01 to 9.7 μmol. These embryos were cultured at 20°C in petri dishes and were assessed daily for viability and gross malformations until hatching. MNU and ENU produced concentration-dependent decreases in viability and increases in malformations. Approximately 30% of the medaka embryos exposed to 2.0 μmol MNU for 2 hours were malformed. MNU was 1.6 times more potent in eliciting malformations than ENU; however, a similar spectrum of malformations was produced following exposure to either alkylating agent. Observed abnormalities included flexures, cardiac, optic, and cephalic malformations. Future studies will correlate the embryotoxic capabilities of these N-nitroso compounds with their chemical properties of methyl or ethyl alkylation. These studies were supported by NIH grants ES-03157 and ES-07032 and a Univ. of Wash. Marine/Freshwater Biomed. Res. Center grant.

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REPRODUCTIVE TOXICITY OF METHYLDOPA IN THE MALE FISHER 344/N RAT. J.K. Donnick, M.W. Harris, L.B. Hall, R.E. Chaplin, and J.C. Lamb, IV. National Toxicology Program, NIAMS, RTP, NC 27709

Methyldopa (L-(α-methyl-3,4-dihydroxyphenyl)alanine) is a drug which is widely used for the treatment of hypertension. Reports in humans and animals have indicated that this drug produces sexual dysfunction in males. To further evaluate the reproductive toxicity, methyldopa was administered for 65 days to male Fischer 344/N rats by oral gavage in corn oil at doses of 0, 50, 100, 200, and 400 mg/kg/day. Interim sacrifices were conducted at day 5, 12, and 19 and animals examined histologically for evidence of testicular change. On days 57-61 the rats were mated to untreated female Fischer 344/N rats. At day 65, 21 animals from each group were necropsied and examined for reproductive toxicity. Fifteen animals in dose groups 0, 100, 200 and 400 mg/kg were kept without dosing for an additional 13 weeks, mated to untreated females, and necropsied and examined for reproductive toxicity. Methyldopa caused a dose related decrease in male fertility, sperm density, sperm motility, and testosterone levels. The male fertility index (% of males with pregnant females) was 62, 81, 67, 14, and 5% at 0, 50, 100, 200 and 400 mg/kg. No histopathologic abnormalities of the testes were seen after 5, 12 and 19 days of dosing. After 65 days of dosing quantitative histology revealed decreased spermatogenesis in the 200 and 400 mg/kg groups. Significant differences could no longer be detected in treated versus control animals after the recovery period.

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REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF AN ALPHA-1-ADRENERGIC BLOCKER IN RATS. F. Andrew, T. Terrell, G. Thacker, M. Onizuka and C. Zustak. Institute of Toxicologic Science, Syntex Research, Palo Alto, CA.

During preclinical toxicity testing, a proposed new α1-adrenergic antagonist antihypertensive agent (9-[2-(indol-3-yi)ethyl]-1-oxa-3-oxo-4, 9-diazaspiro[5.5]undecane hydrochloride), was evaluated for reproductive and developmental toxicity. Rats were gavaged at 0, 10, 30 or 60 mg/kg/day, males for 80 days and females for 14 days prebreeding until pup postnatal day 21. No clinical signs of toxicity were seen in the dams and sires. Dose-related significant effects included infertility, decreased fecundity and pup mortality. Pregnant rats were gavaged at 0, 10, 30 or 60 mg/kg/day on days 7-16 of gestation (mating detected day 1). Dose-related significant effects included maternal toxicity, embryonic mortality, fetal growth retardation and orofacial malformations. A similar dosing regimen to pregnant rabbits did not induce significant maternal or embryo-fetal toxicity. The spectrum of reproductive and developmental toxicity seen in rats dosed with this agent has not been reported with other drugs of this class.
FAILURE OF PERINATALY ADMINISTERED BARBITURATES TO AFFECT MATING BEHAVIOR IN MALE HAMSTERS. F.R. Alleva and T. Balaze, CDB, FDA, Washington, DC.

In hamsters the critical period of sexual differentiation of the hypothalamic center that later controls sex behavior occurs at about birth to 4 days of age (Alleva et al., Endocrinology 85: 312, 1969). Pentobarbital (PB) has been reported to interfere with this differentiation in male hamsters (Clemens et al., Dev Psychobiol 12: 49, 1979). To further study this phenomenon, we injected 5 male golden hamsters with PB (Na salt) at 16 mg base/kg sc once daily at 1, 2 and 3 days of age and another 7 males with 16 mg/kg at 0 (birth) day of age followed by twice daily injections at 1, 2 and 3 days of age. Control groups received saline (N=5) or nothing (N=7).

We also injected 8 pregnant hamsters with sodium phenobarbital (PhB) at 60-80 mg base/kg sc on days 11-15 of pregnancy and 20 of their male offspring with single daily injections of 12-30 mg/kg on days 0-3 of age. Five other pregnant hamsters and 17 of their male offspring received saline. At adulthood, male behavior was assessed by housing PB- and PhB-treated and control males with estrous females and recording the latency time to the first mount and intromission as well as the number of intromissions within 15 min after the first. Data reveal no significant differences in male behavior between treated and control groups. Thus we failed to demonstrate the effect of PB and PhB on sexual differentiation of male hamsters under our experimental conditions.

EFFECTS OF EXPOSURE TO BENOMYL OR CARBENDAZIM ON MALE REPRODUCTION IN PREPUBERTAL AND ADULT SPRAGUE-DAWLEY RATS. S.D. Carter (Laws), and J.W. Laskey. USEPA, NERL, Research Triangle Park, NC. Sponsor: M. Chernoff

Previous studies indicate that the reproductive system of the prepubertal male rat is less sensitive than that of the adult to exposure to the fungicide, benomyl. This study further investigates the age response after exposure to benomyl (B) or its metabolite, carbendazim (C). Prepubertal (33 days of age) or adult (75 days of age) male rats received 10 doses of corn oil or 400 mg/kg/d B or C by gavage. Measurements were obtained 30 days postexposure for selected tissue weights, total epididymal sperm count, vas deferens sperm concentration and testicular histopathology was evaluated for alterations in the spermatogenetic cycle. In adult animals which received B, epididymal weights, total epididymal sperm counts, and vas deferens sperm concentrations were reduced. No such effects were seen in the animals which received C during prepuberty. These same parameters were reduced in both prepubertal and adult animals which received C. Semicrural testicular atrophy was observed in testes from both prepubertal and adult treated animals, with the adults being affected more severely. The data demonstrate effects resulting from C exposure in both prepubertal and adult animals, while effects resulting from B exposures were observed primarily in the adult.


WR 6026.2HCL [6-methoxy-8-(6-diethylamino hexylamino) lepidine dihydrochloride] is a candidate antihistaminal drug. As part of a safety testing program, a study to evaluate teratogenic and embryo/fetotoxic effects was done in rats. Based on pharmacokinetic data showing a relatively long half-life (approximately 6 days) of the drug, an oral dosing regimen designed to produce stable drug levels during treatment was used. This consisted of giving loading doses 4, 3, and 2 times the maintenance doses on gestation days 6, 7, and 8, respectively. Maintenance doses given days 9-15 were 5, 10, or 20 mg/kg per kg. Minimal maternal toxicity as shown by a decrease in weight gain occurred at the low dose. Mid and high doses were markedly toxic. One mid dose and the majority of the high dose rats died. There were adverse effects on dams appearance, body weight, and water or food consumption at mid and high doses, with splenic and/or adrenal enlargement. Cesarean data showed embryo/fetal toxicity in the mid and high dose groups as evidenced by increased resorptions and decreased fetal viability and size. There was no evidence of teratogenic effects. (Sponsored by USAMRDC No. DAMD 17-81-C-1138.)


As part of a comprehensive safety assessment, a three generation reproduction study was conducted with monosodium cyanurate. The test material was dissolved in tap water at concentrations of 400, 1200 and 5375 ppm (based on cyanuric acid content) and offered ad libitum in drinking water. One control group was offered tap water adjusted to pH 7.4 as drinking water. The second, a sodium control group, was offered tap water with ionic sodium (Na+) equivalent to that of the high dose group. Each treatment group consisted of 12 males and 24 females. Treatment was initiated at 36 days of age for the parents and continued for a minimum of 100 days prior to mating. Treatment with 5375 ppm or less of cyanurate did not induce significant fluctuations in reproductive or litter parameters compared to controls. As previously reported (1984 SOT meeting) for the first generation of this study, the highest dose level, 5375 ppm was also determined to be the no-effect level for reproductive and litter parameters for the second and third generations of this study.
Influence of Perinatal Pre-exposure on the Toxicity of Diphenylhydantoin to Adult Rats and Mice.


Battelle Columbus Labs, Columbus, OH and National Toxicology Program, NIEHS, Research Triangle Park, NC.

The possibility that diphenylhydantoin (DPH) exposure during early development may alter response to subsequent DPH dosing was examined. Rat and mouse dams received dosed or control feed during breeding, gestation, and lactation. Fertility, pup survival, and neonatal litter weights were similar in dosed and unexposed groups. However, continued (postnatal) exposure to DPH resulted in delayed physical development (eye opening, vaginal opening and growth) at 8 weeks of age. The young were assigned to chronic dose groups. The growth of rats and mice given DPH as adults was depressed compared to that of control adults. At 11 mos., high-dose mice exhibited elevated spontaneous motor activity. At necropsy (11 mos.), hepatic hypertrophy was apparent in high dose rats and mice and preliminary histopathologic diagnosis indicated centriflobular cytomegaly with increased hepatocellular cytoplasmic vacuolation (rats) and hypernucleation (mice). In general, the toxic effects of adult exposure to DPH were similar, whether the animals had been reared by control or dosed dams. Thus, perinatal exposure to DPH retarded development but did not significantly alter susceptibility to other toxic effects. (Supported by NIEHS Contract No. N01-ES-8-2151.)
A MODEL FOR TRIMETHYLLEAD NEUROTOXICITY IN THE RAT. J. Caldwell-Kenkel and M.R. Krigman. The University of North Carolina, Chapel Hill, NC

The present report describes the development of a model which demonstrates trimethyllead (TMBp) toxicity. The intragastric (PO) route of administration was found to be the most dependable for producing neurotoxicity in the rat. Intraperitoneal (IP) administration of TMBp produced a profound lethal chemical peritonitis and intravenous (IV) dosing was problematic. Using a 14 day survival time, the LD_50 was 40, 25 and 35 mg/kg for PO, IP and IV exposure, respectively. Neurotoxic doses (IV and PO) produced a delayed neurotoxic syndrome characterized by aggression, tremors, hyper-reactivity and a prolonged status epilepticus. There was a dose-dependent decrease in body weight and a corresponding decrease in food consumptions. Liver was the only organ that exhibited a change, which consisted of a decrease in weight in anorexic rats. Electrolyte and organ enzyme panel studies were undertaken on rats (1,3,5 and 7 days post-dosing) that received a 0.5, 3.0 or 10 mg/kg dose PO. There were no consistent changes in non-target organs by these parameters. Histological sections revealed widespread neuronal changes, which were most prominent in the hippocampal formation and the large pyramidal neurons in the brainstem and spinal cord. (Supported by P01 ES01104, T32 ES07126 and T32 ES07017.)

INHIBITION OF Na^+, K^+-ATPase AND OF [3H]GABA UPTAKE BY ORGANOTIN COMPOUNDS IN MOUSE BRAIN IN VITRO. L.G. Costa, Dept. of Environmental Health, University of Washington, Seattle, WA 98195.

Trimethyltin (TMT) has been reported to inhibit the uptake of [3H]GABA into mouse brain synaptosomes both in vitro and after in vivo administration, while other organotins inhibited the uptake of [3H]GABA in rat brain explants (Toxicology 25, 213, 1982; 29, 39, 1983). To determine whether this effect was due to inhibition of Na^+, K^+-ATPase, the effects of various triorganotins on the uptake of [3H]GABA and its possible relationship with their reported inhibition of Na^+, K^+-ATPase, were investigated. All triorganotins tested inhibited the uptake of [3H]GABA into mouse brain synaptosomes in vitro. Triphenyltin (TPHT) and tributyltin were the most potent, and TMT the least potent, inhibitors. Inhibition of [3H]GABA uptake was prevented by some sulfhydryl reagents: DTT and BAL (TPHT) and Na_2S (TPHT and tributyltin). There was a good correlation between the ability of organotins to inhibit Na^+, K^+-ATPase (measured as specific binding of ouabain and as hydrolysis of ATP) and their potency as inhibitors of [3H]GABA uptake. Ouabain also caused inhibition of [3H]GABA uptake. However, unlike organotins, ouabain-inhibition required a preincubation in the presence of sodium and never exceeded 60%. These results suggest that other mechanisms may contribute to the inhibition of [3H]GABA uptake by organotins.

TRIETHYLTIN INHIBITION OF ATPase ACTIVITIES IN TISSUE HOMOGENATES FROM ADULT AND NEONATAL RATS. K.E. Stine1, L.W. Reiter2, and J.J. Lemasters3. 1Dept. of Management, Clemson Univ., Clemson, SC, 2Neurotoxicology Division, US EPA, RTP, NC and 3Lab. for Cell Biology, Dept. of Anatomy, Univ. of North Carolina, Chapel Hill, North Carolina.

The effects of the neurotoxicant triethyltin (TET) on ATPase activities in brain and liver homogenates and subcellular fractions from adult and neonatal (postnatal day 5) rats were compared. Mitochondrial ATPase in postnatal day 5 brain homogenates was much more sensitive (IC50= 2.5M) than the same activity in adult brain (IC50=260 M). Since isolated mitochondria from adult and neonatal brains were equally sensitive to TET, it was hypothesized that competitive binding of TET to myelin reduced the sensitivity of adult brain homogenate mitochondrial ATPase to TET inhibition. This was supported by the observation that addition of myelin to isolated mitochondria reduced TET-induced ATPase inhibition. Furthermore, by postnatal day 10, following the onset of myelination, the IC50 for TET inhibition of brain mitochondrial ATPase increased to 71M. Brain tin concentrations following a neurotoxic dose of TET are expected to be sufficient to inhibit brain mitochondrial ATPase in the postnatal day 3 rat, but not the adult. This age-related difference in ATPase inhibition may be a major contributing factor to the difference in toxic effects seen between TET-treated adults and neonates. (Supported by EPA CR809644-02, NIH GM-28999, NIH AM-30874)
765 TEMPERATURE-DEPENDENT ACCUMULATION OF METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MNT) IN BRAIN SLICES OF BOTH RATS AND MICE.
P.A. McGinley, J.B. Morris and G. Gianutsos.
Section of Pharmacology and Toxicology, School of Pharmacy, University of Connecticut, Storrs, CT.

In previous experiments from this laboratory, we have demonstrated in the rat the phenomenon of temperature dependent accumulation of the pulmonary toxicant methylcyclopentadienyl manganese tricarbonyl (MNT) in slices of lung tissue incubated in vitro. Mouse lung slices do not display this effect (McGinley, et al. Toxicologist 4, 115, 1984.) To further study cellular MNT uptake, slices of whole brain were incubated in Krebs-bicarbonate buffer which contained 1.7 μl/liter MNT for 30 minutes at either 0 or 37°C. Slices kept at 0°C showed some increase in manganese concentration compared to slices incubated at 37°C. Differences in manganese uptake, attributable to MNT, in excess of 200% of that seen at 0°C. Unlike pulmonary uptake, brain uptake of MNT was observed in both the rat and the mouse. The mechanism of the MNT uptake process remains obscure. (Supported by NIH Grant ES02511, P.A.M. is recipient of Richardson-Vicks fellowship.)


Our recent reports demonstrated that cadmium chloride (CdCl2) inhibited ATPases in various tissues both in vitro and in vivo. The present studies were initiated to study the effect of CdCl2 on brain ATPases and catecholamine uptake when administered by i.p. for 15 days. Male Sprague-Dawley rats were treated daily with CdCl2 by i.p. for 15 days. Brain synaptosomes were prepared by ficoll-sucrose gradient method. Na+K+, oligomycin-sensitive (O.S) and unsensitive (U.S) ATPases were determined. H-dopamine (H-DA) and H-norepinephrine (H-NE) uptake by synaptosomes were determined by filtration method. The data obtained showed that all three ATPases were reduced by 50% at 1.0 mg/kg dose. At lower doses the ATPases were reduced in a dose dependent manner. H-DA and H-NE uptake by synaptosomes were also reduced in CdCl2 treated rats and the reduction was significant and parallel to the reduction of ATPases. These results suggest that CdCl2 treatment by i.p may alter synaptic function. (Supported by NIH/NBS grant RR08169.)

767 NEUROTOXIC EFFECT OF MANGANESE ON BIOGENIC AMINE LEVELS AND TURNOVER IN DIFFERENT REGIONS OF MOUSE BRAIN. B. Sriscuhat and R.P. Sharma.
Toxicology Program, Utah State University, Logan, UT.

Male CD-1 mice were exposed with daily ip injections of manganese chloride (20 mg/kg) for 2 weeks. Levels of biogenic amines and their selected metabolites, norepinephrine (NE), dopamine (DA), 5-Hydroxytryptamine (5-HT), vanillylmandelic (VMA), dihydroxphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5HIAA) were measured using HPLC and electrochemical detection. Their turnover rates were estimated in different brain regions by using DL-2-monofluoromethylindole (MFM), an irreversible inhibitor of aromatic L-amino acid decarboxylase. Manganese treatment caused an increase in the steady state level of NE and its turnover in cerebellum, that of 5-HT in cerebellum and midbrain; and DA in midbrain. Increase in levels of NE and DA were observed in hypothalamus and medulla oblongata, respectively. The turnover rate of 5-HT increased in medulla oblongata, whereas that of DA and HVA in striatum, and 5HIAA in midbrain. The results suggest that exposure to manganese caused an elevation of steady state levels of biogenic amines and their turnover rates in different regions of the mouse brain.

768 IN VITRO AND IN VIVO EFFECTS OF VANADATE ON K+-DEPENDENT PHOSPHATASE ACTIVITIES FROM SUBCELLULAR FRACTIONS OF BRAIN, KIDNEY AND LIVER. R.J. Keller and R.P. Sharma.
Toxicology Program, Utah State University, Logan, UT.

Vanadium is widely distributed in nature and the increased usage of fossil fuels has led to its amplification in the environment. Vanadium in the orthovanadate form is a powerful inhibitor of the sodium, potassium-adenosine triphosphatase and several other enzymes. Inhibition of K+-dependent phosphatases by sodium orthovanadate was studied in subcellular fractions from brain, kidney and liver of male Sprague-Dawley rats. Concentrations of vanadate needed to produce 50% inhibition occurred in the submicromolar range for most fractions studied, with a low of 2.0 x 10⁻⁷ in the kidney 900xg fraction. Phosphatase activity was assayed for 1, 6 and 25 hr after rats were injected ip with 8 mg/kg of sodium vanadate dissolved in isotonic saline. The inhibitions measured were not as pronounced as those produced in vitro. Further studies with molecular exclusion chromatography showed the vanadate to be loosely bound, providing a possible explanation for the discrepancies in the phosphatase inhibition found in the treated animals and the in vitro study. (Supported in part by USHS ES07907).
An aliquot (~250 μg protein) of "purified" rat striatal synaptosomes (P2g equivalent), rapidly isolated using a modification of the Gray and Whittaker procedure, was suspended in a physiological HEPES-buffered solution (118.5 mM NaCl, 4.75 mM KCl, 1.19 mM MgSO₄, 2.54 mM CaCl₂, 11.1 mM glucose, 1 mM ascorbic acid, 0.6 mM nialamide, 17 mM HEPES, adjusted to pH 7.5 with Trizma base). The synaptosomes were loaded with 3H-dopamine (37°C, 30 minutes), and then gently layered onto a 0.65 μm cellulose ester filter. The filter with the synaptosomes was superfused with HEPES buffer (37°C) at a rate of 2 ml/min. After a 10 minute washout, 6 superfuse fractions were collected to establish a baseline, after which Pb acetate (concentrations ranging from 0.1 μM to 10 μM) was added to the buffer. Following the collection of 6 fractions, the Pb was removed, and another 6 fractions collected. Then the synaptosomes were depolarized by a 30 sec. exposure to 61 mM KCl. Fractions were collected for another 3 minutes. The amount of 3H in the fractions and remaining on the filter was quantified using LSC. A concentration dependent decrease in 3H-dopamine release was observed for Pb in the 1 to 10 μM range. This Pb-evoked release was independent of concentration of calcium in the extracellular buffer. (Support ed by ES03399).

The neurotoxic effects of inorganic lead on brain lipids were investigated in neonatal rats. Lead acetate (7.5 mg/kg i.p.) was administered from 3-9 days of age and 6 brain regions analyzed for lipid peroxidation (LP, TBA method), phospholipid (PL) and cholesterol (C) at selected ages. Behavior was videotaped at 9 and 19 days of age and analyzed for quantitative changes in activity patterns. Statistical correlations between biochemical and behavioral effects were measured. Brain lipid changes were found in cerebellum (Ce) and brainstem (B) but not in striatum, cortex, hippocampus or midbrain. LP increased over control at 10 days and decreased at 20 days in Ce and B but not in forebrain. PL and C were measured to explain the unexpected LP decrease at 20 days. PL in Ce and B were the same as control at 10 days but decreased at 20 days while cholesterol was decreased at 10 and 20 days. Lower PL content in membranes at day 20 may explain the decrease in LP. Thus, LP is a marker for cell change whether elevated or depressed. Behavioral patterns present at 9 and 19 days correlated with certain regional brain lipid changes. This biochemical and behavioral approach shows that low dose lead toxicity persists throughout the neonatal period.

In previous studies it was shown that the inhibitory effects of ascorbic acid on the binding of dopamine antagonists to neurostriatal membranes are due to lipid peroxidation. The present studies were carried out to determine whether the degree of lipid peroxidation would correlate with changes in membrane lipid fluidity since the dynamic state of membrane lipids has been implicated in membrane receptor function. Neurostriatal membranes from male Sprague Dawley rats were peroxidized in vitro by incubation with 0.05-0.5 mM sodium ascorbate and FeSO₄. The thiobarbituric acid (TBA) test was used to determine lipid peroxidation and the fluorescence polarization technique using all trans-1, 6-diphenyl-1,3,5-hexatriene (DPH) as the lipid probe was used to assess membrane lipid fluidity. The DPH polarization (P) of non-peroxidized membranes varied from 0.334 to 0.368 at 25.0±0.1°C in individual membrane batches. Membranes peroxidized progressively as measured by the TBA test showed increasingly higher P values, with increases up to 0.025 P units. The addition of peroxidation inhibitors during the ascorbate incubation decreased lipid peroxidation and also inhibited changes in DPH polarization.

Regional and Temporal Selectivity of Lead Induced Neurochemical Changes in Neonates: Correlation with Behavioral Abnormalities. B. Callahan, D. Brown, B. Schutz and M. Cleaves. Toxicology Program, Northeastern University, Boston, MA.

The brain cell reaggregate culture system can be used to study normal and altered CNS development. Undifferentiated fetal brain cells aggregate and form histiotypic spheres of mature neurons, astrocytes and oligodendrocytes. Biochemical development in reaggregates closely mimics that found in vivo. In this study, brains from 16 days gestation rats were minced and subjected to low concentration tryptic digestion. A single cell suspension was obtained after tituration. The isolated cells were suspended in a modified minimal essential medium containing 10% Nusserum at a density of 4x10⁶ cells/ml. Aggregation enhanced by gentle rotation began within a few hours and was complete in two days. Reaggregates initially contained a uniform distribution of cells and after one week, took on histiotypic organization. Time of onset of myelination in controls, indexed by 35S-sulfate incorporation into sulfatides, was identical to that found in vivo. Myelination was delayed in reaggregates exposed to between 250 and 1500 Rads at 12 days in vitro (DIV). Sulfate incorporation in the low dose groups approached that of controls within 34 DIV. High dose groups did not recover but retained the ability to undergo protein synthesis and could not be distinguished from controls histologically. (Supported by NIEHS grants ES07026 and ES01248).
The effect of the metabolite inhibitor, piperonyl butoxide (PB), on tri-o-tolyl phosphate (TOCP) toxicity and delayed neurotoxicity in rats was measured as a means of evaluating methods in rats for increasing the sensitivity of rats to organophosphate-induced delayed neurotoxicity. PB (400 mg/kg, i.p.), 1 hour prior to TOCP failed to alter TOCP oral lethality. The oral LD50 (95% confidence limits) values in rats with and without PB pretreatment were 911 (699-1186) mg/kg and 804 (636-1017) mg/kg, respectively. Slopes of the lethality curves were not significantly different. Brain neurotoxic esterase inhibition by TOCP was similar with and without PB pretreatment. No rats pretreated with corn oil or PB showed clinical evidence of delayed neurotoxicity up to 4 weeks after 500 or 750 mg/kg TOCP. Both groups given 750 mg/kg TOCP showed an incidence (38-43%) of mild axonal swelling in dorsal funiculi of spinal cord that was not exacerbated by PB. No other spinal cord or bilateral sciotic nerve changes were suggestive of neurotoxicity. In conclusion, 400 mg/kg PB pretreatment failed to alter the toxicity or neurotoxicity of TOCP in rats. However, a single subcutaneous injection of 20 mg/kg TOCP produced histologic changes in cervical spinal cord suggestive of delayed neurotoxicity.

DOSE-RELATED BENEFICIAL AND ADVERSE EFFECTS OF DIETARY CORTICOSTERONE ON ORGANOPHOSPHATE-INDUCED DELAYED NEUROPATHY (OPIDN) IN CHICKENS.

M. Ehrich, W. B. Gross and B. S. Jortner, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Triorthotolyl phosphate (TOTP), 360 mg/kg po, and 0.01-dimethylphosphorofluoridate (DFP), 1 mg/kg sc, were administered to adult White Leghorn chickens 24 hr after birds were placed on diets containing levels of corticosterone which provide physiological and nonphysiological changes in blood parameters (<50 and 200 ppm, respectively). Supplemented diets were continued until clinical signs of delayed neuropathy appeared. In contrast to beneficial effects of lower doses, clinical signs of neuropathy were magnified in groups of birds given 200 ppm corticosterone with TOTP (score = 2.7±0.2, mean±SD, N=4, vs. 1.5±0.4 at 12 days for TOTP controls; 0.4 nmol/kg of body weight) and with DFP (score = 3.5±0.2 vs. 2.3±0.3 at 10 days for DFP controls). Neurotoxic esterase activity was less than 20% of values measured in birds not given organophosphates. Birds given 200 ppm corticosterone without TOTP or DFP had significantly elevated activity of plasma cholinesterase and significantly inhibited activity of liver carboxylesterase. Degenerating myelinated fibers were noted in distal peripheral nerves of birds given TOTP + corticosterone or given DFP. Indications are that continuous exposure of birds to high levels of their natural corticosteroid increases severity of OPIDN.
0-ethyl-0'-(2-diisopropylaminoethyl) methylphosphonite (QL), an intermediate in the formation of ethyl S-2-diisopropylaminoethyl methylphosphonothioate, has been evaluated for neurotoxicity in the adult hen. Birds were given oral doses of QL ranging from 635 to 6080 mg/kg. The QL-treated hens were observed for up to 24 hr after dosing for acute toxicologic effects and over a 24-day period for evaluation of delayed neurotoxicity. The oral LD₅₀ in hens is 1186 mg/kg. Neurologic dysfunction, as evidenced by motor incapacitation, was observed at six days and thereafter following QL treatment.

Neurotoxic esterase activity was not altered at 24 hr post QL exposure. Neural damage, multifocal in nature, was noted in the peripheral nervous system of QL-treated hens at dose levels ≥ 635 mg/kg oral dose. These findings indicate neural damage tendencies following QL exposure.

The subchronic toxicity of sodium azide (a biocide and an inflating component for auto air bags) was studied by administering 0, 1.25, 2.5, 5.0, 10 or 20 mg of the chemical/kg body weight in water by gavage once daily, 5 days per week for up to 13 weeks to groups of 10 F344 rats and 10 B6C3F₁ mice of each sex. Chemically induced deaths occurred only in rats at the 20 mg/kg dose level. Decrease (P < 0.05) in body weight gain was observed in male rats at 10 mg/kg dose level as compared to controls, but no such decrease was noted in the remaining dose groups of rats or mice. Increases (P < 0.05) in liver/body weight and liver/brain weight ratios occurred in female rats and mice at all dosage levels. The kidney/body weight ratio was also increased (P < 0.05) in female rats at 5 and 10 mg/kg dose levels. Compound-related lesion were noted in the brain (neurois of the cerebrum in the area of the caudate nucleus/putamen, and in some cases involving the thalamus) of male and female rats that died during the study at the 20 mg/kg dose level. No compound-related histological responses were observed in mice of either sex.

ABSENCE OF DELAYED NEUROTOXICITY AND INCREASED PLASMA BUTYRYLCHOLINESTERASE ACTIVITY IN TRIALLATE TREATED HENS. Daniel Lapadula, Frederick Johansen, and Mohamed B. Abou-Donia. Duke University Medical Center, Durham, NC and Monsanto Company, St. Louis, MO.

Triallate is a thiocarbamate that had been reported to produce organophosphorus induced delayed neurotoxicity (OPIDN). Since this class of chemicals had not been previously reported to produce OPIDN, a thorough investigation of this chemical was undertaken. Hens were given single oral doses ranging from 312.5 to 2,500 mg/kg of triallate, 750 mg TCP (tri-o-cresyl phosphate), or empty gelatin capsules on day 1 and 21, killed on day 42. In a second experiment animals were administered daily oral doses of 25-300 mg/kg triallate or 10 mg/kg TCP for 90 days. In a third experiment animals were given single oral doses of 2,500 mg/kg triallate, 750 mg/kg TCP or empty gelatin capsules, and killed after 24 hrs. Delayed neurotoxicity was observed only in TCP treated animals. Animals given daily doses of 300 mg/kg triallate became moribund after 30 days, however histological examination revealed no lesions characteristic of OPIDN. NTE was not significantly altered in triallate treated animals while it was 95% inhibited in TCP treated animals. BuChE increased significantly 24 hrs after treatment with triallate in a dose dependent manner.

In summary, the previous report of possible acutely induced OPIDN by triallate could neither be reproduced nor could it be confirmed following subchronic triallate treatment.

COMPETITIVE INHIBITION OF NEUROTOXIC ESTERASE ISOZYMES BY PARAXON, C.D. Carrington, and H.B. Abou-Donia. Department of Pharmacology, Duke University Medical Center, Durham, NC

Neurotoxic esterase (NTE) is a proposed target site for the initiation of organophosphorus compound-induced delayed neurotoxicity (OPIDN). The activity and inhibition of NTE may be assayed by comparing phenylvalerate (PV) hydrolysis following preinhibition with non-neurotoxic (para-oxon) and neurotoxic (mipafox) OPs. A recent report has described two NTE isoforms in hen brain which may be distinguished by their sensitivity to mipafox. We found that we could also distinguish two isoforms if sequential preinhibition, where the paraoxon is removed prior to inhibition with mipafox, was used. However, with concurrent preinhibition, where the paraoxon and mipafox were added simultaneously, there was only a single NTE component. By varying the concentrations of the OP it was found that paraoxon is able to reversibly decrease the rate of inhibition of both NTE isoforms by mipafox. The rate of phosphorylation of the higher affinity component was decreased to a greater extent so that the two isoforms could not be distinguished in the presence of even relatively low concentrations (40 µM) of paraoxon. Concentrations of 0.8-diisopropyl phosphorofluoridate which were capable of irreversible inhibition of both NTE isoforms, and a number of other non-neurotoxic and neurotoxic OPs, were found to have no effect on the rate of phosphorylation of the isoforms by mipafox. (Supported in part by NIEHS Grant ES02717).
THE EFFECT OF TRI-O-CRESYL PHOSPHATE (TOCP) ON 2',3'-CYCLIC NUCLEOTIDE 3'-PHOSPHOHYDROLASE ACTIVITY IN RAT NEURAL AND NON-NEURAL TISSUES. M.K. Schluger, P.A. Pleban, and E.J. Olajos, Dept. of Chemical Sciences, Old Dominion Univ., Norfolk, Va. 23508 and Toxicology Div., Chemical Rch. and Development Ctr., U.S. Army, AMC, Md. 21010

Chemically-induced demyelination was assessed in TOCP-treated rats by monitoring changes in activity of the myelin-specific enzyme 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP). Atropinized Long-Evans rats were given divided oral doses of TOCP over a 5-day period (total dose, 9.2 or 11.4 g/kg). CNP activity was monitored in crude brain and spinal cord homogenates as well as in red blood cell (RBC) ghosts preparations at maximal neurologic dysfunction, evaluated as locomotor impairment. Enzyme assays of CNP revealed a decrease in spinal cord CNP activity at the time of maximal locomotor incapacitation after treatment with TOCP. Brain and RBC-gHOSTS CNP activities were not altered following TOCP treatment. Neurotoxicant-induced demyelination can be monitored biochemically by assaying CNP activity.

ORGANOPHOSPHATE ACTIVATION, ISOENPHOS, AND AN IN VITRO ASSAY FOR DELAYED NEUROPATHIC POTENTIAL E. Chow, M. M. McChesney, B.W. Wilson. University of California, Davis, CA 95616.

Organophosphorus esters (OPs) that induce a delayed neuropathy (OPDN) generally inhibit neurotoxic esterase (NTE). However, the assay itself, when conducted in vitro, misses OPs that are converted into OPDN-causing agents in the body. We report a rapid way to detect activations of OPs with a preparation of liver mixed function oxidases and brain NTE. Compounds to be tested (at 0.1mM or less) were incubated with a mixture of microsomes isolated from livers of phenobarbital-treated chick embryos (pH 50 content averaged 1.8 nmole/mg protein) and NTE (average of 1.9 umole/min/g wet wt.) from untreated chick embryo brains. The NTE was separated by calcium precipitation and its activity assayed as usual. Compounds that were not neurotoxic (parathion, diazinon) did not increase their low inhibitions of NTE in the presence of NADPH; compounds that required activation (leptophos, DEF and TOCP) greatly increased their inhibitions of NTE with NADPH. In the case of the recently identified neuropathic OP isofenphos (IFP), both it and its oxon required activation to inhibit NTE (inhibitions of 21% and 75% respectively). Evidence is presented that one of the active metabolites of IFP (and possibly the neuropathic metabolite) is des-N-isopropyl IFP oxon. Supported in part by ES 00202.


To develop new drugs for the treatment of organophosphate (OP) poisoning, we have studied competitive AChE inhibitors as inhibitors of AChE phosphorylation. Used as pretreatment antidotes, such compounds could protect against OPs by competing with them for the active site on AChE, preventing irreversible phosphorylation, analogous to pyridostigmine antagonism of OP poisoning by carbamoylation of AChE. We evaluated compounds in a semi-automated AChE assay in which the competitive inhibitor and the OP are added to human RBC AChE and substrate, and the rate of decline in AChE activity is determined. The results show that the potency of compounds as inhibitors of phosphorylation by GD (womam) can be significantly greater than their potency as competitive inhibitors, possibly by selectively competing with GD for pre-equilibrium binding sites on AChE. For Ix-72601, 2-(3-hydroxyphenyl)-1-pyroridino-cyclohexane mech-chronide, a very high affinity slowly-reversible substrate-governed binding to AChE occurs which may result in a high therapeutic ratio because competitive AChE inhibition would increase as an OP raises ACh levels. The selective inhibition of phosphorylation by competitive inhibitors shows promise for the development of a useful pretreatment antidote. (Supported by U.S. Army Medical Research and Development Contract DAMD17-83-C-3106.)

EFFECTS OF TRICRESYL PHOSPHATE ON SPINAL CORD, SERUM, AND BRAIN CHOLINESTERASE IN F344 RATS Randy Deskin, Larry Marsh, Arthur Peters, and Richard Trwin*. Battelle-Columbus, Ohio, and *National Toxicology Program, RTP, NC

Commercial tricresyl phosphate (TCP) is primarily composed of tri-ortho, tri-meta, and tri-para cresyl phosphate isomers. The tri-ortho-isomer (TOCP) is a known neurotoxic, producing a delayed peripheral neuropathy. A 13-week subchronic study was conducted in F344 rats to determine effects of TCP on spinal cord (lower 1/3), serum, and brain cholinesterase activities. Animals received TCP at doses of 800, 400, 200, 100, 50, or 0 mg/kg by gavage in corn oil 5 days per week for 13 weeks. Spinal cord and serum cholinesterase activity was determined on Study Days 10, 45, and 90. Brain cholinesterase activity was determined on Study Day 90. Serum cholinesterase activity was significantly (p<0.05) reduced in males and females at all three time periods. Cholinesterase activity determined in the spinal cord exhibited a pattern of progressive inhibition with time; male rats (800 mg/kg) and female rats (800 and 400 mg/kg) exhibited significant (p<0.05) reductions beginning on Day 10 with females in all TCP groups and males in the 800, 400, and 200 mg/kg group exhibiting significant reductions by Day 90. Male and female rats in all TCP treatment groups exhibited statistically significant (p<0.05) reductions in brain cholinesterase on Day 90. (Supported by Contract NO1-S1-95653-03 from the NTP.)

This study was done to determine if the greater sensitivity of cows to DYFONATE was due to sensitivity of the target acetylcholinesterase (ACHE) to inhibition by DYFONATE-oxon (DX). Brain homogenates and erythrocytes (RBC) lysates from cows and male and female rats were compared to determine in vitro sensitivities to inhibition by DX, paraaxon (PX), and malaoxon (MX). The 60 min. IC50's for male rat brain were 0.93, 0.53 and 3.65 x 10^{-5} M for DX, PX, and MX, respectively. Similar values were obtained for female rats, but the corresponding values for cow brain were 1.92, 1.66, and 6.36 x 10^{-5} M. Kinetic constants for ACHE inhibition also indicated that cow brain ACHE was less sensitive than rat brain ACHE to inhibition. Results of IC50 studies with RBC's were in excellent agreement with results of the brain ACHE studies. Results indicated that DX was equal to or slightly lower in potency than PX and more potent than MX. In general, ACHE from brain or erythrocytes of cows was less sensitive to in vitro inhibition by organophosphates than was that from male or female rats. Thus, the greater susceptibility of cows to DYFONATE, in vivo, cannot be explained on the basis of a greater target enzyme (ACHE) sensitivity to inhibition by DX.

TOLERANCE DEVELOPMENT TO THE CHOLINERGIC TOXICITY OF MALATHION IN CD-1 MICE. P.M. Bartholomew, G. Glanvilles and S.D. Cohen, Toxicology Program, Sch. of Pharm., Univ. of Conn., Storrs, CT 06268.

Tolerance to various organophosphate cholinesterase (CHE) inhibitors (OP) has been reported. OP tolerance induction regimens require prolonged CHE inhibition. The ability of malathion (MT) to induce tolerance was in doubt due to the reversibility of MT's tissue esterase inhibition. In this study, brain CHE activity was 40 and 83% of control 2 and 24 hours after 400 mg/kg MT, i.p. and 23 and 48% of control 2 and 24 hours after a second daily dose. Other tissue esterase activities were similarly inhibited. At 24 hours after 14 daily doses of MT, brain CHE was 30% of control. During the initial course of the dosing regimen, body weights declined but growth rates appeared normal during the 2nd week. There was a decrease in the observable signs of cholinergic toxicity during this period. In addition, 24 hours after the final MT dose, survival after an LD50 challenge with the cholinomimetic carbachol (4.2 mg/kg, i.p.) was greater in the MT-dosed group (60%), 18/30 mice versus controls (17%, 5/30 mice). These data support the conclusion that repeated dosing with MT induced tolerance to the cholinergic toxicity of this compound.

(Supported, in part, by fellowships from Procter and Gamble, Inc., Sandoz, Inc. and Richardson-Vicks, Inc. and USAMRDC DAMDL-84-C-4151.)

TISSUE DIFFERENCES IN CHOLINESTERASE INHIBITION AND MUSCARINIC RECEPTOR CHANGES IN CD-1 MICE MADE TOLERANT TO MALATHION. P.M. Bartholomew, G. Glanvilles and S.D. Cohen, Toxicology Program, Sch. of Pharm., Univ. of Conn., Storrs, CT 06268.

The lethality of organophosphate cholinesterase (CHE) inhibitors (OP) is thought to result from depression of the respiratory center in the brain stem, constriction of and increased secretion by the airways and paralysis of the respiratory musculature. While tolerance to the cholinergic toxicity of OP's has been well documented, no investigations of the brain stem and extra pulmonary airways have been conducted. We have recently demonstrated tolerance to the insecticide malathion. At 24 hours after 14 daily doses of malathion (400 mg/kg, i.p.), CHE activities were 27, 25 and 37% of control in striatum (ST), hippocampus (HI) and cortex (CX), respectively. Brain stem CHE was inhibited only 50%. In addition, the numbers of muscarinic receptors (Bmax) decreased 36, 19, 16 and 40% in ST, HI, CX and trachea, respectively. There was no change in brain stem Bmax. The lack of parallelism between CHE inhibition and Bmax effects in brain stem as compared to all other tissues tested raises questions as to the mechanism(s) by which the respiratory system adapts to OP's.

(Supported, in part, by fellowships from Procter and Gamble, Inc., Sandoz, Inc. and Richardson-Vicks, Inc. and USAMRDC DAMDL-84-C-4151.)

THE PROTECTIVE ACTION OF GLUTATHIONE ON THE EFFECTS OF DIETHYLTHIOCARBAMATE (DDC) ON ASTROCYTES IN CULTURE. M. Toulon, L.D. Trombetta, I. Jamail Department of Pharmaceutical Sciences, St. John's University, Jamaica, N.Y. Sponsor: S. Carson

Cultured rat astrocytes, in the log phase of growth, exposed for 1 hour to 38ig DDC/ml of medium showed severe cytology after a 24 hour period. More than 50% of the DDC-treated cells detached from the culture flasks and the remaining adherent cells appeared to lose their processes. DDC-treated cells were unable to survive further passage. Cells were again treated with DDC for 1 hour, washed and selected groups were refed medium containing 0,1,10, and 100mM of glutathione (GSH). Cells post-treated with 1mM of GSH appeared similar to cells treated with DDC alone. Cells post-treated with 100mM of GSH remained attached to the culture flasks but appeared retracted from one another. Cells post-treated with 10mM GSH appeared similar to normal controls and easily survived further passage. Electron microscopy of the 10mM GSH post-treated cells revealed numerous lysosomal inclusions and mitochondrial changes. Cilia filamentous material and microtubules appeared normal. Results indicated that GSH prevented the lethal effects of DDC.
TREMOR AND HYPERTHERMIA PRODUCED BY p,p'-DDT ARE ASSOCIATED WITH DOSE- AND TIME-RELATED CHANGES IN BRAIN BIOGENIC AMINE AND AMINO ACID LEVELS IN RATS
H.A. Tilson, P.M. Hudson, P.H. Chen, and J.S. Hong.
Laboratory of Behavioral and Neurological Toxicology, N.I.E.H.S., Research Triangle Park, NC

Rats were given various doses of p,p'-DDT by gavage and spectral analysis of motor movement and rectal temperature were measured prior to sacrifice. p,p'-DDT (50, 75 and 100 mg/kg) produced dose-related increases in temperature 12 hrs later; tremor was seen at 25 to 100 mg/kg. Dose-related increases in MHPG (75 to 100 mg/kg) and 5-HIAA (50 to 100 mg/kg) were seen in the hypothalamus (HYP) and brain stem (BS); striatal (STRA) and hippocampal (HPC) 5-HIAA (50 to 100 mg/kg) and DOPAC (75 to 100 mg/kg) were also increased. Six amino acids were assayed in the BS, HYP, and STR; aspartate (ASP) and glutamate (GLU) were increased in the BS (25 to 100 mg/kg); no consistent effects on taurine, glutamine, glycine or GABA were seen. A time course study of the effects of 75 mg/kg found that the onset (5hrs) and time of peak effect (12hrs) were associated with increases in MHPG in the HYP and BS; increases in SHIAA in the BS, HYP and hippocampus (HPC) were also seen. HYP-DOPAC was increased 12hrs postdose. ASP and GLU in the BS, but not HYP or HPC were increased; GABA in the BS and HPC were increased. Various doses of o,p'-DDT (25 to 100 mg/kg, 12hr postdose) or Mirex (75 mg/kg, 24hr) had no effect on biogenenic amines, amino acids, tremor or temperature.

STUDIES ON THE BIOACTIVATION OF THE SELECTIVE NIGROSTRIATAL TOXIN 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)

MPTP is metabolized by rat brain mitochondria to the corresponding pyridinium species (MPP) in a process which is blocked by type B MAO inhibitors (Chiba et al. Biochem. Biophys. Res. Commun. 120:574, 1984). Suspecting that this 4-electron oxidative transformation proceeds by two 2-electron steps, we synthesized the predicted 2,3-dihydroxyphenylpyridinium intermediate MPDP and showed that its chromatographic and diode array UV spectral characteristics were identical to those of a polaron metabolite of MPTP. As expected, sodium cyanide trapped the dihydroxyphenylpyridinium intermediate as a mono-cyano adduct. An examination of the rate of formation and disappearance of MPDP and MPP showed MPDP to be an obligatory intermediate in the biotransformation of MPTP to MPDP. MPDP undergoes rapid disproportionation to MPTP and MPP in mitochondrial incubation mixtures. Since this reaction is not inhibited by pargyline only the conversion of MPTP to MPDP appears to be catalyzed by MAO. The possible interaction of MPDP/MPP with dopamine to form cytotoxic products will be discussed.

(Work this is supported by NIH Grant #CA 3405-01)

IMPAIRMENT IN BIOCHEMICAL PROCESSES IN RAT BRAIN MITOCHONDRIA INDUCED BY CHLORPHENERINE
L. Zychlinski and M.R. Montgomery. Colleges of Medicine and Public Health, University of South Florida and VA Hospital, Tampa, FL

The effects of chlorphenerine (CP) on bioenergetics and monoamine oxidase (MAO) activity in rat brain mitochondria were examined in vitro. Oxidation rates of glutamate and succinate were investigated in the presence of CP (0.1-5.0 mM). With 0.1-1.0 mM CP, respiratory control ratio (RCR) and ADF/O ratio were decreased and state 4 respiration stimulated. State 3 respiration and uncoupled state were unaffected. CP also increased respiration in state 3 that had been inhibited previously by oligomycin. These data indicate that CP is an uncoupler of oxidative phosphorylation. In the presence of higher concentrations of CP (2.0-5.0 mM), respiration in states 4,3, and in uncoupled state, as well as RCR and ADF/O, were decreased. Oxidation of norepinephrine, serotonin, octopamine, tyramine and dopamine by MAO, an enzyme marker of the outer mitochondrial membrane, was inhibited by 0.01-0.1 mM CP. Tryptamine and benzylamine oxidation was unaffected. Examination of serotonin oxidation kinetics in the absence and presence of CP (0.01-0.1 mM) indicated that both the Vmax and Km were affected. CP is an inhibitor of brain mitochondrial MAO with mixed type inhibition. These combined data show CP affects biochemical processes in both inner and outer mitochondrial membranes. Supported by VA Medical Research Funds and NIH Grant ESO2846. 198
Reserpine depletes biogenic amines and thus may induce postnatal changes in the functional development of monoaminergic systems. Neurotransmitter (NT) receptor binding and monoamine concentrations were measured in rats prenatally exposed to either 0, 0.275, 1.50 mg/kg/day reserpine s.c. on days 12-15 of gestation. Brains from pups on postnatal day (PND) 1 and 8 were bisected, while regional areas were dissected from PND 21 brains for analyses.

Prenatal reserpine treatment produced both age- and sex-related alterations in dopamine (DA) and serotonin (5-HT) receptor binding. On PND 1, DA receptor binding was decreased in both low and high dose females (by 35 and 59%, respectively). Reserpine treated offspring of both sexes showed a slight reduction in 5-HT receptor binding. On PND 8, 5-HT receptor binding was further reduced in male pups but a 50% increase in DA receptor and a slight increase in 5-HT receptor binding was seen in females. On PND 21, DA receptor binding was decreased, DA concentrations decreased and DOPAC concentrations increased in caudate nucleus in both a dose and sex-specific manner. Thus, prenatal reserpine exposure appears to result in a complex pattern of postnatal neurochemical alterations that may mediate several behavioral changes noted in these offspring.

It is hypothesized that conversion of amino groups to alkyler pyrrole adducts in neurofilament (NF) or other axonal proteins may represent the critical initiating event in β-diketone neuropathy. This investigation was designed to examine CNS axonal cytoskeletal proteins from rats receiving 0.5% 2,5-hexanediol (2,5-HD) in the drinking water for up to 8 weeks. 2,5-Dimethylpyrrole adduct was detected in serum and axonal proteins during 2,5-HD exposure, reaching plateau levels by 2 weeks and by 5 weeks, respectively. Conversion of approximately 1% of lysine ε-amino groups in axonal protein was observed after 2 weeks. Gel electrophoresis revealed discrete new protein bands in CNS axonal preparations from treated animals, along with high-MN, non-migrating protein probably derived from NF protein. Concentration of this non-migrating material increased progressively with exposure. Partial reversal of these changes was observed 9 weeks after cessation of dosing. In vitro incubation of axonal protein (pH 7.2, 37°C) with 2,5-HD resulted in the formation of extra bands and high-MN protein identical to that seen in vivo. These findings provide evidence for pyrrole formation and possible covalent crosslinking in CNS axonal cytoskeletal proteins from 2,5-HD-treated rats. (Supported by NIH/DCD grant OH-01972)

Lack of neuropathological changes after exposure to butyl benzyl phthalate. E.C. Robinson, F.R. Johannsen, and D.K. Branch, Monsanto Company, St. Louis, MO.

While phthalate esters have been alleged to have neurotoxic effects on workers, concurrent exposure to numerous substances confounds interpretation of the data. Butyl benzyl phthalate (SANTICIZER® 160 plasticizer) was fed to 3 groups of 10 male and 10 female Sprague-Dawley rats for 6 weeks at target doses of 500, 1500 and 3000 mg/kg/day. Control groups of 6 males and 6 females received untreated diets for the same period. At the end of 6 weeks, half of the rats in each group were killed and examined grossly. The remainder were shipped to another facility where they were examined grossly and perfused with fixative, followed by removal of selected nervous tissue and their examination by light microscopy. Body weight gains were decreased at the 1500 and 3000 mg/kg/day levels in both sexes. Hindlimb stiffness was noted at the 3000 mg/kg/day level, and was more prevalent in males. The affected high dose rats withdrawn from test material before shipping appeared clinically normal on examination the next day. Microscopic examination of central and peripheral nervous system tissues did not reveal any compound-related pathological changes. In summary, high doses of butyl benzyl phthalate appear to produce reversibility of hindlimb stiffness that is unassociated with any detectable morphological changes in the nervous system.

Sickles DW, Goldstein BC and Pearson JK, Department of Anatomy and Pharmacology and Toxicology Time course and specificity of acrylamide inhibition of oxidative enzymes. Acrylamide (ACR) is a neurotoxic chemical which produces a central-peripheral distal axonopathy. We are investigating the hypothesis that inhibition of oxidative enzymes involved in energy transformations is responsible for producing the neuropathy. We have previously shown that chronic (5 and 10 day) ACR injections decreased NADH-TR activity in soleus (SOL) motor neurons (MN) which normally have high activity of this enzyme. We have used an in vivo quantitative histochemical assay to show that 30 minutes to one hour after a single 50mg/kg dose of ACR, this enzyme activity is decreased from 0.425 (control 0.66) to 0.345 and 0.273, respectively. Tensor fascia lata MN were unaffected. Both MN pools showed a short term compensatory increase in activity (6-12 hours); the TFL MN enzyme activity remaining elevated over 96 hours, the SOL returning to normal levels at 72 hours. Equimolar concentration of the nonneurotoxic analogue methylene bis ACR decreased SOL MN NADH-TR activity to 0.406 as compared to 0.273 for ACR. Further studies showed cytochrome c reductase and 1 lipoprotein dehydrogenase activities significantly reduced when exposed to 3-5 mM ACR. Therefore, ACR causes a significant, rapid and specific inhibition of certain oxidative enzymes. Supported by OH02020 and NS 18664.

Six groups of 9 Sprague-Dawley rats were fed for 60 days a diet containing 0.1% HCB and 0.5, 1.5 and 4.5% Ca++ with 2,200 U.S.P. units of vitamin D/kg rat for periods of 1, 2 and 3 and 0.5% Ca++ with 8,800, 35,200 and 160,000 U.S.P. units of vitamin D/kg rat for groups 4, 3 and 5, respectively. Body weights, mortality, excretion of total urinary porphyrins and serum thyroid hormones were monitored for 110 days. Then, liver microsomal enzyme levels (cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase, benzo[a]pyrene hydroxylase, ethoxyresorufin-o-de-ethylase, aminopyrine N-demethylase and UDP-glucuronosyltransferase) were determined. Both Ca++ and vitamin D increased the induced mortality, but delayed the onset of porphyrin in survivors of the various groups. Levels of thyroxine (T4) but not triiodothyronine (T3) were reduced below detection limit by HCB. Enzyme induction was independent of Ca or vitamin D status. These results show that toxicity of HCB is thyroid hormone, Ca++ and vitamin D dependent, whereas liver microsomal enzyme induction is not. Thus, causative correlations between toxicity and enzyme induction by halogenated aromatic hydrocarbons should be viewed with reservations.

979 REDUCED HEPATIC LOW DENSITY LIPOPROTEIN BINDING AND ADIPOSE LIPOPROTEIN ACTIVITY IN RABBITS TREATED WITH 2,3,7,8-TCHELOROBENZENE (HCB).

D. W. Bombeck, D. W. Brewer, and F. Matsumura. Pesticide Research Center, Michigan State University, East Lansing, MI

Hepatic low density lipoprotein (LDL) binding and adipose lipoprotein lipase (LPL) activity were examined in hepatocyte and adipocyte preparations from rabbits administered HCB. The reduction in these parameters may explain the hyperlipidemia in treated rabbits since they are key events in regulating serum lipids and, we have shown that LDL binding and LPL activity is reduced in HCB treated guinea pigs, an animal which has a similar TCB-induced hyperlipidemia as rabbits. Rabbits administered single i.p. dose (50 g/kg) of HCB after 10 days exhibited an approximate 50% reduction in hepatocyte LDL binding compared to paired controls. At the same dose and time regimen adipose LPL activity was barely detectable. In addition, electron micrographs of arterial endothelium from HCB-treated rabbits show a loss of integrity which may result from the increased serum lipoprotein levels. These findings may have human health implications since the rabbit is regarded as a good model for investigating hyperlipidemia in humans.


(Supported by NIEHS grant no. ES01963, USDA contract 59-3244 427, and MI Agricultural Experiment Station Environmental Toxicology Program)

979 ROLE OF THYROID HORMONES IN THE TOXICITY OF 2,3,7,8-TCHELOROBENZENE (HCB). K. Rozman, H. Greim, T. Pazdernik and A. Parkinson. Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS and Abt. für Toxikologie, Gesellschaft für Strahlen- und Umweltforschung mbH München, Neuberger, F.R.G.

The effect of 100 µg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) I.p. on indices of toxicity (lethality, body weight, food intake and immunosuppression) and liver microsomal enzyme induction (cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase, benzo[a]pyrene hydroxylase, ethoxyresorufin-o-de-ethylase, UDP-glucuronosyltransferase) was determined in mothyroidecomized, thyroidectomized, thyroxine (T3)-treated thyroidectomized and triiodothyronine (T3)-treated thyroidectomized rats. Thyroidectomy provided protection from TCDD induced lethality, body weight loss, food intake reduction and immunosuppression. Administration of both T3 and T3 restored toxicity to control (mothyroidecomized) levels. Enzyme induction was independent of thyroid status. It is concluded, therefore, that although the toxicity of several classes of halogenated hydrocarbons correlates well with their ability to induce benzo[a]pyrene hydroxylase and UDP-glucuronosyltransferase activity, toxicity and microsomal enzyme induction are differentially affected in rats by changes in thyroid status. Thus, liver microsomal enzyme induction probably should not be viewed as a causative indicator of the toxic action of TCDD.
801 EFFECT OF COLD EXPOSURE ON 2,3,7,8-TCDD AND DIOXIN (TCDD) TOXICITY. K. Rozman, Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS and Abt. für Toxikologie, Gesellschaft für Strahlen- und Umweltforschung mbH, Muenchen, Neuberger, F.R.G.

Rats were adapted to 4°C and the effect of TCDD (60 or 100 µg/kg i.p.) on mortality and mean time to death as well as on the time course of body weight, food intake, rectal temperature, serum thyroxine (T₄), serum triiodothyronine (T₃), oxygen consumption of whole animals and selected tissues (brown adipose tissue, heart, muscle, kidney, liver) was compared to rats maintained at 25°C. Cold exposure increased mortality and decreased mean time to death. Body weight loss was significantly faster at 4°C than 25°C, but food intake was reduced by TCDD to about the same extent at both temperatures. Rectal temperature was significantly reduced by TCDD only in the cold exposed rats. Hypothermia started at about 3 days after dosing and persisted in non-survivors of TCDD, while the body temperature of survivors returned to normal levels after 24 to 48 hrs. Similarly, oxygen consumption of whole animals as well as of tissues was impaired in non-survivors of TCDD only. The onset of reduced oxygen consumption occurred at about 3 days after dosing. Cold exposure increased serum thyroid hormone levels and TCDD led to reduced T₄ but not T₃. Serum levels within 4 days after dosing in rats maintained at either ambient temperature. Data support the view that TCDD's primary toxic effect is an impairment of energy metabolism.

802 TERATOGENICITY OF TCDD IN MICE: ENHANCEMENT BY HYDROCORTISONE. L.S. Birnbaum, M.W. Harris, C.P. Miller, R.C. Pratt, and J.C. Lamb, NIEHS, Research Triangle Park, NC

TCDD is a potent teratogen in mice causing hydronephrosis (HN) and cleft palate (CP) at doses below that resulting in maternal or fetal toxicity. Adrenocortical steroids such as hydrocortisone (HC) can also cause CP. The purpose of this study was to examine the interaction of TCDD and HC in the induction of fetal malformations. Pregnant C57BL/6J mice were treated on gestation days 10-13 with TCDD (0, 3 µg/kg, po), HC (0.25, 50, 100 mg/kg HC, sc), or a combination of TCDD and HC. The dams were sacrificed on d.18 and analyzed for maternal and fetal toxicity and soft tissue malformations. TCDD alone had no effect on litter size, fetal mortality or weight, or maternal weight gain. Although this dose failed to produce CP, all TCDD-treated fetuses had HN, the most sensitive indicator of TCDD teratogenicity. HC alone caused dose-related decreases in fetal weight and maternal liver/body weight ratios, and dose-related increases in CP (0.5, 10, 30%). No effects of HC were seen on litter size or fetal mortality, but maternal weights were affected. Combination of all doses of HC with TCDD resulted in a 100% incidence of CP. This was accompanied by a HC dose-related decrease in litter size and fetal weight, and an increase in fetal mortality. TCDD tended to reverse the decrease in liver/body weight ratio seen with HC alone. The mechanism of this synergism is under investigation.


Evidence is accumulating that thyroid hormones (TH) play a role in TCDD toxicity. Recent biological studies combined with theoretical modeling work (this laboratory) have suggested that TCDD is a thyroxine (T₄) agonist. TCDD treatment results in a depletion of T₄ in rat tissues. We modified existing methods for isolating solubilized nuclear receptors for T₄ in rat liver and demonstrated competition for specific T₄ binding sites by soluble, polar adenosine derivatives of dioxin and furan. The binding results were similar to those found earlier (McKinney et al., J. Med. Chem. In press) for thyroxine binding prealbumin, a model for the nuclear receptor. These binding events require lateral halogenation also a requirement for toxicity. We also observed an increase in the nuclear T₄ receptor levels in liver from TCDD treated rats. A similar modulation of nuclear receptors by TH has been observed previously. In separate studies, polar derivatives shown to compete for T₄ specific nuclear sites were also shown to antagonize TCDD immunotoxicity in cell culture systems. TH activity mediated by nuclear receptors can regulate gene expression. We propose that dioxin toxicity is the expression of potent and persistent TH activity.

804 MEMBRANE STRUCTURAL/FUNCTIONAL PERTURBATIONS INDUCED BY GOSSYPOL. R.D. Sauerheber, Rees-Steady Research Foundation; P.A. Hylas, Scripps Clinic and Research Foundation; A. de Peyster, San Diego State University, San Diego, CA Sponsor: H.-W. Leung

Gossypol, a constituent of cotton plants proposed for use as a male contraceptive and anticancer drug, was examined for its effects on membrane order and functional properties. ESR studies of natural membranes and phosphatidyl choline (PC) vesicles labeled with the 5-nitroxide stearic acid spin probe showed that 0.05-0.4 mM gossypol causes a dose-dependent ordering or 'condensing' of membrane lipid, reflected by an increase in the order parameter (S). Spectrophotometric studies of PC vesicles showed that gossypol dramatically increases the permeability of the phospholipid bilayer to glycerol when incubated at a 1:10 gossypol/PC ratio.

Basal and insulin-stimulated 2-deoxy-D-glucose transport is also inhibited in isolated rat adipocytes incubated with gossypol at 37°C. Half maximal inhibition occurs at approximately 0.2 mM gossypol for glucose uptake in both the presence and absence of 40 mg/ml insulin. Microscopic examination (40X) confirmed that this inhibition is not due to cell swelling.

We suggest that perturbation of lipid regions of the plasma membrane may mediate gossypol's toxic and pharmacologic action on various cellular processes. Cells with a high glucose requirement may be preferentially affected by gossypol.
807 THE EFFECT OF SODIUM THIOUSULFATE (ST) AND ALPHA ADRENERGIC ANTAGONISTS ON PLASMA THIOCYANATE (SCN). J.C. Patterson and S.D. Cohen, Univ. CT, Toxicology Program, Sch. Pharm., Storrs, CT.

ST antagonizes cyanide (CN) by accelerating its conversion to SCN. Several alpha adrenergic antagonists have been reported to protect against CN poisoning. Chlorpromazine (CPZ) and phenoxybenzamine (POZ) have been reported to potentiate the anti-cyanide action of ST, but when administered alone they are of very limited value. Phenolamine (PDA) has also been reported to protect against CN poisoning. This study was undertaken to investigate the effect of these agents on SCN formation in CN-challenged mice. Male C57 mice were pretreated with POZ, PDA, CPZ and/or ST and challenged with KCN 4 or 20 mg/kg. Plasma SCN concentrations, 10 min after 4 mg/kg KCN, in ST, POZ/ST, PDA/ST and CPZ/ST pretreated mice were 170, 126, 199, and 131.6 μmole/l, respectively. Plasma SCN concentrations, 10 min after 20 mg/kg KCN, in ST, POZ/ST, PDA/ST and CPZ/ST pretreated mice were 339, 366, 370, and 301, respectively. At 4 mg/kg KCN, POZ/ST and CPZ/ST pretreated mice had lower SCN concentrations as compared to ST alone. These results, and the lack of a difference in effects of the pretreatments on plasma SCN at the 20 mg/kg dose, indicate that the anti-cyanide action of alpha adrenergic antagonists cannot be explained by enhancement of SCN formation. (JCP was supported by a Stauffer Chemical Co. Fellowship in Toxicology.)

806 PITUITARY AND THYROID FUNCTION IN RATS AFTER PERINATAL THIOCYANATE EXPOSURE. W. Heydens, Monsanto Co., Environmental Health Laboratory, St. Louis, MO; R. Hartung, W. Schramm and N. Khalessi, The University of Michigan, Ann Arbor, MI.

Thiocyanate (SCN) is present in tobacco smoke, edible plants and agricultural and industrial products. It is also the major metabolite of cyanide and some nitriles. This compound has antithyroid activity in adults, but the effects of SCN in developing organisms have not been extensively studied. Therefore, an investigation was undertaken in rats to determine the effects of SCN on postnatal pituitary and thyroid function. Thiocyanate exposure during gestation did not significantly alter postnatal serum levels of pituitary and thyroid hormones. However, continued thiocyanate administration throughout lactation resulted in significant hormone changes after the compound was withdrawn. On the 56th postnatal day, serum thyroid hormones were elevated and thyrotropin was decreased. By Day 91, thyrotropin was depressed even further, and thyroid hormones returned to normal in most animals. The observed hyperthyroidism may have resulted from: (1) hypersensitivity of the thyroid to thyrotropin; and (2) insufficient suppression of thyrotropin secretion by the pituitary in response to elevated thyroid hormone concentrations.

(Supported in part by the NIH grants ES-00040 and ES-00210)

808 EFFECT OF SOMAN ON AMINOPHOSPHOLIPID (APL) DISTRIBUTION IN ELECTROPLAX (E); USE OF 2,4,6-TRINITROENZYMESULPHONIC ACID (TNBS). B.A. Weber and P. Rosenberg, Univ. of Conn., Toxicology Program, Sch. of Pharm., Storrs, CT. Sponsor: S.D. Cohen.

The membranal effects of organophosphorus compounds that are independent of, or triggered by, initial cholinesterase (ChE) inhibition, are not well characterized. TNBS-labeling of phosphatidylethanolamine (PE) and phosphatidylserine (PS) was used to monitor alterations in membrane organization. Isolated single E from Electrophorus electricus were exposed to 0.1 mM soman for 30 min and then to 0.5 mM TNBS under conditions in which TNBS was impermeable. The non-internalized (NI) and internalized surfaces (I) of E were separated and fractionated into plasma membrane (PM) and mitochondrial membrane (M) fractions. ChE activity, total lipid phosphorus (TPL), phospholipid (PL) distribution, and TNBS-labeled APL were measured. ChE was inhibited 100% in both I and NI crude homogenates. TPL and PL distributions in both I and NI crude homogenates, PM and M fractions were not changed after soman exposure, however, TNBS-labeling of PE (30%) was increased (40%) in the I PM fraction. PS was not labeled in control or soman treated E. In contrast to the I PM fraction, the labeling of PE in the NI PM fraction was not changed by soman. These data suggest that soman may alter PE distribution within the I PM. (Supported by DAMD 17-62-C-2066)
In vitro incubation of DCCP with hepatic microsomes has been reported to result in formation of chemically reactive metabolites, and DCCP has been reported to be mutagenic in S. typhimurium only in the presence of S-9 activation. In this study the rat hepatic microsomal metabolism of 14C-DCCP has been investigated in vitro by determination of free bromide (Br\(^-\)) and protein bound 14C. Both parameters were increased in the presence of oxygen (O\(_2\)) or an NADPH generating system, and were decreased by the addition of metyrapone or dimethylformamide. Phenobarbital (PB) pretreatment increased both parameters while 3-methylcholanthrene had no effect. The pH maxima of the two parameters were different, and ethanol inhibited Br\(^-\) release with little effect on 14C binding. PB, but not control microsomes, were able to metabolize DCCP in the absence of O\(_2\) if NADPH was present. The addition of 3,3,3-trichloropropene oxide did not increase 14C binding suggesting the absence of a reactive epoxide metabolite. Glutathione (GSH) inhibited 14C binding to protein by 95% and increased Br\(^-\) release in both PB and control microsomes. These data suggest that the hepatic microsomal metabolism of DCCP involves the PB inducible form of cytochrome P-450, and may also involve other, as yet unidentified, enzyme activities.

810 NITROSAMINES IN TOBACCO SMOKE, DIMETHYLNITROSAMINE AND N-NITROSOPYRROLIDINE, AND THEIR EFFECTS ON CELL INJURY. B. V. R. Sastry, J. H. Patton, M. J. Surber and E. J. Landon, Department of Pharmacology, Vanderbilt University, Nashville, TN

Dimethylnitrosamine (DMNA) and N-nitrosopyrrolidine (NNP) represent 90% of total volatile nitrosamines (VNA) in both mainstream and sidestream cigarette smoke. They cause cell injury. Increase in intracellular Ca\(^++\) is a potential mechanism for tissue injury. Therefore, the effects of DMNA and NNP on the rat liver Ca\(^++\) levels, cell necrosis and membrane microviscosity (\(\eta\)) were studied. Rats received DMNA (26 mmol/kg) or NNP (10-26 mmol/kg) orally and were sacrificed 24 hours later by decapitation. Liver homogenates, heavy and light microsomes and histological sections of the treated and untreated rats were prepared. Total free Ca\(^++\) in the homogenate and \(\eta\) of microsomal membranes were measured by fluorescence polarization. Centrilobular necrosis was graded from 0-4 from the histological sections. Both DMNA and NNP (26 mmol/kg) increased Ca\(^++\) levels (DMNA, 22 times; NNP, 7 times) and centrilobular necrosis (Control, 0; DMNA, 2.6; NNP, 1.5). They also decreased \(\eta\) of heavy (DMNA, 41%; NNP, 48%) and light microsomal (DMNA, 14%; NNP, 29%) membranes. These observations indicate that DMNA is more potent than NNP in increasing Ca\(^++\) levels, causing cell necrosis and altering membrane fluidity than NNP. (Supported by grants from The Council for Tobacco Research, U.S.A., Inc., and US PHS NIH ES-03172 and ES-02504.)


Previous studies from this laboratory have revealed that following iv administration of [2\(^\text{H}\)]N-nitrosodimethylaniline ([14C]NDA) to mice, radioactivity was retained in liver, and in nasal, bronchial, esophageal and salivary duct epithelium. In an attempt to elucidate the nature of this retention of radioactivity, adult, male, C57BL/6J mice were injected iv with 5 µCi of [14C]NDA (0.60 mg/kg) and sacrificed 24 hr later by cervical dislocation. Small pieces of liver, esophagus, nose, lung and salivary gland were removed and fixed in 10% formalin. Routine paraffin sections were prepared from each tissue. Prior to coating these sections with Kodak NTB2 emulsion, they were deparaffinized, dipped through a series of solvents and stained with H and E. Since this technique removes all soluble radioactivity, silver grains visible on the slides represent covalently-bound material. Heavy labeling of bronchial epithelium was observed; silver grains appeared to be most intense over nuclear and plasma membranes of these cells. Specific localizations were seen in salivary gland and esophagus; only epithelial cells were labeled in these tissues. These results are consistent with metabolism to the proximal, reactive carcinogen within the epithelial cells. (Supported by Pharmacorn Research Foundation, Inc.)

812 A STUDY OF THE TRANSPORTABILITY OF HYDROXY-ALKYL-NITROSAMINES INTO INTACT LIVER NUCLEI. B. Gold and L. Hines, Eppliy Institute for Research in Cancer and Department of Bio-medical Chemistry, University of Nebraska Medical Center, Omaha, NE

The metabolism of N-nitrosamines into reactive electrophiles is mediated by hydroxylation of the carbon adjacent to the N-nitroso moiety. The initial metabolism is thought to take place in the cytoplasm and therefore the hydroxylated nitrosamines must be sufficiently stable to diffuse through the cytoplasm and penetrate the nuclear envelope before decomposing. The transportability of the hydroxy compounds has been evaluated using covalent binding to nuclear DNA of intact rat liver nuclei as an endpoint, after incubation with the acetoxyl derivatives of N-nitrosopyrrolidine (NP) and N-nitrosodi-methylamine (DMNA). These esters are rapidly hydrolyzed to the hydroxy compounds by base or esterase catalysis. Intact nuclei are high in esterase activity, which can be inhibited by disopropyl fluorophosphate. The hydroxylated derivative of DMNA was able to efficiently penetrate the nuclear envelope regardless of where it was generated. However, the NP derivative gave significant binding only when it was generated directly on the nuclear envelope. (Supported by NIH Grant R01 CA29088)
Glutaraldehyde (GA), a protein cross-linker and biocide, has a potential for human exposure. The acute toxicity and irritancy of aqueous solutions has been studied. Rat peroral LD50 values (with 95% confidence limits) in ml/kg are: 50% (w/w) GA, 1.30 (0.87-1.94): 25%, 1.87 (1.26-2.76): 10%, 1.62 (1.01-2.62): 5%, 3.3 (2.4-4.4): 1%, 12.3 (9.1-16.7). Rabbit percutaneous LD50 values (ml/kg) are as follows for 24-hour occlusion: 50%, 1.59 (0.70-3.59): 25%, 8.0 (1.9-33.5): 5%, >16.0. Single exposures (6-9 hr) of rats to statically degenerated saturated vapor atmospheres produced only signs of sensory irritation to the eyes and respiratory tract. Measurements indicated an initial GA concentration of 11 ppm, decreasing to 2 ppm at 6 hr, and an average of 4.3 ±3.4 (SE) ppm. The 4-hr LC50 values for dynamically-generated GA vapor to rats were 24 (17-33) ppm for males, and 40 (15-105) ppm for females. GA solutions of >5% caused severe conjunctivitis and corneal injury. The no-effect concentration for corneal injury was 0.5%, and for conjunctivitis was 0.1%. A 4-hr occluded contact on intact rabbit skin produced severe local inflammation and punctate necrosis with solutions of 25% and above. A concentration-related decrease of dermal inflammation occurred below 25%, with a threshold for erythema at 1% GA.

The stability of Acroclor 1254-induced rat liver S-9 over a 5 year period was investigated in a retrospective study. Samples of S-9 were prepared by a standard procedure at 6 month intervals and stored at -75°C for periods ranging from 6 months to 5 years. Protein and cytochrome P-450 content and aryl hydrocarbon hydroxylase activities for these samples were very similar. A two-fold variation in ethoxyresoruvin O-deethylase or aniline hydroxylase activities among the samples was not attributable to duration of storage, but may reflect variability in enzyme induction among these S-9 preparations. Ames testing (TA93) indicated that frozen storage for 5 years had no effect on the ability of the S-9 to activate 2-aminoanthracene to its mutagenic metabolite(s). However, a 50% decrease in the mutagenic response to benzo(a)pyrene (BP) was observed when using S-9 stored for 5 years. Decreases (>10%) in the mutagenic response to BP were evident when using S-9 that had been stored for 1.5 years or longer, but only when the concentration of S-9 in the assay was lowered. Therefore, the maximum duration of frozen storage that still maintains S-9 suitable for use in genotoxicity assays may vary, depending on the concentration of S-9 and the test compound to be used. We suggest that S-9 may be stored at -75°C for at least one year for use in in vitro genotoxicity assays.

The inhibition of protein synthesis by diethylmaleate (DEM) depletes glutathione (GSH) from various tissues. DEM, however, has been shown to possess other actions, some of which are a consequence of, and some of which are separate from, GSH depletion. We found that DEM, at the dose of 1 ml/kg, commonly used in toxicological studies, inhibited protein synthesis in brain and liver of mice by 25-30%. In vivo protein synthesis was measured as the incorporation of [3H]-valine in TCA-precipitable material. This dose of DEM decreased GSH levels in brain and liver by 40 and 80%, respectively. At an ambient temperature of 22°C DEM also caused a dose-dependent hypothermic effect. After 1 ml/kg, colonic temperature decreased by 2.9-3.6°C. To determine whether the effect of DEM on protein synthesis was due to hypothermia, mice were kept at an ambient temperature of 35°C. At this temperature hypothermia was reduced to 0.3-1.0°C. However, GSH levels were decreased by 84 and 36% in liver and brain, respectively, and protein synthesis in these two organs was inhibited by 19-23%. The results suggest that inhibition of protein synthesis by DEM is not entirely due to its hypothermic effect and the latter is not related to GSH depletion. However, both effects should be taken into account when DEM is used to modify the metabolism of a chemical by "selectively" depleting GSH in vivo. (Supported in part by grant ES-03624 from NIEHS).

Benzyl chloride (BCI) is extensively used in industry in the manufacture of dyes, perfumes, pharmaceutical products and in the preparation of many organic chemicals. This study was conducted to investigate the kinetics of BCI in rats after intramuscular injection (i.m.). A single dose of 200 mg/kg of the body weight was administered i.m. to Sprague-Dawley rats. The peak plasma level was reached at 15 min with an absorption half-life of 18 minutes. Two hours after administration a rapid elimination of BCI from plasma was observed with a half-life of 20 min (α-phase), while the β-phase of elimination was 30 hr. The distribution of BCI study revealed that approximately 62% of the administered dose localized at the site of administration followed by blood and fat, while it fairly distributed in a uniform manner of a very small concentration throughout pancreas, carcass, liver, stomach, brain, testes, kidney, bone marrow and lung. About 4% of the initial dose was excreted by kidney as benzyl mercapturic acid, meanwhile 1% of the initial dose was excreted as the parent compound through expired air. In vitro study revealed that the incubation of 1 nM BCI with the 9000 mg liver supernatant fraction revealed that approximately 45% of the glutathione was decreased after 15 minutes from the incubation.
Amiodarone (AD) is an antiarrhythmic drug which has been shown to induce pulmonary and hepatic toxicity in humans following chronic administration. The pathological picture resembles a drug-induced phospholipidosis similar to that produced in animals by numerous other cationic amphiphilic drugs (CADs). We administered AD to male Long Evans hooded rats (100 mg/kg daily for 6 weeks) and examined lung and liver for response. Pulmonary alveolar macrophages (AMs) did not accumulate in the alveoli as they do with other CADs. However, the cells did contain a 2-fold increase in total phospholipid, and lamellar inclusion bodies characteristic of the disorder were present. As a measure of AM function, we examined their chumiluminescence response to zymosan particles. No change in this activity was found. Phospholipid levels in liver were increased significantly following AD treatment. The results indicate that daily administration of a high dose of AD to rats leads to the development of phospholipidosis in lung and liver. While one aspect of AM activity appears normal under these circumstances, the functional consequences of the disorder on both organ systems is unclear at present. (Supported by WVU Senate Grant).

The glucocorticoid, dexamethasone (DEX), decreased the lethality of T-2 mycotoxin (T-2) in mice. Efficacy of DEX was accessed by measuring both the mean time to death (MTD) (mean ± S.E., hrs) and LD$_{50}$ (48 hr). The MTD was determined for mice injected with an LD$_{50}$ dose of T-2 (5 mg/kg, sc) and with either saline (CON) or DEX (13 mg/kg, sc). DEX was injected either 1 hr prior to (+), at the same time, or at 1, 2, or 3 hr after (-) exposure to T-2. The MTD for the different DEX injection times were 56.2 ± 5.70, -1 hr; 44.0 ± 5.92, 0 hr; 45.0 ± 5.75, +1 hr; 42.5 ± 5.49, +2 hr; and 42.3 ± 5.99, +3 hr. These times were significantly different (p<0.01) from the CON MTD of 14.4 ± 0.81. The LD$_{50}$ for T-2 was determined at different doses of DEX. When injected at the same time as T-2, DEX, at 0, 0.125, 1.25, and 12.5 mg/kg, gave LD$_{50}$ values of 2.46, 2.77, 3.09, and 4.04 mg/kg, respectively. In conclusion, DEX, given 1 hr prior to or after T-2 exposure, was effective in delaying the time to death and lessening mortality.

The effects of saline perfusion on tissue levels of Zinc (Zn), Iron (Fe), Copper (Cu), and Lead (Pb) were studied in the neonatal rat. On the day of parturition, 4 dams and their litters (8 pups/litter) were assigned to control (C) and Pb-treated groups. On days 6, 9, 12, 15 and 18 postpartum, Pb-treated pups were intragastrically exposed to 50 mg Pb/kg BW. Animals were sacrificed at day 20 and 4 pups from each dam were anesthetized and then perfused with isotonic saline through the left ventricle. The 4 remaining C pups of each litter were not perfused. Liver, kidneys, and brain were removed, weighed, and the concentration of Zn, Fe, Cu, and Pb quantified by atomic absorption spectroscopy. Kidney Pb levels of perfused animals were significantly lower (1.78 ± 0.52 μg/g wet wt.) in comparison to non-perfused controls (3.20 ± 1.04 μg/g). The concentration of Pb in liver (0.78 ± 0.27 vs. 1.31 ± 0.37) and brain (0.33 ± 0.15 vs. 0.52 ± 0.15) was likewise lower in the perfused animals. No effect of perfusion was observed in either the tissue concentration of Zn or Cu, whereas kidney (20.26 ± 4.11 vs. 41.14 ± 15.31) and brain Fe (8.87 ± 1.66 vs. 11.48 ± 1.58) were lowered.

Failure to incorporate this routine procedure may lead to erroneous data.

Tissue citrate accumulation has been described as the hallmark of SFA poisoning presumably by blocking the conversion of citrate to citraconate. We examined the relationship between serum citrate concentrations, tissue ATP and citrate levels in a primary target organ, the heart. ATP and citrate levels were measured in the same heart sample following excision with a pneumatic biopsy drill and rapid freezing. These values were compared with corresponding serum samples which were analyzed for citrate. After oral administration of 3 mg/kg of SFA, groups of male CR/CD rats (N = 4-5) were bled and sampled at 0, 5, 1, 2, 4, and 8 hrs. At 2 hrs after dosing, heart citrate had increased 8-fold and serum citrate, 5-fold, with a 41% decrease in heart ATP concentration. By 8 hrs, heart and serum citrate had increased over control values by 15 and 9-fold respectively; however, ATP depletion began to level-off after 4 hrs with a further decrease of only 9% by 8 hrs. Over the time course tested, there was a highly significant linear correlation (r = .97, p<.001) between serum and heart citrate elevations. Conversely, a significant inverse correlation was found between mean heart ATP and mean serum citrate (r = .93, p<.01) or mean heart citrate (r = .91, p<.05). The use of serum citrate as an indicator of toxicity was further demonstrated in dogs given toxic doses of SFA (100-150 μg/kg IV) or fluorocitrate (2-32mg/kg) where the onset of serious clinical signs was preceded by 2 to 3-fold elevations in serum citrate. In conclusion, these results demonstrate the value of serum citrate as a valid peripheral marker of cis-aconitate inhibition.
Thiourea (TU) is toxic to the lung and thyroid. It is also a suspected animal carcinogen. These effects are believed to be associated with covalent binding to tissue macromolecules. Certain sulphydryl compounds, notably GSH and cysteine, can protect against the toxicity of TU. To characterize the conditions under which TU could undergo tissue binding, rat lung and liver microsomes were incubated with 3H-TU. Little binding was observed with heat-denatured or CO-saturated microsomes, or in the absence of NADPH. Binding increased 2-3 fold when TU was incubated aerobically with active microsomes and NADPH, indicating that TU is likely activated by the cytochrome P-450 monooxygenase enzyme system. Microsomal binding was antagonized by the addition of GSH, cysteine or N-acetylcysteine but not GSSG, suggesting that a reduced sulphydryl group might be needed. However, this was not an absolute necessity, as ascorbic acid could also inhibit binding partially. Similar results were observed with L-4-MU, indicating that binding might involve a thionyl group, possibly through the formation of a protein-bound polysulfide. In another experiment, cysteine decreased binding even when it was added after microsomes were pre-incubated with TU for 30 min, suggesting that microsomal binding to TU could be reversed. It is possible that antioxidants can antagonize TU binding by donating electrons to disrupt the polysulfide linkage.


The rat hindgut flora maintained in vitro under conditions of continuous flow possesses bacteriological and metabolic properties similar to the native bacterial population of the cecum (J. Gen. Microbiol. 1961 123, 103; Appl. Environ. Microbiol. 1983 46, 591). Addition of 75mM sodium cyclamate (Cyc) to this system did not induce Cyc metabolism (sulphamates activity) over periods of 8-10 wk. However, when the concentration of the nutrient medium supplied to the culture was decreased, conversion of Cyc to cyclohexylamine (CHA) was induced within 4 wk, attaining a maximum 8 wk after the start of cyclamate addition (equivalent to 2-3Z molar conversion of Cyc to CHA). During adaption the recovery of viable cells from the culture decreased, although the relative proportions of the major bacterial types remained unchanged. Concurrent with the induction of sulphamates activity, bacterial 6-glucosidase, 6-glucuronidase, nitrate reductase and nitrate reductase activities per 10^11 bacteria were greatly decreased. The ability to convert Cyc to CHA was therefore induced in the hindgut microflora independently of other bacterial enzymes, and was not associated with any gross taxonomic changes.

We thank U.K. Ministry of Agriculture, Fisheries & Food for financial support.

PROBLEMS ASSOCIATED WITH THE USE OF RADIOLabeled DNA PRECURSORS TO ASSESS CHEMICALLY INDUCED DNA DAMAGE AND REPAIR. P. L. Ebbeiro, R. S. Mitra and I. A. Bernstein, Program in Toxicology, Dept. of Env. and Ind. Health, The University of Michigan, Ann Arbor, MI 48109.

Radiolabeled DNA precursors are extensively used in the study of DNA damage and repair. Not much attention has been paid to the contribution of radionuclide precursors to DNA damage. After exposure to 1μM Gd3+ (known to produce DNA single strand breaks) murine L5178Y cells were found to recover the ability to grow after a long lag. To study this recovery in terms of DNA repair, the DNA of these cells was radiolabeled by growing cells for 10 hrs in medium containing 3H Tdr (2μCi/ml). However these radiolabeled cells did not recover. It was found that the incorporated precursor did produce deleterious effects of its own in the form of growth inhibition and DNA single strand breaks with no detectable double strand breaks. Both the extent of growth inhibition and single strand breaks seemed to be closely related to the amount of radiolabeled precursor added to the media as well as its specific activity. Cells exposed continuously to 3H Tdr in the medium alone could accommodate to the radioactive stressor. In this accommodative response, DNA repair was followed by a concomitant return to a growth rate similar to that of unexposed cells. One should consider DNA damage induced by radiolabeled precursors in the study of DNA repair.


In previous studies we found that the carcinogenic activities of complex mixtures are substantially different from that predicted from data for individual compounds. In the present study the 800-850°F (CL-1) and the >850°F (CL-2) fractions from the solvent refined coal (SRC) process were evaluated. Skin tumor initiating activity for CL-2 was 2.5 times greater than for CL-1 even though both CL contained about 1700 ppm benzo(a)pyrene (BaP). When BaP was applied to mouse skin alone or in the presence of CL-1 or CL-2 at doses identical to those used in initiation studies (50 ug BaP and 25 mg CL), binding to DNA in the presence of CL was not detected. When BaP (50 ug) was applied in the presence of CL (500 ug), the binding of BaP to DNA was reduced by 80%, relative to the binding of BaP alone; there were no detectable differences in BaP binding between the CL. These data indicate that binding of BaP to DNA is substantially reduced in the presence of the CL suggesting that the carcinogenic activities of these complex mixtures are determined primarily by components other than BaP. (Supported by the U.S. Department of Energy under Contract No. DE-AC06-76RL01830 and DE-AM06-76-RL02225.)
The spectrum of solar ultraviolet (UV) radiation penetrating the ozone layer and reaching the earth's surface is in the region of 290-405 nm. Chronic exposure to this radiation is a major factor in the etiology of human dental cancer. Radiation of these wavelengths is known to produce tissue injury through interactions with nonDNA chromophores (photosensitizers) and molecular oxygen, resulting in the production of reactive oxygen species. We have detected the production of superoxide anion (O_2^-) as measured by cytochrome c reduction after irradiation of the pyridine nucleotides NADH and NADPH by 270-405 nm UV (with and without the presence of superoxide dismutase during the irradiation). At 270, 334, 365, and 405 nm, 0.080, 0.017, 0.004, and 0.006 mmole O_2^- per kJ m^-2, respectively, were formed. Quantum yields were all in the region of 10^-3. We have shown previously that O_2^- is mutagenic to mammalian cells (Mutation Res. 121: 299-304, 1983) and data presented here suggest that O_2^- may participate in solar-UV-induced dental carcinogenesis. (Work supported by the U.S. Department of Energy under contract No. W-31-109-EN-38 and by NIH Grant No. CA 35456).

Hydrazine (H_2) induces methylation of target-organ DNA guanine. We proposed (Tox. Appl. Pharm. 70: 324, 1983) that the methylation mechanism involved reaction of HZ with endogenous formaldehyde (HCHO) to yield HCHO hydrazine which could be oxidized to diazomethane. This proposal was tested by pretreating animals with methanol, ethanol, cyanamide, or pyrazole to alter endogenous hepatic HCHO levels and then administering HZ. HCHO levels proved refractory to the pretreatments. Ethanol administration resulted in measurable levels of acetaldheyde in liver. HZ administration increased HCHO levels and, in ethanol-cyanamide pre-treated animals, acetalddehyde levels. The pretreatments inhibited the methylation of DNA guanine induced by HZ2. Ethyglycinic acid were not detected. DNA incubated with hamster liver homogenates metylenated only when both HZ and HCHO were in the system. Both micromolar and soluble protein were active in HZ-induced DNA methylation. Substitution of HCHO by acetaldehyde did not result in DNA alkylation. Alcohol and aldehyde dehydrogenases, in addition to micromolar enzymes, may be involved in activation of HZ-HCHO reaction products; one such product is tetraformylirisine (TFT). Neurotoxic doses of TFT produced methylguanines in liver DNA. The rate of formation and time to maximal methylguanine levels for TFT are more rapid than those observed after HZ administration. The data suggest a more complex metabolic pathway for HZ-induced methylation of DNA, where TFT may be a more proximal intermediate than HZ. (Supported by Air Force Contract F33615-80-C-0512).
The purpose of this study was to evaluate the cytotoxic effect in vitro of various concentrations of diesel particulate matter (DPM), benzo(a)pyrene (BaP) and 1-nitropyrene (1-NP) on rat alveolar macrophages, a mouse macrophage-like cell line (J774A1) and a human lung epithelial cell line (A549). Following 24- and 48-hour incubations, the cells were observed under phase contrast microscopy for changes in cell morphology, cell counts made using an electronic cell counter, and viability measured using trypan blue. From these data, a viability index was calculated to reflect changes in both cell number and viability. All three cell lines showed a dose-dependent reduction in the viability index above 10 µL DMSO/mL in the epithelial cell line and above 25 µL/mL in the primary and macrophage-like cell lines. Incubations with BaP, 1-NP and DPE had no effect on the viability index in either the primary or macrophage-like cell lines. Neither cell line showed any significant reduction in cell viability even at the highest concentration and only a slight reduction in cell number at the highest concentration tested. However, the epithelial cell line showed a significant dose-dependent reduction in viability index to BaP and 1-NP and a moderate reduction to DPE. These data indicate a difference in sensitivity to injury by these agents with the epithelial cells being the most sensitive cell type and a relative cytotoxicity of diesel particle hydrocarbons with BaP > 1-NP > DPE.

Effect of Ozone (O₃) on Prostacyclin (PGI₂) Synthesis in Pulmonary Endothelium (E) in Vitro

O₃ is a major component of oxidant air pollution. O₃ could alter arachidonic acid (AA) metabolism by formation of hydroperoxides, toxic oxides, or due to sulfhydryl interactions. The major AA metabolite of E, prostacyclin (PGI₂), is a potent pulmonary vasodilator and inhibitor of platelet aggregation. We have already reported a dose-dependent inhibition of PGI₂ by E after O₃ exposure (Fed. Proc. 42:871A, 1983). To further examine in culture the site of PGI₂ inhibition, bovine pulmonary artery E were grown to confluence in roller bottles and exposed to O₃ for 2H. We examined the capacity of E to convert AA to PGI₂ by measuring the amount of the stable PGI₂ metabolite, 6-keto-PGF₁α, in the media by radioimmunoassay after incubating the E with 20 µM AA for 5 min immediately after O₃ exposure. The data are expressed as % of the 0.00 ppm control group:

<table>
<thead>
<tr>
<th>Ozone (ppm)</th>
<th>0.00</th>
<th>0.03</th>
<th>0.10</th>
<th>0.30</th>
<th>1.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>12</td>
<td>12</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>R(SEM)</td>
<td>0.11(15)</td>
<td>+1(28)</td>
<td>-36(22)</td>
<td>+64(14)</td>
<td></td>
</tr>
</tbody>
</table>

(significant difference, *p<0.05, **p<0.01, from control group)

These data suggest that O₃ decreases the ability of E to synthesize PGI₂ in a dose-dependent manner. This may be due to an O₃-induced inhibition of cyclo-oxygenase and/or PGI₂ synthetase. (Supported by UNC Center for Environmental Medicine.)

ASBESTOS AND BENZO(a)PYRENE INDUCED CHANGES IN TRACHEAL ORGAN CULTURES. ENHANCED SENSITIVITY IN TISSUES FROM YOUNGER ANIMALS. M.E. Placke, M.J.W. Chang and C.L. Fisher. Toxicology and Health Sciences Section. Battelle Columbus Labs.

Hyperplastic and metaplastic lesions of tracheal organ culture mucosa are well documented changes resulting from in vitro exposures of asbestos and benzo(a)pyrene (BaP). This study was designed to assess the sensitivity of tracheal explants, obtained from donor animals of various ages, to these changes. Syrian golden female hamsters; 30 days, 90 days and 180 days were killed, tracheas removed and 4mm² sections explanted into media. Explants were exposed to crocidolite asbestos 4 mg/ml or 8 mg/ml or BaP 0.05 mg/ml or 0.5 mg/ml. Mucosal changes were morphometrically quantified and histochemical techniques were used to detect changes in specific cytochemical functions of epithelium sampled 1 and 4 weeks after exposure. Morphometry supported the qualitative observations that lesions were more severe in tracheas from 30 day old animals than those from the 90 and 180 day donor hamsters for all treatment groups. The predominant changes in the 30 d.o. group were widespread hyperplasia and squamous metaplasia with keratinization of both surface and basal epithelia. Lesions from older animals were more focal with fewer architectural disruptions and less keratinization. A loss of mucin production was associated with these lesions. Supported by Corporate Technical Development of Battelle Memorial Institute.
LONGTERM ORGAN CULTURE OF ADULT PERIPHERAL LUNG AND IN VITRO DEVELOPMENT OF INTERSTITIAL FIBROSIS FOLLOWING EXPOSURE TO SILICA DUST. M.E. Placke and C.L. Fisher. Battelle Columbus Laboratories. Columbus, Ohio 43201.

The use of fetal and neonatal lung cultures has provided important contributions to understanding histogenesis and acute disease processes of lung. However, attempts to maintain adult peripheral lung for periods beyond 7 days or to examine respiratory disorders in such a model have been unsuccessful. We have cultured 1-2 mm thick cross sections of lung lobes for periods beyond 4 weeks. Normal morphologic and macromolecular composition are maintained. Eight different enriched, serum free media, with heated liquid agarose were perfused into the airways of hamster and rat lungs via a tracheal cannula at a pressure 20 cm HzO. Thin sections were explanted onto squares of porous surgical packing material and floated on media. The ability of each media to maintain normal lung was assessed by determining tissue protein, RNA, DNA, histopathology and automated morphometric analysis. The optimal media for each species was determined and varied concentrations of silica dusts or parquat were incorporated into the perfusion mixture. Exposed sections showed degeneration and necrosis of alveolar pneumocytes, thickening of alveolar walls, alveolar macrophage accumulation and early interstitial fibrosis. Supported by Corporate Technical Development, Battelle Memorial Institute.

ROLE OF MITOCHONDRIAL GLUTATHIONE (GSH) IN PROTECTING AGAINST CELLULAR TOXICITY OF FORMALDEHYDE (CH₃O) IN ISOLATED RAT HEPATOCYTES. R.H. Ku and R.E. Billings. Univ. of Texas Med. Sch., Dept. of Pharmacol., P.O. Box 20708, Houston, TX 77225

Decreased cellular concentrations of GSH below a threshold level are associated with CH₃O toxicity in isolated rat hepatocytes. Since it has been suggested that the mitochondrial (mito) GSH pool is essential for maintaining cellular integrity, the effects of CH₃O and diethyl maleate (DEM) on mito GSH were investigated. Mito were rapidly isolated from CH₃O and DEM treated cells using di-glonin (0.2 mg/ml). The mito fraction contained >95% of the glutamate dehydrogenase activity and 11% of the total cellular GSH. Cytotoxic contamination of the mito fraction, as determined by lactate dehydrogenase activity, was <5%. CH₃O (2.5 mM - 7.5 mM) decreased cytosolic GSH from 85% to 50% of control whereas mito GSH remained unaltered (>90% of control). DEM (0.0 mM-2.5 mM) caused a dose-dependent decrease in both cytosolic GSH (from 21.9 to 2.8 mmol/10⁶ cells) and mito GSH (from 2.8 to 0.7 mmol/10⁶ cells). Neither CH₃O nor DEM at these concentrations were cytotoxic. When CH₃O was added to cells pretreated with 1 mM DEM, a dose-dependent increase in toxicity was observed when CH₃O exceeded 5 mM. However, CH₃O had no additional effect on mito GSH beyond the level depleted by DEM alone. These results dissociate depletions of mito GSH from CH₃O toxicity. In addition, it was found that DEM decreased mito GSH without causing toxicity. (Supported by NIEHS grants ES02868 and ES07090).

ENTRY AND EXIT OF 2,2',4,4',5,5' HEXABROMOBENZPHENYL IN CULTURES OF MOUSE 3T3L1 ADIPOCYTES A. L. Kraus and I.A. Bernstein. Dept. of Biochemistry and Env. and Ind. Health, Toxicology Program, The University of Michigan, Ann Arbor, MI 48109.

In this study the distribution of ¹⁴C 2,2',4,4',5,5' hexabromobenzophenyl (HBB) between cells and medium was studied during lipogenesis and lipolysis in cultured 3T3L1 adipocytes. During lipogenesis increases in ¹⁴C HBB incorporation paralleled increases in cellular triglyceride. A linear relationship was found between ¹⁴C HBB incorporation and triglyceride levels in the cells (correlation coefficient 0.97). Lipolysis was induced by the addition of norepinephrine (1x10⁻⁶ M) to low serum culture medium containing in glucose. During lipolysis 3T3L1 adipocytes pretreated with HBB showed decreases in cellular HBB levels. Cellular triglyceride levels decreased greater than cellular HBB levels. A linear relationship was found between cellular triglyceride and HBB levels during lipolysis with a correlation coefficient > 0.98. Evidence supports the view that cellular triglyceride levels have strong influence on HBB movement into, and out of, the 3T3L1 adipocyte. The ratio of cellular HBB/triglyceride increased during lipolysis indicating some retention of chemical in the cell. This increase was less when culture medium serum levels were higher. Higher serum levels allow more HBB to exit the cell concomitant with a given triglyceride decrease. Further work will identify those serum components which facilitate HBB exit.


Mirex (MX) is a hepatic carcinogen in rodents and is shown to cause marked hepatic ploidy changes. Differential uptake of ¹⁴C-MX by mouse hepatocytes was studied after the isolation of hepatocytes by in situ collagenase perfusion. Diploid (DP) and polyplid (PP) enriched cell fractions were separated on a ficoll gradient and kinetics of ¹⁴C-MX influx were measured under controlled conditions. A linear and rapid ¹⁴C-MX uptake shown by DP cells was 2-3 times more than PP. Influx of ¹⁴C-MX into both DP and PP cells exhibited single saturable high-affinity systems with different Km and Vmax values, respectively, i.e. 4.95x10⁻⁵ M and 310 mmoles/min/mg for DP and 3.47x10⁻⁵ M and 102 mmoles/min/mg for PP. The uptake of ¹⁴C-MX was temperature-dependent, and partially sensitive to Na⁺ ions, ouabain and ph, but not to Ca²⁺ ion concentrations. Early saturation and increased affinity of PP, compared to DP, were interesting findings contrary to our earlier observations in vivo (Toxicologist: 4,42,1994). Although this disparity may possibly be due to the effect of certain unknown humoral factors (?) missing in perfused hepatocytes, the preferential early saturation of PP and its possible relationship with the imbalances observed in hepatic polyplid as a result of chemical insult remains to be investigated. (Supported by Amer. Cancer Soc.#5A Shell Internationale, Maatschappij B.V.)
Although modification of dietary Fe has long been associated with altered Pb absorption, tissue levels, and toxicity, relatively little attention has been given to the effect of Fe on cellular Pb metabolism and toxicity. The effect of Fe on Pb metabolism was studied in hepatocytes by kinetic analysis. Cells were loaded with 210Pb (3 μM) with and without additional Fe Cl3 in media containing 3.2 μM Fe. After 20 hrs, 210Pb efflux was monitored and pool sizes, rate constants, fluxes and half-times were estimated by analysis of 210Pb washout curves. The effect of 5 μM to 40 μM Fe on lead metabolism was concentration dependent. 40 μM Fe as compared to 5 μM Fe (18 μM is normal serum Fe) decreased total cellular 210Pb 20%, increased the rapidly exchanging pool (S1) 5-10%, had no effect on the intermediate pool (S2), and decreased S3, which includes mitochondrial Pb, 5-10%. Flux between S1 and S2 also decreased 50%. The decrease in the size of S2 and of 210Pb flux between S1 and S2 suggests a competitive interaction or a common pathway for Pb and Fe across the mitochondrial membrane, and because total Pb decreased, there may be an interaction between Pb and Fe at the plasma membrane. Because most cellular Pb is in the mitochondria, the decrease in S2 with increasing Fe may be important in reducing body Pb or in the protective effects of Fe against Pb toxicity.

The toxicity of aflatoxin B1 (AFB1) is several fold greater in primary fetal bovine kidney (PFBK) cells as compared to the established cell line, Madin Darby bovine kidney (MDBK) cells. The uptake, subcellular distribution, macromolecular binding and metabolism of AFB1 were investigated in these two cell systems. [3H]AFB1 (2.5-50 μM/1 μL) was added to the culture and cells were harvested after 24 hrs. The cellular uptake and macromolecular binding depended upon the concentration of AFB1 in the medium, however, PFBK cells accumulated a substantially higher amount of radioactivity in the total or the acid-insoluble fraction. During a 24 hr incubation period, PFBK cells accumulated an equivalent of 250-350 pCi/10^6 cells in the two fractions, respectively, whereas the values for MDBK cells were 184 and 81, respectively. Most of the radioactivity was observed in the cytosolic fraction. HPLC analysis of the culture medium suggested a definite qualitative and quantitative difference in the metabolism of AFB1 in the two cell systems. Supported in part by NSHS grant B80341U.


Toxicity often limits the application of otherwise useful drugs and other substances. Therefore, an understanding of the possible mechanisms by which these compounds exert their toxic effects at a cellular level is of importance. Many compounds produce toxic effects on biological tissues by interaction with specific cellular receptors. In these cases, the mechanisms available to explain toxicity are dependent upon the prevailing ideas of receptor function. In this regard, recent electron microscopy studies have revealed "coated" structures (pits and vesicles) that appear to provide a mechanism by which cell surface receptors can be internalized in a process of endocytosis. The intracellular fate of these receptors appears to be destruction or recycling to the cell surface. Some of the ramifications of such a process to Toxicology include: (1) a mechanism by which toxic compounds can be internalized to intracellular targets; (2) a mechanism by which cell surface membranes are cleared of toxic compounds; and (3) a mechanism by which receptor number or density can be modified during exposure to toxic compounds. The present report summarizes some of the information on coated structures available from cell biology literature and proposes an expanded view of the possible mechanisms by which drugs and other chemical compounds can exert their toxic actions.
THE TESTING OF DI(2-ETHYLHEXYL)PHTHALATE (DEHP), MONO(2-ETHYLHEXYL)PHTHALATE (MEHP), DI(2-ETHYLHEXYL)ADIPATE (DEHA) and 2-ETHYLHEXANOIC (2EH) IN
A BATTERY OF GENOTOXICITY ASSAYS. E.B. Barber (Eastman Kodak Co.), A. Malholland (Ethyl Corp.) and D.R. Jagannath, M. Cifone, M. Cirino, R. Hydr
and J. Rundell (Litton Bionetics, Inc.).
Sponsor: B.D. Astill.

DEHP, DEHA, MEHP and 2EH were tested in five genotoxicity assays as part of a program sponsored by the Chemical Manufacturers Association. All four compounds gave negative results when tested in the Ames Salmonella/microsome assay in the presence and absence of rat liver metabolic activation. DEHP and DEHA gave negative results in the L5178Y Mouse Lymphoma assay and in the rat hepatocyte UDS system. All four were tested in the in vivo mouse micronucleus assay using the B6C3F1 mouse. DEHP, DEHA and 2EH gave negative results while MEHP produced equivocal results. All four compounds gave negative results in the Balb 3T3 transformation assay in the presence and absence of a co-cultivated rat hepatocyte activation system. This testing confirms and extends the work of many others and we conclude that these compounds show no clear evidence of genotoxicity.

REDUCTION OF CADMIUM-INDUCED CYTOTOXICITY IN CULTURED RAT LIVER CELLS BY 5-AZACYTIDINE PRE-

Recent work indicates the exposure of cultured cells to the pyrimidine analog, 5-azacytidine (AZA), enhances cadmium (Cd)-induced expression of the metallothionein (MT) gene. This study was designed to assess the effect of AZA pretreatment on Cd cytotoxicity. Cultured rat liver cells (TRL 1215) in log phase of growth were first exposed to AZA (8 μM). After 48 h, Cd (10 μM) was added and MT was measured (by the Cd-Hemoglobin assay) 24 h later. AZA alone caused a modest increase in MT concentration (1.7-fold) over control, while Cd alone caused a 10-fold increase. The combination of AZA pretreatment followed by Cd, however, caused a 23-fold increase in MT levels over control. AZA pretreated cells were also harvested and incubated in suspension with Cd (250 μM) for 0 to 90 min. After incubation intracellular and extracellular fluids were separated by centrifugation through an oil layer. AZA pretreated cells, as compared to controls, showed marked reductions in intracellular potassium loss, glutamic-oxalacetic transaminase loss and lipid peroxidation following Cd exposure. Results indicate that AZA pretreatment induces tolerance to Cd cytotoxicity which appears to be due to an increased capacity to synthesize MT rather than high levels of pre-existing MT at the time of Cd exposure.

VARIABLE TOXICITY OF TRICHTHOCENE MYCOTOXINS IN CELL CULTURE SYSTEMS. W.L. Thompson, J.C.

Studies on 19 different trichothecene mycotoxins (TMa) using a cell culture protein synthesis inhibition assay have shown the importance of structure-function relationships. Removal of side groups in the C15 and C4 position of the basic ring structure or addition of an acetyl group at C3 or a second epoxide across C9 and C10 greatly reduces toxicity of the molecule. Although some cell systems are more sensitive to the TMa than others, the order of toxicity remains the same. The extent of TM metabolism between cell systems varies significantly. After 4h exposure, % metabolism in vero cells, lymphocytes and hepatocytes was 10%, 50% and >90%, respectively. Uptake of T-2 mycotoxin into tissue culture cells and lymphocytes is rapid and complete within 30 min, but little of the less toxic TMa, tetraol and triol, are taken up. This, coupled with the additive rather than competitive effect of combined TMa, indicates that the variable toxicity of some of the mycotoxins is due to cellular uptake and not differences in binding to the active site. The macrocyclic TMa, Noridin A and Verrucarin A, while equally toxic to T-2 mycotoxin, are the only TMa tested whose effects are not easily reversed. Whether this is due to cellular release or binding at the active site is yet to be determined.

INHIBITION OF PROTEIN SYNTHESIS IN ISOLATED RAT HEPATOCYTES AS AN INDEX OF CELLULAR TOXICITY.
J.M. Frazier, W.S. Din, L. Collins. The Johns Hopkins University, Baltimore, MD.

Inhibition of the synthesis of both intracellular and secreted proteins was investigated as a potential index of cellular toxicity in isolated rat hepatocytes. Primary suspension cultures of isolated rat hepatocytes were prepared from male Wistar rats (150-200g). Viability was assessed by both trypan blue exclusion and lactate dehydrogenase leakage. Hepatocyte preparations of viability >90% were exposed to cadmium (0-100μM), zinc (0-1500μM) and ethanol (0-800mg/100ml) In the presence of 3H-lucine. After 2 h of incubation, cells were lysed and proteins precipitated with 10X TCA. Proteins in the media were similarly precipitated. TCA precipitable proteins were counted for 3H incorporation and data are expressed as % of control. EC50's were <20μM for Cd, >450μM for Zn, and >800mg/100ml for ethanol. Both intracellular and secreted proteins demonstrated dose-response relationships. These data indicate that inhibition of protein synthesis in isolated rat hepatocytes can be used as a sensitive index for evaluation of cytotoxicity.

This report describes the development of an in vitro system to evaluate the irritancy potential of compounds that contact the skin and to study mechanisms of cutaneous toxicity. Primary rat cutaneous keratinocytes are maintained as submerged cultures for 7 days and then raised to the liquid-air interface where they are maintained for periods up to 3 weeks. Two types of substrata, collagen and synthetic membranes, have been investigated for the attachment and proliferation of keratinocytes. Cells formed from a mixture of Vitrogen 100 and rat tail collagen supported keratinocyte growth that was 220% of the plastic culture dish controls. Of the 12 synthetic membranes studied, a nylon membrane supported growth 200% of that on plastic culture dish controls. After 14 days cultivation at the liquid-air interface on either substratum, the raised cultures exhibited morphological features, such as desmosomes, tonofilaments, keratohyalin granules and cornified cells similar to the in situ epidermis. Preliminary investigations include in vitro assays of agents with known irritancy values as determined in animal and human skin irritancy tests. (Partially supported by USDA/USC Contract DOG-DAMD 17-82-C-198)

MUCOSAL LESIONS IN URINARY BLADDER ORGAN CULTURE FOLLOWING EXPOSURE TO NAPHTHYLAMINE ANALOGUES. M.E. Placke and A.J. E. Chin. Battelle Columbus Laboratories; Columbus, Ohio.

8-Naphthylamine (8-NA) induces transitional cell carcinomas in animals and is presumed to be a human bladder carcinogen. N-phenyl naphthylamine (PNA) is an analogue with questionable carcinogenic activity, particularly when responses between species are examined. These differences are proposed to reflect differential metabolic pathways of the two compounds among species. We have explored the response of bladder organ culture to these two chemicals. This in vitro model has previously been shown to exhibit preneoplastic changes after treatment with nitroamines. Small cross-sections (3-4 mm in diameter) of Syrian golden hamster bladder were explanted and maintained in serum-free media. Explants were singly or repeatedly exposed to concentrations of 10 µg/ml, 100 µg/ml or 1000 µg/ml of 8-NA and PNA dissolved in 0.5% DMSO. The spectrum of dose-related effects included focal hyperplasia, multiple expansive proliferations of mucosa into the muscularis, squamous metaplasia with keratin formation, and anaplastic papilloid extensions of transitional cells after exposure to low and intermediate concentrations and anastrous degeneration followed by necrosis following concentrations of 1000 µg/ml. Supported by Corporate Technical Development, Battelle Memorial Institute.

USE OF RAT HEPATOCYTES OBTAINED FROM PERCOLL GRADIENT CENTRIFUGATION IN DNA DAMAGE AND REPAIR SYNTHESIS STUDIES. M.T.S. Hsia, B.L. Kraemer and S.K. Duddy. Env. Tox. Ctr. and Dept. of Entomol., Univ. of Wisconsin, Madison, WI.

One of the technical problems encountered in using suspensions of freshly isolated hepatocytes for screening potential carcinogens has to do with the relatively short half-life of these cells preparations. This is believed to result from the proteolytic activities released from cells partially damaged during the isolation procedure. We have now used density gradient centrifugation with Percoll, a synthetic colloidal silica, to enrich viable hepatocytes for DNA damage and repair-synthesis studies. Pellets of freshly isolated rat hepatocytes obtained via situ collagenase perfusion were suspended in L-15 medium (pH 7.4, saturated with 95% O2-5% CO2). A suspension of hepatocytes was then diluted to 106 cells/ml with a 38% (v/v) Percoll solution in L-15, and subjected to density gradient centrifugation. The Percoll-purified cells exhibited much higher viability and a substantially prolonged half-life than untreated cells as determined by the trypan blue exclusion test and enzyme leakage. After incubation with selected chemical carcinogens, the cells were analyzed for unscheduled DNA synthesis by a filter retention method (Hsia, Kraemer & Dolara, 1984. Mutation Res., 122: 177) and for single-strand breaks by the alkaline elution technique. Our data indicates that Percoll-purified hepatocytes provide an excellent in vitro model for the study of carcinogenicity.

MACROMOLECULAR COVALENT BINDING OF 14C-NITROPYRINE IN NORMAL AND ANTIBIOTIC-TREATED RATS. P.H. Ayres, J.D. Sun, and J.A. Bond. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Nitropyrene is a direct acting mutagen which contributed to the observed mutagenicity of diesel exhaust and fly ash particle extracts. This study investigated the contribution of gut microfloral metabolism to the macromolecular covalent binding (MCB) of NP and/or its metabolites in lungs and liver of F-344 rats in vivo. Normal (N) and antibiotic-treated (AT) rats were administered 14C-NP (0.04 nmoles/kg, 50 nCi/mole), and MCB was quantitated at various times in lungs and liver. MCB in lungs of AT animals sacrificed 4 hr after oral administration of NP (0.15 nmoles NP equivalents/g) was decreased (p < 0.05) to less than one-half of that of the N rats (0.42 nmoles NP equivalents/g). One day after administration of 14C-NP, MCB was significantly increased in lungs of AT rats (0.86 nmoles NP equivalents/g) to two-fold greater than the N rats (0.38 nmoles NP equivalents/g). MCB in lungs of AT rats was no different from the N rats one week after NP administration (0.1 nmoles NP equivalents/g). Comparison of livers from N and AT rats demonstrated the same pattern of MCB as the lungs but the differences were not statistically significant. These results reveal that gut metabolism plays a role in the time course of covalent binding of active metabolites of NP to macromolecules. (Research supported under U.S. Department of Energy Contract No. DE-AC04-76EV01013.)

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CN's lethality has been attributed to inhibition of CYT OX. Despite the universal acceptance of this statement, few investigators have studied the in vivo inhibition of CYT OX by CN. In this study, we investigated the dose-response relationship between CN and CYT OX activity, in vivo and in vitro. Male C3H mice were treated with KCN 1-15 mg/kg. Brain (B) and heart (H) and diaphragm (D) CYT OX activities were determined spectrophotometrically. At 20 mg/kg B and H CYT OX were inhibited by 35 and 60%, respectively, at death. However, at 4 mg/kg, a non-lethal dose, B and H CYT OX were inhibited by 35 and 44%, respectively. To address the possibility that CN was reversibly inhibiting CYT OX, mice were administered 6 mg/kg KCN. Tissue homogenates were dialyzed against buffer and the CYT OX activity of dialyzed and nondialyzed tissues were compared. In all instances, CYT OX activity was not increased after dialysis. Lastly, we examined the in vitro dose-response profile of CYT OX. H and D CYT OX was linearly inhibited from 10-100 M. Control 100 M H and D CYT OX were inhibited by 6 and 23% at 10-10 M and by 26 and 74% at 10 M. Little or no additional inhibition occurred from 10-10 M. Since in vivo CYT OX activity did not correlate with CN lethality, CYT OX may not be a good monitor of CN toxicity. (JCP was supported by a Stauffer Chemical Co. Fellowship in Toxicology.)

PRODUCTION AND DEGRADATION OF HYDROGEN PEROXIDE IN LIVER HOMOGENATES PREPARED FROM RATS OR MICE TREATED WITH DI-(2-ETHYHEXYL)PHTHALATE (DEHP). K.E. Tomaszewski, D.K. Agarwal, J.L. Melnick, E.J. Rau cm, M.M. Klume. National Toxicology Program, NIH, NIH, RNC.

In vitro steady state levels of H2O2 derived from peroxisomal fatty acid oxidation were determined in F344 rat and B6C3F1 mouse livers following treatment with DEHP for 7 or 14 days. Steady state levels of H2O2 in liver homogenates were calculated from measurements of velocities of H2O2 production by peroxisomal palmitoyl-CoA oxidase (PCO) and of H2O2 degradation by catalase (CAT) according to:

\[ [\text{Palmitoyl-CoA}] [\text{PCO}] [\text{PCO}] [\text{CAT}] = [\text{CAT}] [\text{CAT}] \]

At 50 µmol palmitoyl-CoA, [H2O2] steady state was 0.20 mmoles/g liver for control rats and 0.47 mmoles/g liver for control mice. Treatment with DEHP (2 g/kg B. Wt. by gavage) for 14 days increased PCO and CAT activities, resulting in a 6.5 fold increase in [H2O2] steady state in rat livers and a 10 fold increase in mouse livers. Therefore, in addition to causing an increase in H2O2 production in vitro, DEHP likely causes an increase in hepatic steady state levels of H2O2 in rats and mice. Glutathione peroxidase and superoxide dismutase activities were largely unaffected by the treatment with DEHP. The relationship of these results to suggested mechanisms of DEHP-induced hepatocarcinogenesis is under study.

EFFECT OF NITROGEN DIOXIDE (NO2) ON ANTI-OXIDANT ENZYME PROFILE IN ENDOTHELIAL CELLS (EC). J.M. Patel and E.R. Block. Univ. of Florida College of Med. and VAMC, Gainesville, FL.

NO2, an environmental pollutant, is toxic to pulmonary epithelial and EC. Since NO2 is an oxidant, changes in the antioxidant enzyme profile of lung cells may occur with NO2 exposure. To test this, we evaluated the changes of antioxidant enzyme activity in pulmonary artery (PA) and aortic (AO) EC in monolayer culture. Confluent porcine PA and AO EC were exposed to 3 or 5 ppm NO2 or air (control) for 3-24 hr. and assayed for GSH-reductase (GSH-red), GSH-peroxidase (GSH-per), and glucose-6-phosphate dehydrogenase (G6PDH) activities as well as for intracellular GSH content. After 3 or 6 hr. NO2 exposure, GSH-red and G6PDH activities were not different from controls in both PA and AO EC. Exposure to 5 ppm NO2 for 12 hr. slightly but not significantly increased GSH-red and G6PDH activities in both PA and AO EC. Exposure to 3 or 5 ppm NO2 for 24 hr. resulted in significant increases in GSH-red (p < 0.05) and G6PDH (p < 0.001) in both PA and AO EC. GSH-per activity and GSH content in NO2 exposed PA and AO EC were not different than controls irrespective of NO2 concentrations and exposure time. We conclude that G6PDH and GSH-red activities are increased in PA and AO EC exposed to NO2, and this response is comparable to that in the lungs from animals exposed to NO2. (Supported by Health Effects Institute 83-6).


The chemical-biological reactions of halocarbon-induced cytotoxicity are not well understood. However, the initial chemical events may involve an activation by a form of active oxygen, •O2-, which is generated intramembranously. The purpose of this study was to determine the interactions of •O2- with selected halocarbons in an aprotic environment simulating membrane conditions. •O2- was generated in DMSO, electrochemically at the platinum electrode, by the univalent reduction of molecular oxygen. The addition of carbon tetrachloride, chloroform, ethylene dibromide, decachlorobiphenyl, and 2,4,7,8-tetrachlorodibenzo- dioxin to aprotic media containing •O2- resulted in a) decrease in both •O2- and halocarbon, b) appearance of free halide and halocarbon breakdown products and c) immediate flashes of chemiluminescence. The decrease in •O2- and increase in halide and halocarbon products are dependent upon halocarbon concentration over a range of 1-100 ppb. These results demonstrate that a reaction does occur in aprotic media between these halocarbons and •O2-. Similar reactions may occur in the hydrophobic, aprotic environment of biomembranes and thus may play a role in the initiation of halocarbon-induced membrane damage. (Supported in part by USPHS Grant ES02824).
Quantitative cytochemistry is potentially of great use in toxicological studies because the biochemical activity or concentration can be directly related to tissue morpholgy. Its usefulness, for example, in studies on the distribution of glutathione and cytochrome P-450 in rat liver has already been demonstrated and an explanation for the centrilobular location of many hepatotoxic injuries provided (1). In the present study we have used quantitative cytochemistry to determine what role, if any, lipid peroxidation (LPO) plays in producing the centrilobular hepatic injury caused by a variety of halogenated hydrocarbons, including CCl₄, bromobenzene (BrB), and the proximal tubule renal necrosis caused by HgCl₂. Three cytochemical assays of lipid peroxidation have been used and their validity tested by comparison to conventional biochemical assays. The results to date suggest an important role for LPO in CCl₄ hepatic injury but no role in BrB or HgCl₂ induced necrosis.


The role of lipid peroxidation in the toxic effects of parquat, dinitro and other bipyriddy herbicides is controversial. In vitro studies have shown that these compounds are potent generators of oxygen radicals and stimulate lipid peroxidation (1). However, in vivo studies have failed to show clear evidence of lipid peroxidation resulting from toxic exposure to these compounds (2). We have used hepatocytes depleted of glutathione to study the toxicity of three bipyriddyls and have correlated superoxide production and lipid peroxidation with cell death. We have also studied the effects of various antioxidants on the toxicity of these bipyriddyls. Our results to date suggest that lipid peroxidation is not a critical event in the toxic effects of these compounds.


Quinone metabolites may be involved in the toxicity and carcinogenicity of a number of xenobiotics including 1-naphthol, benzene, eillipticine, diethylnitrosamine, and benzo[a]pyrene. These quinone metabolites are often difficult to detect because of their low concentrations and moderately low yields. The application of HPLC with reductive electrochemical detection (HPLC-RED) makes it possible to detect small (pmolar) quantities of quinones with minimal interferences. Using this technique we have shown that a simple superoxide (O₂⁻)-generating system (xanthine oxidase + hypoxanthine) converts various xenobiotics, including 1-naphthol, to their respective quinone metabolites. In experiments with 1-naphthol, 1,4-naphthoquinone was the principal oxidation product with yields up to 8%. The mechanism of quinone formation in this system appears to involve oxidation by O₂⁻ since superoxide dismutase (0.2 mg/ml) totally inhibited quinone formation. This mechanism of quinone formation may be important in vivo because other physiological O₂⁻-generating systems, such as those of polymorphonuclear leukocytes and NADPH-cytochrome P-450 reductase, could catalyze similar reactions.

Coxydation of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) by prostaaglandin synthetase (PGS). D.C. Thompson, M.A. Trush, D.G. Lange, and Y.N. Cha, Dept. Env. Health Sci., Johns Hopkins Univ., Baltimore, MD 21205

PGS is capable of activating a number of compounds to reactive intermediates. We now report the in vitro metabolism of the antioxidants BHA and BHT by PGS isolated from rat seminal vesicles. BHA is metabolized by PGS to two products as determined by HPLC and were identified as BHA dimers by mass spectrometry and NMR. Arachidonic acid and hydroperoxide oxide (DMPO) and observed ESR. If no spin trap is present, a stable radical is formed which can also be detected by ESR. BHT is also metabolized to a free radical which can be trapped with DMPO. However, in the absence of a spin trap, no visible radical was detected. No products of BHT metabolism by PGS were detected by HPLC, perhaps because the reactive BHT-quinone methide may be formed which then binds to macromolecular proteins rather than forming a stable metabolite. We are presently investigating the ability of these free radical intermediates to covalently bind to cellular macromolecules via a PGS-catalyzed reaction. (NIDSH #001833-02).
Aromatic nitro compounds are important as drugs, pesticides, and intermediates in chemical synthesis. Recent reports have indicated that mitochondria may be an important target for toxicity by these compounds, especially from nitro reduction and superoxide generation. Using the structure-activity approach, we studied the effects of nitrofurantoin (NF), nitrofurazone semicarbazone (NSC), nitromidazole (NI), and nitrobenzoic acid (PNB) on mouse (C57B6/J, male) liver mitochondria. Oxidative phosphorylation and oxygen consumption were determined with an oxygen electrode. Mitochondrial membrane swelling and Ca\(^{2+}\) flux were measured by spectrophotometric methods. At 1 mM, NF caused a pronounced reduction of State 3 (ATP synthesis) and the uncoupled state rates with glutamate and malate as substrates. There was no effect on these states with succinate as the substrate. In the presence of NADH and ADP, only NF and NFA at 1 mM caused an increase in oxygen consumption, which was not blocked by rotenone. Additionally, NF and NFA inhibited calcium influx into mitochondria. These effects were not due to any gross alterations in the membrane since no swelling was observed. Thus, the nitrofurans, NF and NFA, were more toxic than NI and PNB to liver mitochondria.

Paraquat is known to deplete NADPH. The role of NADPH in maintaining cellular reduction capacity and membrane integrity suggests its depletion may be critical during the oxidant-mediated insult from paraquat. Rat lung slices were used to determine if NADPH levels could be maintained during paraquat intoxication by increased glucose. NADPH and NADPH assays were based on rates of dye reduction. DNA content was used to monitor edema. Control lung slices had NADPH-to-NADP+ \((H^+/H^-)\) ratios of 1.59, regardless of glucose concentration. With \(10^{-3}\)M glucose, 0.01mM paraquat had no effect, but 0.1mM and 1mM decreased NADPH by 40% with \(H^+/H^-\) ratios of 0.69 and 0.59 respectively. At 0.01mM paraquat, stepwise increases in glucose from 0 to 22mM resulted in incremental increases in \(H^+/H^-\) until at 22mM the ratio (1.57) was nearly identical to control. Total NADPH plus NADP+ with 0.01mM paraquat and no glucose was 60% of control. With any added glucose from 1.1 to 22mM, the total was 92% of control. The results indicate that increased glucose alleviated NADPH depletion during paraquat poisoning. Supported by NIH Grant ES02846 and VA Medical Research Funds.
861 IN VITRO TOXICITY OF PARAQUAT, CHLORODECON, AND HEXACHLOROPHENE FOR RAT LUNG FIBROBLASTS. G.N. Cosma and D.G. Wenzel, Dept. of Pharmacol. and Toxicol., Univ. of Kansas, Lawrence, KS 66045.  
Sponsor: G.J. Traiger

Primary monolayer cultures of neonatal rat lung fibroblasts were used to evaluate the toxicity of 3 environmental toxicants: paraquat (PQ), chlorodecon (CD), and hexachlorophene (HCP). Cell cultures were prepared from 2-4 day old Charles River rats and maintained in Eagle's minimal essential medium + 5% fetal bovine serum. Toxic effects of PQ, CD and HCP on the cultures were measured by a quantitative metabolic inhibition test (QMIT), supplemented with morphologic evaluation (Wenzel and Cosma, Toxicology 29:173, 1983). Exposure of the toxicants to cultures for 7 days produced concentration-related injury for all 3 agents as measured by the QMIT. TTC values (obtained by integration of time-concentration effects) for PQ, CD and HCP were 60, 16 and 2.5 μM, respectively. Co-treatment of cultures with the antioxidant tert-butyldihydroxyanisole (BHA) provided significant protection against PQ injury, but none against CD or HCP toxicity. Treatments with ADP-Fe²⁺ potentiated PQ toxicity, but not that of CD or HCP. Co-treatment of cultures with superoxide dismutase (SOD), catalase, or mannitol failed to provide any protection against PQ toxicity. Although results with BHA and ADP-Fe²⁺ suggest a role for free radicals in PQ toxicity, this possibility could not be confirmed with free radical scavengers.


The economical and logistical characteristics of in vivo studies permit the design and execution of more experimentally complex studies than in vitro. As such, in vitro test systems should be useful tools to identify and characterize joint actions, and to develop and refine hypotheses regarding toxicant joint actions prior to extensive in vivo investigation. The objectives of this study were to 1) define in toxicological and mathematical compatible terms the principle joint actions of toxicants; 2) develop a statistical approach for the detection and characterization of these joint actions; and 3) demonstrate the principle of statistical analysis using Cd²⁺ and Hg²⁺ in cultured rat hepatocytes. A method was developed to statistically distinguish between Concentration Additivity (simple similar joint action and Response Additivity (simple independent joint action). The fraction of LDH released in each cell culture was transformed by the angle transformation to linearize the concentration-response function and to make the variance of the transformed response homogeneous, and fit by linear regression analysis. The assumption of either concentration or response additivity was accepted or rejected from the predicted mathematical joint response function.

862 CELLULAR METABOLISM OF LEAD: A KINETIC ANALYSIS IN CULTURED OSTEOCLASTIC BONE CELLS. J.F. Rosen and J.G. Pounds. Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY and National Center for Toxicological Research, Jefferson, AR.

Characterization of lead metabolism in bone is necessary to understand the role of skeletal lead in the expression of its clinical and biochemical toxicity. The metabolism of lead in bone is also important because it is the major site of chelation by CaNa₂EDTA and d-penicillamine. Experiments were conducted to characterize the steady-state kinetic distribution of 210Pb and to identify the biological structures associated with the kinetic pools. Bone cells, derived from mouse calvaria, were enriched for osteoclasts by a collagenase digestion and maintained in primary culture for 1 week. Cultures were labeled with 210Pb as 5 μM lead acetate for 20 hr and the kinetic parameters were obtained by analysis of 210Pb washout curves. Cellular metabolism was defined by three kinetic pools of intracellular lead containing ~10% (S₁), ~15% (S₂) and ~75% (S₃) of total cellular lead (1.7 - 2.2 mmol/mg cell protein). The half-times for isotope exchange were 1, 40 and 1000 minutes, respectively. KCN, DNP and DBcAMP decreased S₃, whereas increasing medium PO₄ to 4 × increased S₃, suggesting that S₃ includes mitochondrial 210Pb. These data indicate that lead is readily mobilized from osteoclastic bone cells and, like soft tissues (hepatocytes), the bulk of cellular lead is associated with mitochondria.


Trimethyltin (TMT) is a potent neurotoxicant, but little is known regarding TMT's systemic toxicity. In this study, mice were administered 2.75 μg TMT/g B.W. or saline and housed in room air or a 40% O₂ hyperoxic atmosphere. Both TMT-treated groups developed generalized tremors within 24 hrs. Serum and liver were collected for analysis 48 hrs post-treatment. Animals exposed to TMT and O₂ developed pan-lobar fatty livers. Hepatic triglycerides (TGs) in O₂ controls and both TMT-treated groups were mildly elevated, while hepatic cholesterol levels were unchanged. Serum chemistries were significantly altered in both TMT-treated groups. Animals exposed to TMT and elevated O₂ demonstrated more pronounced serum changes than treated animals in room air. SGPT was elevated in both TMT and TMT + O₂ groups relative to controls (70.3 ± 24.9 vs 27.0 ± 1.0 and 229.3 ± 13.6 vs 31.9 ± 2.8 IU/ml, respectively). TGs were significantly elevated relative to controls (room air: 436 ± 123 vs 156 ± 10 and O₂: 547 ± 157 vs 156 ± 12 mg/dl), as was cholesterol (room air: 159 ± 6 vs 96 ± 2 and O₂: 167 ± 5 vs 92 ± 9 mg/dl). Serum glucose was markedly decreased in both treated groups compared to their controls (room air: 23 ± 5 vs 124 ± 2 and O₂: 14 ± 4 vs 107 ± 7 mg/dl). These data suggest a complex interaction between TMT and hyperoxia.
**EFFECT OF Selenium ON Cadmium-INDUCED ALTERATIONS IN delta-AMINOLEVULINIC ACID HYDRATASE ACTIVITY IN Male Rats**

Marcus B. Izard and J. L. Early, College of Pharmacy and Pharmaceutical Sciences Florida A&M University, Tallahassee, FL Sponsor: R. Craig Schnell

Cadmium, a heavy metal, is known to produce a variety of toxic manifestations in man and animals. Selenium, a trace element, has been shown to protect against heavy metal toxicity. The purpose of this investigation was to examine the effect of selenium on cadmium-induced alterations in the activity of hepatic delta-aminolevulinic acid dehydratase (ALAD) in male rats. Sprague-Dawley derived albino rats (151-175g) were treated with sodium selenite (1.6mg Se/kg), cadmium acetate (84 mg Cd/kg) or both selenium and cadmium administered in opposite quadrants via the intraperitoneal route. Animals were sacrificed at 2, 6, 12, and 24 hrs following treatment. ALAD activity was quantitated spectrophotometrically. Selenium decreased ALAD activity to 68% of control at 2 hr. Cadmium increased ALAD activity to 247, 192 and 144% of control at 6, 12, and 24 hrs, respectively. In contrast, the administration of both selenium and cadmium resulted in ALAD activity of 102, 125, and 84% of controls at 6, 12, and 24 hrs, respectively. Thus, selenium may prevent cadmium-induced decreases in cytochrome P-450 levels and drug metabolism by preventing alterations in ALAD activity. (Supported by NIH Grant No. RR-08111)

**DISPOSITION OF Cadmium FOLLOWING ORAL ADMINISTRATION IS DOSAGE DEPENDENT.**


This study was designed to determine if the disposition of Cd after oral administration varies with dosage. Initial experiments indicated that 7 days after administration of a single oral dosage of Cd (10, 100, 1000, 10,000 µg/kg) to rats, the percent of dose retained increased from 0.40% at the 1 µg/kg dosage to 1.65% at the 100 µg/kg and higher dosages. To determine whether dosage-dependent retention of Cd is due to differences in absorption of Cd from the gastrointestinal tract, rats were given an oral dosage of Cd (1 or 10,000 µg/kg) and 3 hrs later organs were removed to determine Cd content. Concentrations of Cd in tissues increased more than the increase in dosage suggesting that absorption is dosage-dependent. To determine the role of metallothionein (MT) in dosage-dependent absorption of Cd, intestinal cytosol (prepared 3 hrs after administration of Cd) was applied to a G-75 Sephadex column. At the 1 µg/kg dosage a higher percent of Cd in intestine was bound to MT than was bound to MT with the 10,000 µg/kg dosage (61.4 and 48.5%, respectively). These results indicate that dosage-dependent retention of Cd following oral administration appears to be due to increased absorption of Cd at higher dosages and that binding of Cd to intestinal MT may be partially responsible for this phenomenon. (Supported by USPHS Grants E5-01142 and E5-07079).

**A COMPARISON OF THE EFFECTS OF INHALATION OF CADMIUM CHLORIDE (CdCl2) AND CADMIUM OXIDE (CdO) IN THE LIVER.**

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The purpose of this study was to determine the toxicity of inhaled cadmium (Cd) on hepatic biochemical function, comparing similar aerosols of CdCl2 and CdO. Male rats were exposed for 2 hr to 0.48 and 4.5 mg Cd/m3. Following exposure to 4.5 mg/m3 CdCl2, decreases in body weight, liver weight, liver to body weight ratio, total protein, total sulfhydryls and non-protein sulfhydryls, as well as decreased activities of glutathione (GSH)-reductase, GSH-peroxidase, and G-6-PDH were observed. Increases in serum bilirubin, and activities of creatine kinase, lactate dehydrogenase (LDH), and aspartateaminotransferase were also evident 72 hr after exposure. Exposure to a similar concentration of CdO only decreased the activities of G-6-PDH, GSH-reductase and peroxidase, total sulfhydryl content and increased the activities of serum alkaline phosphatase and LDH. At 0.48 mg/m3 exposure to CdCl2 also produced more effects than did a similar exposure to CdO. This differential response between CdCl2 and CdO is probably due to a more rapid transport of CdCl2 from the lung to the liver via systemic clearance as compared to CdO. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
The influence of age upon the induction of liver MT by Zn was examined in 6 and 24 month old male C57Bl/6J mice. At 24, 48 and 96 hrs. post 10 mg Zn (as ZnCl₂)/kg sc, mice were sacrificed and livers taken for Zn analysis and to prepare 104,000 xg supernate and pellet.

Supernate MT was separated by Sephadex G-75 gel chromatography. The pattern of Zn accumulation was similar for both groups. Peak supernate Zn concentration occurred at 24 hr. post dosing in 24 month old mice and at 48 hr. post dosing in 6 month old mice. Peak liver MT contents occurred in conjunction with peak liver Zn concentrations. For both age groups, linear relationships were found between supernate Zn concentrations and amounts of Zn bound to MT. These results indicate that age dependent differences in the kinetics of Zn accumulation by liver influence the induction of MT synthesis by Zn treatment. (Supported in part by USPHS grant ES02881).

At 2 days of age, Ha/ICR mouse pups received 10 μg LPSW ip or sterile physiological saline ip and the pattern of Zn and Cu accumulation in liver was examined for the subsequent 26 days. Primary emphasis was placed on the role of MT in hepatic metabolism of these metals. In both groups, there was a strong linear relationship between total Zn or Cu concentration and the concentration of these metals in the 104,000 xg supernate. Further, for both control and LPSW-treated mice, strong linear relationships were found between the amount of Zn and Cu bound to MT and the concentration of these metals in liver supernate. These results suggest that changes in liver contents and concentration of Zn and Cu produced by LPSW treatment do not specifically affect the relationship between liver burden of Zn and Cu and the amount of metal bound to MT. Rather, similar regulation of MT synthesis exists in both groups. (Supported by PHS grants RR05808 and ES02881).

A number of inorganic metals, as well as certain organometals and metalloporphyrine are capable of increasing de novo synthesis of heme oxygenase, the rate-limiting enzyme in heme degradation. Among the most effective metals in inducing heme oxygenase and depleting cytochrome P-450 in the liver is cobalt, both in its chloride form, as well as chelated within the protoporphyrin macrocycle. The effects of inorganic cobalt (250 μmol/kg) on heme oxygenase and cytochrome P-450 in male Sprague-Dawley rats are found to be partially prevented by the simultaneous administration of an equimolar dose of zinc chloride. Furthermore, when zinc is administered in a single dose as low as 1/5 the molar equivalent of inorganic cobalt, a substantial reduction (∼50%) in the subsequent tissue levels of cobalt as measured by graphite furnace atomic absorption spectroscopy is found to occur. The decrease in cobalt tissue levels is dose- and time-dependent with respect to zinc administration. These studies demonstrate that inorganic zinc blockade of hepatic heme oxygenase induction by inorganic cobalt is associated with a marked displacement of the latter element from the tissue in which its enzyme inducing action is expressed.

Isolated rat hepatocytes provide a useful in vitro model for the investigation of hepatotoxicity. Organometallic compounds are known to be biotransformed in mammalian liver. We are investigating the hepatic metabolism and toxicity of the alkyl derivatives of lead and tin (potent neurotoxins). Tetrathylethyllead (Et4Pb) was dealkylated in hepatocyte cultures which yielded ethene and ethylene in a ratio of approximately 1:15 during a 4 h incubation. The production of 2-C-hydrocarbons from 5,10 or 20 μM Et4Pb metabolism represented ca. 25% monodealkylation. Coincident with hydrocarbon evolution, triethylylead (Et3Pb) appeared in media and hepatocytes. The incubation and assay conditions did not result in dealkylation of Et4Pb in the absence of cells. Preincubation of hepatocytes for 12 h with metyrapone, a monoxygenase inhibitor, decreased the dealkylation of Et4Pb by approximately 80%. Et3Pb is dealkylated in vitro at a slower rate than Et4Pb, which corresponds to in vivo metabolism of these compounds. Equinolar Et4Pb was more hepatocytoxic than Et3Pb as determined by release of lactate dehydrogenase into the culture medium. Additional studies will examine the effects of other cytochrome P–450 inhibitors and inducers on the metabolism and toxicity of alkylleads and tins. (Supported by T32 ES07017 and PO1 ES01194.)

AN ESTIMATION OF THE HEALTH RISK TO CHILDREN FROM ARSENIC–TREATED WOODEN PLAYGROUND STRUCTURES. R.D. Schlag, E.C. Berteau, and L.A. Garcia. California Department of Health Services, Berkeley, CA 94704

The use of arsenic as a wood preservative is of concern because of the perceived possibility that children might ingest arsenic due to hand–to–mouth activity when playing on arsenic-treated wooden structures. We calculated that in a typical situation of playground activity, a child might be exposed to an amount equal to the daily permissible level for arsenic in drinking water from each hand–hold. Analysis of wipe samples from wooden playground equipment treated with chromated copper arsenate revealed that surface arsenic residue concentrations ranged from less than 1 μg/cm² to 314 μg/cm². Preliminary studies with adult human volunteers who handled arsenic-treated playground equipment and then ingested residues from their hands, revealed urinary levels of arsenic that were less than those encountered from eating small amounts of fish. About 85% of the arsenic from the preservative is reportedly bound to the wood fibers as an insoluble complex and much of the arsenic will be expected to pass through the gastrointestinal tract unchanged. However some will be absorbed producing a small risk to the playing child. It is recommended that sealants be applied to protect against this hazard.


Male, Sprague-Dawley rats were injected 6 times over a period of 6 weeks with 10 mg/kg of paraquat ion i.p. In week 7 the dose was doubled and during week 8 rats showing little response were given 35 mg/kg paraquat while the remaining rats received 15 mg/kg. Using a whole body plethysmograph, the respiration of the rats was monitored during air breathing and breathing 10% CO2, 20% O2, and 70% N2 prior to and during 36 wks following the first injection. After the 2nd injection, the exposed rats showed an increase in respiratory rate (f) when breathing air or the CO2 mixture. Three days after the 3rd injection, small increases in f were seen only when breathing the CO2 mixture but not when breathing room air. However, no f changes were produced by next 3 paraquat injections indicating tolerance. Increases in f were again seen after the last 2 injections at higher doses. In conclusion, our method allowed the study of respiratory toxicities and development of tolerance. (Supported by NIEHS grant 5–R01–ES52747)
Male and female Fischer 344 rats and B6C3F1 mice were exposed to 0, 10, 30, 90, or 150 ppm TELONE II vapors (corresponding to 0, 9.1, 27.3, 81.8 and 136 ppm 1,3-dichloropropene; DCP); 6 hr/day, 5 days/week for 13 weeks. Depressed growth rates were observed in both sexes of rats and mice exposed to 90 or 150 ppm TELONE II resulting in 18-20% and 10-12% lower terminal body weights in rats and mice of the high exposure groups than control animals, respectively. Degeneration of the nasal olfactory epithelium and/or hyperplasia of the respiratory epithelium occurred in all animals exposed to 90 or 150 ppm, and 2 of 10 male rats exposed to 30 ppm TELONE II vapors. Lesions of the olfactory epithelium in the high exposure level mice were occasionally accompanied by some focal areas of respiratory metaplasia. A diffuse, moderate hyperplasia of the transitional epithelium was also observed in the urinary bladder of female mice exposed to 90 and 150 ppm TELONE II. Submucosal aggregates of lymphoid cells, associated with some areas of bladder epithelial hyperplasia, were also observed in the bladders of female mice of the 30 ppm exposure group. A no-observable-effect-level of exposure observed in this study was between 10 and 30 ppm TELONE II vapors.

The enantiomers of O-aryl O-methyl phenylphosphonothioates are shown to have different potencies in causing delayed neuropathy in hens. However, comprehensive in vivo data, so far, are still lacking due to the unavailability of enough resolved isomers to run thorough tests. In order to acquire further insight into the relationship of chirality and potency of phosphorus esters towards acute and delayed neurotoxicity, isomers of desbromoletoephos oxon [O-(2,5-dichlorophenyl) O-methyl phenylphosphonate] were synthesized and their toxicological properties were examined. Phosphonate ester was chosen as the test compound because of its higher delayed neurotoxicity and enhanced effectiveness in vitro over phosphonothioate. The (+) isomer of desbromoletoephos oxon was more toxic, in vivo, to houseflies and mice than was the (-) isomer. The in vitro assays using bovine erythrocyte and housefly head acetylcholinesterase qualitatively supported the in vivo data. The inhibitory activity of the isomers against neurotoxic esterase (NTE) of hen brain followed a reverse order of potency as expected compared to the two cholinesterases studied. In contrast, however, in vivo ED50 values of hens deviated from the expected order of potency derivable from in vitro NTE assay.
The percutaneous absorption of DIFOLATAN Technical and DIFOLATAN 80 WDG was determined by topically applying single doses of either 0.5 or 5.0 mg of each test material containing [14C]-Captafol in 0.2 ml of saline onto a 12 cm2 application site on the backs of four male adult Sprague-Dawley rats each. The rats were placed in Metrap restraining metabolism chambers for eight hours during which urine, feces, CO2, and volatiles were collected. At the end of eight-hours, the animals were sacrificed, necropsied, and the skin from the application site, blood, carcass, and excreta were analyzed for 14C content. After an eight-hour exposure period, more than 94% of the 14C-Captafol remained at the skin application site. Almost all (>96%) of the 14C-material at the skin site was removed by washing with acetone. The mean percent percutaneous absorption of the recovered dose was 1.2% and 0.2% for 14C-Captafol (Technical) and 0.5% and 0.7% for 14C-Captafol (80 WDG) at 0.5 and 5.0 mg/rat, respectively. The results indicated that DIFOLATAN Technical and DIFOLATAN 80 WDG were poorly absorbed when applied dermally to rats.

Environmental toxicant concentrations are often time variable. Acid precipitation associated depression of pH and elevation of metal concentrations in natural waters are prime examples. We examined the role of duration of exposure in acute and subchronic responses of fish to H2SO4 and HAC. Mortality, reduced growth, and depletion of whole body electrolytes (sodium, potassium, and calcium) quantitated toxicities of exposures. Conditions of exposures were as follows: water hardness of 8-12 mg/l as CaCO3, pH 3.7 to 7.0, aluminum concentrations of 0 to 1200 ppb, control water was provided between toxicant pulses in intermittent exposures. Fish were resistant to H2SO4 and HAC exposures of short durations (i.e., 12 hr toxicant followed by 12 or 36 hr control water for 4 to 24 cycles) but with longer durations (i.e., 48 hr toxicant followed by 48 or 96 hr control water for 4 to 6 cycles) little difference from continuous exposures was apparent. Depletion of electrolytes correlated well with but was more sensitive than other indices of toxicity. These data indicated repetitive episodic exposures of trout to H2SO4 and HAC were nearly as toxic as continuous exposures when durations exceeded 24 to 48 hr. (Supported by USEPA CR-810157-020, National Acid Precipitation Assessment Program).

A lifespan dosing of gentian violet (hexamethyl-p-rosaniline and pentamethyl-p-rosaniline) in the diet of B6C3F1 mice (C57Bl6 x C3H) at dose levels of 100, 300, and 600 ppm was conducted to determine its toxicity and carcinogenicity. Sacrifices were conducted at 12, 18, and 24 months. A total of 720 males and 720 females were allocated to the study. Food consumption was equal among the 3 dose groups and the control and there was no dose effect on body weight gain. A dose effect was noted for mortality in all dose groups and was greater in females. Mortality in the controls of both sexes was less than 15% at 24 months, but was approximately 62% in the females and 25% in males in the high dose. A positive response for hepatocellular carcinoma was noted in males at 24 months and in females at 18 and 24 months. The females also exhibited dose related responses at a low incidence for type A reticulum cell sarcoma in the uterus, the bladder, the ovaries, and the vagina, and for atrophy of the ovaries and erythroplasia in the spleen. Gentian violet appears to be a carcinogen in mice at several different organ sites.
CINCH Herbicide is a novel cinoeole herbicide which has shown promise as a soil-applied treatment for several broad-leaved crops. Studies were undertaken in Fischer 344 rats and B6C3F1 mice to determine the subchronic toxicity of dietary CINCH at doses up to 1000 ppm. After 7 or 13 weeks of dietary treatment, rats and mice were necropsied for pathologic evaluation. After 7 weeks of dietary exposure, indices of hepatic drug metabolizing activity were also assayed. Dietary exposure to CINCH revealed no macroscopic or microscopic evidence of compound related toxicity. With respect to control animals, rats and mice exposed to 1000 ppm of CINCH had significantly higher liver weights after 7 and 13 weeks of exposure. After 7 weeks of exposure, the high dose liver weight effect was associated with higher hepatic cytochrome P-450 linked enzyme activities. Moreover, these activities were higher in rats and mice exposed to lower doses of CINCH that did not result in higher liver weights. Acute oral exposure to CINCH did not result in changes in hepatic non-protein sulphydryl levels.

The paucity of appropriate toxicological data on Pilocrom (herbicide and potential water contaminant) necessitated an evaluation of its toxicity in drinking water. Acute oral, 14- and 90-day studies were conducted in male and female Charles River CD rats using standard protocols. The acute oral LD50 in males was 954 (812-1120) mg/kg and in females it was 886 (599-786) mg/kg. Depression, prostration, ataxia, tremors and clonic convulsions preceded death; the severity was dose dependent. Times to death were 1.3 to 12.4 hours. Ten male and 10 female rats received doses of 0, 60, 190 or 600 mg/kg/day in their drinking water for 14 days. There was no evidence of toxicity and no mortality. In the 90-day subchronic study, 20 males and 20 females per group received 0, 60, 190, 600 or 1070 mg/kg/day in their drinking water. At 1070 mg/kg, 80% of males and 70% of females died and at 500 mg/kg/day, 20% of the males and 10% of the females died. The only compound related effects were depressed body weight at the highest dose, altered albumin/globulin ratios in both sexes and depressed SGT and SGOT in males. There were no other consistent compound related and dose-dependent effects noted. (Supported by EPA Contract No. 88861010. This report does not necessarily reflect EPA views.)

Blood serotonin and histamine, plasma 11-hydroxycortisol and the urinary norepinephrine metabolite, 4-hydroxy-3-methoxy mandelic acid (HMA) were determined in 80 male persons, comprising 20 healthy normal control subjects, 20 bilharzial patients and 40 workers who were occupationally exposed to pesticides. Twenty of these workers were bilharzial and the other were non-bilharzial. All the selected subjects were of similar age ranging from 25 to 35 years. Results indicated that, blood serotonin and histamine and urinary HMA levels were significantly higher in bilharzial and non-bilharzial workers as well as in the bilharzial patients as compared to their corresponding control group levels. The plasma cortisol level was lower in bilharzial patients and bilharzial workers, while it was significantly higher in non-bilharzial workers as compared to the control group. These results suggested that, the effect of bilharziasis on the levels of the tested biogenic amines and their metabolic product was similar to that developed by being occupationally exposed to pesticides. This similarity was not the case in the effect on the plasma cortisol level.

The presence of cholinesterase (ChE) in virtually all species is well recognized. Until recently, no physiological role could be ascribed to plasma ChE, and similarly none could be ascribed to acetylcholinesterase (AChE) beyond that of the enzyme role in neurotransmission. However, certain publications in the more contemporary literature provide evidence for the existence of extensive physiological roles for these enzymes. Many pesticides by design inhibit ChE. The EPA lists 70 compounds in the class of ChE inhibitors for which food tolerances have been established. Plasma ChE and/or erythrocyte AChE assays are often employed as indicators of exposure to anticholinesterase. More basic research into the possible diverse consequences of ChE inhibitors is encouraged.
The responses were examined of the L5178Y mouse lymphoma cell tk"tk" mutation assay (Cleve and Spector, Mutation Res. 31(1975)17-29) to chemicals metabolized through a variety of mechanisms, in an attempt to identify an optimal, general purpose metabolic activation system. The basic system which was manipulated for this investigation consisted of Aroclor 1254-induced male rat liver S9, NADP and glucose-6-phosphate in Fischer's medium. Addition of Mg++ is not necessary since this reduces cell survival and the ton is already present in mammalian cell culture media. Over at least a 4h incubation period, NADPH generation was not a limiting factor. S9 toxicity was variable, but in some preparations 50 ul/ml decreased survival and increased mutation frequency. S9 at 30ul/ml was the highest concentration that could be used and reliably avoid these unwanted effects. With added, single concentration substrates and avoiding relative total growth 10%, significant increases in mutation frequencies occurred at the following S9 concentrations: 3-methylcholanthrene, 2.5ug/ml S9 at 2.5 - 5 ul/ml cyclophosphamide, 3.0ug/ml S9 at 5 - 10 ul/ml procarbazine, 3.0ug/ml S9 at 5 - 30 ul/ml methanol, 7.9mg/ml S9 at 10 - 15 ul/ml dimethylsulfoxide, 2mg/ml S9 at 10 - 15 ul/ml 2-acetylaminofluorene, 40ug/ml S9 at 15 - 30 ul/ml Effects of S9 on assay sensitivity are currently being investigated and must be considered before final recommendation of the activation system.

CHO-K1, BR4 cells were exposed to either N-hydroxy-2-aminofluorene, N-hydroxy-N'-acetylbenzidine or 1-nitrosopyrene, each of which forms one major DNA adduct, substrate through the C-9 position of deoxynucleosine. Thus, the effects of different adduct structures on the induced biological responses and their relationships could be assessed. Allquots of the exposed cells were then used to measure SCE frequency, mutation induction at the HGPRT locus and cloning efficiency. When SCE formation and mutation induction were compared, the correlation coefficient obtained was low, indicating the lack of a predictive, quantitative relationship between these two responses. A weak correlation was also obtained when mutation induction was compared to cell survival. However, when SCE formation was compared to reduced cell survival, a strong correlation (r=0.93, p<0.0001) was observed and there were no significant differences among the slopes and intercepts of the linear regression lines of the individual chemicals. The strong, chemical-independent, quantitative relationship described here, similar to that described in CHO cells exposed to simple alkylating agents (Morris et al., Mutation Res. 105:163-168, 1982), again suggests a common factor in the induction of these latter two endpoints.

Cytochalasin B (CB) is one of a large group of cytochalasins isolated from fungal extracts. CB is cytotoxic for cells in culture and is recognized as a microfilament inhibitor. We have examined the effects of CB on differentiation in B16 melanoma cells in culture. In these cells, differentiation is associated with the production of the pigment melanin which is synthesized intracellularly and secreted into the extracellular culture fluids. We found that CB, in concentrations ranging from 1-10 µM, was a potent inducer of melanogenesis in B16 melanoma cells. The concentration of CB required to inhibit growth of the cells by 50% was in the range of 3 µM. At 10 µM, CB completely inhibited cell growth. B16 cells became multinuclear and spread on the culture plates. This was associated with increased melanin production by the cells. 1 µM CB had no effect on cell growth or morphology but did induce differentiation, as evidenced by the release of melanin into the culture medium. These results indicate that growth inhibition is not required for CB to induce differentiation in B16 cells. Furthermore, it appears that CB induced melanogenesis is independent of microfilament inhibition.

(Supported by NIH grant CA 33212)

A rapid and reliable alkaline elution assay was developed to detect single-stranded DNA breaks in cultured bovine kidney epithelial cells (MDBK). Cells were seeded 22 hrs prior to labeling with [3H]thymidine for 22 hrs. The cells were then washed and exposed to either the test compounds for 4 hr or x-ray. DNA was eluted with 42 ml of elution solution (pH 12.4) by gravity flow. Elution was generally complete by 2 hr. DNA was quantitated in eluates and filters by LSC. Cell viability was estimated by trypan blue dye exclusion. The extent of strand breakage was measured by two ratios: 1) elution fraction (DNA/total DNA) and 2) DNA on filter plus filter wash DNA/total DNA was effectively calibrated by x-irradiation (200, 400, 800 and 1500 R) showing a reproducible dose response. The assay proved to reliably detect carcinogetic activity of seven carcinogens at concentrations of 0.03 mM, 0.3 mM and/or 3 mM. All carcinogens tested showed dose-responsive increases in strand breaks. Six non-carcinogens were shown to induce no detectable strand breaks over control cultures by this method. These results indicate the gravity flow alkaline elution technique is a rapid, sensitive and reliable method to determine DNA strand breaks in a cell culture system. Supported in part by USPHS grants ES03410 and ES07097.
COMPARATIVE METABOLISM AND TOXICITY ASSESSMENTS USING HUMAN HEPATOCYTES. C. E. Green, C. A. Tyson, J. C. Mirtsalis, and W. F. Blazak, SRI International, Menlo Park, CA 94025

Hepatocytes were isolated by collagenase perfusion from several species including humans and characterized as to their functional and metabolic competence for use in in vitro toxicity studies. Human liver tissue was obtained from heart/lung transplant donors and the cells were isolated by the biopsy perfusion method within 2.5 hr of organ removal. Hepatocytes from rodents, rabbits, dogs, monkeys, and humans averaged 86, 83, 88, 82, and 90% viability (trypsin blue exclusion), respectively. The rate of urea synthesis varied with species from 4.23 for human to 22.6 nmole/min/10^6 cells for dog hepatocytes. Benz(a)pyrene hydroxylase activity was highest in squirrel monkey (5.90 nmole/10^6 cells) and lowest in human (0.47 nmole/10^6 cells) hepatocytes; p-nitroanisole O-demethylase activity was highest in rabbit hepatocytes (47.7 nmole/10^6 cells vs. 14.1 nmole/10^6 cells for human). The metabolic profiles of amphetamine produced by hepatocytes from 5 species including humans were very similar to those obtained in vivo. Cultured human hepatocytes also metabolized acetaminophen (APAP) to reactive intermediates and lost viability with increasing APAP concentrations characteristic of resistant species. Human hepatocytes activated genotoxins as evidenced by the detection of unscheduled DNA synthesis and by induction of sister chromatid exchanges in Chinese hamster cells cocultured with them. Thus, in vitro studies using human hepatocytes should have significant potential for bridging the gap between laboratory animals and humans. Supported in part by GM28158.

PROPOSED TERATOGENIC MECHANISM OF ACTION FOR PROCARBAZINE HYDROCHLORIDE IN 13-14 DPC EMBRYONIC RAT NUCLEI. D.S. Johnson, I.A. Muni, G.N. Wogan, and M. Newberne, Bioassay Systems Corporation, Woburn, MA; Massachusetts Institute of Technology, Department of Nutrition and Food Science, Cambridge, MA.

The purpose of this study was to examine a possible teratogenic mechanism of action for procarbazine hydrochloride. In vivo and in vitro administration of procarbazine was studied using rat embryo nuclei to investigate what effects this drug may have upon nuclear DNA-dependent RNA polymerase activities. For 13 dpc nuclei the Mg++ and Mn++/(NH4)2SO4-activated RNA syntheses were inhibited 50% of control values, showing no 24-hour recovery patterns. Recovery was observed for 14 dpc nuclei. Regional nuclei (head) showed higher polymerase activity than carcass nuclei. Polymerase prepared from head nuclei also showed a greater decrease in activity after procarbazine treatment. Soluble enzyme preparations of RNA polymerase and 14dpc rat embryonic chromatin were prepared in order to evaluate which fraction was more affected by drug treatment. A greater effect was observed on the polymerase activity, suggesting that procarbazine may interact with an embryonic translational process. A mechanism of action is proposed for this teratogen in rats, indicating a possible nuclear site of action upon RNA processing.

MATERNAL AND FETAL 14C EXCRETION AND TISSUE DISTRIBUTION PATTERNS FOR LABELLED PROCARBAZINE HYDROCHLORIDE ADMINISTERED DURING ORGANGENESIS IN RATS. D.S. Johnson, I.A. Muni, G.N. Wogan, P.M. Newberne. Bioassay Systems Corporation, Woburn, MA; Massachusetts Institute of Technology, Department of Nutrition and Food Science, Cambridge, MA.

The drug-induced malformations in rat embryos have been established using procarbazine hydrochloride. The dose-response character for various skeletal and other connective tissue defects was estimated for 10-14 dpc using singel i.v. injections. Whole 21 dpc tissues were observed for malformations by specimen clearing and histological examination. Total maternal 14C-excretion and embryonic 14C distribution studies were undertaken using 14C-1-methyl procarbazine. Maternal 14C recovered dose by 24 hours, with the total embryonic (13 dpc) compartments receiving less than 0.5% of the maternal dose. There were also differential embryonic 14C tissue distribution patterns, suggesting selective 14C specific activities for limb buds and amniotic fluid. Pharmacokinetic studies suggested that amniotic fluid may be an important maternal compartment for sustaining embryonic drug exposure. Chemical fractionation of embryonic tissues showed most of the 14C is incorporated into RNA of limb buds and embryos, with maximum specific activity attained by 4 hours. 14C-DNA specific activity increased steadily over 24 hours and was nearly 10 times its 1 hour value for limb buds at 24 hours. Placental fractions showed no differential specific activities over 24 hours.


The potential long-term effects of materials employed in intracocular lenses (poly(methylmethacrylate), PMMA; titanium wire, TiW; silicone glass, SIC) were evaluated in groups of albino rats for 2 years following implantation. Additional groups served as negative controls (USF reference standard plastic for implants), sham controls or untreated controls. All implants were into the right globular muscle. There was no systemic toxicity or ophthalmic effects. Male rats implanted with SIC showed increased incidence of skin nodules during the last 3 months of study. Pathologic evaluation revealed no non-neoplastic lesions attributable to the test materials. All groups receiving implants, including negative controls, showed inflammatory and degenerative responses at implant sites. Survival rates were similar and allowed adequate statistical evaluation of tumor data. Most common spontaneously occurring tumors in both sexes were pituitary adenomas and lymphomas. Average latency, tumor rates and types showed no significant variations between treated and control groups. Tumors arising at the implantation site were not observed. Thus the intracocular lens materials tested evoked no overt toxic effects and showed no evidence of carcinogenic potential.
The biologic (hypocalcemic) & toxicologic effects of intranasally instilled salmon calcitonin (sCT) were measured to evaluate this route of administration for the peptide.

Treatment of rats with sCT, 10 U/kg in 1% gelatin vehicle reduced serum calcium by 17% 1 hour later; calcium returned to baseline by 3 hours. When surfactants were added, sCT at 1 U/kg lowered calcium by 18% - 24% at 1 hour; 10 U/kg decreased calcium by 18% - 29% & 27% - 31%, respectively, at 1 & 3 hours. When acetate or citrate buffers were used as vehicle, similar decreases were observed.

Dermal, ocular, oral & intranasal safety studies in rats & rabbits demonstrated that these formulations produced no signs of toxicity or local irritation.

These results indicate: 1) sCT can cross the nasal mucosa in rats, 2) the use of surfactants enhances this absorption, as judged by the biologic effect of the peptide, 3) these preparations were safe when administered to animals.

Chrysotile, anthophyllite, forsterite, quartz, and tantalum particles in the 1 μm size range were physicochemically characterized to determine their bulk and surface properties. The effects of these dusts on alveolar macrophages lavaged from inbred rats were studied. Cultures were inoculated with characterized dusts at 50, 100, and 200 μg/ml. At various times macrophages were counted and tested for viability, phagocytosis and killing ability, and esterase activity. Studies of particle effects encompassed 14 treatment durations ranging from 2 min to 61 hrs. Methods for selective extraction of the cytoplasmic matrix using Triton X-100 and glycerol in PHEM buffer were developed to facilitate cytoskeletal studies. Unseparated cells were fixed for stereo scanning and high voltage electron microscopy with glutaraldehyde-tannic acid and OsO₄ in PHEM buffer. Dust effects on cytoskeletal morphology and function reflected decreases in adherence, phagocytosis, and spreading. The chrysotile and forsterite were found to induce irreversible damage by 4 hrs. Quartz and anthophyllite were less damaging and tantalum was biologically inert. Cytoskeletal damage was not restricted to areas in which particles were located. The relationships between particle characteristics and biological activity will be discussed.

Somatic cell mutation arising as a result of exposure to inorganic lead in vivo--a measure of the effect of the metal on the integrity of DNA--was quantified by determining in vitro the percentage of rat peripheral lymphocytes which were resistant to 6-thioguanine (TG) as compared with the response of cells from control rats. Resistant cells are mutant with respect to the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). A modification of the method of Albertini and Strauss was used to quantify by autoradiography and scintillation counting the amount of 3H-thymidine uptake in the presence of TG. Male, CPAI rats, 6-7 weeks old, were exposed to lead acetate at 0.5, 1.5 and 2.0 mg/ml lead acetate in drinking water ad libitum for up to 10 weeks. Exposure to lead caused an abnormal increase in 3H-thymidine incorporation of lymphocytes in the presence of TG as well as an increase in the percent of labelled nuclei. This TG resistance response appeared to be dose dependent. These results indicate that lead causes somatic mutation in rats and suggest that studies should be carried out to examine this phenomenon in humans.

(Supported in part by Research Grant 15-16 from the March of Dimes Birth Defects Foundation)

The antineoplastic drug BIDA was given intravenously to mice, rats and dogs as a single dose (x1) or five consecutive daily doses (x5). In mice, the LD90, LD50 and LD10 were 236, 207 and 182 mg/m2 for x1 and 98, 85 and 75 mg/m2/day for x5. In the toxicity studies, mouse equivalent LD50, LD10 and the derived doses of LD10 were used. Clinical signs exhibited in dogs and rats immediately after dosing suggested toxic effects on the central nervous system and gastrointestinal tract. Many of the dogs also excreted reddish-colored urine. This color was attributed to the drug, not blood. Changes in clinical pathology were decrease in MBC in dogs and rats and decreases in reticulocytes, platelets, MCT, HGB and RBC in rats. Histopathological lesions were observed only in rats in the hematopoietic, lymphatic, digestive and reproductive systems. Toxicity was dose-related. Males and females were similarly affected. Reversibility was evident but incomplete in the x5 rats. Very few indicators of toxicity were observed in dogs at the 1/10 MELD.

Supported by NCI Contract No. NO1-CM-17365.


Adult female mink were fed diets that contained 0.01 or 0.05 ppm 3,4,5,3',4',5'-hexachlorobiphenyl (3,4,5-HCB) from two months prior to breeding through parturition (135 days). In addition to an ad libitum fed control, both treatment groups had corresponding pair-fed control groups. Fifty percent of the females fed 0.05 ppm 3,4,5-HCB died during the exposure period while 0.01 ppm had no effect on mortality. Body weight gains were significantly depressed only in the 0.05 ppm group, while food consumption was not consistently affected. Liver weights were significantly greater in both treatment groups than in the controls. 3,4,5-HCB fed at 0.05 ppm caused a significant increase in kidney and adrenal weights. PCB treatment had no significant effect in hepatic aminopyrine N-demethylase and benzo(a)pyrene hydroxylase activities. Free T4 was not affected by PCB treatments but bound T4 was significantly lower at the 0.05 ppm level and free and bound T3 concentrations were significantly reduced at both 0.01 and 0.05 ppm HCB.


Kathon® NAR biocide was administered in the drinking water to 3 groups of Sprague-Dawley rats (25/sex/grp), for 15 wks, at concentrations of 25, 75, and 225 ppm of active ingredient. Two additional groups of 5 rats/sex/grp were given tap water or the water containing the same inorganic ion concentration as the high dose group. All animals were observed daily. Body weights, food consumption, and water consumption were determined weekly. After 4 and 15 weeks of treatment 10/sex/grp were bled for hematological and clinical chemistry analyses. After 13 wks, 15 rats/sex/grp were killed, necropsied, and selected organ weights recorded. The remaining 10 rats/sex/grp were treated for an additional 2 wks then narcotized. Maternal water consumption and body weights were recorded. The progeny were monitored until Day 21 of lactation. No deaths occurred and no toxic signs were seen during the toxicity or reproductive phases. Transient decreases in bodyweights and food consumption observed in the high dose group. A concentration-related decrease in water consumption was attributed to palatability of the Kathon. No hematological effects were seen. A decrease in globulin and an increase in A/G ratio, seen in males at 225 ppm and in the low control group. Decreased total protein was seen only in the high dose males. SGOT was increased in females at 225 ppm. No treatment-related gross pathological changes were seen at necropsy; however, histopathologic examination of the animals killed after 15 wks showed a low-level irritation of the stomach mucosa of the stomach at 225 ppm. No treatment-related effects were seen at 75 ppm, no adverse effects were seen on reproductive capability or on pup survival.


A battery of acute toxicity studies was conducted on (1) raw extracted bitumen, (2) raw naphtha (0-290°C), and (3) synthetic crude oil (6-500°C), derived from athabasca tar sands. In an acute oral study (5 male/5 female rats), all three test samples exhibited a low order of toxicity (95.0 g/kg). The acute dermal LD50 in rabbits (3 male/3 female) was also low (35 g/kg) for each test material. All three materials were judged to be "slight" irritants in eye irritation studies as evidenced from maximum Draize scores of 4, 8 and 6 respectively. Single concentration acute inhalation studies (6 hour exposure, 10 rats/mice, 5/sex) produced varied responses. Raw bitumen administered as an aerosol/vapor (1.46 mg/l) did not cause mortality in either test species; lung discoloration was the only necropsy finding of note. Raw naphtha administered as aerosol/vapor (10.6 mg/l) was lethal to essentially all the mice during the exposure period, while only 2 rats died. Necropsy findings indicative of toxicity in both species were lung, liver and kidney damage. Synthetic crude oil administered primarily as aerosol (4 mg/l) resulted in the death of 5/10 mice and 0/10 rats. Severe hair loss was noted in the surviving mice with some alopecia in rats. Both species exhibited liver and lung toxicity. Acute effects of comparable petroleum and asphalts materials are similar and will be discussed.
Bactobolin was evaluated in beagle dogs using both the X1 and X5 schedules. Single doses of 0.75 mg/kg (0.57MELD10) produced mild, reversible toxicity while single doses of 1.31 mg/kg (MELD10) produced lethality in 4/4 dogs. Bactobolin was slightly more toxic on the X5 schedule with lethality noted in 4/4 dogs given five intravenous doses of 0.45 mg/kg/day (1/2MELD10). Mild, reversible toxicity was observed in 4/4 dogs given 0.18 mg/kg/day (0.20MELD10) on the X5 schedule. All deaths occurred between days 2 and 5. All dogs vomited within one hour of dosing regardless of the size of the bactobolin dose given or the dose schedule used. Bactobolin produced an inflammatory vasculitis and a drug-induced alteration in vascular fragility and hemodynamics. Prothrombin times (PT) were markedly prolonged on day 2 for MELD10 dogs on the X1 schedule, but this effect was short lived. Changes noted in various hematology parameters were consistent with bactobolin-induced necrosis and/or depletion of bone marrow and lymphoid tissue which were noted during histological evaluation. Other tissues affected included: pancreas, gastrointestinal tract, liver, spleen, testes and uterus. Supported by Contract No. NO1-CM-17365.

The subacute oral toxicologic profile of a novel anxiolytic agent, (4-2-chlorophenyl)-1,6-dihydro-1,3,9-trimethylimidazo-(1,2-a)pyrazolo-(4,3-f) diazepine (CI-918), was evaluated in rats and dogs. CI-918 was well tolerated by Wistar albino rats when given as dietary admixture at dose levels of 80, 40 and 20 mg/kg/day for 13 weeks with no clinical signs of drug toxicity. Females dosed at 80 mg/kg had significant reduced body weight gains over the study period. No drug-related clinical laboratory, gross or histopathologic changes were identified. CI-918 administered orally in gelatin capsules at daily dose levels of 30, 15 and 7.5 mg/kg for 13 weeks produced no significant alterations in food consumptions or body weights in Beagle dogs. Transient behavioral changes of aggression and apprehension were the main clinical signs in all treated groups. Drug-associated microscopic pancreatic lesions characterized by degenerative and atrophic changes of the exocrine acinar cells were observed at all dose levels. Pancreatic islet cells appeared normal in all dogs. The pancreatic changes had not been observed in a previous 2 week oral subacute study in dogs or rats.

An insulin formulation was evaluated for toxicity in beagle dogs by a series of fourteen consecutive i.v. administrations. Three formulations were used: a sodium acetate buffer, pH 7.8, a vesicle carrier containing the acetate buffer and a vesicle carrier containing insulin. Thirty six dogs were divided into the following six groups of six animals of equal sex: The control; vesicle containing buffer at 0.13 and 0.40 mg/kg (lipid); and, the vesicle containing insulin at 0.12, 0.25 and 0.39 units. Each dog, fasted overnight, was infused daily via the cephalic vein using a 2 inch saline indwelling catheter attached to a Harvard or Sage infusion pump, regulated to deliver the dose over a one hour period. Blood was withdrawn prior to initiation of treatment and again on day 15. Complete cell count (9 parameters) and serum chemistries (21 parameters) were conducted. Additionally, urinalysis was performed at these intervals and microscopic evaluation of tissues was made. The repetitive i.v. infusions were well tolerated by the dogs. The animals remained clinically normal during and after each infusion period. Blood, serum chemistry and urinalysis parameters were not significantly altered. It was concluded that i.v. administration of insulin-containing vesicles at this dose level and infusion rate was well tolerated by beagle dogs.

SCH 34343 is a new broad spectrum penem antibiotic which is highly active against Gram-positive and Gram-negative (excluding Pseudomonas) organisms. Acute and subchronic perinatal toxicity studies of SCH 34343 have been conducted in dogs, mice and rats. The ID50 in I.M. and I.V.-dosed mice and I.M.-dosed rats ranged from 3500 to 4000 mg/kg with no apparent target organs evident. The I.V. ID50 in rats ranged from 1544 mg/kg (males) to 2280 mg/kg (females); clonic convulsions were observed in males at 1189 mg/kg and higher and in females at 1679 mg/kg and higher. No target organs were identified in an I.V. rising-dose tolerance study in dogs at doses up to 2000 mg/kg. No mutagenic activity was observed in mouse bone marrow micro-nucleus, mouse lymphoma or Salmonella/mammalian microsomal mutagenicity bioassays. In an I.V. rat teratology study (200, 400 and 800 mg/kg), no compound-related changes were observed. Compound-related changes in 1-month studies in rats (I.P. - 160, 320 and 640 mg/kg, b.i.d.) and dogs (I.V. - 160, 240 and 480 mg/kg, b.i.d.) were limited to minimal renal changes in rats and erythrocyte, platelet and hepatic changes in dogs. Minimal to mild gastrointestinal changes also were observed in both species. These studies demonstrated that SCH 34343 possesses a very low order of toxicity.
Proliferative Liver Lesions in Rats Induced by Methyl Carbamate. P.C. Chen, and J.A. Quest, National Toxicology Program, NIH, Research Triangle Park, NC. Sponsor: W.M. Kluwe.

Methyl carbamate (MCB) is used to make dimethyl methyl carbamate-based resins which are applied in the textile industry as durable-press finishes for polyester/cotton blend fabrics. The toxicity of MCB was investigated because of its potential for long-term human exposure and its structural relationship to urethane. Groups of 10 Fischer 344/N rats were administered MCB in distilled water by gavage 5 times/week for 13 weeks. The doses given were 0, 50, 100, 200, 400, 800 mg/kg for male rats and 0, 62.5, 125, 250, 500, 1000 mg/kg for female rats. The highest dose of MCB for male and female rats respectively reduced survival; the lower doses had no effect on survival. Clinical signs of toxicity included: lethargy, rapid breathing, rough coats and incoordination. Dose related reduction in body weight and increase in incidence of hepatic lesions were observed in rats of both sexes. The lesions included foci of cytologic alterations (basophilic, clear cell, and acidophilic), Feulgen-positive cytoplasmic inclusions, atypical mitoses, cytomegaly, necrosis, and pigmentation. Other treatment related changes were testicular atrophy, bone marrow atrophy, and hepatic pigmentation (hepomiderin). The proliferative nature of the hepatic lesions resembled those observed in early stages of hepatocellular carcinogenesis. The carcinogenic potential of MCB is being investigated.


Prechronic toxicity studies of 4-nitrotoluene (a chemical intermediate) in F344 rats and B6CF1 mice were conducted. In the 14-day repeated-dose studies, groups of 5 animals/sex/species were given 0, 93, 187, 375, and 750, or 1500 mg chemical/kg body weight in corn oil by gavage daily, 5 consecutive days per week for 2 weeks. Compound-related deaths occurred in male rats and mice at 1500 mg/kg and in females of each species at >750 mg/kg. In the subchronic studies, the chemical was administered to groups of 10 male and 10 female rats in corn oil by gavage at dose levels of 0, 25, 45, 90, 180, or 360 mg/kg daily, 5 consecutive days per week for 13 weeks. Similarly, dose levels of 0, 10, 20, 40, 60, 80, and 160 mg/kg were administered to mice. No compound-related deaths occurred. Kidney lesions (hyaline droplets) were noted only in male rats at the 360 mg/kg dose level. Organ weight to body weight ratios of brain, heart, kidney, liver, lung, and testes were affected in rats while only liver was affected in mice. From these results, it appears that 4-nitrotoluene has a sharp dose-response curve. A separate guinea pig sensitization study using the Buehler closed patch technique showed that 4-nitrotoluene was a potent sensitizer as judged by incidence (9/10) and severity (1.3 at 24 hours) indices. Rechallenge resulted in no response.

Toxicity of Microencapsulated Trichloroethylene (TCE) in Rats. R. Melnick, T. Goehl, B. Collins, C.W. Jameson, R. Maronpot, A. Greenwell, F. Harrington, R. Ntson, K. Tomaszewski, D. Agarwal National Toxicology Program, NIH, RTP, NC.

TCE, stabilized in gelatin-sorbitol microcapsules (45% TCE, wt/wt), was mixed in NIH-07 diet at microcapsule concentrations of 0 (untreated or 5% placebo capsules), 1.25, 2.5, 5, and 10%, and provided to groups of 10 male F344 rats for 2 weeks. Additional groups of male rats were administered TCE in corn oil by gavage for 14 days at dose levels comparable to those in the feed study. Deaths occurred only in the gavage groups at the two highest dose levels. Body weight gain and feed consumption were reduced at the two highest dose levels of both the feed and gavage groups. During the study, the measurements of TCE content in the feed samples varied from 97 to 113% of the initial TCE concentrations. Dose related increases in organ (liver and kidney) weight/body weight ratios and in hepatic peroxisomal palmitoyl CoA oxidase and microsomal NADPH cytochrome C reductase activities were found in both the dosed feed and gavage groups. The liver and kidneys are being examined microscopically. The demonstration of no significant loss of TCE from the feed and of similar toxic effects produced by microencapsulated and neat TCE indicates that microencapsulation can provide an alternative oral route of administration to study the toxicological properties of TCE.


Prechronic toxicity studies of 4-nitrotoluene (a chemical intermediate) in F344 rats and B6CF1 mice were conducted. In the 14-day repeated-dose studies, groups of 5 animals/sex/species were given 0, 93, 187, 375, 750, or 1500 mg chemical/kg body weight in corn oil by gavage daily, 5 consecutive days per week for 2 weeks. Compound-related deaths occurred in male rats and mice at 1500 mg/kg and in females of each species at >750 mg/kg. In the subchronic studies, the chemical was administered to groups of 10 male and 10 female rats in corn oil by gavage at dose levels of 0, 25, 45, 90, 180, or 360 mg/kg daily, 5 consecutive days per week for 13 weeks. Similarly, dose levels of 0, 10, 20, 40, 60, 80, and 160 mg/kg were administered to mice. No compound-related deaths occurred. Kidney lesions (hyaline droplets) were noted only in male rats at the 360 mg/kg dose level. Organ weight to body weight ratios of brain, heart, kidney, liver, lung, and testes were affected in rats while only liver was affected in mice. From these results, it appears that 4-nitrotoluene has a sharp dose-response curve. A separate guinea pig sensitization study using the Buehler closed patch technique showed that 4-nitrotoluene was a potent sensitizer as judged by incidence (9/10) and severity (1.3 at 24 hours) indices. Rechallenge resulted in no response.


A comparison was made of the health status of Sprague-Dawley rats housed in Hazeltone ventilated stainless steel caging and nonventilated stainless steel caging. Twenty male and female rats were housed in each type of caging for 28 days. No test chemical was administered. Airflow in the ventilation exhaust trunk, airflow into selected cages, and exhaust system static pressure were recorded.

There were no significant differences in mean body weights between groups. Mean body weight gain was significantly increased in male and female rats in ventilated housing during the first week as compared to rats in conventional housing. There were no meaningful differences in food consumption, urinalysis, organ weights, organ/body ratios, or gross pathological changes at necropsy. Of 12 hematology and 20 blood chemistry parameters examined, hemoglobin and hematocrit were decreased and alkaline phosphatase and cholesterol were increased in males in ventilated caging; however, the values were within the observed historical range. In conclusion, housing in ventilated caging appears to produce no significant adverse health effects in rats.

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AN EVALUATION OF THE HEALTH HAZARDS FROM PESTICIDES IN WATER SUPPLIES OUTSIDE OF THE UNITED STATES. R. Scofield and D.P.H. Baier.
Department of Environmental Toxicology, University of California, Davis, CA

American military personnel stationed abroad are dependent on local water supplies which occasionally contain high levels of pesticides and other contaminants. The purpose of this study was to identify those pesticides which are most likely to be present in water supplies at health-threatening levels. A database of over 1500 measured pesticide concentrations was collected. These concentrations were then evaluated for their health significance with respect to their long-term and short-term hazards. A simultaneous search for epidemiological evidence of health hazards attributable to pesticides in drinking water was also undertaken. The information we collected showed that the chlorinated hydrocarbons (e.g., DDT and lindane) are the most commonly detected pesticides in water outside of the United States. It also appears that pesticides in drinking water are not a common health hazard.


The use of orbital sinus bleeding (OSB) was evaluated to determine if adverse effects are potentiated after serial OSB in rats exposed to known toxicants. Groups of 18 rats/sex were orally gavaged daily for 6-7 weeks with CCl₄ (0.25 ml/kg), uranyl nitrate (UN, 5.0 reduced to 2.5 mg/kg) or imipramine HCl (IH, 50 mg/kg). These treatment groups were divided into 3 subgroups: terminal bled (TB) from the vena cava after 1 week of dosing; TB during the 7th week; or serially bled by OSB during weeks 1 (pre-test), 1.3.4 and 6, plus TB at week 7. Treatment effects were compared in anesthetized rats bled serially by OSB and anesthetized rats bled once by TB. Clinical signs and weight gain were monitored. Blood samples were taken for serum chemistry and hematology and urine samples collected for urinalyses. Histopathology was done on target organs. Serial OSB decreased weight gain in controls. Severe toxicity was noted in IH rats only after OSB. There were interactive effects on weight gain between serial OSB and treatment with CCl₄ (addition) or UN (antagonism). Since OSB-induced effects or OSB-compound interactions can occur, aspects of toxicity other than clinical pathologic changes should be evaluated in nonserially bled rats.

COMPARATIVE SUBCHRONIC EFFECTS OF CHLOROFORM ADMINISTERED TO MICE IN CORN OIL OR 2% EMULPHOR IN WATER. J.M. Brown, E.F. Mielrhenry, C.J. Rushbrook, T.A. Jorgenson, J.R. Meier, and R.J. Bull. SRI International, Menlo Park, CA and HERL, USEPA, Cincinnati, OH

In previous chronic bioassays, chloroform (CHCl₃) in corn oil by gavage was carcinogenic in the mouse liver, whereas CHCl₃ in drinking water was noncarcinogenic to mice. The current study was undertaken to investigate the comparative hepatotoxicity of CHCl₃ administered with a lipid (corn oil) and with an essentially non-lipid vehicle (2% emulphor in water). Ten 86g/31 mice of each sex were treated by gavage for 90 days with 0, 60, 130, or 270 mg/kg/day of CHCl₃ in either vehicle. Mice receiving CHCl₃/lipid were much more susceptible to the toxic effects of CHCl₃ than were mice receiving CHCl₃/non-lipid. Male and female mice receiving CHCl₃/lipid exhibited significant dose-related reductions in body weight, increases in food consumption, decreases in serum triglyceride, and increases in liver weight. Other organ weights and numerous clinical chemistry and hematology parameters were also affected but to a lesser extent. Lipid content of the liver was significantly increased at the low-dose level, whereas more advanced hepatic degeneration, with initial stages of cirrhosis, was seen at the high-dose level in both sexes. Mice receiving CHCl₃/non-lipid showed very little evidence of CHCl₃ hepatotoxicity. (This work was supported by EPA Contract No. 68-03-1880 but does not necessarily reflect official EPA policy).


This laboratory has previously reported that low concentrations of methylmercury (MM) potentiated human platelet secretion by a prostaglandin (PG) dependent mechanism. Further studies carried out with rat platelets demonstrated that MM inhibited arachidonate 12-lipoxygenase. We have extended these studies to examine the effects of MM on vascular and platelet (PG) biosynthesis. MM at nanomolar concentrations stimulated coronary prostacyclin (PG1) biosynthesis (RIA of 6-keto-PGF₁α), aorta PG1₂ biosynthesis (bioassay and inhibition of platelet 5HT secretion) from endogenous precursor. Similar concentrations of MM potentiated platelet aggregation and PG biosynthesis from exogenous [3H] arachidonic acid. In vivo MM enhanced PG biosynthesis could alter cellular function (via PGs), damage membranes (PGD₂ or PGH₂) or deplete arachidonic acid pools.
A TIME- AND DOSE-DEPENDENT SUPERSENSITIVITY TO NOREPINEPHRINE IN THE RAT CAUDAL ARTERY FOLLOWING PRETREATMENT WITH BCNU. C.L. Rawson and R.E. Larson. College of Pharmacy, Oregon State University, Corvallis, OR

Studies with the carcinostatic drug 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) demonstrated that this drug had profound effects on the cardiovascular system of the rat that had not previously been described. Caudal artery segments taken from BCNU-pretreated (15-25 mg/kg ip) and control rats were isolated, cannulated, and perfused intraluminally with Kreb’s bicarbonate (37°C). Arterial segments were stimulated via bipolar platinum ring electrodes at 1.4-10.0 Hz (.3 msec duration) for 10 seconds at supramaximal voltage to generate frequency response curves (FR). Arteries were then perfused with norepinephrine (5.6 x 10^-6 to 10^-5 M) to generate concentration response curves (CR). FR of arteries from treated rats were not significantly displaced from those of controls but CR were shifted significantly (p<.05) to the left of control artery curves. CR generated with methoxamine showed no significant difference in treated versus control rats. These findings suggest that the supersensitivity of the caudal artery to norepinephrine is most likely not caused by a change in alpha-adrenergic receptor dynamics or transduction but may have its origin in an alteration of the metabolism or uptake of the neurotransmitter, norepinephrine, in BCNU treated rats.

ADJUNCT FORMATION OF TOXIC ALPHA, BETA-UNSATURATED ALDEHYDES WITH 2-THIOBUTARIC ACID. G. Witz, A. Laccaria, N.J. Lawrie, H.J. Perman, Jr.,* and B.D. Goldstein. UMDNJ-Rutgers Medical School, Piscataway, NJ and University of New England, Biddeford, ME.

In the thioleptic acid (TBA) test for lipid peroxidation, the three-carbon dialdehyde malonaldehyde (MDA) or MDA precursor formed during lipid peroxidation react with TBA to give an adduct which absorbs maximally at ~530 nm. The presence of the present work has to date been if other biologically reactive aldehydes were capable of adduct formation with TBA. The compounds examined were: acrolein (ACR) and crotonaldehyde (CRO), two environmental air pollutants; trans-4-hydroxynonenal (4-OH-N), a toxic lipid peroxidation product; and trans,trans-2-muconaldehyde (HDO), a six-carbon hematoxinic diene-dialdehyde which might be a ring-opened metabolite of benzene. The aldehydes were incubated with TBA at 100°C for 15-120 min and the formation of absorption maxima at 450, 495 and 530 nm was studied. After reaction, the mixtures were purified using a Boker-10 solid phase extraction system. The spectral characteristics of the methanol eluants of the purified mixtures showed that 4-OH-N, similar to MDA, is a TBA adduct with a 530 nm absorption maximum. The TBA adducts of HDO, ACR and CRO exhibited the major absorption maximum at 490 nm. These studies show that alpha,beta-unsaturated aldehydes can form a 490 chromogen with TBA which might be used for their detection in a variety of systems.

TOXIC INTERACTION OF DOXORUBICIN AND UBIQUINONE-10 ANTAGONISTS. O. Tabara and A.B. Combs, Div. of Pharmacology and Toxicology, College of Pharmacy, Univ. of Texas, Austin, TX

Administration of ubiquinone-10 (CoQ) can reduce the acute toxicity of doxorubicin (DOX) in rats and mice. Three in vitro antagonists of CoQ, 2-hydroxy-3-n-dodecylmercapto-1,4-naphthoquinone (I), 2,3-dimethoxy-5-mercapto-octadecyl-1,4-benzoquinone (II), and 2,3-dimethoxy-5-beta-naphthylmercapto-1,4-benzoquinone (III), were used in combination with DOX and with DOX + CoQ to determine whether CoQ's protective action might be coenzymatic or antioxidant in nature. Inhibitor II had very little effect on survival in mice in combination with DOX, inhibitor I increased the lethality moderately, and inhibitor III greatly increased the lethality. DOX alone, or pretreatment with CoQ before DOX did not change glutathione reductase and glutathione peroxidase activities in the heart and liver 48 hours after DOX. The inhibitor III, by itself, appeared to reduce heart and liver glutathione reductase and peroxidase activities. These effects of Inhibitor III were not prevented by CoQ pretreatment. (Supported in part by HL29463 from the National Heart, Lung, and Blood Institute).

ANALYSIS OF 4-METHYLPYRAZOLE PLASMA AND URINE LEVELS BY HPLC AND USE IN THERAPY OF ALCOHOL POISONINGS. R.E. McMartin, T.D. Collins, and T.P. Reelert. Department of Pharmacology, Section of Toxicology, L.S.U. Medical Center, Shreveport, LA.

4-Methylpyrazole (4-MP), a potent competitive inhibitor of alcohol dehydrogenase activity, has potential usefulness as a treatment means for methanol and ethylene glycol poisonings. Further study of the safety and metabolism of 4-MP in human subjects is needed before it can be used in such cases. An HPLC assay has been developed to measure 4-MP levels in plasma and urine samples. 4-MP was eluted on a reverse phase column using a mobile phase which was composed of 40% methanol; 60% potassium phosphate buffer (5 mM, pH 7.5). Detection was by UV absorbance at 220 nm. The method was sensitive enough to quantitate 4-MP in an amount as low as 0.1 nmol. Recovery of 4-MP from spiked urine and plasma samples was greater than 90%. 4-MP levels in the plasma and urine of rats injected with an oral dose of 50 mg/kg were determined; the detectability limit in such samples was about 3 µM. The method is easy to perform and thus has practical application for research laboratories dealing with ethanol metabolism and clinical laboratories desiring to monitor 4-MP levels. (Supported in part by a research grant from the Distilled Spirits Council of the United States, Inc., and by Biomedical Research Support Grant N.I.H. 05822).
Selective analysis of thiocyanates may be of interest in the biological monitoring of exposure to specific electrophilic substances undergoing conjugation with glutathione to yield ultimately cysteine and acetyl-cysteine derivatives. We have tested a simple analytical method based on the HPLC separation and fluorescence detection of derivatives of de-acetylated thiocyanates. Mercapturic acids present in urine are first de-acetylated enzymatically or by acid hydrolysis. Cysteine conjugates thus formed are reacted with ortho-phthalaldehyde and mercaptoethanol yielding fluorescent derivatives further separated by HPLC in a reverse phase system, in a procedure derived from amino acid analysis. Cysteine conjugates initially present in urine may be analysed directly without the de-acetylation step and their contribution subtracted. This method permits specific analysis of various synthesized thiocyanates: S-2-hydroxyethyl cysteine, N-acetyl-S-2-hydroxyethyl cysteine, S-carboxymethyl cysteine and N-acetyl-S-carboxymethyl cysteine, all potential metabolites of ethylene oxide and halogenated hydrocarbons such as vinyl chloride and 1,l-dichloroethane. This procedure is simple, specific and has micromolar sensitivity. It may prove to be applicable to the specific analysis of other types of thiocyanates. (Supp. by IRSST, Q.)

Specific analysis of the spectrum of potential bromobenzene metabolites is of interest to the study of toxic mechanisms of compounds biotransformed into epoxides. We have tested an analytical method based on the HPLC separation and ultraviolet detection of metabolite derivatives after alkaline plus acid hydrolyses of urine. Alkaline hydrolysis (NaOH 2.5 N, 100°C, 30 min.) liberates bromochloroethene from bromophenylmercapturic acids, while the subsequent acid hydrolysis (HCl 1.5 N, 100°C, 10 min.) deconjugates bromophenols and bromocatechol. After extraction (ethyl acetate, pH 4), evaporation to dryness and dissolution in acetonitrile, the sample is analysed by HPLC with a C18-reverse phase column and a water (pH 3.2)-acetonitrile gradient (70:30 to 50:50) using ultraviolet detection (225 nm). This method allows the simultaneous analysis of o-, m- and p-bromophenylmercapturic acids (synthesized from 2-acetamidoacrylic acid and each bromophenol) together with 3,4-bromocatechol, o-, m- and p-bromophenols. This procedure may prove to be applicable to the rapid analysis of the various metabolites of other ultraviolet absorbing substances which, as bromobenzene, are biotransformed to epoxides. (Supported partially by Institut de recherche en santé et en sécurité du travail, Québec.)

Two capillary gas chromatographic methods using a 50 m glass column coated with OV 101, and a FID detector were developed for the analysis of n-hexane and related chemicals. Gas flow rates (m³/min): N₂, (carrier gas); 3, capillary make up 27; H₂, 33; and air 355. Temperatures (°C) were 220 for the injector and 280 for the detector. Chromatography was used as internal standard. Column temperature programming for the first method was: 50°C for 30 min, raised at a rate of 10°C/min to 180°C then held for 7 min. The following chemicals were separated: n-hexane, 2,5-dimethylfuran (2,5-DMP), 3-hexanone, 2-hexanone (MeBN) hexanal, 2-hexanol, 3-hexanol, 1-hexanol, 5-hydroxy-2-hexanone, 2,5-hexanedione (2,5-D), 1,2-cyclohexanedim. In the second method the following programming was used: 70°C for 15 min, increased at a rate of 40°C/min to 220°C then held for 5 min. All previous chemicals were separated except hexanal, 3-hexanol, and 3-hexanone. Normal phase HPLC was used to analyze MeBN and its metabolites 2,5-DMP; 5-hydroxy-2-hexanone, 2,5-hexanedione, and 2,5-HD. The mobile phase was a linear gradient of 3-55% of 2-propanol in n-hexane in a period of 8 min at a rate of 0.8 ml/min. Quantitation was achieved by monitoring the UV absorbance at 254 nm. Recovery from chicken plasma ranged from 35-79%. (Supported by NIOSH Grant OH00823.)
IN ORDER TO DETERMINE THE EXTENT OF NICOTINE RACEMIZATION DURING THE SMOKE PRODUCTION PROCESSES, SMOKE CONDENSATE FROM THE CIGARETTE MADE FROM DIFFERENT TOBACCO TYPES WAS GENERATED VIA A MOTOR-DRIVEN SYRINGE-TYPE SMOKER. CONDENSATE WAS COLLECTED IN COLD TRAPS, DIVIDED INTO 100 G. AMOUNTS AND FROZEN AT -80°C. NICOTINE WAS ISOLATED FROM THE CONDENSATE BY FIRST SOLVING IT IN CHLOROFORM AND THEN EXTRACTING WITH 1N NAOH AND THEN 1N HCl. THE HCl LAYER WAS MADE BASIC BY ADDITION OF 1N NAOH AND EXTRACTED WITH ETHER. THIS FRACTION (BASIC FRACTION) WAS VACUUM DISTILLED TO GIVE A SAMPLE OF NICOTINE WHICH WAS THEN CONDENSATE FROM THE CIGARETTE HPLC, AND NMR. POLARIMETRY DATA INDICATED THAT IN SOME CASES AS MUCH AS 90% RACEMIZATION OF S-(-)-NICOTINE HAD OCCURRED WHILE IN OTHER CASES THE RACEMIZATION OBSERVED WAS LOWER. PURE S-(-)-NICOTINE TAKEN THROUGH THE SAME EXTRACTION SCHEME SHOWED NO RACEMIZATION. ADDITIONAL ANALYSIS OF THE NICOTINE SAMPLES WAS PERFORMED BY NMR SPECTROSCOPY UTILIZING A CHIRAL LANATHANE SHIFT REAGENT. SINCE S-(-)-NICOTINE BUT NOT R-(+)-NICOTINE, HAS BEEN SHOWN TO BE METHYLATED BY GUINEA PIG LUNG CYTOSOL FRACTION TO FORM R-(+)-METHYLNICOTINUM. THE RACEMIZATION OF S-(-)-NICOTINE MAY RESULT IN SIGNIFICANT AMOUNTS OF THE METHYLATED METABOLITE IN THE BODY WHICH COULD BE OF TOXICOLOGICAL IMPORTANCE. (AIDED BY GRANT NO. 4CO18, TOBACCO & HEALTH RESEARCH INSTITUTE, UNIVERSITY OF KY.)

Mycotoxin foodborne contaminants (i.e. fungal elaborated secondary metabolites) encompass a widely diverse class of chemicals. These include highly oxygenated heterocyclics with substituted furanofuran, sesquiterpenoid alcohols, toxic lactones, anthraquinoid pigments and cyclic polysubstituted peptides, etc. Novel and sensitive techniques for the detection and analysis of a variety of mycotoxins and metabolic congeners have recently been developed in our laboratory employing GC/MS. These involve splitless injection of non-derivatized samples on a 12.5 m microbore, cross-linked methyl silicone capillary column, cold trapping and temperature ramping to enhance resolution, and fragmentography on a quadrupole mass spectrometer (electron impact at 70 eV). For example, a trichothecene mixture (deoxynivalenol, aminde, zearalenone, T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetrol and verrucarol) has been successfully resolved and confirmed. This method has proven useful for hyphenated techniques such as LC/MS and in conjunction with studies designed to elucidate the molecular mechanism of action of these agents. (Supported by USDA Project 84CRS-2-2434 and DoD Project DAAG29-83-G-0088).


The advent of microcomputers and quartz crystal microbalance cascade impactors has enabled the inhalation toxicologist to utilize a "real time" aerosol monitor during exposures to insure constant chamber concentrations. Unfortunately, interfacing these instruments can be a difficult and time consuming task to the non-computer oriented toxicologist. This presentation will detail both the hardware and software needed to interface a model HP-85 (Hewlett-Packard) microcomputer with a California Measurements PC-2 Quartz Crystal Microbalance cascade impactor. The information will be presented in such a manner that a scientist with no electronic or computer hardware experience could easily make the adaptations. A statistical program will be included in the presentation. The following parameters will be outputted by the program: total concentration (particles/meter), geometric mean diameter, geometric standard deviation, median aerodynamic diameter, particle size (each stage), particles per cu. meter (each stage), normalized relative frequency and normalized cumulative frequency. This program was written for use in the PC-2's continuous mode so data can be collected, collated and updated every two minutes.

APPLICATION OF AN ELECTROCHEMICAL DETECTION METHOD FOR THE ANALYSIS OF DEOXYNIVALENOL (VOMITOXIN) IN FUNGAL CULTURES. V.L. Sylva, J.L. Green, N.D. Hedelbaugh and T.D. Phillips. Dept. of Veterinary Public Health, Texas A&M University, College Station, TX 77843.

Vomitoxin, or deoxynivalenol (DON), is the most frequently encountered trichothecene mycotoxin contaminating grain products. Feed refusal, emesis and reproductive disorders in swine have been attributed to DON residues in Fusarium infected feeds. DON was produced in liquid cultures of Fusarium moniliformis, three different media and two strains of fungi. Inoculum from 7-day growth of either F. moniliformis ATCC strain 28114 or a soil isolate was added to flasks containing glucose-yeast extract-peptide (GYEP), corn steep or rice steep media and incubated at 28°C for 34 days. The cultures were then homogenized, lyophilized, and extracted with 90:10 acetonitrile-water. Purification was by thin layer chromatography and high pressure liquid chromatography with ultraviolet detection (UV) at 224 nm and electrochemical detection (ECD) using a constant applied voltage of -1.4. Production of DON was greatest by the ATCC strain in GYEP media and least by the soil isolate in corn steep media. Mean DON values ranged from 4.3 - 146.3 ppm by UV detection and 3.9 - 146.2 ppm by ECD. (Supported by DoD Project DAAG29-83-G0088 and USDA Project 84CRS-2-2434).

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Systemic toxicity of T-2 mycotoxin in a variety of laboratory and farm animals is well documented. However, there are few documented toxicity data following respiratory exposure of T-2 toxin. The methodology for generating aerosols of T-2 mycotoxin as solution, suspension and as a dry powder was developed. Rats and mice were exposed to T-2 mycotoxin both by inhalation and by intratracheal instillation. Toxicity data from both routes of administration are similar. These data indicate that when T-2 mycotoxin is in solution (5% ethanol or 5% dimethylsulfoxide), toxicity following respiratory tract exposure is similar to that of systemic administration of T-2 mycotoxin. With respiratory tract exposure, T-2 powder is more toxic than T-2 in saline suspension; which, in turn, is more toxic than T-2 in solution (5% ethanol or 5% dimethylsulfoxide). Time to death following respiratory exposure of T-2 mycotoxin is dependent on both T-2 dose and vehicle and may vary from <0.5 hrs to >168 hrs.


Intratracheal injection of silica into the lungs of rats caused marked hyperplasia and hypertrophy of alveolar Type II cells as indicated by both light and electron microscopy. These effects of silica were investigated by using centrifugal elutriation of Type II cells dispersed from the lungs of silica-treated rats by trypsin digestion. Type II cells were separated primarily according to size by using flow gradients during elutriation. Type II cells released from the lungs by trypsin were increased 7.3-fold 28 days following a single intratracheal injection of silica (10mg). Based on elutriation profiles, Type II cells from the lungs of silica-treated rats consisted of two major populations, whereas untreated lungs contained a single population. Treated lungs contained Type II cells of approximately normal size (Type IIA) (67%) and a new larger variety (Type IIB) (33%). Induction of Type IIB cells by silica was both dose- and time-dependent. Electron microscopy of the isolated cells confirmed the hypertrophic nature of the Type IIB cells. These data indicate that silica has a marked hypertrophic effect on Type II cells in the lungs of rats and that these cells may be separated from normal Type II cells.

* Supported by ES 07126.

FOUR-WEEK InhalATION STUDIES OF TALC: EFFECTS. S.C. Brown, NIEHS, National Toxicology Program, Tucon, AZ, and J.A. Pickrell, J.M. Benson, R.K. Jones, R.L. Carpenter and R.L. Hanson, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Preliminary to the conduct of a 2-year inhalation exposure study, whole-body inhalation exposures to talc were conducted in F-344 rats and B6C3F1 mice for a period of 4 weeks (6 hr/day, 5 days/wk). Target chamber concentrations were 0, 2, 6 and 18 mg/m³. Talc selected for test was shown to be free of asbestos contaminants. MMAD was 3.25 and 2.7 μm in the rat and mouse studies, respectively. Animals at all dose levels tolerated the treatment well. Clinical signs were minimal for both species. Histological evaluation of lung tissue showed only a modest diffuse increase of free macrophages within alveolar spaces in both species at the highest concentration. Results of this study are compared with lung burdens and effects observed in studies with other relatively nontoxic insoluble particles. Based on these data, concentrations of 0, 6 and 18 mg/m³ were chosen for the chronic study. The higher level is expected to exceed the capacity of the clearance mechanism and produce lung changes. The lower levels should permit clearance to proceed by normal processes and produce no abnormalities. (Research performed for the National Toxicology Program under Interagency Agreement 22-V-01-ES-20088 under DOE Contract No. DE-AC04-76EV01013.)


The administration of 4-ipsomolen (0, 10 (ID) and 25 (ID) mg/kg, i.p.) to rats, resulted in dose dependent degeneration and necrosis of the nonciliated (Clara) and ciliated epithelial cells of the terminal bronchioles. More extensive necrosis of the terminal bronchiolar epithelium, with exposure of the basement membrane, was produced in the HD group. Alveolar clearance of 51Cr labeled polystyrene latex microspheres was analyzed through 40 days postinstillation using nonlinear regression for a double exponential model. Alveolar clearance during phase 1 (day 2-6) was delayed and significantly decreased in both the ID and HD groups. Alveolar clearance during phase 2 (day 10-40) was significantly decreased only in the HD group. The decreased alveolar clearance in HD subjects was long term and did not correlate with the return of morphologically normal appearing Clara and ciliated cell structure. (Supported in part by U. S. Air Force Contract F33615-80-C-0512.)
EFFECT OF INTRATRACHEAL T-2 MYCOTOXIN ON RESPIRATORY GAS EXCHANGE IN THE RAT. D.G. Martin, D. Creasia, and G.W. Parker. Pathophysiology Division, US Army Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701
Sponsor: R.W. Mahfete

T-2 mycotoxin (T-2) causes perturbations in arterial blood gases and cardiopulmonary function following parenteral administration, however, the effect of T-2 exposure via respiratory route is unknown. In this study, arterial PCO2, PO2 and pH were evaluated before and over time after intratracheal (IT) administration of T-2 in saline suspension (T) (1mg/kg in 1mg/ml) or saline (1ml/kg) (S) in sedated, unanesthetized rats. Sedated (0.0100mg/kg fentanyl and 0.06mg/kg droperidol) control rats (C) were also evaluated. Acutely, (5-20 min post IT) S and C rats demonstrated a mild respiratory acidosis. By 2 hr blood gases returned to baseline and were stable through 24 hr. Initially (5-20 min) T rats had significantly lower pH and PO2, with a higher PCO2 than S and C. T rats demonstrated low pH with normal PO2 and PCO2 at 2 hr progressing to hypoxemia, hypocapnia and a return to near normal pH by 6 hr. Nine of 10 T rats were dead at 24 hr. These data were consistent with respiratory acidosis initially, followed by metabolic acidosis with respiratory compensation in T rats. The data suggest IT T-2 has acute deleterious effects on respiratory gas exchange. Subsequent effects (2-6 hrs) appear to be similar to other routes of perenteral administration.

BIOSYNTHESIS OF RADIOLABELED DEOXYNIVALENOL BY FUSARIUM ROSEUM IN LIQUID CULTURE. J.L. Green, V.L. Sivia, N.D. Heidelbaugh and I.D. Phillips. Dept. of Veterinary Public Health, Texas A&M University, College Station, TX 77843.

Deoxynivalenol (DON), a trichothecene mycotoxin commonly occurring as a fungal contaminant of cereal grains, has been implicated in animal feed refusal and emesis. A cost-effective biotechnological method for the production of radio-labeled DON is needed to aid in the mechanistic evaluation of DON toxicity. Two strains of Fusarium roseum, ATCC #28114 and NCPRL-A, were incubated separately on PDA plates at 28°C to achieve confluent growth. Flasks containing 100 ml of synthetic GYEP, corn and rice liquid media were separately inoculated with 10 agar punches (5 mm dia.) from the confluent plates and incubated for 7 days at 28°C. On day 7, an aliquot of 100 uCi of [1-14C] acetate was added to each flask and the cultures were incubated for 30 days, homogenized, lyophilized and extracted three times with acetonitrile:water (90:10). Successive extracts were pooled and then purified by TLC and HPLC. Purified fractions of radio-labeled DON were collected by HPLC, taken to dryness under nitrogen and confirmed via GC quadrupole mass spectrometry. Specific activities of 119 uCi/mmol (NCPRL-A on corn), 67.5 uCi/mmol (NCPRL-A on rice) and 21.5 uCi/mmol (ATCC 28114 on rice) were obtained in the present study and represent the first reported incorporation of 14C into this mycotoxin. (Supported by DOD Project DAAG29-83-0086 and USDA Project 84CRSR-2-2434).

SUBACUTE EFFECTS OF CYCLOPIAZONIC ACID IN RATS. R.E. Morrissey, W.P. Norred, D.M. Hinton, R.J. Cole, and J. Dornier. Russell Center, USDA-ARS, Toxicology Unit, Athens, GA and National Peanut Research Laboratory, USDA-ARS, Dawson, GA.

Cyclopiazonic Acid (CPA) is a mycotoxin produced by strains of Aspergillus flavus and other fungi that occur on corn, peanuts, and other commodities. To determine the potential subacute toxicity of CPA, groups of male Sprague-Dawley rats received po doses of CPA on four consecutive days at levels of 0.0, 0.2, 2.0, 4.0, or 8.0 mg/kg/day. Clinical signs of toxicity were observed only in the two highest dose groups. Liver and spleen were more severely affected than other organs in the two highest dose groups. Livers contained diffuse pycnotic nuclei and, in some high dose rats, focal areas of coagulative necrosis. Hepatocytes from all dose groups had ultrastructural changes that included dilatation and vesiculation of the endoplasmic reticulum, with subsequent loss of ribosomes from rER in some rats. In the high dose group, aspartate and alanine aminotransferase activities were elevated, cytochrome P-450 concentration was decreased, and glutathione-S-transferase activity was unchanged. Spleens were hemorrhagic and white pulp contained necrotic lymphocytes. White cell counts were decreased in a dose-related manner in the two highest dose groups. Pathological changes in conjunction with decreased food intake and water intake probably contributed to the general deterioration of high dose rats that resulted in death.


In order to study early indices of lung damage, male Balb/C mice were given a single dose of 100 mg/kg bleomycin IV or of 4 mg/kg bleomycin intratracheally (IT) and killed 1-21 days later. An increase in proliferation of the cells in the alveolar zone, visualized by autoradiography, was observed beginning 9-11 days after IV injection of bleomycin and peaked at day 17. After IT instillation, cell proliferation peaked on days 5 and 9. The activities of lactate dehydrogenase (LDH), acid and alkaline phosphatase and of angiotensin converting enzyme (ACE) measured in bronchoalveolar lavage fluid (BAL) were variable and not always higher than in controls in the first few days after IT or IV bleomycin. At later timepoints, the enzyme activities roughly followed parallel patterns regardless of the route of bleomycin administration. On days 11 through 17 they reached much higher activities in a more consistent pattern than seen earlier. It is concluded that increased enzyme activities measured in BAL may not only accompany initial cell injury, but may be associated with cell proliferation during the recovery period. (Operated by Martin Marietta Energy Systems, Inc. with US Dept. of Energy. *Oak Ridge Assoc. Univ. Student, Summer 1984. †Oak Ridge Grad. Fellow supported by ORAU.)
937 CELL KINETICS IN ACUTE LUNG INJURY PRODUCED BY ANTINEOPLASTIC AGENTS. H.P. Witzchi and R.C. Lindenschmidt.1 Biol. Div., ORNL2, Oak Ridge, TN 37831

The antineoplastic agents, cyclophosphamide (Cxa) and busulfan (Bu) produce acute lung injury. In order to study the cellular events associated with the development of the toxic lesions, male mice were injected ip with 100 mg/kg of Cxa or with 30 mg/kg of Bu. One to 21 days later the animals were given tritiated thymidine ip, killed 60 min. later and the lungs prepared for autoradiography. In animals treated with Cxa, proliferation of the cells in the alveolar wall began 5 days later and remained elevated until day 14. Peak amounts of covalently bound radioactivity derived from radiolabelled Cxa were found in the lung within the first 24 hours of Cxa administration. Practically all proliferating cells were capillary endothelial or interstitial cells; type II alveolar epithelial cell proliferation was significantly lower than in controls. In animals treated with Bu, a significant increase of alveolar labeling indices above controls was only observed 13 and 17 days after Bu. It is concluded that lung damage caused by the two antineoplastic agents does not follow the usual pattern seen with many other lung toxicants, i.e. early proliferation of alveolar epithelial cells. (Oak Ridge Grad. Fellow supported by ORAU, 2Operated by Martin Marietta Energy Systems Inc. with US Dept. of Energy.)

939 PYRAZINAMIDE DISPOSITION IN THE MALE WISTAR RAT. L.W. WHITEHOUSE, K. BAILEY, B.H. THOMAS, Drug Toxicology Division, Health Protection Branch, Ottawa, CAN.

Pyrazinamide (PZA), a key drug in the short term chemotherapy of pulmonary tuberculosis, has only limited studies reported on its disposition and pharmacokinetics in both animals and humans. The blood profile, urinary excretion, and metabolite profile in urines were examined in male Wistar rats following administration of 150 mg/kg po of 14C-PZA. Comparable T1/2 for radiolabel 14C (1.45 ± 0.06 hr) and unmetabolized PZA (1.39 ± 0.04 hr) in the blood compartment were observed. Cumulative 48 hr excretion in urine and feces accounted for 82.6 ± 3.2 and 11.0 ± 1.3%, respectively, of the dose administered. In the 0-6 hr urines pyrazinonic acid (PA), 5-hydroxy-pyrazinonic acid (5-HO-PA), metabolite II, and PZA, respectively, accounted for 25.4 ± 1.2, 17.7 ± 1.2, 11.6 ± 0.8 and 2.7 ± 0.2 % of the administered dose. In the 6-12 hr urines, the proportions of PA and 5-HO-PA statistically increased whereas metabolite II and PZA decreased. Metabolite II was identified spectrometrically as hydroxy-pyrazinonic acid and the hydroxyl group was tentatively assigned to the 5-position.

This metabolite was found to chromatograph identically to compound II eluted from human urine by Auscher et al. (Biomedicine 28, 129 1978). Since the urinary metabolite profile in the rat resembles that reported by Auscher et al. for human (PA, 25%; 5-HO-PA, 12%; Compound II, 13%; PZA 5%) rat appears to be an appropriate animal model for PZA metabolic studies.

940 THE EFFECT OF DEUTERIUM SUBSTITUTION ON PHENELZINE METABOLISM. M.O. Watanabe and P.R. Ortiz de Montellano. Department of Pharmaceutical Chemistry, University of California, San Francisco, CA

Phenelzine (PEn), a monoamine oxidase inhibitor still in use as an antidepressant, undergoes NADPH-dependent oxidative metabolism in rat liver microsomes to yield significant amounts of phenylacetdehyde, benzaldehyde, ethylbenzene and 2-phenylethanol. The latter three metabolites arise, at least in part, by cytochrome P-450-dependent formation of the 2-phenylethyl free radical. Phenylacetdehyde is produced in the absence of NADPH via a mechanism that does not necessarily involve a radical intermediate. The mechanisms by which 2-phenylethanol and benzaldehyde are produced are under investigation. Total deuterium substitution on the methylene carbons of PEn, which has been reported to potentiate its monoamine oxidase inhibitory activity, also alters the distribution of metabolites. Incubations of this analog, d2-PEn, with rat liver microsomes result in a two-fold increase in benzaldehyde formation and a two-fold decrease in phenylacetdehyde formation when compared to parallel incubations containing PEn. A compensating, if more modest, increase in the formation of 2-phenylethanol is seen in the d2-PEn incubations. Deuterium substitution thus favors the free-radical metabolic pathway.

940 STRATEGIES TO PROMOTE REMOVAL OF PERSISTENT CHEMICALS FROM TISSUE STORES. K. Rozman, T. Rozman and H. Greim. Abt. für Toxikologie, Gesellschaft für Strahlen- und Umweltforschung mbH München, Neuberberg, F.R.G., and Dept. of Pharmacol. Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS

This paper uses 3 representative lipophilic compounds to illustrate the rationale for decontamination of individuals exposed to halogenated hydrocarbons. The rate limiting step in the disposition of hexachlorobenzene is nonbiliary, intestinal excretion of the parent compound in the large intestine. Therefore, successful decontamination entails stimulation of this process which can be accomplished by dietary administration of aliphatic hydrocarbons (mineral oil, hexadecane). The rate limiting step in the disposition of pentachlorophenol is biliary excretion of its glucuronide. Consequently, trapping of this metabolite in the intestine with a suitable resin (cholestyramine) is the key to promote removal of this compound from the organism. Heptachlor is rapidly metabolized to the epoxide which has an even higher affinity to fat than the parent compound. Thus, the rate limiting step in the disposition of heptachlor is conversion of the epoxide to excretable metabolites which occurs by conjugation. Therefore, a phase II enzyme inducer (trans-stilbenexide) is required to reduce the half-life of this chemical. In summary, the key to successful promotion of elimination of chlorinated hydrocarbons is the determination of the rate limiting step in their disposition.
PHARMACOKINETICS OF $^{14}$C-CYCLOPIAZONIC ACID IN MALE FISCHER 344 RATS. W.P. Norred, R.E. Norrissey, R.J. Cole, and J.E. Dorner. Russell Research Center, ARS, USDA, Athens, GA and National Peanut Laboratory, ARS, USDA, Dawson, GA

Cyclopiazonic acid (CPA) is a fungal metabolite produced by a number of Penicillium sp. molds, and may be involved in cases of animal mycotoxicoses. Recently it has been identified as a metabolite of aflatoxin-producing strains of Aspergillus flavus, and has been isolated, along with aflatoxin, from corn and peanuts. 14C-labelled CPA was produced by cultures of P. griseofulvum NRRL 3523 grown in the presence of 14C-tryptophan, isolated and purified. The labelled toxin was dissolved in 1.0 mM sodium bicarbonate and administered to male Fisher 344 rats either orally (PO) (5.0 mg/kg, 0.64 μCi/kg) or intraperitoneally (IP) (1.0 mg/kg), 0.13 μCi/kg). The rats were killed 1, 3, 6, 12, 24, 48 and 72 hr after treatment. Orally dosed rats excreted 50% of the radioactivity in feces within 72 hr, and 17% in urine. Rats treated IP excreted 40% in feces, indicating biliary excretion, and 28% in urine within 72 hr. Blood levels of radioactivity reached a peak of 7% in PO-dosed rats after 6 hr, and declined to 2% by 72 hr. In IP treated rats blood contained 11% of the dose within 1 hr, and 3% after 72 hr. Other tissues containing significant quantities of radioactivity included liver (up to 7% of the dose) and skeletal muscle (up to 30%).

BIOTRANSFORMATION OF PARAOXON AND P-NITROPHENOL BY ISOLATED PERFUSED MOUSE LIVERS. L.G. Sultatos. Dept. of Pharmacology, LSU Medical School, New Orleans, LA.

Single-pass perfusion of mouse livers with the organophosphate paraoxon (PO) resulted in formation of p-nitrophenol (PNP), p-nitrophenyl sulfate (PNPS), and p-nitrophenyl glucuronide (PNPG). Following initiation of the perfusion steady-state conditions with respect to PO were achieved in 20 to 30 min, at which time the extraction ratio ranged from 0.42-0.52. At all concentrations of PO examined (1μM-100μM) the amount of PNPS produced exceeded that of PNPG. However, as the concentration of PO increased the relative proportion of PN to PNPS and PNPG increased. Single-pass perfusion of mouse livers with PNP resulted in production of PNPS and PNPG. As with PO, steady-state conditions were achieved in 20-30 min. The extraction ratio of PNP, as well as the metabolite profile, changed markedly with varying concentrations of PNP. At PNP concentrations of 8μM or less the extraction ratio of PNP was 1, with all PNP metabolized to PNPS. As PNP concentrations increased (up to 100μM) both unchanged PNP and PNPG appeared in the effluent. These results suggest the capacity of mouse liver to biotransform PO is not as great as previously thought. Moreover, the metabolic profile of PNP in livers perfused in situ is clearly dependent on substrate concentration. (Supported by a grant from the Edward Schlieder Foundation).
A DRUG METABOLISM PROGRAM IN SUPPORT OF DRUG TOXICITY STUDIES. J.M. Jaffe*, Sandoz Research Institute, Sandoz, Inc., East Hanover, N. J. Sponsor: R.E. Bagdon

An integrated drug metabolism (DM) program with toxicity (Tox) studies has been implemented following suggested guidelines (Glocklin, V.C., Drug Metabol. Rev. 13, 929-939, 1982). DM studies are performed in rats and dogs from sub-chronic Tox studies as well as in supplemental dogs. The dosage form, dose level, route of administration, and dose duration in the DM studies are the same as those in the Tox studies to allow correlations. Blood levels, urinary and fecal excretion as total radioactivity and unchanged drug are determined in both species. Tissue distribution and biliary excretion as total radioactivity are determined in rats. Metabolic patterns in blood and urine are assessed in rats and dogs. The effect of route (oral vs. i.v.), sex, dose level, and single vs. multiple doses on the ADME parameters are assessed. Pharmacokinetic parameters (peak, time of peak, half-life, AUC) in blood are determined. The degree of accumulation after multiple doses is assessed. Blood level and other ADM data are correlated with the toxic and non-toxic dose level as well as target organs determined in the toxicity phase of the coordinated program. Evaluation of the interdependence of drug metabolism-toxicity data obtained on several drug candidates are presented.

METABOLIC STUDIES WITH DI-(2-ETHYLPHENYL)ADIPATE (DEHA) IN THE MOUSE. D. Guest1, F. Pallas1, S. Northup1, E. Moran2 and M. El-hawari2. Phthalate Esters Panel, Chemical Manufacturers Association1 and Midwest Research Institute2.

The absorption, disposition and metabolism of DEHA was studied in male and female B6C3F1 mice. Doses were oral with 50, 500 or 5000 mg/kg [hexyl-2-14C]-DEHA. For absorption studies, 14C was determined in blood, liver and GI tract, and metabolites in liver and GI tract compared by HPLC. Absorption of 14C from the GI tract was very rapid. At 6 hrs, the GI tracts of females contained 30-38% of the dose, compared with 56-70% in males. 14C in the GI tract consisted largely of DEHA, monoester (MEHA) and 2-ethylhexanol (EH), while the liver contained components more polar than EH. For metabolism studies, urine, feces and expired air were collected for 24 or 48 hrs and tissues were removed for 14C assay. In mice receiving 50 or 500 mg/kg DEHA, urine, feces and expired air at 24 hr contained 91%, 7% and 1-2% of the dose, respectively. Mice receiving 5000 mg/kg excreted 65-75% of the 14C in the urine by 24 hr and 3% in the feces; 20% of the 14C was found in the GI tract and was excreted by 48 hr. Only 0-3.1% of the dose remained in the tissues at 24 hr. Urine contained metabolites of EH, including ethylhexanoic acid (EHA) and its glucuronide, 5-hydroxy-EHA and 2-ethyl-1,6-hexanediol acid (EHDA). The proportion of the highly oxidized metabolites, 5-hydroxy-EHA and EHDA, increased with dose. No EH, DEHA, MEHA or oxidized metabolites of MEHA were detected.

DISPOSITION AND METABOLISM OF DIISONONYL PHthalATE (DINP) IN FISCHER 344 RATS: SINGLE DOSING STUDIES. M. El-hawari1, E. Murrill1, M. Stoltz1, F. Pallas1, A. Lington2 and J. Baldwin; Midwest Res.Inst., Kansas City, MO1 and Exxon Corp., East Millstone, NJ2.

14C-DINP was administered orally to male and female rats at 50 or 500 mg/kg. Excreta were collected and rats were sacrificed at 1, 4, 8, 24, or 72 hr (males) or 24 hr (females). Livers were subjected to subcellular fractionation and urine, feces, GI tract and tissues were analyzed by HPLC. 14C in blood and tissues were highest at 1 hr. Liver 14C was localized primarily in the cytosol. Limited liver uptake was shown after the high dose. Prolonged tissue retention was not apparent. Most of the dose was eliminated during the first 24 hr. After the low dose, 14C was equally distributed in urine and feces, but feces had greater 14C at the high dose. Urine contained major amounts of phthalic acid, (up to 28%) and side-chain oxidation products of the monoester, MNIP (58-83%). PA decreased after the high dose while the oxidation products increased. Feces contained DINP (8-41%), MNIP and oxidation products. The GI contained primarily oxidation products. Livers, testes and fat contained major amounts of MNIP and oxidation products, lower amounts of PA, and traces of DINP (high dose). Lower amounts of oxidation products were recovered in feces and GI tracts of male rats.
The disposition and metabolism of single oral doses of DInP was previously reported. To assess the effects of repeated exposure, 14C-DInP was administered to male rats in 5 daily oral doses at levels of 50, 150, and 500 mg/kg/day. Excreta were collected and animals were sacrificed at 1, 4, 8, 24, and 72 hr for tissue sampling. Livers were subjected to subcellular fractionation, and urine, feces, GI tracts and tissues were analyzed by HPLC. 14C in blood and tissue were highest (7-8%) of doses) at 1 hr but declined to 0.3-0.5% by 72 hr. 14C levels were highest in liver, kidney, and blood. Liver 14C was localized primarily in the cytosol. At all doses, 14C was excreted primarily in urine. Most of 14C in urine (79-91%) was side-chain oxidation products of the monooester, MInP. Smaller amounts of phthalic acid (up to 13%) were also detected. Fecal 14C was divided between DInP, MInP and oxidation products. The G1 contained primarily oxidation products. Liver, testes and fat contained major amounts of MInP and oxidation products. Compared to single dosing, increased formation and elimination of MInP-oxidation products were observed following repeated DInP exposure.


7-14C-Di(2-ethylhexyl)phthalate (DEHP) was given by gavage at 100 mg/kg to male Cynomolgus monkeys, F-344 rats, and B6C3F1 mice. Urine and feces were analyzed by HPLC, and major urinary metabolites were characterized by GC/MS. Urinary and fecal excretion of radioactivity was almost complete after 24 and 48 hr, respectively. By 96 hr most tissues contained 0.01% or less of the radioactivity administered. Radioactivity in urine and feces was resolved into as many as 12 components. The monoester derivative of DEHP was found in monkey and mouse but not rat urine. Metabolite V, an o-1 oxidation product, was a major component of monkey and rat urine. Metabolite IX, an o-1 oxidation product, was a major component in urine of all 3 species; metabolite VI, formed from IX, was a major component in rodent but not monkey urine. Metabolite I, a S-oxidation product, was a major metabolite in rodent but not monkey urine. Thus, S-oxidation appears to be a major metabolic pathway in rodents, but not in a primate species.


7-14C-Di(2-ethylhexyl)phthalate (DEHP) was fed to male F-344 rats at 1000, 6000, or 12000 ppm for 24 hr, after 0, 6, or 20 days of feeding of these concentrations of unlabeled DEHP. Urine and feces were analyzed by HPLC, and major urinary metabolites were characterized by GC/MS. DEHP was well absorbed at all dietary levels. Urinary excretion of radioactivity increased while fecal excretion decreased with dose. Less than 12 of the radioactivity consumed was detectable in tissues at 112 hr after 14C dosing. Radioactivity in excreta was resolved into 14 to 15 components. With no prior exposure metabolite V, an o-oxidation product, and metabolite I, an o- and S-oxidation product, increased disproportionately in urine with dose. This was partially offset by a decrease in o-1 oxidation product IX in feces. At every dietary level, prior exposure caused a disproportionate increase in I in urine. In summary, there are quantitative differences in the disposition and metabolism of DEHP in rats between the dietary levels of 1000 and 6000 ppm.

ORAL ADMINISTRATION OF THE 2-ETHYLHEXYL (ISOOCTYL) ESTER OF 2,4-D TO FISCHER 344 RATS. S. W. Frantz, B. E. Kropscott. Mammalian and Environmental Toxicology Laboratory, Dow Chemical USA, Midland, MI 48640. Sponsor: J. C. Ramsey.

The 2-ethylhexyl (EH) ester of 2,4-D was orally administered to 11 week old male and female Fischer 344 rats to investigate its disposition relative to 2,4-D acid itself. Animals in 8 groups (3/sex/group) were given a single oral dose of 130 mg EH ester per kg (equivalent to 86.3 mg 2,4-D acid per kg) in corn oil and serially killed up to 72 hr to obtain blood samples. Urine was collected every 12 hr from the 72 hr group. A control group (3/sex) was dosed with only corn oil. The absence of any 2-ethylhexyl ester (0 ppb or above) in either blood or urine (72 hr post-dosing) was the most significant finding; 2,4-D acid was detected in both blood and urine. Blood 2,4-D acid concentration vs. time curves demonstrated similar handling for both male and female rats, peaking at 2 hr for females and 4 hr for males, with no detectable level (100 ppb or above) of the acid found at 72 hr. Urine 2,4-D acid levels peaked at 12 hr for both sexes and clearance was nearly complete by 36 hr. Cumulative recovery of 2,4-D acid equivalents in urine was 94.8±9.2% for males and 84.3±4.5% for females. These data indicate that this ester of 2,4-D is rapidly converted to 2,4-D acid, is excreted as acid in the urine, and is expected to be toxicologically equivalent to 2,4-D acid.
MIFOBATE PHARMACOKINETICS AFTER SINGLE INTRAVENOUS AND ORAL DOSES IN CYNOLOMUS MONKEYS.
E.K. Hwang, J. Battor, C.L. Yong, D. Drea and R. Brown. Department of Metabolism, Marion Laboratories, Inc., Kansas City, MO.

Mifobate is a new compound being investigated for lipid altering properties. The purpose of this study was to determine mifobate pharmacokinetics in cynomolgus monkeys. Four male and five female healthy, young, adult animals received a single intravenous and oral solution dose of 25 mg/kg mifobate at separate times. Serial blood samples were taken over a 48-hour period and the plasma assayed by HPLC. The initial estimates of the pharmacokinetic parameters were obtained by using standard curve stripping techniques. Curve fitting was then performed by least-squares regression analysis using the NONLIN program. The time course after intravenous and oral administration of mifobate can be described by a one-compartment pharmacokinetic model with the following major parameters: t1/2 = 1.66 hr (IV), 1.65 hr (po); Vd = 1.087 L/kg (IV), 1.121 L/kg (po); CL = 0.458 L/hr/kg (IV), 0.463 L/hr/kg (po); ke = 0.42 hr⁻¹ (IV), 0.44 hr⁻¹ (po); AUC(0→) = 57.31 μg/ml x hr (IV), 48.3 μg/ml x hr (po). Peak plasma level, 12.85 μg/ml (po), was obtained within one and a half hours after oral dosing. Mifobate was rapidly absorbed with an absolute bioavailability of 87.5%. There was no significant sex difference for the pharmacokinetic parameters for mifobate in the cynomolgus monkeys.

AGE-RELATED CHANGES IN THE METABOLISM AND EXCRETION OF ALLYL ISOTHIOCYANATE. S.J. Borghoff, L.S. Birnbaum. NIEMHS, Research Triangle Park, NC.

Allyl isothiocyanate (AITC) is a volatile constituent of oil of mustard and an important food additive. Long term oral administration of AITC caused transitional-cell papillomas of the male rat urinary bladder. The objective of this study was to examine the effect of age on metabolism and excretion of AITC. Male Fischer 344 rats, 3.16 and 27 mos old, were administered 25 mg/kg C-AITC, po. Urine, feces, volatiles and expired air were collected for 72 hrs. Biliary excretion was also examined after 10mg/kg of C-AITC, iv. Urine was the major route of excretion of AITC-derived radioactivity with an increase being observed in older rats. The percentage of dose excreted as volatiles decreased in the 15 mos animals, then increased in the 27 mos rats. These animals also showed a decrease in the production of CO₂. An age-related decrease in fecal excretion of AITC-derived radioactivity was observed. However, the percentage of dose excreted in bile increased at 16 mos and then decreased in the senescent rats. The age-related decrease in 14C₀₂ production and biliary excretion suggests a change in the metabolism of AITC in senescent animals. However, there was no change in the relative amounts of mercapturic acid excreted in urine suggesting that glutathione conjugation was unchanged with age. Thus, age-related changes in the minor routes of excretion observed could be due to both physiological and metabolic alterations in older organisms.


Hydroquinone (HQ) is used as a photographic developer. The percutaneous absorption of [U-¹⁴C]HQ was determined in Beagle dogs by excretion analysis. The urinary excretion of ¹⁴C was determined after dermal exposure to iv dosing. After iv dosing, ¹⁴C-HQ was injected and blood, urine and breath were collected for 8-12 hr under anesthesia. After recovery, dogs were housed in metabolism cages for collection of blood, urine and feces. Blood ¹⁴C concentrations declined slowly, with 3 apparent phases. Half-life values for the α, β and γ phases were 0.50, 0.77 and 6.55 hr for 1 mg/kg and 0.52, 1.52 and 8.64 hr for 10 mg/kg. Urinary excretion of ¹⁴C was rapid initially with 14.5% and 42.4% of the doses appearing in 4 hr for 1 and 10 mg/kg doses, respectively. Values for urine, feces and total recovery of ¹⁴C at 5 days were 33.2%, 7.1%, 40.3% for 1 mg/kg and 65.7%, 8.12, 68.8% for a 10 mg/kg dose. Fecal ¹⁴C accounted for 7.1% and 3.1% for 1 and 10 mg/kg doses, respectively. No ¹⁴C was detected in the breath. After dermal application of ¹⁴C-HQ no ¹⁴C was detected in blood. Urinary excretion of ¹⁴C accounted for only 0.03% of the applied radioactivity at 2 days and 0.04% at 5 days. Urinary ¹⁴C was still detectable at 5 days. The percutaneous absorption rate was 0.18 mmol/cm²/hr. Using these data to estimate human uptake, after immersion of both hands for 1 hr, only about 0.9 mg of HQ would be absorbed through the skin.

DISPOSITION OF O-BENZYL-P-CHLOROPHENOL IN RATS. L.R. Kao and L.S. Birnbaum. NIEMHS, Research Triangle Park, NC.

O-Benzyl-p-chlorophenol (BCP) is a broad spectrum germicide for household and hospital use. BCP was nominated for toxicity testing because of its potential for widespread human exposure and lack of knowledge concerning its toxicity. Its absorption, metabolism, distribution and excretion were studied in F344 male rats which had been shown to be more sensitive than female rats or B6C3F1 mice to BCP induced renal toxicity. Rats were treated orally with 10 to 1000 mg/kg BCP. At the high dose, excretion occurred very slowly in the first 24 hr, but by 3d 90% of the ¹⁴C-BCP radioactivity had been excreted in both urine and feces at all dose levels. After iv administration of 10 mg/kg, 90% of the total dose was also excreted after 3d. However, the feces contained less BCP-derived radioactivity after iv than after oral treatment (5% vs 76%). The fact that more BCP derived radioactivity was secreted in bile than in feces suggested a role for enterohepatic circulation. The radioactivity in feces was determined to be BCP, BCP-glucuronide and glucuronide conjugates of oxidized BCP metabolites. In urine, both glutathione and glucuronide conjugates of BCP were present. In vitro metabolism using liver and kidney extracts produced mainly BCP glucuronide. Although BCP was widely distributed throughout the body, the highest concentration occurred in kidney and liver.
957 EFFECT OF ENVIRONMENTAL CHEMICALS ON TOXICANT METABOLISM AS DETERMINED BY IN VITRO ENZYME ACTIVITY AND A MODEL SUBSTRATE ASSAY. R. W. Chadwick, G. F. Carlson, B. A. Trela, and M. F. Copeland. U.S. EPA, Research Triangle Park, NC and Purdue University, Lafayette, IN.

The effects of a number of drugs and chemicals on xenobiotic metabolism has been evaluated by comparing results from a variety of in vitro enzyme assays with those from a model substrate assay involving in vivo metabolism of lindane. Dose-response studies employing phenobarbital, ethanol, allyl alcohol and carbon tetrachloride were conducted by treating female Fischer 344 rats i.p. for 7 days with either the vehicle (controls) or one of three doses of the chemical under investigation. Dose levels employed were: phenobarbital (0, 1, 10, 80 mg/kg); ethanol (0, 0.12, 0.6, and 3.0 ml/kg); allyl alcohol (0, 3, 10, 30 mg/kg); and CCl₄ (0.2, 20, 200 µl/kg). Chemical-induced alterations in both phase I and phase II pathways were investigated. Results from the model substrate assay and the in vitro enzyme assays were comparable for phenobarbital, ethanol and allyl alcohol whereas there were some discrepancies between the results obtained in vitro and in vivo for CCl₄. Results from this study indicate that the model substrate assay employing lindane may serve as a useful, sensitive, and noninvasive index of chemically-induced alterations in phase I and phase II pathways in the whole animal.


Experiments were performed to study the glucuronide metabolites of DCB, a representative polychlorinated biphenyl. [14C] DCB (4 µM) was incubated with monkey liver microsomes (1 mg protein/mL), NADPH, and 3 mM UDPGA. After removal of unmetabolized DCB, extraction of incubation mixtures separated metabolites into primary (organic) and secondary (aqueous) metabolite fractions. The secondary metabolite fraction was incubated with β-glucuronidase and reextracted. The organic extract contained 50% of the secondary metabolites. Liquid chromatography (Cl₈) of the organic extract gave radiolabelled fractions coeluting with recognized primary metabolites of DCB: dichlorobiphenyls (75% of chromatographed radiolabel) and 4'-chlorobiphenyls (11%); thus the glucuronides of these primary metabolites were formed in the original incubation. Alternatively, glucuronides were demonstrated by adjusting incubation mixtures to pH 2 and extracting with ethyl acetate. The extract (containing 95% of secondary metabolites) was evaporated and the residue treated with β-glucuronidase and reextracted. The extracts (30% of secondary metabolites) gave chromatographic fractions coeluting with the mono- (2%) and dichlorobiphenyls (89%). These results, along with those above, confirm that the compounds in incubation mixtures were glucuronides of these primary metabolites. (Supported by 5-T 32-ES07091 and GIS 2-12800.)


Studies of the disposition and metabolism of 2-14C-TCP, the major component of the flame retardant FYRROL® PCF, revealed dose-related changes in the pattern of excretion of radioactivity in male CD® rats following administration of a single oral 20 or 200 mg/kg dose. After 8 days, an average of 48.6% and 70.1% of the dose was recovered in the urine at the 20 and 200 mg/kg dose levels, respectively. Fecal excretion of radioactivity also appeared to be dose-dependent. BPAP was identified as a major metabolite of TCP in both the urine and feces, accounting for over 50% of the dose at both the low and high dose levels. At the 20 mg/kg dose level, BPAP was excreted approximately equally in the urine and feces of male rats (27.3% of dose in urine, 27.4% of dose in feces), whereas in males at the 200 mg/kg dose level, this metabolite was excreted predominantly in the urine (43.8% of the dose in the urine, 13.4% of the dose in the feces). The dose-dependent excretory pattern of this metabolite in the urine and feces corresponds well with the dose-dependent changes in excretion of total radioactivity observed at the 20 and 200 mg/kg dose levels.

960 URINARY METABOLITES OF FURFURAL AND FURFURYL ALCOHOL IN F344/N RATS. R. D. Irwin, S. B. Enke*, J. D. Prejean*, TRTP, National Toxicology Program, NIEHS, RTP, NC, 5031, Birmingham, AL. Sponsor: R. Chhabra

Male and female F344/N rats were catheterized with urinary catheters and administered furfuryl alcohol (100 mg/kg body weight or 25 mg/kg body weight) or furfural (60 mg/kg body weight or 15 mg/kg body weight) by gavage in corn oil. Urinary output from each animal was measured over a 24 hour period after dosing, and the urine analyzed by gas chromatography for the presence and the concentrations of fururylglycine, total fururic acid, free furacic acid and furan acrylic acid. The results reveal that fururylglycine is the major urinary metabolite of both furfural and furfuryl alcohol in both sexes of F344/N rats. Fururic acid and furanacrylic acid were also identified as urinary metabolites of both furfural and furfuryl alcohol in both sexes of rat. These results indicate that the major route of metabolic transformation of furfural and furfuryl alcohol in F344/N rats involves oxidation to fururic acid and conjugation with glycine. A secondary route involves condensation with acetic acid to form furanacrylic acid. Therefore, furfural and by analogy furfuryl alcohol, are metabolized in F344/N rats in a manner similar to the metabolism of furfural by humans.
Biliary excretion is the major route of elimination of aflatoxin B1 (AFB) in rats, and the glutathione (GSH) conjugate of AFB is a major biliary metabolite (Biochem. J., 210: 227, 1983). To identify other possible biliary metabolites of AFB, rats were administered 2.5 mg/kg AFB i.p. and bile was collected and analyzed by reversed-phase HPLC. Polar AFB metabolites were eluted with a gradient of methanol and phosphate buffer. Ten distinct polar metabolites were routinely separated. Of these, 2 peaks with elution times of 11.7 (peak 3) and 18.0 (peak 5) minutes were dominant and together accounted for about 50% of total polar metabolites. Peak 5 was ninhydrin-positive on TLC and was not hydrolyzed by glucuronidase (G-ase) or sulfatase (S-ase), suggesting that it is the GSH adduct. Peak 3 was hydrolyzed by G-ase, but not by S-ase, and the hydrolyzate peak co-migrated with aflatoxin P under conditions which resolve all major AFB phase I metabolites. Direct probe mass spectrometry of the G-ase hydrolyzate peak gave a mass spectrum identical to that of authentic AFB. These data demonstrate that numerous metabolites of AFB are excreted in bile, and that AFP-glucuronide is a major biliary metabolite of AFB. (Supported by Am. Cancer Soc. Grant IN-261).

Inhibition of glucuronidation by depletion of hepatic UDP-glucuronic acid impairs the hepatobiliary transport of glucuronidated xenobiotics. However, it is not known if the enhancement of hepatic glucuronidation increases the biliary excretion of these compounds. Therefore, mice were fed 1% BHA in their diet for 10 days which elevated hepatic UDP-glucuronic acid levels (+131%) and UDP-glucuronosyltransferase activities toward phenolphthalein (PP; +94%), iupanoic acid (IOP; +50%) and valproic acid (VPA; +120%). BHA did not affect the biliary excretion of unmetabolized organic acids but enhanced that of PP (+108%) and IOP (+63%) as glucuronides. However, BHA increased the biliary excretion rates only during the initial 15-minute period after administration and it decreased the biliary excretion of VPA (-43%). Simultaneously, BHA increased the urinary excretion of the glucuronates of PP (+48%), IOP (+45%) and VPA (+150%). Thus, enhanced glucuronidation does not facilitate the biliary excretion of all glucuronidated compounds and only transiently increases others. It is likely that this phenomenon is the result of the glucuronides readily entering the plasma and being excreted by the kidney. (Supported by USPHS Grant ES-03192).

In our previous findings indicate that the in vitro capacity for hepatic glucuronidation is elevated in mice fed BHA, in that both glucuronosyltransferase activity and UDP-glucuronic acid levels are increased. The present study examined the effects of BHA on acetaminophen-induced hepatotoxicity and metabolism in vivo. Female Swiss Webster mice received BHA in the diet (1% w/w) for 12 days (700 mg/kg/day). Hepatotoxicity (histopathological and biochemical evaluation at 24 hrs) and acetaminophen pharmacokinetics (HPLC of blood and urine) were assessed following ip injection of 500 mg/kg of acetaminophen. The major findings were: (1) BHA prevented acetaminophen-induced hepatotoxicity, (2) acetaminophen clearance was increased ten fold in BHA-treated mice (49 ml/min/kg) compared to controls (4 ml/min/kg), (3) the rate constant for glucuronide formation was increased seven fold in BHA-treated mice (0.041 min⁻¹) compared to controls (0.006 min⁻¹), and (4) UDP-glucuronic acid levels in BHA-treated mice decreased to a lesser extent following acetaminophen administration and returned to control values more rapidly than in untreated animals. These findings suggest that increasing the glucuronidation of acetaminophen enhances its elimination and prevents hepatotoxicity. (Supported by USPHS Grants ES-03192 and ES-07079).

This study was undertaken to resolve conflicting reports on the inducibility of liver microsomal B[a]P hydroxylase activity in rats treated with PCN. Studies with one-month-old male Long Evans, Sprague Dawley, Wistar and Holtzman rats failed to reveal an anticipated strain difference in the inducibility of B[a]P hydroxylase activity by PCN. Studies with immature and mature male and female Long Evans rats revealed that the inducibility of B[a]P hydroxylase activity decreased with age in male but not female rats, i.e., PCN induced B[a]P hydroxylase activity 5- to 8-fold in immature male, immature female and mature female rats but only 2-fold in mature male rats. The loss of inducibility by PCN in adult male rats coincided with an age-dependent increase (2.4 fold) in basal B[a]P hydroxylase activity. These sex-dependent developmental changes can be explained by an age-dependent increase in the constitutive levels of the major PCN-inducible form of cytochrome P-450 in male but not female rats. Electrophoresis of liver microsomes and spectral studies with metyrapone provided additional support for this explanation. Moreover, this explanation resolves the conflicting literature reports. (Supported by Flossie West and Speas Foundation and USPHS Grant ES-03192).
DEPLETION OF HEPATIC UDP-GLUCURONIC ACID BY COMPOUNDS SUBJECT TO GLUCURONDATION. S.A. Howell, G.A. Hazleton and C.D. Klaassen. Dept. of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

Glucuronide formation is a major biotransformation pathway for numerous xenobiotics. This study was conducted to determine if drugs that undergo glucuronidation lower levels of UDP-glucuronic acid, the cosubstrate for this reaction. Valproic acid (VAL), chloramphenicol (CHLOR), salicylamide (SAL), or clofibrate (CLOF) were administered ip to mice. Liver UDP-glucuronic acid was determined by a radiometric method which measures the formation of [3H]-diethylstilbestrol (3H-DES) glucuronide from [3H-DES and UDP-glucuronic acid (limiting substrate). Liver UDP-glucuronic acid was maximally depleted by 15 min after injection of all compounds. Levels subsequently increased and were near control levels by 60 min after injection. VAL, CHLOR, SAL, and CLOF significantly depleted UDP-glucuronic acid at dosages of 0.25, 0.5, 0.5, and 4.0 mmole/kg, respectively. Liver UDP-glucuronic acid was decreased from control values by 91% by VAL, 91% by CHLOR, 98% by SAL, and 72% by CLOF (after dosages of 4, 2.5, 1.0, and 7 mmole/kg, respectively). These results indicate that compounds subject to glucuronidation can cause a transient, dose-dependent depletion of liver UDP-glucuronic acid. (Supported by USPHS Grants ES-03192 and ES-07079).

DIFFERENCES IN THE ACTIVATION AND INDUCTION PROPERTIES OF UDP-GLUCURONOSYLTRANSFERASE ACTIVITY TOWARD DIGITOXIGINEN-MONODIGITOXOSIDE IN RAT AND MOUSE LIVER MICROSONES. G.A. Hazleton and C.D. Klaassen, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

Glucuronidation of the cardiac glycoside digitoxigenin-monodigitoxoside (DIG) is mediated by a microsomal enzyme, UDP-glucuronosyltransferase. The present studies examined the activation and induction of UDP-glucuronosyltransferase activity toward DIG in rat and mouse liver microsomes. Enzyme activity in native and detergent-solubilized microsomal preparations was assayed radiochemically by measuring [3H]-DIG-glucuronide formation. Enzyme activity in native microsomes from rats (0.104 ± 0.010 nmole/min/mg) was 70% lower than in mice (0.379 ± 0.044 nmole/min/mg). After solubilizing microsomes with ionic and non-ionic detergents, enzyme activity in rats remained unchanged but in mice was activated 2-3 fold. Enzyme activity in rats was induced with pretreatment with pregnenolone-3α-carbonitrile (900μg) and dexamethasone (4000μg) but not with 3α-methylcholanthrene or phenobarbital. Pretreatment with these inducers had no effect on mouse enzyme activity. These findings suggest that the capacity as well as the activation and induction of the UDP-glucuronosyltransferase(s) for DIG from rat and mouse livers are markedly different. (Supported by USPHS Grants ES-03192 and ES-07079).

INHIBITION OF ENERGY METABOLISM AND ITS EFFECT ON HEPATIC UDP-GLUCURONIC ACID AND ADENOSINE 3'-PHOSPHATE 5'-PHOSPHOSULFATE (PAPS) CONCENTRATIONS IN VIVO. R.L. Dills and C.D. Klaassen, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

The hepatic conjugation of xenobiotics with sulfate and glucuronic acid is known to be decreased in vitro by compounds that impair energy metabolism. The proposed mechanism is that depletion of ATP in metabolically compromised cells causes a decreased synthesis of the high-energy cosubstrates, PAPS and UDP-glucuronic acid. This proposal was examined in vivo by quantitating hepatic adenosine nucleotides and cosubstrates in rats treated with the following inhibitors of energy metabolism: rotenone (0.6, 1.4 and 2.0 mg/kg), antimycin A (0.8, 1.6 and 3.2 mg/kg), m-chlorophenylhydrazine carbonyl cyanide (4, 5 and 6 mg/kg) and 2,4-dinitrophenol (7.5, 15 and 30 mg/kg). Hepatic ATP levels 30 and 60 min after administration of the inhibitors was about 30% of control. Hepatic energy charge (ATP + 0.5 ADP) / (ATP + ADP + AMP) was significantly reduced by each inhibitor when given at the two highest dosages. Unexpectedly, UDP-glucuronic acid and PAPS concentrations were not reduced at 30 or 60 min. Thus, it does not appear possible to deplete hepatic ATP in vivo to the extent necessary to affect basal levels of UDP-glucuronic acid and PAPS. (Supported by USPHS Grants ES-03192 and ES-07079).

CAPACITY-LIMITED CONJUGATION OF ACETAMIDOPHEN IN MICE. J.J. Hjelle, G.A. Hazleton and C.D. Klaassen, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS.

The goals of the present study were to characterize the dose dependency of acetaminophen (AA) elimination in mice and to determine whether sex-related differences in AA biotransformation exist. The half-lives of AA in plasma of male Swiss Webster mice increased as the dosage was increased (18 ± 2, 25 ± 2 and 44 ± 4 min at 100, 400 and 600 mg/kg, respectively). The apparent rate constants of AA-sulfate, AA-glutathione and AA-mercapturate formation showed the greatest decline with increasing dosage whereas that of AA-glucuronide exhibited the least. A significant difference in the half-life of AA was noted between male and female mice (44 ± 4 vs 71 ± 6 min) at 600 mg/kg. The apparent rate constants for metabolite formation were similar for most metabolites except AA-cysteine which was two-fold higher in male than female mice. Also, the amount of H-AA-derived radioactivity bound to liver protein was significantly higher in male mice. Hepatic UDP-glucuronic acid and glutathione levels were decreased after AA administration in both sexes. These data in mice show that (1) AA elimination is dose-dependent, (2) male mice rate AA more rapidly than females, and (3) AA depletes UDP-glucuronic acid as well as glutathione. (Supported by USPHS Grants ES-03192 and ES-07079).
Acute treatment with sodium selenite (Se) can ameliorate acetaminophen (AAP) and bromobenzene (BB) hepatotoxicity. Studies were undertaken to determine if this protective effect of Se was related to alterations in AAP metabolism. Se was injected ip in a dose of 12.5 umole/kg 24 hr before AAP (1 g/kg, ip) or in a dose of 12.5 or 30 umole/kg 72 hr prior to BB (7.5 umole/kg, ip). Profiles of AAP or BB and the respective metabolites were measured in urine collected over 72 hr. Se increased the 72 hr excretion of AAP plus metabolites as compared to controls (59% vs 71%). This increase was primarily in the AAP-glucuronide with some reduction in the excretion of AAP and AAP-sulfate. AAP-GSH and AAP-mercapturate metabolites were unaffected by the Se treatment. With BB, neither the cumulative urinary excretion nor the pattern of BB and metabolites was affected by the Se treatment over 48 hr. Likewise, the plasma decline of C-BB over 46 hr was not affected by Se treatment. The results of these experiments indicate that the protective effect of Se on AAP is related to an enhanced glucuronidation which reduces AAP toxicity. However, with BB, the Se protective effect is not mediated by alterations in BB metabolism. (Supported by NIH Grant ES-02425 and Burroughs-Wellcome Toxicology Scholar Award).

Previous work has suggested that the quantitative differences in the in vitro and in vivo metabolism of mononitrotoluene isomers are a result of differences in the hepatic conjugation and oxidation of the first metabolic intermediates, the mononitrotoluene alcohols (NBAIc). This study was designed to determine the steady-state kinetic parameters (Vmax, mmoles/min/mg protein; Km, um; V/K, mmoles/min/mg protein/um) for the metabolism of NBAIc by rat hepatic glucurononyltransferase (GT), sulfotransferase (ST) and alcohol dehydrogenase (ADH). 4-NBAIc was the best substrate for ST (Vmax=1.7; Km=48; V/K= 0.0372). 2-NBAIc had a similar Vmax, while that for 3-NBAIc was slightly lower. V/K for both 2- and 3-NBAIc was 10% of that for 4-NBAIc. 2-NBAIc was the best substrate for GT (Vmax=3.6; Km=373; V/K=0.0113). Vmax for 3- and 4-NBAIc was similar to that for 2-NBAIc, but V/K for 3- and 4-NBAIc was about half that for 2-NBAIc. 3-NBAIc was the best substrate for ADH (Vmax=1.5; Km=504; V/K=0.0391). Vmax and V/K for 4-NBAIc were slightly less than those for 3-NBAIc. 2-NBAIc was not metabolized by our preparation of ADH. These data are in qualitative agreement with those found when nitrotoluenes are incubated with isolated rat hepatocytes.

Marked interspecies variability exists in the acute toxicity of TCDD, with the guinea pig being the most sensitive species to TCDD's acute toxicity. The metabolism of pure 14C-TCDD (3mu) was examined in hepatocytes isolated from control and TCDD pretreated (50ug/kg ip, 72 hr earlier) animals over an 8 hr period. The rate of 14C-TCDD metabolite formation in hepatocytes from TCDD pretreated guinea pigs was unchanged from the control rate (0.25-0.07 pmol/mg cell prot/hr), while the rate in hepatocytes from TCDD pretreated rats was 3.2-fold greater than control (0.70±0.10) and 9 times greater than in the guinea pig. TCDD pretreatment (50ug and 500ug/kg) also significantly induced the metabolism of TCDD in the hamster. The rates of metabolism were similar to that observed in the rat and were dependent upon the dose of TCDD pretreatment. However, phenobarbital pretreatment (800mg/kg 3 days; 24 hr earlier) produced little change in the rate of 14C-TCDD metabolism in the rat. Thus, TCDD may be metabolized by a TCDD inducible form of cytochrome P-450 which is expressed in the rat and hamster but not in the guinea pig. Quantitative and qualitative differences in the metabolism of TCDD may play a major role in explaining the unique sensitivity of the guinea pig to TCDD toxicity. (NIH grant ES02693)
973 METABOLISM OF (14C-2,3) ACETIC ACID AND (14C-2,3) ETHYL ACETATE BY MALE SPRAGUE-DAWLEY RATS. J.D. deBeeth, J.R. Udinsky, K.L. McCarthy, J.M. Smith, and H.E. Schleimer. Toxicology Department, Rohm and Haas Company, Spring House, PA 19477.

The metabolism and excretion of ethyl acetate (EA) and acetic acid (AA) were studied in rats following single oral doses of 2, 20, or 200 mg/kg and 4, 40, or 400 mg/kg, respectively. Both EA-14C and AA-14C were rapidly cleared as CO2 with cumulative 14CO2 excretion reaching a maximum at 5% (4 mg/kg) to 42% of the dose (400 mg/kg) for AA and at 5% (2 mg/kg) to 45% of the dose (200 mg/kg) for EA. By 24 hr, urinary elimination of total 14C accounted for less than 5% of the dose of AA and for EA varied from 20% of the dose at 2 mg/kg to 65% of the dose at 200 mg/kg. Fecal elimination of 14C was less than 5% of the dose for both compounds at all doses. The balance of the administered 14C at each dose was in the tissues (AA, 28% of the dose; EA, 15% of the dose). Adipose is a major depot for AA-derived 14C accounting for half of the 14C retained in tissues. The 14C in adipose may represent incorporation of 14C-ascorbate into fatty acids since 14C-ascorbate was identified as a urinary metabolite of AA. Less than 0.05% of administered AA or EA was eliminated unchanged in expired air. Metabolism of EA was qualitatively similar to AA with 4 major peaks by HPLC appearing in the urine. EA is rapidly cleared via CO2 at a rate similar to the clearance of AA indicating that the hydrolysis of EA to AA is not a rate limiting step in the metabolism of orally administered EA.


The O2 requirements for the reductive defluorination of halothane (CF3CHClBr) was examined in adult male Sprague-Dawley rats, in rat hepatocyte suspensions (HS), and in rat hepatic microsomes (HM). Only rats which were pretreated with phenobarbital (PB) or Aroclor 1254 (PCB) had significant serum fluoride (F) levels (1500 and 1744 μg respectively) immediately following exposure to 1% O2 for 2 hr at 37°C. Serum F was also elevated after 1% O2 exposure at 21% O2 (with PB) and at 40% O2 (with PCB). HS (6 x 106 cells/ml media) of control and PB-treated rats defluorinated H (3 ul) linearly for 2 hr at 10% O2 producing 12 and 20 nmoles F/106 cells, respectively. At 55% O2, no defluorination occurred in control HS and 5 nmoles F/106 cells were released in PB HS. HM (3 mg protein/ml) from control, PB, and PCB treated rats were incubated with 2 ml H and NADPH. 2% O2 or less was required for F evolution from control HM, while PB and PCB HM defluorinated H at 5% O2 or less. Maximum defluorination of G, F2, and F6 HM was at 5% O2 (2.1, 5.5, and 5.9 nmoles F/mg protein/30 min, respectively. The extremely low O2 levels required for the reductive metabolism of H by isolated HM would indicate that during H exposure in vivo and in HS, a localized hypoxia exists within the hepatocytes in the vicinity of the endoplasmic reticulum. (NIH AM 10717).

975 METABOLISM OF THE PYRROLIZIDINE ALKALOID METABOLITE TRANS-4-HYDROXY-2-HEXENAL (t-4HH) BY LIVER CYTOSOLIC AND MITOCHONDRIAL ALDEHYDE DEHYDROGENASE (ALDH). M.W. Lane and H.J. Segall. VM/Pharmacology & Toxicology, University of California, Davis, CA

The metabolism of t-4HH by ALDH was compared with that of propionaldehyde (PAL) and t-2-hexenal (t-2H) using inbred BALB/c mice. Kinetic experiments with hepatic cytosolic ALDH(s) resulted in linear Lineweaver-Burk plots when t-4HH (0.5 mM-20 μM) was used as a substrate. Values of 43.3 μM and 9.9 nmoles NADH/min/mg of protein were obtained for Km and Vmax, respectively. Higher concentrations of t-4HH, above 0.5 mM, resulted in substrate inhibition. Identical studies using t-2H resulted in linear kinetics with Km=3.1 μM and Vmax=7.9 nmoles/min/mg. Use of PAL as a substrate resulted in biphasic plots; Km=5.0 μM, KmII=0.8 μM, VmaxI=12.8 μM and VmaxII=18.7 nmoles/min/mg. DEAE-52 celluose chromatography of the cytosol resulted in the separation of two isoenzymes of ALDH; both metabolized PAL but only the isoenzyme eluted with 10 mM phosphate buffer metabolized t-4HH. Mitochondria as a source of ALDH(s) activity produced biphasic plots for both PAL and t-4HH. Studies with PAL yielded values of KmI=2.6 μM, KmII=4.0 μM and VmaxI=4.0 and VmaxII=49.5 nmoles/min/mg. The use of t-4HH produced values of KmI=52.4 μM, KmII=0.2 μM, VmaxI=3.2 and VmaxII=5.7 nmoles/min/mg. It appears that ALDH could be a factor in t-4HH detoxification and may play a role in pyrrolizidine alkaloid metabolism. (Supported by NIH, ES03343)

976 TRANS-4-HYDROXY-2-HEXENAL (t-4HH): A REACTIVE ALDEHYDE ISOLATED FROM THE MACROCYCLIC PYRROLIZIDINE ALKALOID (PA) SENECTIONINE. H.J. Segall, D.W. Wilson, J.L. Dallas and W.F. Hadden. VM/Pharmacology, Biological Chemistry, University of California, Davis, CA and WARC, USDA, Albany, CA

The hepatotoxicity of macrocyclic PAs has been attributed to the formation of reactive pyroles from dihydroxyalkenines. A metabolite, t-4HH, has been isolated from the macrocyclic PA seneconine using an in vitro hepatic microsomal system plus C labelled seneconine. Using previously developed HPLC systems t-4HH was isolated and its structure confirmed by mass spectrometry and 1H-NMR. Prior investigators studying the lipid peroxidation of hepatic microsomal lipids due to COCl2 or BrCCL3 intoxications (or NADPH-Fe2+ induced lipid peroxidation) have isolated numerous 4-hydroxyalkenals. Injection of t-4HH or the parent compound senecione into the portal vein of rats as a suspension in a carrier lipid exhibited coalescing regions of coagulative hepatocellular zone 3 necrosis. The pathology caused by t-4HH appears to be identical to that previously described for a reactive PA "pyroles" as necrosis of zone 3 hepatocytes is an established acute response to PA administration as well as organic solvents such as CC14. The isolation of another 4-hydroxyalkenal (t-4HH) from the macrocyclic PA seneconine indicates that these aldehydes may be a more common factor in lipid peroxidation and hepatotoxicity than previously believed. (Supported by NIH ES03343)
Dimethylaminoethanol (DMAE) is a widely encountered xenobiotic, both clinically and industrially. Currently, DMAE or Deanol is used to treat tardive dyskinesias, memory loss, and depression, although its efficacy is a matter of debate. Industrial exposure can occur via the percutaneous route in the plastics, insulation, and metal industries. Experiments were performed using 10-200 mg DMAE/kg in buffered saline. Male heterogenous stock mice were injected i.p. for 7 days with a volume of 0.1 ml/10 gm. Studies were performed to measure the effects of DMAE on hepatic alcohol and aldehyde metabolizing enzymes and enzymes of the pentose phosphate shunt. Significant increases were observed with both mitochondrial and cytosolic low-Km aldehyde dehydrogenase (ALDH). No treatment effects were seen on the high-Km ALDHs or alcohol dehydrogenase. A significant increase in mitochondrial glucose-6-phosphate dehydrogenase was seen. No other treatment effects were observed on pentose phosphate shunt enzymes. DMAE was also examined as a possible substrate and found to exhibit similar kinetic properties as ethanol except with a 40% lower Vmax. Earlier studies in the central nervous system have shown enzyme induction effects of DMAE. This study shows similar results in the liver. Chronic exposure to DMAE simultaneously with bioactivated toxic xenobiotics could diminish their toxicities.

(Supported by NIAAA Grants 05213 and 03527.)

Aflatoxin B1 (AFB1) has been found in high levels in airborne, respirable grain dusts, and thus is a potential respiratory tract carcinogen. Previous work in this laboratory has shown that AFB1 is metabolized in rodent tracheal explants to stable metabolites and to species which alkylate DNA. In the present study, we compared the pharmacokinetic disposition of intratracheally (i.t.) and orally (p.o.) administered [14C]AFB1 in male S/D rats. Blood, urine and feces were collected at selected intervals following a single dose of AFB1 (600 µg/kg) in saline. Blood concentration data from both groups approximated a two-compartment open model. Although the time-to-peak of the i.t. group (1 hr) was slightly less than that from the p.o. group (3 hr), disappearance of label from blood followed a nearly identical course in both groups. The plasma half-lives (t 1/2) were 87.7 and 91.8 hr for i.t. and p.o. groups, respectively. In addition, urinary and fecal excretion profiles were similar. At 23 days, urinary excretion of label was 16.4 and 15.0% of the dose for the i.t. and p.o. groups, respectively. Fecal excretion at the same time interval was 56.0 for i.t. 54.6% for p.o. At this time, however, significantly more label was recovered from the lungs and upper airways from the i.t. group relative to the p.o. group. Supported in part by USPHS grant ES03410.


While the biotransformation of MBK in animals is well characterized, little is known about MBK's effects on endogenous compounds. This study provides information concerning the quantitative relationship between plasmatic (P) and hepatic (H) MBK concentrations and the ketogenic state it induces in rats. Elimination of MBK was followed for 24 hr after an oral administration (0.95, 1.9 or 5.7 mmol/kg in corn oil) to male Sprague-Dawley rats. Two of its metabolites (2-hexanol (2-HOL) and 2,5-hexanedione (2,5HD)) and their kinetics were also monitored. These data were compared to ketone body (KB) levels found in P and H for the same period. Plasma concentrations of MBK and 2,5HD correlated well with their hepatic concentrations. This was not the case for 2-HOL. MBK, 2-HOL and 2,5HD were not detected in P and H, 16 hr after dosing. Meanwhile, a marked ketosis was established between 12 and 24 hr. This ketogenic state was due to an increase in beta-hydroxybutyrate (BOHB), acetacetate and lactate and a decrease in pyruvate. These data indicate that MBK can induce ketosis in rats and suggest that BOHB might be used as a biological monitor of MBK exposures at high concentrations.

(METABOLIC DISPOSITION OF 14C-LODOXAMIDE TROMETHAMINE (LT) BY INHALATION (IN), INTRAVENOUS (IV), AND ORAL (PO) ROUTES OF ADMINISTRATION IN THE MONKEY. E.N. Petzold, A.J. Hanchar, B.J. Leong, J.K. Coombs, and H.C. Johnson, The Upjohn Company, Kalamazoo, MI 49001.

LT, which is intended for prophylaxis of asthma and allergic rhinitis, has shown pharmacologic activity (inhibition of IgE mediated release of histamine from mast cells) when administered by IN or IV to rhesus monkeys, but was not as active when given PO. The recently developed endotracheal nebulizer can deliver 14C-LT as an aerosol directly into the lungs, thus avoiding many problems encountered with other IN techniques; e.g., radiation safety, radiolabel accountability, and multiple routes of drug uptake. A comparison of 14C-LT disposition by IN vs. IV and PO administration showed likeness between IN and IV administration both resulting in >90% of the urinary drug-related materials (DRM) as pharmacologically active components dioxamate (L) and monoxamide (M). In contrast, LT following PO administration was metabolized more extensively, most urinary products being pharmacologically inactive phase II conjugates. L and M collectively accounted for <10% of the urinary DRM.)
FORMATION OF GLUTHIONE CONJUGATES OF BENZO(a)PYRENE EPOXIDES IN RATS AND ITS INHIBITION BY GLUTHIONE S-SULFONATE. K.H. Leung and D.B. Mangel. Depts. of Pharmacology and Medicine, Comprehensive Cancer Center, Duke Univ. Med. Center, Durham, NC 27710.

Incubation of benzo(a)pyrene-4,5-oxide (BP-4,5-oxide), anti or syn isomer of benzo(a)pyrene-7,8-diol-9,10-epoxide (BPDE) with glutathione (GSH) in the presence of dialyzed cytosolic fraction of rat liver, rat lung and human lung tumor-derived A549 cells resulted in the formation of GSH conjugates of the corresponding BP epoxides. BP-4,5-oxide was the best substrate with Vmax at 4.4, 5.8 and 1.5 μM conjugate formed per min per mg protein from rat liver, lung and A549 cells, respectively. Conjugates of anti and syn BPDE were formed at approximately 30% and 10%, respectively, the level of that of BP-4,5-oxide. On a per mg protein basis, rat lung and liver contained similar enzyme activities while A549 cells contained about 10-30% of the activity in the rat lung. Glutathione S-sulfonate (GSSG-4H), a reaction product of glutathione disulfide and sulfide (hydrated form of HS- and syn S2-) was found to inhibit competitively the formation of all three BP epoxide conjugates. Values of Ki ranged from 0.07 mM in A549 cells with BP-4,5-epoxide to 0.9 mM in rat liver with anti BPDE. These results suggest that S2- may affect the detoxication of BP by inhibiting, via generation of GSSG-4H, the conjugation of GSH and BP epoxides. (Supported by NIH grant ES02916.)


Oral acetone (AC) exposure delays and potentiates acetonitrile (MeCN) toxicity in rats. Results of pharmacokinetic studies suggested that AC exerted a biphasic effect on MeCN metabolism to cyanide (CN); the presence of AC in vivo appeared to inhibit MeCN metabolism to CN, whereas the disappearance of AC from serum was followed by stimulation of MeCN metabolism. The current experiments were designed to further investigate the mechanisms of MeCN metabolism and the effects of AC on MeCN metabolism to CN. Liver microsomes were isolated and pooled 24 hr after pretreatment of the Sprague-Dawley rats (180-230 g) with AC (2.5 mL/kg) as a 25% aqueous solution or water. Microsomal metabolism of MeCN to CN was found to be NADPH-dependent and heat inactivated tissue was unable to catalyze the reaction. Following a characteristic lag period of 10 minutes, the reaction was linear from 0 to at least 30 min. AC pretreatment in vivo results in a 4-5 fold increase in the Vo max (127 to 55 nmol CN/mg protein/20 min) and a slight decrease in the Km (24 vs 27 μM). Addition of AC in vitro inhibits metabolism of MeCN, with 50% inhibition at 3.6 mM. These data are in agreement with the observations made in vivo and indicate that the effect of AC on MeCN toxicity can be attributed to its biphasic effects on the metabolism of MeCN.

DERMAL ABSORPTION AND TISSUE DISTRIBUTION OF PHTHALATE DIESTERS. A.E. El-Sisi, D.E. Carter, and L.G. Sipes, Dept. of Pharm./Tox., Coll. of Pharmacy, Univ. of Arizona, Tucson, AZ

This study examined the extent of dermal absorption of phthalate diesters in the rat. The phthalate diesters tested were dimethyl-, diethyl, dibutyl-, di-isobutyl-, dihexyl-, di(2-ethylhexyl)-, di-isodecyl- and butyl benzyl-phthalate. Hair from a skin area on the back of male F344 rats was clipped, the compound applied in a dose range of 30-40 mg/Kg and the area of application covered with a perforated cap. The rats were restrained and housed for 7 days in a metabolic cage allowing separate collection of urine and feces. The restraining technique allowed the animal to walk freely and to have free access to food and water. Urine and feces were collected every 24 hrs. and the amount of 14C excreted was taken as an index of the Percutaneous absorption. Percutaneous absorption increased as the alky chain length increased up to four carbons. Then, as the alky chain length increased beyond four carbons, the percutaneous absorption decreased. Most of the unabsorbed dose remained at the site of application. Analysis of tissue levels showed that there was no specific body tissue accumulation of phthalate esters and the absorbed species were excreted rapidly from the body. (Supported by NIH #ES-82130.)


To evaluate rate of dermal absorption of T-2, 0.1 ml of solution containing 0.5 mg of unlabeled and 10 μC of 14C T-2 in either methanol (M) or DMSO was painted on the shaved back of GP. A barrier was applied, and GP were killed at 0, 5, 60, 300 min; 1, 2, 3, 7 and 14 d. At each time point, the treated skin (S) of 5 GP per group was removed and radioactivity (RA) determined, both with or without prior washing with 10% soap solution. By 1 d, in the DMSO group, 50% of the skin RA disappeared at a linear rate, with little additional A through 14 d. 70-80% of the S-associated RA could be washed off through 14 d. In contrast, in the M group, disappearance of RA from S gradually increased to 25% by d 7, with little additional A through 14 d. Through d 3, 95% of the RA in S could be removed by washing. This decreased to 30% by 14 d. It could be concluded that T-2 was slowly absorbed through the S of the GP and that it was being held up in the S, which might suggest the dermal layer was acting as a reservoir for T-2. The rate of A was faster when toxin was applied in DMSO compared to M.
It was suggested that dermal tissue would not be a major barrier to absorption of phthalate esters. However, quantitative data on the dermal disposition of these esters are limited. This study was performed to determine the absorption, distribution, and elimination of $^{14}$C-DINP following dermal application to rats. $^{14}$C-DINP (neat) was applied dermally to male rats at 2 dose levels (0.1 and 0.2 ml/rat); urine and feces were collected for up to 7 days and animals were sacrificed at 1, 3 or 7 days for tissue sampling. Some rats were pre-treated with nonlabeled DINP before application of the $^{14}$C dose. Absorption of $^{14}$C was slow; only 0.3% of the dose was recovered in urine, feces, GI tract, blood and tissues within 24 hr. Total absorption in 7 days did not exceed 4% of the applied doses. Similar amounts were absorbed following the low and high doses and in the rats pre-treated with nonlabeled DINP. Most of the applied $^{14}$C (910%) was recovered from the application area (wash and skin). The poor absorption via the dermal route contrasts with the significant absorption demonstrated after oral administration of DINP to rats.

Hydrofluoric acid (HF) burns are characterized by progressive tissue necrosis and severe pain. Numerous topical treatments have been proposed yet few have been studied experimentally. The present study compared the efficacy of recommended treatments. Hair on the hind legs of rats was removed and 48 hr later 70% HF was applied for 60 sec. Within 6 hr the wound surface was 4-fold larger than the area of HF application, and histologically, edema and coagulative necrosis down to the dermal-muscular junction was observed. Calcium gluconate (CaG), magnesium ointment, Zephiran, A+D ointment and Aloe gel were applied topically to the burn site after a 5 min water wash. A single application of CaG reduced the surface area of the burn within 1 hr, and it remained smaller for a week. Histopathological changes during wound development and healing for CaG-treated burns were identical to untreated burns. Neither increasing the CaG irrigation from 1 to 15 min nor reducing the delay in application from 5 min to 10 sec enhanced CaG effectiveness. Magnesium ointment, Zephiran, A+D ointment and Aloe gel were not effective in treating HF burns. The results indicate CaG ointment to be the most effective topical treatment for HF burns.

A test regimen was designed which is a composite of separate guidelines adopted by the US Dept. of Trans. (DOT), US CPSC, US EPA, and European Economic Community (in accord with OECD). Using one set of animals, one can assess the likelihood of a corrosive action on skin, and also define any irritation response observed in relation to contact time of the test material with the skin, abrasion of the test sites, and occlusion of the skin during exposure. The method provides for greater efficiency in data-gathering and in the use of animals, more realistic simulation of worker exposure, and applicability of test results across various segments of multinational corporations.

Data-in-hand from testing of petroleum-related products indicate marked variability in the effects of contact time and site occlusion on the dermal response; scarification of the treatment site generally plays an insignificant role.
989 IN VIVO AND IN VITRO TOXIC RESPONSES OF MOUSE SKIN. R.C. Helman, J.W. Hall, and J.Y. Kao. University of Tennessee, Knoxville, TN and Oak Ridge National Laboratory, Oak Ridge, TN

Mouse skin was exposed topically to solutions of dinitrochlorobenzene (DNCB), croton oil (CO), epoxyethylbenzene (EEB), ethanolamine (EtNH2), and isopropanylisocyanate (IPM), and the response evaluated 20 hrs. later with light microscopy. In a parallel series of experiments, discs of mouse skin were collected, exposed in vitro to the same chemicals and examined histologically after 20 hrs., in culture. Additionally, cellular enzyme leakage was determined in the culture medium. DNCB was the most toxic to the skin followed by CO and EEB. IPM and EtNH2 were toxic only at the highest concentration used (10%). Epidermal lesions were similar for the same chemical under in vivo and in vitro conditions. The lesions varied from isolated cell swelling to necrosis of the epidermis with separation of the basement membrane. There were good correlations between the magnitude of histological lesions and the levels of enzyme activity in the culture media. The results show that morphologic responses of skin maintained in vitro is comparable to in vivo conditions. Moreover, enzyme leakage provides an important means to detect sublethal cell injury which might not be observed histologically. (Research sponsored by OHER, U.S. DOE, under contract DE-AC05-84OR21400 with the Martin Marietta Energy Systems, Inc.)

990 STERELOGIC ANALYSIS OF SKIN IN DERMATOOTOXICITY STUDIES. W.C. Cullen, R.C. Helman, and J.Y. Kao. University of Tennessee, Knoxville, TN and Oak Ridge National Laboratory, Oak Ridge, TN

To better understand the relationship between skin structure and function, we have developed a method for quantifying the component structure of skin. A mouse strain with 3 phenotypic variants due to hair density were examined (+/+ normal; NN nude; N+ intermediate). Skin samples from 10 mice in each group were prepared for routine light microscopy. Volume densities of skin components were estimated by point counting volumetrically while thickness was measured by electronic planimetry on 5 mm transverse sections. The absolute volumes of tissue components in mm³/cm² of skin area were calculated from volume density data and statistically analyzed. Colagenous connective tissue occupied about 1/3 of the skin volume in the +/+ and N+ mice and was significantly reduced in the NN strain. Adipose tissue occupied about 1/3 of the skin volume in the NN and NN mice and was significantly reduced in the +/+ mice. The hair follicle volume was significantly increased and sebaceous gland volume significantly decreased in the NN strain. The results show that stereologic analysis can be used to quantify skin structure and can, therefore, be employed as a tool in dermatotoxicity evaluation. (Research sponsored by the OHER, U.S. DOE, under contract DE-AC05-84OR21400 with the Martin Marietta Energy Systems, Inc.)

991 MEDIATORS OF CHEMICALLY INDUCED SKIN IRRITATION E. Patrick and A. Burkhalter, Department of Pharmacology, University of California, San Francisco California. Sponsor: H.I. Waisbach

We reported comparisons of time course, dose response, and histology of inflammatory responses produced by irritants that suggested chemicals do not produce skin irritation by the same mechanism. Studies were conducted to evaluate the involvement of putative mediators in inflammatory responses to 3 mg methyl salicylate, 0.05 mg croton oil, and 2 mg ethyl phenylpropionate. Solutions containing the irritants in 10 µl acetone or ethanol were applied to one pinna of female SW mice 9-12 weeks of age. Inflammation was quantified by measurement of ear thickness before and at multiple times after application with a micrometer. Responses of mice pretreated with agents to block mediator effects were compared to the responses of age matched controls. The methyl salicylate response was significantly suppressed by aprotinin and indomethacin, inhibitors of kinin and prostaglandin synthesis. The EPP response was significantly suppressed by indomethacin, aprotinin, mechloroethane which reduces the number of neutrophils, cobra venom factor which depletes complement, methysergide a 5-HT antagonist, and the H1 and H2 histamine antagonists, cimetidine and mepyramine. The response to croton oil was significantly suppressed by aprotinin, indomethacin, and mechloroethane. These results demonstrate differences in mediators of irritant responses produced by three chemicals.

992 SERUM CREATINE PHOSPHOKINASE (CPK) ACTIVITY AND TISSUE DAMAGE IN RABBITS AND MONKEYS AFTER INTRAMUSCULAR INJECTIONS OF CLINDAMYCIN HYDROCHLORIDE AND CLINDAMYCIN PHOSPHATE. S.C. Halladay, D.L. Thomas-Laurie and D.B. Fairchild, Syntax Research, Palo Alto, CA

This study provided data supporting animal models for evaluation of injectable formulations. Injections (1 ml) of Clindamycin Hydrochloride (CH), Clindamycin Phosphate (CP) and saline (SAL) were evaluated in albino rabbits and cynomolgus monkeys. Concentrations of CP and CH injected were: Dose A - 50 mg/ml; Dose B - 100 mg/ml. In rabbits, CPK activity 24 hours after CH, CP and SAL injection was: Dose A - 40, 6, and 2; Dose B - 59, 33, and 2 times above predose enzyme activity, respectively. SAL injections caused none to minimal muscle damage, whereas, CP caused moderate to marked, and CH caused marked to severe damage. In the monkey, CPK activity 24 hours after CH, CP and SAL injection was: Dose A - 26, 4, and 2; Dose B - 40, 5 and 2 times above predose enzyme activity, respectively. Monkeys injected with SAL had none to minimal muscle damage, whereas, CP caused minimal to marked and CH caused severe damage. Muscle damage after CH injection was greater than after CP injection in the rabbit and monkey. The elevated CPK activity measured in both animal models, although not of the same magnitude, did correlate with the degree of muscle damage.
EVALUATION OF ACUTE HEMATOXICITY OF HYDROXYLAMINE SULFATE BY DERMAL ROUTINES IN RATS AND RABBITS. S.C. Gad, M.J. Derelanko, F.A. Gavigan, and F.C. Babich. Department of Toxicology, Allied Corporation, Morristown, NJ.

The hematotoxicity of hydroxylamine sulfate (HS) and phenylhydrazine hydrochloride (PHZ) were compared in the rat and rabbit following single subcutaneous or 24 hour dermal exposures. Dermal HS exposures were applied at doses ranging from 1.0 to 0.001 g/kg under either a plastic or gauze cover. As a previous study identified the blood as the principle target for HS dermal toxicity, extensive hematological evaluations were performed, with PHZ being used as a comparison. HS and PHZ proved to be more toxic in the rabbit than rat, although similar effects were seen in both species. HS and PHZ were strikingly more toxic when administered under plastic than under gauze. HS and PHZ produced similar responses at equivalent doses, including anemia with methemoglobin formation, reticulocytosis, cyanosis, hyperthermia, and a depression in weight gain, and (at higher doses) necrosis at the exposure site. The study confirmed that HS can be lethal to the rabbit following dermal exposure under plastic covers at levels as low as 0.1 g/kg. Moreover, significant hematological effects can occur at lower dose levels (as low as 0.01 g/kg) without dermal irritation. PHZ was lethal to rabbits at 0.5 g/kg under both plastic and gauze, but not at lower levels. No mortality occurred in the rats exposed to either agent. The results of subcutaneous dosing provide an indicator of dermal absorption of both materials in both species. All effects were reversible.

A MODIFIED WALKER BURN MODEL TO EVALUATE THERAPEUTIC TOPICAL PREPARATIONS. K.N. Smith, I.A. Muni (Bioassay Systems Corporation, Woburn, MA).

The Walker Burn model in rats was modified for clinical evaluation of therapy for Pseudomonas aeruginosa burn infections. Eschar formation was induced in anesthetized rats by immersion for 10 seconds of 20% body surface area in 98°C water. Inocula of 10^4/ml Pseudomonas were spread on the burn sites. Silver sulfadiazine was prepared as 1% in hydroxyethylcellulose, or in alginate. These treatments were compared to each base alone, to Alcide gel, and to Silvadene. Pseudomonas infection was evaluated quantitatively from tissue and blood. Weighed burn site tissue samples taken at days 2, 8, and 14 were macerated in a known nutrient broth volume, which was serially diluted onto growth plates. Dilutions of aseptically taken cardiac blood were serially plated. Quantitative evaluation was made of colony formation. Mortality, morbidity, clinical signs, wound appearance, healing rate, body weights and food consumption were evaluated concurrently. Groups treated with silver sulfadiazine in hydroxyethylcellulose had the lowest mortality (6.7%), and had no septicemia. Groups treated with bases alone had high mortality (41.7% to 75%), and almost all died with septicemia. Animals treated with Silvadene consistently gained weight, while all other groups lost weight for one or two weeks after treatment. Chemical analyses revealed sulfonamides and silver in blood and urine of appropriately treated groups.

A MODEL FOR THE STUDY OF CHEMICALLY INDUCED HAIR LOSS. R.J. Stead, R.B. Bradfield, and H.I. Maibach. Exxon Corporation, East Millstone, NJ; Nutrition & the Law, Orinda, CA; University of California Hospital, U.C. Medical Center, San Francisco, CA.

Routine acute toxicological screening procedures of two industrial aliphatic and aromatic resins when administered orally or dermally to rats and rabbits revealed no hair loss. Hair loss occurred in rats, mice, and guinea pigs exposed to both resins by the inhalation route. A protocol was developed to investigate whether the hair loss resulted from hair shaft trauma or from treatment-induced changes in the hair growth (anagen-telogen) cycle. Guinea pigs, rats, and mice were exposed to 500 mg/m² of an aliphatic/aromatic resin and to 650 mg/m² of a predominantly aromatic resin. The rats and mice exhibited hair loss during the telogen phase. In addition, the anagen phase was initiated prematurely. This was demonstrated with both resins, not only by immediate telogen effluvium, but also by the new growth of hair earlier than would be expected. The guinea pigs did not exhibit hair loss or a shift to the telogen phase. The application of this methodology allows the determination of hair loss by phase.

THE EFFECT OF A DAMAGED SKIN BARRIER ON PERCUTANEOUS ABSORPTION IN RATS. R.L. Bronaugh and R.P. Stewart. Division of Toxicology, Food and Drug Administration, Washington, DC.

Since exposure to a potentially toxic chemical can occur on diseased or damaged skin, we have examined the effect of the resultant decreased barrier properties on percutaneous absorption. Permeation (% of the applied dose absorbed) was measured using in vivo and in vitro (diffusion cell) techniques. In some studies, the skin was abraded with a hypodermic needle or the stratum corneum was completely removed by repeated stripping with tape. Abrasion (in vitro) caused permeation increases of 15-fold for water but only 1.5-fold for benzoic acid. The in vivo vs in vitro absorption of cortisone was not significantly different through normal (19.9% vs 20.1%) or abraded skin (55.4% vs 41.7%). Cortisone permeation through tape stripped skin (in vitro) was 49.5%. A greater penetration increase with damaged skin was observed with the more slowly absorbed nicotinic acid. Again, the in vivo and in vitro absorption was similar with normal (6.8% vs 5.3%) or abraded (47.4% vs 50.9%) skin. U.V. irradiation of skin prior to in vivo studies caused increased absorption of nicotinic acid that was dependent on the irradiation time. After the highest U.V. dose, eschar formation was observed and skin permeability was similar to abraded skin. The effect of damaged skin on absorption varied with the penetrant; in vitro and in vivo values were in good agreement.
A new occlusive patch test system employing a plastic chamber (Million Chamber MTC) was evaluated as a method of application in rabbit dermal irritancy testing. Standard guinea pig skin was painted with 20% and 10% dinitrochlorobenzene (DNCB) solutions. The DNCB was applied to an intact and abraded skin site on the dorsal flanks, clipped free of hair, of six New Zealand albino rabbits (2.0 ± 4.0 g). The solutions were painted with the guauche patch on one side of the animal and by MTC on the other side. The trunk of each animal was then wrapped with an impermeable cover which was left in place for a 24 hour period. After 24 hours, the wrap, patches, and chambers were removed. The application sites were scored for erythema and edema upon patch removal and examined at 72 hours according to the standard Draise scale and the Primary Irritation Index (PII) for each solution by both methods was calculated. The PIIa for 20% and 10% dinitrochlorobenzene (DNCB) using the guauche patch was determined to be 6.21, 5.08, 2.09, 1.54, and 0.63. Using the MTC the PIIa are respectively 5.46, 4.51, 2.15, 2.54, and 1.04. The PIIa for 20% and 10% dinitrochlorobenzene (DNCB) using the guauche patch was determined to be 5.21, 5.09, 4.08, 2.59, 1.91, and 0.96. Using the MTC the PIIa are respectively 5.09, 4.12, 3.25, 2.54, and 1.21. Both methods of application produced levels of irritation that were regarded as comparable for each solution. Based upon this work, a number of advantages become apparent for the MTC. The PIIa does not require the preparation of the guauche patch. The PIIa were used in place of the guauche patch and the rabbits were used in place of the rabbits. As a result, the irritation with the chamber was confined more to a distinct area making it easier to evaluate compared to the guauche patch. The MTC thus appears to be applicable as a method of application for liquid materials in the guauche rabbit dermal irritancy test and offers advantages over the standard guauche patch. Its use with other forms of test materials however, remains to be determined.

The excited skin syndrome (ESS) with the characteristics of a spontaneous flare of dermatitis and increased reactivity to irritants was developed in mice. Female SW mice ten weeks of age were divided into two groups. One group was treated with 2,4-dinitrochlorobenzene (DNCB). The other group was treated with 6.0 mg of DNCB. The reactivity to both DNCB and croton oil to either the abdomen or pinnae of one ear for 6 months. The reactivity to both DNCB and croton oil was quantitated as change in ear thickness measured with a micrometer. Mice sensitized to DNCB by two abdominal treatments showed normal reactivity to croton oil. Reactivity to DNCB increased in mice given bimonthly doses of DNCB. After 6 months, DNCB was applied to the abdomen of these mice and spontaneous flares of inflammation developed in ears previously treated with DNCB or croton oil. Croton oil was applied to the uninvolved ear of mice developing the flare and to the ear of age matched controls. Mice which developed a flare response were significantly more reactive to croton oil than controls. Micronuctically, the characteristics of the response to croton oil in mice developing the flare response were similar to those of controls, but the characteristics were more intense. The spontaneous flare response itself was characterized by a higher percentage of monocytes in the cellular infiltrate than either the irritant response to croton oil or the sensitization response to DNCB.


Data previously generated from this laboratory indicated that Carbowax® MPEG, of molecular weights 370, 550 and 750, were of low acute toxicity orally, and by skin absorption, and only very slightly irritating to skin and eyes. In a continuation of these experiments, 15 male New Zealand SPF rabbits (5/group) were subjected to 6 hrs of uncovered dermal exposure to Carbowax® MPEG-350 for 9 days. Animals received 1 ml/day of one of the following test material concentrations: 0.0% (vehicle; 0.1% methyl cellulose in distilled water), 50% (v/w) and 100%. Body weight gain, food consumption, clinical signs, organ weights (liver, kidneys, brain, adrenals, testes) and Draize skin irritation scores were evaluated. No treatment-related effects on body weight gain, food consumption or organ weights were observed. Grade 1 (mildly perceptible) erythema was recorded for 2/5 animals at both 100% and 50% Carbowax® MPEG-350. Erythema was first seen after 7 days and did not appear to increase in severity. Based on these results, the ability of Carbowax® MPEG-350 to penetrate rabbit skin is poor, and little irritation is produced.
1001 ISOCYANATE DOSE-RELATED DERMAL IRRITATION AND DELAYED CONTACT HYPERSENSITIZATION IN GUINEA PIGS


A study was conducted to compare the potency of seven isocyanate compounds in inducing and eliciting delayed dermal sensitization. Initially, acute dermal irritation effects were determined. Groups of six animals were treated with 6 hr occluded exposure to 0.03 ml of six concentrations (1.0-0.003 molar) in olive oil:acetone (1:1 mixture). One molar for all compounds caused slight to moderate erythema which persisted to day six. For all compounds, 0.03 molar caused no dermal effects. Three concentrations of each compound were selected as sensitization induction concentrations. The highest concentrations produced minimal erythema. Groups of 15 animals were treated with occluded dermal exposure to 0.05 ml for 6 hours, once per week for three weeks. Two weeks later, each animal was challenged with 0.025 ml of 5 non-irritation concentrations followed by dermal observations at 24 and 48 hours. All isocyanate compounds caused delayed contact hypersensitization. Compounds with isocyanate groups attached to saturated carbon atoms were more potent for this effect. Sensitization incidence was directly related to both induction and challenge concentration for most compounds. These results are useful for evaluating the dermal hazards of materials containing isocyanates at various concentrations.

1002 EFFECT OF SOLVENTS ON THE EXPRESSION OF PHOTOTOXICITY POTENTIAL OF FRAGRANCE OILS IN AN IN VITRO YEAST ASSAY. S.T. Springer and P.C. Merker. Vicks Research Center, Shelton, CT. 06484.

The objective was to study the effect of solvents on the expression of phototoxicity potential using the in vitro yeast assay described by Weinberg, et al (1981). The method uses ultraviolet (UV) irradiation during diffusion of test chemicals from a paper disc placed upon agar seeded with S. cerevisiae. Light-specific zones of growth inhibition reflect phototoxicity. Solvents tested with UV and in the dark were: methanol; ethanol; dimethyl formamide; ethylene glycol; dimethyl ether; 1-methyl,2-pyrrolidone; glycerol formal; formamide; tetrahydrofurfuryl alcohol; 2-phenoxyethanol; acetanilide; cyclohexanone; dimethyl sulfoxide; dioxane and acetone. At concentrations of 25%, 2-phenoxyethanol and 1-methyl,2-pyrrolidone were found to be toxic to yeast. The others were non-toxic even at 100%. 8-methoxypsoralen (8MOP), lime oil, rue oil and angelica root oil were tested in the non-toxic solvents. At concentrations ranging from 10^-5g to 10^-3g, 8MOP gave equivalent zones of phototoxic inhibition in all solvents, ranging from 1 mm to 17 mm, resp. Lime oil at 10% generated essentially equivalent zones of phototoxic inhibition (8 mm to 12 mm) in all solvents. Rue oil and angelica root oil at 10% produced varying sized zones in each solvent ranging from 1 mm in dimethyl sulfoxide to 12 mm in methanol. Results indicate the solvent can affect the expression of phototoxicity potential and determinations of potencies of materials require testing in a series of solvents.

1003 BIS-(3-CHLOROETHYL) SULFIDE ALTERS CARBOHYDRATE METABOLISM IN CULTURES OF RAT BASAL CELLS. Brubec, M.J., Hsu, T. and Scavarelli, R. Program in Toxicology, Dept. Environ. Ind. Hlth, The University of Michigan, Ann Arbor, MI 48109.

Bis-(3-Chloroethyl) sulfide (BCES), a potent alkylating agent, produces blistering in topical application. The mechanism of toxicity is unknown. The effect of BCES on lactate production, glucose consumption and protein synthesis was examined in cultures of basal cells prepared from neonatal rat epidermis. BCES, dissolved in methylene chloride: ethanol (4:1), was applied to cultures of 7-9 day old basal cells purified by centrifugation through 10% Ficoll gradients. After 15 min exposure, the media was replaced and the concentration of lactate and glucose monitored in the media for up to 6 hr. Incorporation of 3H-leucine was determined in the final 30 min of incubation. Rates of lactate production were stimulated by concentrations of BCES greater than 100 uM. The stimulation of lactate production at lower concentrations may be antagonized by an independent metabolic effect of the carrier solvent. Glucose utilization rates were stimulated by all concentrations of BCES tested. BCES did not alter either the morphology of the cells or the rate of incorporation of 3H-leucine at concentrations up to 300 uM. Thus, BCES may have a selective and acute effect on carbohydrate metabolism in cultured rat basal cells.

(DAMD17-82-C-2198).

1004 A TOPICAL ORAL MUCOSAL IRRITATION MODEL: A GROSS AND HISTOPATHOLOGICAL STUDY IN GUINEA PIGS. D. Doughty, T.E. Murchison* and P.C. Merker. Vicks Research Center, Shelton, CT and *Dawson Research Corporation, Orlando, FL.

The objective of this study was to more fully describe the spontaneous microscopic lesions of the gingiva in healthy untreated guinea pigs and evaluate the gross and histopathologic responses to topical irritants as originally described by Guarnieri (Thesis, 1971). The anterior lower jaws of 33 untreated guinea pigs were collected intact, fixed in 10% buffered formalin and then decalcified. Sections were made through the gingiva and incisors. Lesions included cellular infiltration of the epithelium adjacent to the gingival crevice, subacute labial gingivitis, suppurative periodontitis and an occasional vacuolization. To compare these with lesions produced by known irritants, irritation was induced by topical application of sodium lauryl sulfate (NaLS) or sodium N-lauroyl sarcosinate (SNLS) to the lower anterior labial gingiva of 3 or 6 guinea pigs, 2 to 3 times per day for five days. A 20% aqueous solution of NaLS elicited ischemia, epithelial sloughing, necrosis and ulceration of the labial gingiva as seen grossly and microscopically. A 2% or 8% aqueous solution of SNLS and a 10% SNLS toothpaste elicited mild irritation. This model is useful to study gross oral mucosal irritation that can also be characterized histopathologically and can differentiate between mild and severe irritants.
EVALUATION OF CORNEAL CHANGES FOLLOWING OCULAR EXPOSURE TO BENZALKONIUM CHLORIDE PRESERVATIVE SYSTEMS IN RABBITS AND MONKEYS.


Screening studies for ophthalmic preparations are customarily carried out in albino rabbits. Many commonly used ophthalmic preparations containing benzalkonium chloride (BAC) preservative systems are known to cause corneal changes in albino rabbits and man. Studies were conducted with BAC to compare the responsiveness of the cornea in albino and pigmented rabbits, and cynomolgus monkeys. Animals were administered (TID) 0.1 ml of various formulations containing BAC (0.01%). Animals were dosed for 28 days then allowed recovery of 28 days, if warranted. Complete eye examinations, including fluorescein staining, were conducted prior to dosing, at 2 and 4 weeks, and throughout the recovery period. No ocular changes were present in either rabbits or monkeys after 2 weeks. At 4 weeks no changes were observed in the eyes of the monkeys. Epithelial thinning was present in both strains of rabbits, while corneal abrasion/ulceration were observed in only the pigmented rabbit. Recovery from the preservative induced corneal changes was observed in both rabbit strains. Our results demonstrate the pigmented rabbit to be a more responsive model than the albino rabbit or monkey for toxicologic evaluation of ophthalmic preservative preparations.

CORNEAL PACHOMETRY IN THE OBJECTIVE EVALUATION OF EYE IRRITANCY. B. Ballantyne, Applied Toxicology Department, Union Carbide Corporation, Danbury, CT

Injury to corneal endothelium may increase its permeability, resulting in corneal edema. The conditions for measuring corneal thickness (CT) in New Zealand white rabbits, and its value for objectively evaluating the eye irrigating potential of chemicals was studied. CT was measured using a depth micrometer attached to a slit-lamp biomicroscope (accuracy 0.02 mm). Preliminary studies showed good reproducibility for individual operators, and little variation between different operators. For 100 rabbits CT was normally distributed with a mean of 0.38 ± 0.02 (SD) mm. There was no correlation between CT and body weight (r=0.16; p=0.1). CT was not different between left and right eyes; mean paired difference 0.001 mm (p=0.15). Diurnal measurements showed a slight increase (4%) at mid-day only. Sequential planimetry tonometry (hourly) caused statistically significant changes in CT; increased after two applications, and decreased subsequently. For materials of differing eye injuring potential, CT increased linearly with concentration. Proportionate increases were larger and occurred at lower concentrations with the more irritant materials. Corneal pachometry is a convenient in vivo non-invasive technique permitting the objective assessment of eye irritating potential at concentrations less than those producing macroscopic keratitis.

OPHTHALMIC TOXICOLOGY OF DIBENZOXAZEPINE.

B. Ballantyne, Applied Toxicology Department, Union Carbide Corporation, Danbury, CT

Dibenz(b-f)-1,4-oxazepine (DBO) is a potent sensory irritant of low mammalian toxicity. This makes it potentially useful as a warning or riot control agent. Rabbit eye irritation tests show 0.5% (w/w in PEG 300) without effect; 1 and 2% cause mild transient conjunctivitis; 5 and 10% produce moderate conjunctivitis and minor reversible keratitis. Sensory irritant thresholds (TC50) are as follows: for blepharospasm 7.88 × 10^-5 M (rabbit), 3.4 × 10^-5 M (guinea pig) and 8.6 × 10^-5 M (human); the TC50 for sensation (human) is 4.93 × 10^-3 M. Supra-threshold concentrations (range tested 0.1-1.0% DBO) in humans cause immediate eye pain, blepharospasm and lacrimation, for up to 30 min. Conjunctival injection occurs for up to 4 hr, without other signs of inflammation. Systolic and diastolic blood pressures increase immediately, returning to control values by 15-30 min. Hypertension is usually accompanied by bradycardia. Intraocular pressure is increased in the contaminated eye (up to 3 hr), with a lesser increase in the contralateral eye (30 min. duration). In rabbits, general anesthesia reduces the intraocular pressure increase, and abolishes the consensual effect. Naphazoline, a sympathomimetic, reduces the peak pressure. These ocular and vascular hypertensive effects may be deleterious in those with glaucoma or cardiovascular disease.


A 3-minute in vitro assay using MRC-5 fibroblasts to predict eye irritation of medicated and non-medicated shampoos, contact lens cleaning solutions and surfactants was studied. Cytotoxicity was evaluated using morphologic criteria previously described (Bagdon and Prince, The Toxicologist, 1982). Ten marketed shampoos were ranked; a pediatric shampoo was the least cytotoxic. Contact lens cleaning and seeking solution (0.05% benzalkonium chloride, 0.02% EDTA) and eye drops (0.05% tetrahydrozoline) were non-cytotoxic. Surfactants (Tween-20, 80), and 0.05% EDTA were relatively non-cytotoxic, confirming results in other cell lines. Benzalkonium chloride exhibited delayed onset; a 30 minute essay was used to assess cytotoxicity. A second stage, 2 hour time course essay using the maximum non-cytotoxic concentration was employed to confirm the cytotoxicity ranking. Results of short term (30 min, 2 hr.) Draize test of shampoos suggested that relatively non-cytotoxic formulations, e.g., pediatric shampoos were also non-irritating to cornea, iris and conjunctiva. Positive fluorescence staining preceded corneal opacity evoked by undiluted formulations. Cell culture assays appear to be useful for predicting ocular irritancy and for decreasing the number of animals needed for testing of products.
Cyclohexanone is widely used by industry as a solvent in pesticide formulations. This chemical has been reported as a severe ocular irritant in the Registry of Toxic Effects of Chemicals and Substances (NIOSH). In order to determine the feasibility of using cyclohexanone in reduced concentrations to lessen ocular irritation, a dose-effect eye irritation study was performed. Various aqueous dilutions ranging from 1% to 100% cyclohexanone were tested for eye irritation. For each dilution a 0.1 ml aliquot was placed in the conjunctival sac of the left eye in each of eight rabbits. The eyes were evaluated for irritation with a slit-lamp biomicroscope for up to 21 days. A dose-effect relationship was established for both the magnitude and duration of the irritation. Irritation ranged from very severe conjunctival and corneal effects at 100% cyclohexanone to only negligible conjunctival irritation for the 1% dilution. This study shows that eye irritation produced by dilutions of cyclohexanone follows a dose-effect relationship. From these results, pesticide formulations involving cyclohexanone can be designed to minimize the potential for severe eye irritation.

A study was conducted to determine the extent of ocular response to 2 chemicals, an organo-titanium compound and a quaternary compound, in the rabbit eye following administration to either the cornea or the conjunctival sac. Male rabbits were treated with 0.01 ml either directly to the cornea or to the conjunctival sac. Eye irritation was evaluated at 1, 4, 24, 48 and 72 hours and days 7, 14, and 21 using the Braze scores for ocular irritation. The organo-titanium compound produced irritation scores of 10.9 (1-hour) to 26.2 (maximal response at 1 day) when placed directly on the cornea, whereas the values for conjunctival sac application were 10.1 and 19.2. Scores returned to 0 on day 21 following corneal application. Day 14 following conjunctival sac application. Similarly, the quaternary compound yielded a maximal mean score of 34.2 one day following application to the cornea compared to 31.9 when instilled in the conjunctival sac. When each tissue component (cornea, iris, conjunctiva) was analyzed separately, the increased response due to corneal application for both compounds is due to the response in the cornea. For these 2 compounds, the effect of site of application on ocular irritation responses, while significant statistically, is not of great magnitude and the irritancy classification is unchanged.

The potential chronic toxicity of different designs of prosthetic lenses (type I, II, IV, V, XI) was evaluated in rhesus monkeys 123 weeks after intracocular implantation. Lenses consisted of polymethylmethacrylate body with metal or plastic foot/loop processes for iris attachment. Aphakic animals served as controls. In test animals only one eye was intervened and the crystalline replaced with a prosthetic lens; the contralateral eye was untreated. Post-operative reactions developed in 7 monkeys (corneal opacities, elevated intraocular pressure, inflammation and iris bombe) and were sacrificed at 8 months. There were no significant systemic effects and ophthalmoscopy during the study revealed no adverse long-term effects on the eye structures. The implanted lens caused iris hyperpigmentation and the design (4 loop lens and 2 loop with safety pla) influenced the iris stromal reaction. The results indicated long-term safety of prosthetic intraocular lenses and that secondary reactions depend on surgical technique and post-operative care.

S734, 2-[1-(2,5-dimethylphenyl)ethylsulfonyl] pyridine N-oxide, a preemergent herbicide discovered by Unichem Chemical was evaluated in several toxicity studies. It is not acutely toxic or mutagenic. In subchronic studies, ocular toxicity was found in the dog, cat, rabbit and monkey, but not in the rat or mouse. Animals with a choroidal structure that contains a tapetum lucidum appear to be particularly sensitive. Hind limb paralysis was found in rats in a chronic feeding study with S734. The ocular and neurotoxic effects found with S734 are very similar to those produced by the pyriholones; for example, zinc pyridinethione (ZPT) and hydroxypridinethione (HPT). The absence of ocular toxicity in dogs fed 2-hydroxypyridine-N-oxide, a major S734 plant metabolite in soybeans, for three months indicates the requirement of a sulfur atom in the 2-position of the pyridine N-oxide ring in compounds of this type.

CGS 14796C, a potential aromatase inhibitor, has been proposed as a therapeutic agent for the treatment of breast cancer in man. As part of the safety evaluation program, a 3-month oral toxicity study was performed in which beagle dogs were administered CGS 14796C by gavage at 5, 15, or 50 mg/kg/day. Ophthalmoscopic exams revealed bilateral, funduscopic changes affecting the tapetum lucidum. Ophthalmoscopically, changes were characterized as diffuse areas of pigmentation varying in appearance from a brownish peppered or mottled to a more uniform brown appearance similar to that of the non-tapetal area of the fundus. Tapetal reflectivity was absent or markedly reduced. Within the pigmented area, multiple islets (yellow, green or orange) of tapetal cells were visible, suggestive of destruction of the tapetum. In no instance was retinal destruction, edema, or detachment observed. No vascular changes were apparent within the retina. Subsequent examinations performed during recovery, revealed changes characterized as a slight increase in tapetal islets, suggestive of a slight progression and organization within the tapetum followed by an arrest of the toxic insult within the tapetal tissue.
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The numerals before each Keyword Title refer to the abstract number.

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80 AFlATOXIN B1-DNA ADDUCTS WITHIN /DEPENDANT FORMATION OF
961 Aflatoxin B1./OXIN P1-GLUCURONIDE IN BILE OF RATS GIVEN
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417 AGE AND SEX./ION OF THEOPHYLLINE METABOLISM: EFFECT OF
465 AGE AS A FACTOR IN MALE REPRODUCTIVE TOXICITY TO DIA-2-
526 AGE DEPENDANT CHANGES IN GASTROINTESTINAL TRANSPORT AND
869 AGE DIFFERENCES IN ZINC DEPENDENT SYNTHESIS OF METALLOT
964 AGE- AND SEX-DEPENDENT INDUCTION OF LIVER MICROSOMAL BE
956 AGE-RELATED CHANGES IN INTESTINAL TRANSPORT,
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274 AGENT GB IN CD_RATS./INESTERASE LEVELS WITH TOXICITY OF
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ANGIOTENSIN CONVERTING ENZYME INH/ATION OF A NON-SULFUR
ANGIOTENSIN CONVERTING ENZYME INH/N OF A NON SULPHHYDRYL
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ATPase ACTIVITIES IN TISSUE Hg/ETHYLENEDIAMINE INHIBITION OF
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AVIAN./EE COMMERCIAL PCBs AND SELECTED CONGENERS IN THE
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AZACITIDINE PRETREATMENT./CULTURED RAT LIVER CELLS BY 5-
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B16 MELANOMA CELLS IN CULTURE/TH AND DIFFERENTIATION OF
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B6C3F1 MICE AND FISCHER 344 RA/CAFFEINE ADMINISTERED TO
B6C3F1 MICE AND FISCHER 344 RA/TREATED TO MALE AND FEMALE
B6C3F1 MICE AND FISHER 344 RA/TREATED TO MALE AND FEMALE
B6C3F1 MICE GIVEN BENZYL ACETATE/SPONES IN F344 RATS AND
B6C3F1 MICE./4-VINYLCYCLOHEXENE TO FISCHER 344 RATS AND
B6C3F1 MICE./AMINE ADMINISTERED TO FISCHER 344 RATS AND
B6C3F1 MICE./AMINE ADMINISTERED TO FISCHER 344 RATS AND
B6C3F1 MICE./CARCINOGENICITY OF CAPTAFOF IN
B6C3F1 MICE./P BENZPYRENE ISOMERS ON HOST RESISTANCE IN
B6C3F1 MICE./THE DISPOSITION OF INHALED 14C-BUTADIENE IN
B6C3F1 MICE./ION OF TRICRESYLPHOSPHATE IN F344 RATS AND
B6C3F1 MICE./MINISTERED 4-NITROTOLUENE IN F344 RATS AND
B6C3F1 MICE./NETHYLNE BROMIDE IN FISCHER 344/N RATS AND
B6C3F1 MICE./TERED SODIUM AZIDE TO FISCHER 344 RATS AND
B6C3F1 MICE./STUDIES OF CHLORENDIC ACID IN F344 RATS AND
B6C3F1 MICE./UDIES OF TELONE II IN FISCHER 344 RATS AND
B6C3F1 MOUSE LIVER./VE CELLULAR ONCOGENE (c-onc) IN THE
BABOON AND THE RAT./ GASES ON ESCAPE PERFORMANCE OF THE
BACILLUS THURINGIENSIS IN SHEEP/TV/INFECTIVITY STUDY OF
BACILLUS THURINGIENSIS VAR ISRA/ON THE TOXIC ACTION OF
BACTERIA IN VIVO; EFFECTS OF TH/PENDENT PHAGOCYTISIS OF
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BACTOBOLIN (NSC-325014) IN CD2F1/CLINICAL EVALUATION OF
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BASAL CELLS AND DIFFERENTIATED/ES-INDUCED DNA DAMAGE IN
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BASEAL CELLS-/E" ACTION ON RATS WHILE
BASEAL CELLS./E" ACTION ON RATS WHILE
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BENZENE (DNB)/ATOOZA AFTER ACUTE EXPOSURE TO M-DINTRO-
BENZENE EXPOSURE./N RAINBOW TROUT FOLLOWING TOLUENE AND
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BENZO(A)PYRENE BY PCB- AND PBB- INDUC/THE METABOLISM OF
BENZO(A)PYRENE DERIVATIVES AND SKIN TUMOR./HYDROGENATED
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BENZODIAZEPINE DEPENDENCE IN MICE./MODEL OF
BENZQUINONE (BQ)/.E ADDUCTS WITH HYDROQUINONE (HQ) AND
BENZPYRENE ISOMERS ON HOST RESISTANCE IN/THE EFFECTS OF
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BHA ON ACETAMINOPHEN HEPATOT/UTYLATED HYDROXYANISOLE (BHA)
BHA ON HEPATIC GLUCURONIDAT/UTYLATED HYDROXYANISOLE (BHA)
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BILE ACIDS ON PROGESTERONE BINDING IN RAT LIV/EFFECT OF
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BILARY EXCRETION OF DRUGS IN M/TIC GLUCURONIDATION AND
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BILARY TREE PERMEABILITY BY MA/ CHLORDECONE -INCREASED
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BILIRUBIN COMBINATION./IN RATS TREATED WITH MANGANESE-
BINDING (CVB) OF 3-H/14-C-BRO/LISM (MET) AND COVALENT
BOUND (CVB) OF BROMOBENZENE (ICAL PROBES ON COVALENT
BINDING AND ADIPOSE LIPASE ACTIVITY/LOW DENSITY LIPOPROTEIN
BINDING AND HEPATOBILIARY DISPO/NE DEPLETION ON THE DNA
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BINDING AND QUANTITATION OF O6-METHYL Guanosine IN C/DNA
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BINDING IN TELOCHY SKIN AND LIV/RENE METABOLISM AND DNA
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S-9 FOLLOWING LONG-TERM TREATMENT OF POTENTIAL (CHLORAMPHENICOL) SAFETY EVALUATION OF A NEW CARDIOSELECTIVE /INTRAVENTRICAL SAFETY EVALUATION OF A NON-SULFUR ANGIOTENSIN CONV/ORAL SAFETY EVALUATION STUDIES OF HUMAN/IN VIVO AND IN VITRO SAFETY EVALUATION STUDY OF SAL/FOURTEEN DAY INTRATHecal SAFETY EVALUATION STUDY OF SAL/FOURTEEN DAY INTRATHecal SAFETY STUDIES WITH RADIATION-STERILIZED CHICKEN MEAT: SALMO GAIREDERI./HYPOTHETIC ORGAN OF THE RAINBOW TROUT, SALMON CALCITONIN IN DOGS./L SAFETY EVALUATION STUDY OF SALMON CALCITONIN IN RATS./L SAFETY EVALUATION STUDY OF SALMON CALCITONIN./IC EFFECTS OF INTRANASYL INSTILLED SANDS PRODUCTS AND INTERMEDIATE TOXICITY BATTERY OF TAR Sanguinarin Extract./Oducts CONTAINING Sanguinarine AND Sanguinarin Extract./OCHNICAL PROFILE OF Sanguinarine AND Sanguinarine AND Sanguinarin EXTRICOLOGICAL PROFILE OF
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