THE TOXICOLOGIST

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Abstracts of the
27th Annual Meeting
Vol. 8, No. 1, February 1988
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Preface

This issue of the *Toxicologist* is devoted to the abstracts of the presentations for the platform, poster/discussion, and poster sessions of the 27th Annual Meeting of the Society of Toxicology, held at the Loews Anatole Hotel, Dallas, Texas, February 15-19, 1988.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 271.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 317.

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DOSE-DEPENDENT DISPOSITION OF D-LIMONENE: RELATIONSHIP TO MALE RAT-SPECIFIC NEPHROTOXICITY. L D Lehmann-McKeeman and D Caudill. Miami Valley Laboratories, Procter & Gamble, Cincinnati, OH.

Exposure to d-limonene exacerabates hyaline droplet accumulation in male rat kidneys. Studies were conducted to evaluate the relationship between the disposition of d-limonene and its nephrotoxicity. Dose-response studies indicated that d-limonene exacerabates hyaline droplets over control at dosages of 0.3 mmol/kg and higher. Disposition studies with $[^{14}C]$ d-limonene indicated that, at 0.1 mmol/kg, renal concentrations of d-limonene equivalents in male and female rats were similar. However, at 1 mmol/kg, renal concentrations of d-limonene equivalents were significantly higher in males than in females. To determine whether retention of d-limonene in male rat kidney resulted from an interaction between d-limonene and α2u-globulin (α2u), rats were dosed orally with $[^{14}C]$d-limonene and 24 hr later, the distribution of radioactivity among renal proteins was determined by reverse-phase HPLC. The results indicated that in male rat kidney, radioactivity co-eluted with the α2u peak, whereas, no radioactivity was seen in the corresponding fraction of female rat kidney proteins. These results indicate that d-limonene is retained in a renal rat kidney at dosages that exacerabate hyaline droplets which may result from an interaction between d-limonene or its metabolites with α2u.

EFFECT OF TRANSPORT AND RENAL TRANSFORMATION ON THE RENAL SITE-SPECIFIC TOXICITY OF DICHLOROVINYLCYSTEINE (DCVC). GHI Wolfgang, AJ Gandolfi, K Breden, RR Nagle. Arizon Health Sciences Center, University of Arizona, Tucson, AZ.

DCVC, a model cysteine conjugate, is known to produce an $S_2$ lesion in vivo and in rabbit renal cortical slices (RCS). The RCS system was utilized to understand the site-specificity of this lesion. RCS from male NZW rabbits were incubated (serum-free DMEM/F12 medium, 22.5 $D_2/O_2$, 25°C) for up to 12 hr with $10^{-7}$-10$^{-5}$ M DCVC. Transport studies (steady state and kinetic) show that DCVC did not use the organic anionic transporter (PAP) nor was its toxicity inhibited by co-incubation with the anionic transport inhibitor probenecid. There, however, does appear to be a role for neutral amino acid transport. When beta-lyase, which is maintained in control slices, is inhibited by aminoacyl-acyl carrier protein, the toxicity of DCVC is inhibited. Thus emphasis was placed on the role of beta-lyase in activating DCVC. DCVC, which has been shown to be a mitochondrial toxin, only causes a 30% decrease in slice $$ consumption. However, ATP content decreases by more than 90%, indicating a disruption of the energy status. EM studies were used to confirm the site-specific ultrastructural changes in mitochondria. The mechanism of the site-specificity of DCVC in RCS appears to be linked to its uptake, bioactivation, and subcellular targets. (J.H. CAAT/GM 38290/NIEHS-ES-070-91)

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Details of how Cd enters cells remain to be clarified. Although satureable and sensitive to inhibition, cellular Cd uptake in rat jejunum appears to involve neither specific carriers nor competition with other metals (Toxicol, 37:117, 1985). It can be conveniently explained by A) non-specific binding of Cd to anionic membrane sites; binding is reversed by EDTA. Reaction A) is followed by B) temperature-dependent internalization (Am. J. Physiol. 253:6134, 1987); internalized Cd is not accessible to extracellular EDTA except after sonication of the tissue. In similar experiments, Cd taken up by rat erythrocytes was extracted by EDTA only after osmotic hemolysis of the cells. When everted sacs of jejunum were suspended in 20 $\mu M$ CaCl$_2$-saline for 5 s at 37°C and rinsed, 60 s further incubation in fresh saline at 37°C sufficed for internal Cd to reach a plateau. The plateau does not reflect saturation of the internal compartment, as this was 3 times higher after an original exposure of 40 s. Although maximum internalization had occurred, the mean of means from 8 expts. showed 45±13% (50) of tissue Cd still accessible to EDTA. It follows that not all binding sites on the membrane filled during process A) participate in the further process B). The sites involved in step B) have not yet been identified. (Supported by NIH grant ES-02416)


Studies have shown that chlorobiphenyl (CB) effects on mammalian monooxygenase activity are structurally determined. We examined effects of environmentally common PCB congeners that are minor to specific inducers on hepatic monooxygenase systems in fish. Scup were given single ip injections and after 5 days were assessed for levels of microsomal cytochromes P450 and β5, ethoxyresorufin-O-deethylase (EroD) and aminopyrine-N-demethylase (APD) activities. Immunodetectable P450 (the major BNF-inducible scup P450) and translatable P450E mRNA, 3,3',4,4'-tetrachlorobiphenyl (TCB) produced a parabolic dose-response for EroD activity, with strong induction at 1 mg/kg and lower activity at higher doses; APD activity was unchanged. By contrast, immunodetectable P450E and mRNA levels were strongly elevated at all doses. 2,3,3',4,4'-penta-CB, 2,3,4,4',5-penta-CB and 2,2',3,3',4,4',5-hexa-CB had only limited effects on the parameters measured. The response of fish to pure MC-type PCB inducers thus involves transcription and translation, but there can be strong inhibition or inactivation of the P450 induced, by as yet undefined mechanisms. The results also show a relative insensitivity of P450E to mixed-type CB inducers. (Supported by USPHS ES-4220 and USEPA CX-813567.)
The toxicity of the cysteine S-conjugates S-(pentachlorobutadienyl)-L-cysteine and S-(2-chloro-1,2-trifluoroethyl)-L-cysteine is associated with their metabolism to unstable thiols. The bioactivation and cytotoxicity of benzyl sulfides I and II were studied to test the hypothesis that both would undergo cytochrome P-450-dependent benzyl hydroxylation and that the intermediate hemimercaptals would eliminate the cytotoxic thiols. Benzyl sulfides I and II were cytotoxic in isolated rat hepatocytes; I was more cytotoxic than II. The corresponding tert-butyloxyl sulfides were also cytotoxic. The cytotoxicity of I was increased after phenobarbital treatment and was greater in cells from male versus female rats. Benzyl sulfide I cytotoxicity was inhibited by CO and by SKF 525-A. Benzyl sulfides I and II were metabolized to benzaldehyde by hepatic microsomal fractions and by purified reconstituted cyt. P-450pg-β systems. These results support the hypothesis that the benzyl sulfides and the corresponding cysteine S-conjugates yield unstable thiols that give rise to acylating agents or to stable, but toxic, terminal products that cause cytotoxicity. (Supported by grants ES03127, HL07475, and AFOSR 86-ML-007.)

Rats exposed chronically to high concentrations of diesel exhaust (DE) have increased levels of whole lung DNA adducts and develop tumors in the lung parenchyma. We examined the relationship between tumor location and the distribution of DNA adducts in the respiratory tract (RT) following exposure to DE. DNA adducts were measured in rat RT following exposure to a carcinogenic concentration of DE (10 mg soot/m³) for 7 hr/day, 5 days/wk for 12 weeks. Controls were exposed to air only. The nasal tissue, trachea, mainstem bronchi, intrapulmonary airway, and parenchyma were removed, DNA was analyzed for DNA adducts using the 32P-postlabeling method. Levels of DNA adducts in tissues from rats exposed to DE were greater than those in controls. Quantities of DNA adducts ranged from 1 adduct in 10⁹ bases (mainstem bronchi) to 2,000 adducts in 10⁹ bases (parenchyma). Thus, a good relationship exists between tumor location (parenchyma) and level of adduct formation (highest in parenchyma) in rats exposed to DE. These data suggest that DNA adduct levels in discrete locations of the RT may be good measures of "effective dose" of carcinogenic compounds. (Research sponsored by the U.S. DOE/DOE under Contract No. DE-AC04-76EV01013.)

SUSCEPTIBILITY TO 1,3-BUTADIENE-INDUCED LEUKEMOGENESIS CORRELATES WITH ENDOGENOUS ECOTROPIC RETROVIRAL BACKGROUND IN THE MOUSE. R D Irans, H P Cathro, W S Stillman, W H Steinshagen, and R S Shah, Chemical Industry Institute of Toxicology, Ben Triangle Pk, NC, and M Wloyd, Univ Texas Med Br, Galveston, TX.

Chronic exposure of B6C3F1 mice to 1,3-butadiene (BD) results in a high incidence of thymic lymphoma/leukemia (TL). Endogenous ecotropic retrovirus (eMuLV) is frequently associated with murine TL. Therefore, the influence of retroviral background on BD leukemogenesis was examined by comparing the incidence of TL between B6C3F1 and NIH Swiss mice. Proviral sequences for eMuLV are truncated in the NIH Swiss, and the virus is not expressed. Exposure to BD (1250 ppm) for up to one yr resulted in marked differences in the incidence of TL between B6C3F1 (45%) and NIH Swiss (14%) mice. This has previously been shown to be preceded by anemia and bone marrow cytogenetic abnormalities which are indistinguishable between the two strains. In contrast, a marked increase in eMuLV replication was observed only in B6C3F1 mice. These findings suggest that retroviral background influences susceptibility to BD leukemogenesis. Selective eMuLV replication may correlate with TL, whereas hematologic and cytogenetic parameters are not adequate predictors of leukemogenicity. The respective roles of eMuLV and BD in this leukemia model system are currently under study.

INSIGHTS INTO THE RELATIONSHIP BETWEEN CARCINOGEN-DNA ADDUCT FORMATION AND TUMOR LOCATION IN THE RESPIRATORY TRACT FOLLOWING EXPOSURE TO DIESEL EXHAUST. J A Bond, J R Harkema, J L Maudery, R O McGiellan, and R K Wolff. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

The male hybrid B6C3F1 mouse (C57BL/6 female X C3H/He male) has a 30% incidence of spontaneous hepatic tumors. The male C3H/He has a 60% incidence and the female C57BL/6 has a negligible incidence. The Harvey-ras oncogene has been implicated in a wide variety of solid tumors, including hepatomas in the B6C3F1 mouse. The objective of this study was to examine a possible point of transcriptional control of this gene in the liver of all three mouse strains. Msp I/Hpa II restriction enzyme analysis was used to determine the methylation state of the Ha-ras oncogene. There is a negative correlation between methylation and expression of a gene. It was found that Ha-ras is hypermethylated in the female C57BL/6 mouse (S/5). By contrast, Ha-ras usually was found to be relatively hypomethylated in both male B6C3F1 (3/4) and C3H/He (3/5) mice. These results indicate that Ha-ras has a lower potential for transcriptional activity in the C57BL/6 mouse as compared to B6C3F1 and C3H/He mice. The Ha-ras oncogene may therefore be "priced" for aberrant expression by virtue of its hypomethylated state in B6C3F1 and C3H/He mice, and this condition may facilitate hepatoma development in these animals.

DIFFERENTIAL POTENTIAL FOR EXPRESSION OF THE HARVEY-ras ONCOGENE (Ha-ras) IN B6C3F1, C3H/He, AND C57BL/6 MICE. R L Vorce and J L Goodman. Dept. Pharmacology & Toxicology, Cent. Env. Toxicol., Michigan State Univ., E. Lansing, MI.

The male hybrid B6C3F1 mouse (C57BL/6 female X C3H/He male) has a 30% incidence of spontaneous hepatic tumors. The male C3H/He has a 60% incidence and the female C57BL/6 has a negligible incidence. The Harvey-ras oncogene has been implicated in a wide variety of solid tumors, including hepatomas in the B6C3F1 mouse. The objective of this study was to examine a possible point of transcriptional control of this gene in the liver of all three mouse strains. Msp I/Hpa II restriction enzyme analysis was used to determine the methylation state of the Ha-ras oncogene. There is a negative correlation between methylation and expression of a gene. It was found that Ha-ras is hypermethylated in the female C57BL/6 mouse (S/5). By contrast, Ha-ras usually was found to be relatively hypomethylated in both male B6C3F1 (3/4) and C3H/He (3/5) mice. These results indicate that Ha-ras has a lower potential for transcriptional activity in the C57BL/6 mouse as compared to B6C3F1 and C3H/He mice. The Ha-ras oncogene may therefore be "priced" for aberrant expression by virtue of its hypomethylated state in B6C3F1 and C3H/He mice, and this condition may facilitate hepatoma development in these animals.
USE OF RAT HEPATOCYTES CULTURED ON EXTRACELLULAR MATRIX AS AN IMPROVED SYSTEM TO STUDY LIVER GENE EXPRESSION IN VITRO. P. S. Guzelian, and E G Scheutz. Medical College of Virginia, Richmond, VA.

Traditional monolayer cultures of adult rat hepatocytes on type I collagen (VIT) lose many liver functions including the cytotoxins P-450 (CYP450) induced by phenobarbital (PB), P450b/6, or by isoaflorid (IF), P450d. When we incubated freshly isolated rat hepatocytes in serum-free medium on matrigel (MgEl), a reconstituted basement membrane, the cells remained rounded as viable clusters for over 30 days in contrast to the flattened, confluent monolayers on VIT. Analyses of RNA by Northern blots and of proteins by immunoblots revealed that MgEl cultures exposed to Pb contained amounts of P-450b/6 mRNAs and proteins comparable to Pb treated rat liver whereas no responses were detected in Pb treated VIT cultures. Moreover, treatment of VIT cultures with IFP, 3-methylcholanthrene or PCBs induced the mRNA and protein only for P450c. The same treatment of MgEl cultures resulted in co-induction of P450c and P450d mRNAs and proteins provided the medium was supplemented with hormones including epidermal growth factor. We conclude that MgEl permits cultured hepatocytes to retain many liver-specific functions including inducible expression of some CYP450 not previously reported in vitro and thus provides a new way to rigorously investigate mechanisms by which xenobiotics affect liver cell physiology and toxicity.


Interferon Y was found to ameliorate the bleomycin (BM)-induced lung fibrosis in mice. The effects of polyinosinic-polyribodic acid (Poly IC), an inducer of interferon, on BM-induced lung fibrosis was studied in hamsters. Poly IC (10 mg/kg IP) was administered for two days and immediately prior to IT instillation of BM (7.5 U/kg) and thereafter, daily for 15 days. The lung hydroxyproline in Poly IC, BM and Poly IC + BM groups were 84%, 149% and 97% of the control (791 μg/lung), respectively. Prolyl hydroxylase activities in the corresponding groups were 83%, 183% and 142% of the control (1x10^9 dpn/lung). Protein in bronchoalveolar lavage (BAL) supernatant in Poly IC, BM and Poly IC + BM groups were 72%, 288%, and 286% of the control (1157 μg/lung), respectively. There was no difference in total leukocyte counts between Poly IC + BM and BM groups but the differential cell counts were changed. The numbers of PMN, Mono, Lymph and Eosin were 50%, 84%, 91% and 10% of the BM group, respectively. The hamsters in Poly IC + BM group had significantly less volume of lesion (1.0cm^2), which was restricted to the intact skin as compared to BM group (1.6 cm^2) where there was also alveolar fibrosis. In addition, lesions in BM group were multifocal and primarily proximal acinar in location, had fewer extracellular fibers, PMN and Mono. (NHBLI Grant # 5RO1 HL 27354-07)

PREDITREATMENT WITH CYCLOPHOSPHAMIDE DOES NOT PROTECT AGAINST THE LUNG DAMAGE AND EIBROSIS OF A SECOND DOSE. R.D. Smith and J.P. Kehrer. Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.

Administration of a single intraperitoneal (i.p.) dose of cyclophosphamide (CP) produces lung damage and pulmonary fibrosis in mice. CP is metabolically activated by the mixed-function oxidase (MFO) system and can reportedly inhibit this system. Other investigators have reported that a 50 mg/kg dose of CP, 7-14 days prior to 250 mg/kg, protected against lung damage in male mice. The objective of the present study was to determine whether this protective effect was evident using a higher preliminary dose and more sensitive indices of lung damage. Male BALB/c mice were injected with 100 mg/kg CP followed by an additional 100 mg/kg on days 3, 7 or 14. Control animals received the same volume of saline. Incorporation of radioactively labeled thymidine into pulmonary DNA has been shown to be an indirect measure of the extent of lung damage. Mice in each treatment group were injected i.p. on various days with 0.5 μCi of 14C-thymidine and sacrificed after 90 min. Thymidine incorporation was maximal on Day 7 in mice receiving only a single dose of CP, and returned to control levels by Day 21. This peak was unaffected by a subsequent CP dose on Day 3. Those mice receiving a second dose of CP on days 3, 7 or 14 experienced a second surge of thymidine incorporation on Days 21-28. Measurement of total lung hydroxyproline (HOP), an amino acid found primarily in collagen, accurately reflects the degree of fibrosis. All groups given a second dose of CP had levels of HOP on Day 35 significantly greater than saline or CP controls. The increases with two 100 mg/kg doses of CP were more than twice those seen with a single 100 mg/kg dose, but less than those with a single 200 mg/kg dose. These results indicate that a preliminary dose of CP delays the lung cell proliferation induced by a subsequent dose, but does not prevent additional lung damage and fibrosis. (JPK is the recipient of Research Career Development Award HL 01435.)

GLUTATHIONE IN HYDROPEROXIDE TOXICITY IN RAT ALVEOLAR MACROPHAGES. H.J. Forman, G.A. Loeb, and D.C. Skelton. Children's Hospital and University of Southern California, Los Angeles, CA.

Glutathione is an essential component of defense against oxidant injury. Release of oxidized glutathione (GSSG) has been used as a semi-quantitative measure of oxidant stress in a number of model systems. In the rat alveolar macrophage, the transport system responsible for the release of GSSG appears to be absent. Therefore, we focused upon another indicator of altered glutathione metabolism in these cells, glutathione-protein conjugate (PSSG) formation. PSSG is formed by the interaction of GSSG with the protein cysteinyi moiety in a reaction catalyzed by glutathione S-transferase. When 10 mM t-butyl hydroperoxide (tBOOH) was incubated with alveolar macrophages there was no release of GSSG above control until the appearance of concomitant lactate dehydrogenase (LDH) release, which began after 30 min. LDH release indicates gross membrane damage. Prior to LDH release however, total reduced glutathione (GSH) and GSSG decreased from 8.6 ± 1.6 to 3.0 ± 0.6 nmol/µg protein with the major fraction converted to PSSG. Under less severe oxidant stress (10 μM tBOOH), GSSG was maintained at control levels of 5% of the GSH + GSSG pool and PSSG did not increase. These results suggest that formation of PSSG can be a sensitive indicator of hydroperoxide stress and a better quantitative measure than is GSSG release.
HALOTHANE-INDUCED INHIBITION OF SUPEROXIDE RADICAL PRODUCTION AND MOBILIZATION OF INTRACELLULAR CALCIUM. J E Ryan-Powell, H J Forman, and R J Bortolino. Children’s Hospital of Los Angeles and University of Southern California, Los Angeles, CA.

Halothane is a routinely used general anesthetic. The incidence of pneumonia, a common post-surgical complication, is directly related to time under anesthesia. We examined the hypothesis that halothane interferes with antibacterial defenses, such as superoxide (O$_2^-$) production by alveolar macrophages (AM). O$_2^-$ production can be stimulated by agonists, which either mobilize Ca$^{2+}$ or activate protein kinase C, such as phorbol esters. In vitro exposure of rat AM to 1 mM halothane induced a 50% inhibition of phorbol ester-stimulated O$_2^-$ production as measured by cytochrome c reduction. Halothane also caused a 50 mM increase in intracellular calcium as measured by fura-2 fluorescence. AM were labeled with myo-[H]$^3$H]inositol. Labelled products were separated by Dowex 1-X8 anion exchange chromatography. Halothane (10 mM) induced a 40% increase in the levels of inositol phosphates (inositol 50 seconds after halothane addition. Inhibition of phorbol ester stimulated O$_2^-$ production has been found with other agents, which elevate intracellular Ca$^{2+}$, such as A23187. Thus, the halothane induced inhibition of phorbol ester stimulated O$_2^-$ production may be related to its effect on Ca$^{2+}$ mobilization.


This study was designed to determine the effect of prolonged ozone exposure (1.5 mg/m$^3$; 7 days; 24 h/day) on cytochrome P-450 (P-450) dependent xenobiotic metabolism in whole rat lung microsomes as well as in isolated bronchial Clara cell preparations. It was demonstrated that the increases in components of the pulmonary microsomal P-450 electron transport system were not accompanied by a concomitant increase in specific model substrate conversions. O-dealkylations of ethoxyresorufin and ethoxyresorufin were decreased, whereas, in contrast, the O-dealkylation of pentoxyresorufin was increased. Clara cell populations isolated from ozone-exposed rats showed a comparable qualitative shift in P-450-linked monooxygenase activities. In vitro experiments revealed that ozone is able to inactivate the various O-dealkylation activities, but the results could not explain the increase in vivo data. Lung morphometrics and Clara cell isolation data supported the view that the in vivo effects of ozone should rather be ascribed to proliferation and/or renewal of P-450 containing cell populations and to intrinsic cellular biochemical changes.


We have investigated the effect of ozone exposure on defense to a respiratory infection with Listeria monocytogenes in the rat. We present data that indicate that continuous exposure of rats to ozone during 7 days impairs phagocytic and lytic activity of alveolar macrophages. A more profound effect is observed on the development of cellular immune responses to Listeria is suppressed. As a consequence control of the respiratory Listeria infection in exposed rats is inadequate. Pathological lesions induced by pulmonary Listeria infections are characterized by multifocal inflammation of the lung parenchyma. These foci comprise infiltration of its feocytic and lymphoid cells. Thecellularity of interstitial tissue is increased, and many alveolar macrophages are observed. Since ozone influences both these types of inflammatory cells, we have investigated the influence of ozone on the pathological lesions associated with pulmonary infection with Listeria. After ozone exposure the severity of the lesions is more pronounced, more extensive. Moreover, the tendency of pulmonary Listeria infection to induce granulomatous alterations is enhanced in ozone exposed rats; in exposed and infected rats severe granulomas can be observed. Consequently ozone exposure adds to the loss of lung functions after respiratory infection.

RAPID INCAPACITATING AND LETHAL EFFECT OF HCL IN GUINEA PIGS DURING EXERCISE. D R Malek, M K Stock and Y Alarie. Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

The guinea pig ergometer (Toxicol. 3, 123, 1987) permits continuous measurement of tidal volume, respiratory frequency, O$_2$ uptake (VO$_2$) and CO$_2$ output and is featured with a clear incapacitation endpoint (collapse) was used for this study. Guinea pigs were exercised on the ergometer at a 25-30% of their VO$_2$ max according to a defined 5 min protocol. Four groups of exercising guinea pigs were exposed to 107, 142, 169 or 586 ppm HCl. All animals collapsed at the 3 higher concentrations after 20, 2 and 1 min of exposure respectively. At 586 ppm they died within 4 min. Collapsed animals were coughing, gasping for air and the dark color of their eyes and skin of the ears and paws indicated asphyxiation. No collapse or death occurred in sedentary animals exposed to 1,300 ppm. In humans exposed up to 100 ppm HCl Matt (Dissertation, University of Wurzburg 1889) concluded that work would be impossible in the range of 50-100 ppm. Thus the guinea pig is less sensitive than man to HCl. These experiments were conducted to investigate escape capabilities of human workers where HCl is released from polyvinylchloride. The direct action of HCl on the respiratory tract was greatly enhanced by an increase in minute ventilation. Supported by NBS 60NAAH84001.
17 MEASUREMENT OF TIDAL VOLUME, RESPIRATORY FREQUENCY, O₂ UPTAKE AND CO₂ OUTPUT IN EXERCISING GUINEA PIGS. Y Alarie and D Maierk. Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

We have built a guinea pig ergometer within an enclosed ventilated chamber which served as a whole body plethysmograph permitting continuous measurement of tidal volume (VT), measured indirectly from pressure change (P). (P) created with each breath, respiratory frequency (f), O₂ uptake (VO₂) and CO₂ output (VCO₂). Guinea pigs were trained to run with no shocking device. The above measurements were made at rest and during 30 minutes of running at twice or three times their basal VO₂. With these increases in VO₂ there was an increase in minute volume (MV) but this increase was mainly due to an increase in f rather than an increase in VT. This is opposite what we have previously found in guinea pigs challenged with 10% CO₂ in inspired air which increased VT much more than f. Adding 10% CO₂ during exercise resulted in a large increase in VT with the increase in f still maintained. This combination of exercise and 10% CO₂ resulted in a final increase in MV of 9 to 12 times above baseline. The large increase in MV was sustained with no evidence of stress on the animal. This protocol could be used in toxicological studies of airborne contaminants. Supported by NIHES 1RO1-EOS2747 and NBS 66092307.

18 DOSE-DEPENDENT CHANGES IN AIRWAY CONDUCTANCE AND EDEMA IN GUINEA PIGS EXPOSED TO UNMALED ENDOTOXIN. J Gordon, J Balmes, J Fine, and D Sheppard. UCSF, CA.

There is evidence that some of the acute airway symptoms associated with exposure to inhaled cotton and grain dusts are correlated with the level of endotoxin (ENDO) in these dusts. In the present study, changes in specific airway conductance (SAGaw) and airway edema were examined in guinea pigs exposed to aerosols of ENDO (1 and 10 µg/ml in saline) or saline for 3 hrs. SAGaw began to drop by 90 min and decreased by 22% and 33% by 3 hrs in animals exposed to 1 and 10 µg/ml ENDO, respectively. Exposure to saline alone did not alter SAGaw. Exposure to ENDO produced a dose-dependent increase in vascular permeability (4% Evans blue dye, 50 mg/kg, was used as a marker of protein extravasation) in the trachea and mainstem and hilar bronchi. The dye content of tracheas from animals exposed to 10 µg/ml ENDO (49±2.4), but not 1 µg/ml ENDO (23.2±2.4), was significantly greater than that of tracheas from animals exposed to saline (20±7.3). Dye leakage was more pronounced in the mainstem (50.5±6.1 and 101.5±12.7) and hilar bronchi (66.1±11.9 and 102.5±14.8) in 1 and 10 µg/ml ENDO animals (0.05<p<0.01), respectively. In saline animals, the dye content in the mainstem bronchi was 24.5±3.4 and in the hilar bronchi was 29.5±3.2. These results suggest that inhalation of aerosols of ENDO (approximately 10 to 100 µg/m³) in concentrations that approach those found in the workplace can produce airway edema as well as airway obstruction. Airway edema may contribute to symptoms experienced by workers during acute exposures to cotton and grain dusts.

19 BRONCHIAL REACTIVITY TO HISTAMINE: TESTING GUINEA PIGS IN BODY PLETHYSMOGRAII. P S Thorne and M H Karol. Dept. of Industrial Environmental Health Sciences, Univ. of Pittsburgh, Pittsburgh, PA.

Bronchial hyperreactivity has been associated with late-onset allergic reactions. The aim of this work was to incorporate a method for assessing bronchial reactivity in guinea pigs into an animal model for late-onset pulmonary sensitivity without interruption of long term pulmonary monitoring. A multiple dose (MD) protocol was developed in which guinea pigs received 15 min exposure to 1.5-fold increasing histamine doses. This protocol was tested with and without breathing augmented by exposure to 10% CO₂. Plethysmograph pressure changes (AP) associated with each breath were monitored. Histamine exposures with CO₂ revealed a dose-dependent decrease in AP from the CO₂-induced increase. The concentration required to reach a 33% decline from the AP increase, PC_{50}(CO₂), averaged 0.68±0.17 mg/ml (±SD, N=38). Exposures to histamine in air produced an increase in AP due to bronchoconstriction with dose-dependent onset. The dose which doubled AP, PC_{2}(AP), was 2.1±0.9 mg/ml (N=20). Parallel tests using this protocol and a head-only system showed that PC_{2}(AP) correlated with a decrease in V_f. Exposures on 3 consecutive days demonstrated repeatability with no significant difference in PC_{2}(AP) or in PC_{50}(CO₂). The intersubject variability with these protocols was half that reported for PC_{20(FEV1)} in humans. Supported by NIHES ES01582.


Formaldehyde (HCHO) is a nasal carcinogen in the rat and is metabolized by FDH (EC 1.2.1.1) to non-genotoxic formate. FDH activity is known to exist in the olfactory and respiratory mucosa of the rat. The cellular localization of FDH activity has not previously been investigated. Cold processed glycol methacrylate (GMA) embedded tissues were used to localize FDH activity in the rat respiratory tract and kidney. Ten µm GMA sections were incubated at 37°C with a reaction mixture containing HCHO, glutathione, NAD, pyrazole, and nitroblue tetrazolium. Dyes were used to inhibit nonspecific aldehyde dehydrogenases. FDH activity yielded a blue formazan precipitate. The kidney was stained intensely at the brush border and in the cytoplasm of the epithelial cells of the proximal tubule. FDH staining was weaker in the respiratory tract, with the exception of the Clara cells. However, FDH activity was detected in the nasal passages, with diffuse cytoplasmic staining of both respiratory and olfactory epithelial cells. Bowman's gland, and seromucous glands. Identical preparations lacking glutathione, a required substrate for FDH, had little or no activity. These results indicate that FDH activity is present in the rat respiratory tract and kidney in specific cell types.
SELECTIVE TOXICITY OF 3-TRIFLUOROMETHYLPIRIDINE (3-FMP) TO RAT OLFACTORY EPITHELIUM. P A Lock and P M Heek. ICF PLC, Central Toxicology Laboratory, Macclesfield, Cheshire, UK.

3-FMP is a semi-volatile by-product from the synthesis of a process intermediate. Toxicological studies showed that via inhalation 3-FMP produced necrosis to the olfactory epithelium (OE) and liver (L) of rats. Whole body autoradiography studies with [14C]-3-FMP showed selective retention of radioactivity in the OE and L. We have examined the distribution of radioactivity from [14C]-3-FMP in rat OE and L following in vivo and in vitro administration. Rats were treated (po) with 125mg/kg 3-FMP and killed 0.5 to 24 hr later and total and protein bound radioactivity determined in the two tissues. Both tissues showed marked accumulation of radioactivity with time. Studies in vitro with isolated OE and L slices showed that these tissues accumulated radioactivity from [14C]-3-FMP. This accumulation of radioactivity was abolished by inhibitors of cytochrome P-450 (eg metyrapone). Prior treatment of rats with metyrapone (150mg/kg ip) followed by [14C]-3-FMP prevented the retention of radioactivity in the OE but not the L and the onset of the lesion in the OE. These studies show that radioactivity from 3-FMP is selectively retained in the rat OE and L by a cytochrome P-450 dependent process and that inhibitors of cytochrome P-450 can prevent the toxicity to the OE.

SUBCHRONIC INHALATION STUDY IN RATS WITH DIBASIC ESTERS (DBE): RECOVERY OF NASAL LESIONS. C M Keenan, M S Bogdanoff, and D P Kelly. E I du Pont de Nemours & Co, Inc, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

DBE is a solvent mixture of dimethyl -succinate, -glutarate and -adipate used in the paint and coating industry. A subchronic inhalation study with a recovery period was initiated to evaluate nasal lesions that occur in rats exposed to DBE. Male and female rats were exposed to DBEs at concentrations of 20, 80, or 400 mg/m3 for 45 or 90 days. An additional group was allowed a recovery period of 45 days. After 45 days of exposure, slight degeneration of the olfactory epithelium was observed in both male and female rats at 80 and 400 mg/m3. After 90 days, degeneration of the olfactory epithelium was present at all DBE concentrations in female rats but only at the mid and high concentrations in male rats. The severity and incidence of the lesions were dose-related for both sexes with female rats being more sensitive than males. Following the recovery period, histological changes compatible with repair in the olfactory mucosa included an absence of degeneration, focal disorganization of the olfactory epithelium, and respiratory metaplasia. Inhalation studies of other esters demonstrate similar pathology in the olfactory epithelium. Since olfactory mucosa is high in carboxylesterase activity, acids may be the toxic metabolites of these compounds.

KINETICS OF NASAL MUCOSAL CARBOXYLASE- MEDIATED HYDROLYSIS OF DIBASIC ESTERS. C A Patterson, C R Ree, and M S Bogdanoff. E I du Pont de Nemours & Co, Inc, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

Ninety-day inhalation exposure of rats to dibasic esters (DBEs), a mixture of dimethyl -succinate, -glutarate and -adipate, causes mild lesions of the nasal olfactory mucosa (OLF) of rats at concentrations that do not affect the nasal respiratory mucosa (RESP). A possible mechanism of toxicity may be metabolism of these compounds in nasal tissue to toxic acids. The purpose of this study was to determine the kinetic constants for carboxylesterase-mediated hydrolysis of DBEs and correlate these findings with lesion formation. Formation of mono- and diacid metabolites of DBEs in nasal mucoosal homogenates were monitored by HPLC. However, none of the diacid metabolites were formed. Vmax values for the formation of monomethyl succinate (MMS), monomethyl glutarate (MMG), and monomethyl adipate (MMA) were approximately 8 to 10 times larger in OLF than RESP. V/K values for the formation of MMG and MMA were approximately 9 and 10 times larger in OLF than RESP. For the formation of MMS, V/K was approximately 2 times larger in RESP than OLF. Thus, hydrolysis of these esters occurs more readily in OLF than RESP and the efficiency of hydrolysis at low substrate concentrations is related to carbon chain length and mucosal type.

DIFFERENTIAL METABOLISM AND MUTAGENESIS OF 2-ACETILAMINOFLUORENE BY HUMAN AND RAT HEPATOCYTES, S9 AND MICROSOMES. K Rudo, W Dauterman, and R Langenbach. CGTB, NIEHS, RTP, NC. Toxicology Program, NCsu, Raleigh, NC.

Human and rat hepatocytes, liver S9 and microsomal fractions were compared by measuring the metabolism and mutagenicity of 2-acetylaminofluorene (AAF). Human tissue mediated mutagenesis with Salmonella typhimurium was highest in hepatocytes followed by S9 and microsomes, respectively, with variation existing from individual to individual. Mutagenesis with rat S9 and hepatocytes were roughly equivalent with rat microsomes being inactive. Human hepatocyte and microsomal activation was higher than rat hepatocytes and microsomes, with human and rat S9 mutagenesis appearing to be similar. Organic-soluble AAF metabolites produced by hepatocytes, S9 and microsomes were qualitatively similar but exhibited quantitative species variation. Ring hydroxylated products, N-hydroxy-AAF and AF were the major metabolites. Human and rat hepatocytes produced only trace levels of the mutagenically active N-hydroxy-AAF in contrast to the high levels produced by subcellular fractions. Human hepatocyte levels of water soluble metabolites were higher than human S9, although human S9 exhibited a capacity to conjugate AAF. Human microsomes, rat S9 and microsomes only produced water soluble products at very low levels. This study illustrates the differences between tissue fractions and that species differences can vary with the tissue preparation utilized.
INDUCTION OF DIFFERENT DRUG METABOLIZING ENZYMES BY CLOTRIMAZOLE IS NOT INITIATED BY THE SAME PARAMETERS OF DRUG EXPOSURE. W L Hopson, and M R Franklin. Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

Clotrimazole, when administered daily for 3 days at 75 mg/kg is a high magnitude (3-4 fold) cytochrome P-450 inducer. The induction exhibits dose-differentiated isozyme induction. Erythromycin demethylase activity, unlike p-nitroanisole demethylase and pentoxysorufin dealkylase activities, is not induced by a lower (25 mg/kg x 3 days) dose. Since overall dose has changed in the two treatments, (225 mg/kg vs 75 mg/kg) the inductive effect of a single 75 mg/kg dose was investigated. The effects, except for the extent of cytochrome P-450 induction (2-2.5 fold) paralleled those of the 75x3 dose, not the 25x3 dose. Changes in Phase II drug metabolizing enzymes were also compared. Microsomal UDP-glucuronosyltransferase (p-nitrophenol) was induced to the same extent (1,5 fold) by all three treatments. Cytosolic GSH transferase (1-chloro-2,4-dinitrobenzene) was induced 3 fold by the 225 mg/kg total dose and only 1.5 fold by the 75 mg/kg total dose given either as a single or divided dose. Depression of cytosolic sulfotransferase (p-nitrophenol) was only seen with the 225 mg/kg dose. Thus, differential cytochrome P-450 isozyme induction appears to be triggered by a bolus concentration, not total dose. UDP-glucuronosyltransferase induction is independent of either bolus concentration or total dose and cytosolic GSH transferase induction and phenolsulfotransferase depression appear proportional to total dose.

ISOZYME-SELECTIVE INHIBITION OF THE PULMONARY CYTOCHROME P-450-MEDIATED BIOACTIVATION OF 3-METHYLINDOLE. G S Yost, J C Hafizur, J D Adams, Jr., and J Y Jaw. Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

The bioactivation of the highly selective pulmonary toxin, 3-methylindole (3MI), by isozymes of cytochrome P-450 was studied through the synthesis and use of isoynzyme-selective amine deaminase and suicide inhibitors of P-450. Both 3MI metabolic turnover and covalent binding of 14C-labeled 3MI were inhibited to an equal extent (50% of controls) by 1-aminobenzotriazole at a concentration of 0.01 mM in goat lung microsomes. Concentrations of the inhibitor greater than 0.1 mM caused a complete loss of 3MI metabolism and covalent binding in a time-dependent manner. The isoynzyme-selective inhibitor, alpha-methylbenzyaminobenzotriazole (AMB), was synthesized and used to assess the involvement of P-450 forms selective for benzphetamine metabolism as contrasted to ethoxyresorufin metabolism. A concentration of AMB of 0.01 mM inhibited approximately 80% of 3MI metabolism or benzphetamine but only 28% of ethoxyresorufin O-deethylase activity. These results show that cytochrome P-450 is responsible for the bioactivation of 3MI to an alkylating intermediate, and that the "phenobarbital-inducible" isozymes are primarily responsible for the activation process. Supported by USPHS Grant HL13645. GSY is a USPHS Research Career Development Awardee (HL02119).

IN VITRO EFFECTS OF ENVIRONMENTAL ALKANES ON RAT PULMONARY CYTOCHROME P450-DEPENDENT MONOOXYGENASE ACTIVITIES. J Rabovsky and DJ Judy. NIHSH/DRO. Morgantown, WV. Sponsor: V Castranova.

Alkanes are environmental contaminants that are metabolized through cytochrome P450-dependent enzyme activities (P450). The reactivities between alkanes and two rat lung microsomal P450 activities, constitutive benzylidihydroxyphenoza dealkylase (BEOP) and 8-naphthoflavone (BEF)-induced ethoxyresorufin dealkylase (ETOP) assay, were studied by measuring the inhibition of resorufin formation. The data are expressed as the concentration of alkanes required for 50% inhibition (I50). I50/BEOP for n-hexane through n-undecane (nC6-nC11) ranged from 2-10µM. Some branch-chain octanes (C8) exhibited decreased I50/BEOP: i.e. I50=-5µM-C8, 6µM-2methylheptane, 1µM-4methylheptane, 0.9µM-2,5dimethylohexane. No effect on I50/ETOP's activity was observed for straight-chain alkanes or branch-chain C8's at concentrations up to 1-10µM. Hydroxyl and carbonyl C8's exhibited increased I50/BEOP'ase (I50=54µM-3-octanol, 85µM-3octanone) and measurable I50/ETOP'ase (I50=0.5µM-3octanol, 1.2µM-3octanone). The data show in rat lung microsomes, the alkanes were more reactive towards BEOP's than towards ETOP's activity. The reactivities depended on structure and functional groups. BEOP's and ETOP's activities are useful tools for the study of specific interactions between environmental pollutants and pulmonary P450-mediated reactions.


Hepatic cytochrome P-450(s) (P-450) and their resultant monooxygenase activities exhibit sexual dimorphism in adult rats. For instance, hepatic testosterone 6b and 16a/21a hydroxylase activities are relatively high in males due to the presence of the P-450o and /h isozymes respectively, but are low or absent in females, where testosterone is predominantly reduced at the 5a position. Furthermore, 7-ethoxyresorufin-O-deethylase activity (EROD) is also present at low activities in (uninduced) adult rat liver due to the low amounts of P-450c. Monooxygenase activities were investigated in liver biopsy samples from adult male and female rhesus monkeys. No significant sex differences were seen in the hepatic metabolism of testosterone which was hydroxylated predominantly at the 6b position in both sexes and was not reduced at the 5a position. EROD activity was 18-fold greater in both male and female monkey liver than in rat liver but these high activities did not correlate with an increased capacity to form benzaldehyde epoxides. These findings suggest that large species differences occur between the rat and rhesus monkey in both quantity and substrate specificity of their analogous P-450 isozymes.
IDENTIFICATION OF A TCDD-INDUCIBLE HAMSTER LIVER MICROsomAL PROTEIN IMMUNOCHEMICALLY RELATED TO RAT CYTOCHROME P-450a BUT WITHOUT TESTOSTERONE 7α-HYDROXYLASE ACTIVITY.
M P Arlotta, S K McMillen and A Parkinson.
Kansas University Medical Center, Kansas City, KS.

Polyclonal antibodies against purified rat liver microsomal cytochrome P-450a were raised in rabbits, and rendered unspecific by absorption chromatography. In Western immunoblots, the antibody recognized a single protein (Mr 48,000) in liver microsomes from rats of different age and sex, or from rats treated with phenobarbital, 3-methylcholanthrene (3-MC) or dexamethasone. The antibody also completely inhibited (>98%) the 7α-hydroxylation of testosterone by these liver microsomal preparations. Immunoblots of liver microsomes from hamsters revealed the presence of two proteins (Mr 48,500 and 50,000) immunologically related to rat cytochrome P-450a. Treatment of hamsters with TCDD or 3-MC caused a marked intensification (>25 fold) of the larger protein recognized by antibody to rat P-450a (Mr 50,000), but slightly decreased the intensity of the smaller protein (Mr 48,500). The role of testosterone 7α-hydroxylase catalyzed by liver microsomes was slightly decreased after treatment of hamsters with TCDD or 3-MC. These results indicate that hamster liver microsomes contain two proteins immunologically related to rat cytochrome P-450a, the larger of which is highly inducible by TCDD and 3-MC, but is apparently devoid of testosterone 7α-hydroxylase activity. Supported by NIH grants ES-03765, ES-00166 and ES-07079.

INDUCTION OF CYTOCHROME P-450 BY PHENOBarBITAL AND 3-METHYLCOLANThRENE; DIFFERENCES BETWEEN RATS AND HAMSTERS.
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Kansas University Medical Center, Kansas City, KS.

Previous studies have shown that treatment of rats with phenobarbital (PB) causes a marked induction (>30 fold) of P-450a, which is associated with a comparable increase in the rate of liver microsomal testosterone 16α-hydroxylation and pentoxysyrosorufin O-dealkylation. In the present study, we show that treatment of hamsters with PB caused a marked increase (>25 fold) in the intensity of a liver microsomal protein immunologically related to P-450b (as determined by Western blot). However, treatment of hamsters with PB caused less than a 5-fold increase in testosterone 16α-hydroxylase and pentoxysyrosorufin O-dealkylation activity. Similarly, previous studies have shown that treatment of rats with 3-methylcholanthrene (3-MC) causes a marked induction (>30 fold) of P-450c, which is associated with a comparable increase in the rate of liver microsomal benzo[a]pyrene 3-hydroxylation and ethoxyresorufin O-dealkylation. Treatment of hamsters with 3-MC caused a marked increase (>25 fold) in the intensity of a liver microsomal protein immunologically related to P-450c, which caused a comparable increase in ethoxyresorufin O-dealkylase activity. However, treatment of hamsters with 3-MC caused a slight decrease in benzo[a]pyrene 3-hydroxylation activity. These results suggest that rat P-450b and P-450c differ catalytically from the corresponding hamster proteins. Supported by NIH grants ES-03765, ES-00166 and ES-07079.

SPECIES DIFFERENCES IN THE OXIDATIVE BIOtransFORMATION AND TOXICITY OF DIGIToxIN.
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The cytochrome P-450-dependent biotransformation of digitoxin (dtg) catalyzed by liver microsomes from various laboratory animals was studied by HPLC. When liver microsomes from male rats were incubated with dtg and NADPH, the predominant metabolite formed was digitoxigenin bisdigitoxoside (dtg₂), which formed by oxidative cleavage of the terminal sugar of dtg. When liver microsomes were incubated with dtg and NADPH, very little dtg₂ was formed. However, hamster liver microsomes converted dtg₂ to 17α-hydroxy-dtg₂, a metabolite not formed by rat liver microsomes. Based on the profile of dtg₂ metabolites formed by liver microsomes, mice resembled rats, whereas guinea pigs resembled hamsters. Liver microsomes from cynomolagus monkeys and rabbits also converted dtg₂ to 17α-hydroxy-dtg₂, but the major pathway catalyzed by monkey liver microsomes was 12β-hydroxylation of dtg₂ to digoxin. Treatment of rats with dexamethasone caused a 4-5-fold increase in the rate of conversion of dtg₂ to dtg₂, whereas treatment of hamsters with dexamethasone caused a 4-5-fold increase in the conversion of dtg₂ to 17α-hydroxy-dtg₂. Treatment with 10 mg/kg digitoxin was lethal to 100% of male rats, whereas male hamsters completely tolerated dosages of 100 mg/kg digitoxin. We are currently investigating whether differences in the pathways of digitoxin biotransformation are the reason for the marked species difference in digitoxin toxicity. Supported by NIH grants ES-03765 and ES-00166.
We have previously reported on the toxicology of MCA in mice (Toxicologist 5:199,1986). In Sprague-Dawley rats injected with MCA (80 mg/kg, iv) followed 15 minutes later by either saline, phenytoin (80 mg/kg, ip) or PB (40 mg/kg, ip), the overall 24 hour survival rate for the three treatment groups was 25%, 38%, and 100%, respectively. Relative to the saline treatment group, the survival rate was significantly higher in the PB but not the phenytoin treatment group. When the order of treatment was reversed, i.e., PB or saline was administered to rats 15 min prior to MCA, the 24 hour survival rate was significantly higher in the PB (87%) group than the saline (53%) group. PB had no effect on plasma or cerebrospinal fluid levels of 14C-MCA or its radiolabeled metabolites. PB had no effect on 14C-MCA equivalents covalently bound to brain proteins. These results suggest that (1) PB is an effective antidote to MCA intoxication in rats and (2) this effect does not result from an alteration in the metabolic disposition of MCA.
IN VITRO BIOACTIVATION OF 2,4-DIAMINOTOLUENE BY AROchlor-1254-INDUCED RAT LIVER S9. M L Cunningham, L T Burka, and H B Matthews, NIEHS, Research Triangle Park, NC.

2,4-Diaminotoluene (2,4-DAT) is used as an intermediate in the synthesis of various dyes and for the production of toluidine blue oxide for the manufacture of polyurethane. 1.6 x 10^7 pounds of 2,4-DAT, a potent bacterial mutagen and rodent carcinogen were produced in 1982. In order to characterize the mutagenic (and by inference, carcinogenic) metabolite(s) of 2,4-DAT, an Arochlor 1254-induced rat liver S9 mix was used as an activation system, and the metabolite(s) separated and purified by HPLC and characterized by mass spectrometry. 2,4-DAT metabolism exhibited Michaelis-Menten kinetics with a K_m of 5.2mM and 18.1 umoles/mg protein/min., respectively. 2,4-DAT was rapidly and quantitatively metabolized to one major metabolite in this S9 activation system. It had a molecular weight of 256 daltons and mass fragmentation pattern consistent with a structure of dimethyl, dimino, dihydroxyphenazine (CH_NH, C_H N,NH+H, N,NH CH). This metabolite was not produced when 2,4-DAT was incubated with boiled S9. The major metabolites of 2,4-DAT found in vivo exposure, N-acetyl conjugates of benzoic acid derivatives, were not found in this in vitro S9 activation system. The noncarcinogenic isomer, 2,6-DAT, did not appear to form a similar dimeric metabolite in this activation system. This dimeric metabolite of 2,4-DAT is planar and may be a mutagenic intercalating agent.

STRUCTURE-ACTIVITY STUDIES OF CYCLIC ANHYDRIDES WHICH CAUSE PULMONARY SENSITIZATION. C L Leach, N S Hatoum, C R Zeis, and P J Garvin. HHT Research Institute, Veterans Admin, and Amoco Corporation, Chicago, IL.

Trimellitic anhydride (TMA) is known to cause pulmonary sensitization and lung pathology, however, there are many structurally similar cyclic anhydrides and carboxylic acid precursors which have not been investigated for their potential to cause sensitization. Inhalation studies were conducted on (I) trimellitic acid (TMAC), a precursor to TMA, and (II) pyromellitic dianhydride (PMDA), a chemical used similarly to TMA. (I) Rats were exposed for 2 weeks to 3000 ug/m^3 of TMAC, rested 2 weeks, and challenged with TMAC prior to termination. There were no lung lesions or TMA antibody at any time point. In vivo studies showed that the TMAC reactivity to protein, a necessary prerequisite for immunogenicity, was minimal compared to TMA. (II) Rats exposed once to 2.2 mg/l of PMDA, rested 2 weeks, and challenged with 500 ug/m^3 showed hemorrhagic lung foci, and increased lung weights, lung volumes, and IgG antibody. Rats exposed to 500 ug/m^3 of PMDA for 5 days, rested 2 weeks, and challenged also showed lung foci, and increased lung weights and IgG antibody. The effects of PMDA inhalation were similar to those caused by TMA, however, there were no effects caused by TMAC. In vivo studies of antibody reactivity to TMA and PMDA coupled to albumin showed substantial cross-reactivity between the two anhydrides. Thus, there appeared to be comparable activity between TMA and PMDA suggesting that other similar anhydrides may also be sensitizers.

CONTACT HYPERSENSITIVITY RESPONSE TO GLUTARALDEHYDE IN GUINEA PIGS AND MICE. M L Stern, J A McCoy, R D Brown and M P Holscapple, Dept. of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA.

Glutaraldehyde (GL) has a wide spectrum of uses which can result in dermal contact with the agent. The low number of reports of hypersensitive reactions to GL indicates a low incidence of sensitization. Female albino Hartley strain guinea pigs and female B6C3F1 mice were sensitized with 0.3, 1.0 and 3.0% GL and challenged with 10% GL. Doses of GL were selected by assays for primary irritancy. Guinea pigs received 100 μl by direct dermal application for 14 consecutive days, and mice received 20 μl by direct dermal application for 5 or 14 consecutive days, to sites prepared by shaving and dermabrading. Rest periods were 7 or 14 days for guinea pigs and 4 or 7 days for mice. Measurement of the contact hypersensitivity response in guinea pigs was by both visual evaluation (scoring) at 24 and 48 hours following challenge and radioisotopic assay at 48 hours, and in mice by radioisotopic assay 48 hours after challenge. Both guinea pigs and mice demonstrated dose dependent contact hypersensitivity responses to GL. The radioisotopic assay appeared to be more sensitive than visual evaluation in detecting contact allergic hypersensitivity to GL. (Supported by NIH contract ES 55594).


The safety and immunomodulatory activity of a synthetic T-Cell Modulatory Peptide (TCPM-80) derived from the human IgG Fc region was assessed in CD rats (12/sex/group) given daily i.v. injections of vehicle or 1, 2, 10 or 100 mg/kg TCPM-80 for 14 days. Routine safety evaluations (body weight, clinical chemistry, hematology, gross postmortem examination) revealed no toxic effects. Splenocytes from 8 rats per group were collected for lymphocyte subset and lectin mitogenesis analyses. Lymphocyte subsets were not affected in females but T helper cells in males tended to decrease at all dose levels after 14 days. The percentage of 1A+ cells increased independently of dose. PHA proliferation was inhibited at the 1 and 2 mg/kg doses while 100 mg/kg enhanced PHA proliferation by 133% in males and by 197% in females. B lymphocyte proliferation (LPS) was enhanced (178-63%) in both sexes at the high dose of TCPM-60. The 1 and 2 mg/kg doses were inhibitory and stimulatory for B cell mitogen proliferation (PHA, LPS). Thus, TCPM-80 did not produce any short term toxicity but did demonstrate strong dose dependent T and B lymphocyte modulation.

In toxicology the most commonly used experimental animal is the rat. Hence, also in the overlapping field with immunology i.e. immunotoxicology, rats are often chosen. We are interested in the study of effects of potential immunotoxicants on local immunity in the intestines, since the alimentary tract is one of the major routes of exposure to toxicants. We have developed a mouse monoclonal antibody, that specifically recognizes rat IgA. This reagent proved valuable in an enzyme-linked immunosorbent assay (ELISA) for the titration of specific IgA in the serum of rats that were immunized via the Feyer's patches with ovalbumin, or that were orally infected with muscle larvae of the parasite Trichinella spiralis. Using this assay we have determined the effect of dietary exposure during 6 weeks to up to 80 mg bis(tri-n-butyltin)oxide (TBT0) per kg food on IgA responses. Whereas no significant effect on IgA responses to ovalbumin was observed, exposure to TBT0 enhanced in a dose-related fashion the IgA response to T. spiralis. As a possible explanation IgA may serve the function that TBT0, known to suppress immune responses, inhibits expulsion of T. spiralis worms from the gut, thus prolonging the stimulus to produce IgA. Thus in this system immuno-suppression by TBT0 can paradoxically lead to immune stimulation. Another explanation may be that TBT0 corrupts immune regulatory mechanisms.

EVALUATION OF SURFACTANT TA FOR SYSTEMIC ANAPHYLAXIS IN GUINEA PIGS. L L Yang, S Tekell, and W K Patterson. Abbott Laboratories, Abbott Park, IL.

Surfactant TA (STA), a pulmonary surfactant extracted from bovine lung, is intended for use in hyaline membrane disease which occurs in premature infants. Intratracheal (IT) administration is an important clinical route. The potential of STA to produce anaphylaxis was assessed in male Hartley guinea pigs weighing 300-450 g. Animals received three IT injections of STA in saline at a dose of 20 mg/animal/injection for injections. Three injections were given on alternate days. Egg albumin at doses of 0.2 and 0.5 mg/animal/injection was used as a positive control. Three weeks after the last IT injection, animals were challenged with an IT test material by an IT instillation. Doses of 0.5 and 1.25 mg/animal were used for egg albumin. A dose of 20 mg/animal was used for STA. None of the animals that received STA or saline showed signs of anaphylaxis or death. Convulsions followed by death occurred shortly after the IT challenge in animals receiving egg albumin. Gross and histologic examinations of the lungs from the animals that died showed various degrees of pulmonary changes which were compatible with the anaphylactic responses of guinea pigs.

ON THE POPLITEAL LYMPH NODE (PLN) ASSAY FOR THE DETECTION OF AUTOIMMUNE AGENTS IN MICE. X Joseph, J P Betrecht, and I Balass. FDA, Washington, DC and Univ. of Toronto, Toronto, Ontario, Canada.

The PLN reaction, a locally induced graft-versus-host reaction, has been used recently as a predictive test system for chemically induced immune disregulation (Kammler et al., Abstr. 6th Int. Congr. Immunol., 699, 1986). We examined the reliability of this assay for identifying the autoimmunity-inducing potential of various chemicals, viz., procainamide (PRG) and its metabolites [nitro (N) and hydroxyamino (HY) derivatives], hydralazine, methyl-dopa, and propranolol in inbred mice. Phenyltoin (PT) was used as a positive control. Groups of 8 each of 6- to 8-week-old male C57B1/6J mice were given a single sc injection of 0.05 ml (2.5 mg) of each drug or its vehicle to one of the hind footpads. Certain irritants (1% acetic acid or 50% ethanol) were also given to other groups of mice. PLNs were removed 7 days after injection and PLN indices (PI) were calculated as the ratio of lymph node weights of the injected over the un.injected side. The N derivative of PRG (PI 4-5) and PT (PI 5-10) gave strong positive reactions, the HY derivative was intermediate (PI 1.5-3), and all other drugs were negative. However, acetic acid and ethanol were positive (PI 4-8), indicating that the PLN reaction is not specific for autoimnunogens.

LOCALIZATION OF HALOTHANE-INDUCED ANTIGEN IN SITU BY SPECIFIC ANTI HALOTHANE "METALOLITE" ANTIBODIES. T P Roth, AK Hubbard, AJ Gandolfi, Department of Anesthesiology, University of Arizona, Tucson, AZ.

Multiple halothane exposures of rabbits induce an antibody cross reactive with trifluoroacetylated (TFA) proteins. The anatomical location of the halothane-induced antigen that induces this immune response was investigated in livers from halothane exposed rabbits. Rabbits, immunized with TFA-rabbit serum albumin were exposed multiple times to halothane anesthesia. Antibodies from these animals will detect TFA-conjugated proteins produced by halothane exposure, using an immune-staining technique, binding by the antibody, to the antigen, was detected in liver tissue from all 6 halothane exposed rabbits. Antigen could be detected only in the central globular area around the central vein where staining intensity was concentrated in an area 7-9 cells deep. Approximately 60-75% of the central veins within each tissue section were positive for antigen detection and staining around one vein became contiguous with staining around another central vein. No necrosis, however, was observed in this animal model. These data suggest that halothane exposure by inhalation generates an antigen in the central globular area of the liver which is antigenically similar to the reactive intermediate, TFA halide. (NIH GM 34788)
ELICITATION OF A CELL MEDIATED IMMUNE RESPONSE TO A REACTIVE INTERMEDIATE OF HALOTHANE. AK Hubbard, TP Roth and AJ Gandolfi, Dept Anesthesiology, University of Arizona, Tucson, AZ

Halothane exposures of rabbits and humans elicit a humoral immune (antibody) response with specificity for the reactive intermediate of halothane, trifluoroacetyl halide moiety (TFA), conjugated to a carrier protein, rabbit serum albumin (RSA). Since T cells could also potentially mediate liver damage, experiments were conducted to determine if a cell mediated immune response for TFA was elicited in halothane exposed rabbits. Male New Zealand white rabbits were exposed 4 times to 1% halothane (80% O2) at 2 wk intervals. Splenic leukocytes were isolated and cultured for 72 hr in the presence of TFA-RSA, RSA, or media. 3H-thymidine was used to measure the 24 hr proliferation of T lymphocytes. Data are expressed as stimulation indices (3H-thymidine cpm antigen/med). TFA-RSA (5,1 μg/ml) stimulated leukocytes from halothane exposed rabbits (3,5,3,7,2,2, respectively) and only mildly stimulated leukocytes from unexposed rabbits. RSA (0,1-5 μg/ml) did not stimulate either source of leukocytes. These studies suggest that multiple halothane exposures induce a cell mediated immune response with reactivity toward a reactive intermediate of halothane (GM 34788).
EFFECT OF ZINC ON THE DISTRIBUTION AND TOXICITY OF CADMIUM IN ISOLATED INTERSTITIAL CELLS OF THE RAT TESTES. T Koizumi and M P Waalkes. Laboratory of Comparative Carcinogenesis, National Cancer Institute-FCCP, Frederick, MD

It is known that pretreatment with zinc prevents the eventual formation of interstitial cell (IC) tumors by cadmium in the rodent testes. However, the mechanism by which zinc reduces the toxicity of cadmium in these target cells of cadmium carcinogenesis is not known. Therefore, in this study we assessed the effects of in vivo pretreatment with zinc on the cytotoxicity and subcellular distribution of cadmium in vitro in ICs isolated by collagenase dispersion from Wistar rats. Such zinc pretreatment resulted in marked reduction of cadmium-induced cytotoxicity, as reflected by a reduced loss of cellular K and glutamic-oxaloacetic transaminase (GOT). Although the overall cadmium uptake was modestly increased the subcellular distribution of cadmium showed a marked alteration in zinc-pretreated cells, including a major shift of cadmium away from the nuclei to other components. Cadmium content in isolated nuclei was also decreased by zinc pretreatment. The uptake of cadmium into nuclei isolated from zinc-pretreated cells also was substantially reduced. Thus, it is speculated that zinc pretreatment against cadmium-induced testicular tumor formation is due to the ability of zinc to decrease cadmium uptake into the nuclei, the presumed target site for the genetic toxicity of cadmium.

CYTOTOXICITY OF SIX METALS TO TESTICULAR CELLS IN VITRO. C D Brown, O Li and M J Brabec. Dept. Chemistry, Eastern Michigan University, Ypsilanti, MI.

The testes is a target organ for some toxic metal cations. This study compared the relative cytotoxicity of metal cations in primary cultures from rat testes. Primary cultures of testicular cells isolated from rats were exposed to one of six divalent metal cations: Cd, Pb, Zn, Cu, Mg or Ca. Two culture systems were used: Sertoli cells from 21 day old rats, and co-cultures of Sertoli-germ cells from 27-30 day old rats. Cytotoxicity was assayed after a 24-hour incubation with the metal cation by neutral red incorporation, reduction of a tetrazolium dye (MTT) and lactate secretion. Germ cell detachment was assayed in co-cultures. Results from the four assays showed strong correlation between the relative cytotoxicities of the 6 metals. Lactate secretion was the most sensitive (PC0.5 with 1 μM Cd) assay. All assays ranked the cytotoxicities in the order Cd > Pb > Zn > Cu > Ca = Mg. The assays used are relatively simple, rapid, and use few animals. Quantitative in vitro assays such as these may be useful for initial screening of suspected testicular toxins. NIEHS R01-04141.

CADMIUM INDUCES TWO SMALL PHOSPHOPROTEINS IN SERTOLI CELL CULTURES. S W Clough, M J Welsh and M J Brabec. Dept. Chemistry, Eastern Michigan University, Ypsilanti, MI.

The hypothesis that cadmium (Cd) alters protein phosphorylation as a mechanism of toxicity was examined in primary cultures of rat Sertoli cells. Sertoli cell cultures were exposed to sub-lethal concentrations of Cd and the changes in 32P-labelled phosphoproteins was examined utilizing autoradiography and two-dimensional gel electrophoresis. The phosphorylation of two acidic proteins of 26 kD molecular weight increased in a time- and dose-dependent manner. The phosphorylation of one protein, GCl, in calcium and g-merin-dependent. The phosphorylation of the other, GASP, was stimulated specifically by Cd, and not by lead, zinc or mercury. Cycloheximide treatment reduced the increased phosphorylation of both proteins to that of control, while actinomycin D caused only a slight reduction in intensity. The alteration in phosphoproteins occurs at concentrations of Cd similar to that which cause an increase in lactate secretion and germ cell release in Sertoli-germ cell co-cultures. Our tentative conclusion is that the apparent induction of the two phosphoproteins represents a primary biochemical event of Cd toxicity, and may be important in understanding how Cd disrupts testicular function. NIEHS 04141.

THE EFFECTS OF SALIGINEN CYCLIC-G-TOLYL PHOSPHATE (SCOTP) ON PRIMARY SERTOLI CELL CULTURES. R E Chapin, S G Somkuti*, J L Phelps, J J Heindel, D M Lapadula*, M Othman*, and M B Abou-Doina*. Developmental and Reproductive Toxicology, NTP, NIEHS, Research Triangle Park, and *Department of Pharmacology, Duke University Medical Center, Durham, NC.

Since in vivo studies identified Sertoli and Leydig cells as targets for tri-g-cresyl phosphate (TOCP) in testis, we investigated the effects of TOCP and its active metabolite, SCOTP, on cultured Sertoli cells. Daily repeat exposure for 5 days to up to 100 μM of TOCP produced no changes in lactate or pyruvate secretion or morphology. But SCOTP produced morphologic effects at 20 μM, and depressed non-specific esterase (NSE) activity at and above 0.1 μM by up to 40%. A 24-hour exposure to SCOTP produced equivocal changes in media lactate, pyruvate, ABP, and cellular ATP, protein synthesis, and FSH-stimulated cAMP. However, 5 daily exposures to SCOTP increased media lactate and pyruvate, and further depressed NSE. The NSE decrease did not recover for 5 days after 24 hr. exposure to SCOTP. These studies define some effects of a neurotoxic organophosphate metabolite on testicular Sertoli cells, and imply that Sertoli cells lack the metabolic ability to transform TOCP to SCOTP.

Phthalate esters which cause testicular toxicity act primarily on the Sertoli cells. To investigate early biochemical effects of these toxicants, protein and RNA synthesis was evaluated in primary rat Sertoli cell-enriched cultures (approx. 85% Sertoli cells), using a 60 min pulse with [3H]-leucine and [3H]-uridine respectively. In cultures treated for 24 hr with MEHP, [3H]-leucine incorporation into Sertoli cell proteins was significantly increased by 15% at 1 µM, up to 50% at 200 µM. At 200 µM this effect was first evident after a 3 hr treatment and was maximal after 18-24 hr. An increase in RNA synthesis was evident after 1 hr, maximal after 3 hr (125% control) and absent by 6 hr. Blocking RNA synthesis with actinomycin D abolished the stimulation of protein synthesis by MEHP. Exposure to MEHP for only 30 min produced a significant stimulation of [3H]-leucine incorporation assessed 24 hr later. None of the above effects were observed with phthalate monoesters which do not affect the testis in vivo. Thus MEHP, at concentrations relevant to tissue levels in vivo, rapidly induces increased transcription and translation in cultured Sertoli cells. The signals involved and their role in the toxicity of MEHP remain to be established. (Supported by the UK Ministry of Agriculture, Fisheries and Food)

DI-N-PENTYL PHthalate (DPP) INDUCED INFERTILITY: CORRELATION WITH SERUM ANDROGEN BINDING PROTEIN (sABP). P. Lindstrom, M. Harris, M. Ross, J. C. Lamb, R. Chaplin. DART, STB, NIEMS/NTF, Research Triangle Park, NC.

We showed that changes in sABP correlate with changes in morphological and biochemical reproductive endpoints in DPP-treated F344 rats. sABP may be a good marker in the rat for germinal epithelial damage. However, it should also indicate changes in total reproductive function. One objective was to study effects of DPP on fertility, then to correlate the results with changes in sABP. Histopathology from the earlier study suggested a very slow recovery; the second objective was to evaluate recovery up to 30 weeks postexposure. Eighty male rats were dosed once via gavage with DPP in corn oil (0.025, 1.0, and 2.0 g/kg BW) then mated to untreated females at 3, 6, and 10 weeks postexposure. Controls and high-dose rats were killed at 14, 18, and 30 weeks after dosing, and the germinal epithelium was evaluated histologically. Two g/kg DPP caused reduction in pregnancies and live pups and increase in preimplantation loss. One g/kg DPP did not change these endpoints. sABP had more than doubled during the first 2 weeks postexposure to 2 g/kg DPP, while after 1 g/kg DPP sABP showed 50% increase during the first week postexposure. No high-dose animal showed signs of recovery. In summary, 115% initial increase in sABP correlated with subsequent infertility, while 48% increase did not. The epithelium did not recover within 30 weeks.

STAGE-SPECIFIC UNSCHEDULED DNA SYNTHESIS (UDS) IN RAT SPERMATOGONIC CELLS. S. Bentley and P. K. Working. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

DNA repair in spermatogenic cells at various stages of maturity was determined by UDS. F-344 rats were exposed to 35 mg/kg (i.p.) methyl methanesulfonate (MMS) or methyl nitrosourea (MNNU). One hour later segments of seminiferous tubules were cut corresponding to stages II, IV, VI, VII, VIII, X, XII, and XIV of spermatogenesis using the unique transillumination pattern of the tubules as a guide. Intact tubule segments were cultured 24 hours in the presence of [3H]-thymidine and UDS was measured by autoradiography as net grains per nucleus (NG). In MMS-exposed animals NG increased as primary spermatocytes (SC) matured from leptotene SC (3.5 NG) up to stages VIII and X pachytene SC (22 NG), after which NG in stages XII pachytene SC and in diplotene SC decreased (16 NG and 8 NG, respectively). A similar stage-specific UDS pattern was observed in primary SC isolated from MNU-exposed animals. Round spermatids of steps 2 to 8 all exhibited approximately the same UDS response (8 NG). Spermatids as mature as step 14 exhibited UDS responses after exposure to MMS or MNNU in vivo, but step 15 and later spermatids apparently lacked the ability to repair DNA. Whether the variation in UDS response observed in primary SC was due to a difference in DNA repair capability or in the degree of DNA alkylation cannot be determined from these data.

CIRCADIAN FLUCTUATION OF GLUTATHIONE (GSH) LEVELS IN THE REPRODUCTIVE TRACT OF THE MALE RAT. H. K. Bates, R. D. Harbison, J. Gandy, Pathology Associates, Inc./WCTR, Jefferson, AR and University of Arkansas for Medical Sciences, Little Rock, AR.

Studies in our laboratory have shown that chemical depletion of reproductive tract GSH increases susceptibility to germ cell mutations. Hepatic glutathione in the rat has been demonstrated to fluctuate on a circadian cycle linked to food intake. Extragonadal pools of GSH have not been adequately investigated. Fluctuations in GSH levels were examined in the male reproductive tract as a possible mechanism for varying individual susceptibility to reproductive toxicants. Male rats 12 weeks old were maintained on a 12:12 hour light-dark cycle starting at 6:00am with ad libitum access to food. Total GSH was measured every four hours in the liver, kidney, testis, caput and cauda epididymis, and the seminal vesicles. Liver, testis, and caput epididymis exhibited significant (p < 0.05) circadian differences in GSH content with maximal levels of 2490 ± 156 (SE), 935 ± 57, and 1604 ± 85 ug/g wet tissue at 1400, 0600, and 0600 hours, respectively. No significant variations in GSH content occurred in the remaining tissues. Fluctuation of the high GSH content in the male rat reproductive tract indicates a potential for variable susceptibility to chemically induced toxicity. (Supported in part by NIH grant HD02258 and the National Foundation of the March of Dimes.)
DECREASED TESTICULAR GLUTATHIONE (GSH) LEVELS DO NOT EXACERBATE THE REPRODUCTIVE TOXICITY OF 1,3-DINITROBENZENE (m-DNB) IN MALE RATS. V Slott, R Linden, L Strader, S Perreau. USEPA, HSERL, Reproductive Toxicity Br., RTP, NC. Sponsor: R Chadwick

The intracellular thiol GSH is known to protect cells against many toxic chemicals. To investigate a protective role for GSH in adult rat tests, GSH was decreased to 30-50% of control levels in one testis of each rat by intratesticular injections of a mixture of diethyl maleate and buthionine sulfoximine prior to oral gavage with the testicular toxicant m-DNB (0, 16 or 24 mg/kg). At 14 days posttreatment the reproductive effects on the GSH-depleted and contralateral control sides were compared to determine if reduced levels of GSH enhance the effects of m-DNB. GSH depletion alone did not affect testis or epididymal weight, sperm motility, testicular spermatid counts, or cauda sperm reserves compared to vehicle-injected controls, but an increase in histologically abnormal tubules localized to the injection site occurred in some GSH-depleted testes. Among the m-DNB-treated rats, 24 mg/kg significantly reduced all of the above parameters while 16 mg/kg reduced all but sperm motility and testicular spermatid counts. Both 16 and 24 mg/kg increased the number of abnormal tubules, however GSH depletion did not enhance any of the effects of either dose of m-DNB. Thus, since depletion of testicular GSH to 30% of control did not exacerbate the reproductive toxicity of m-DNB, GSH may not protect against this toxicant.

TESTICULAR TOXICITY OF THE CHLORONITROBENZENES. K L Mohr and P K Working. CCIT, Research Triangle Park, NC.

Many nitroaromatic compounds, including nitrobenzene and 1,3-dinitrobenzene (DNB), are testicular toxicants in rodents. We have assessed the testicular toxicity of the chloronitrobenzenes (CNN) in Fischer 344 rats. Males (n=5 or 6) received a single oral dose of one-half the LD50 of 2-, 3- or 4-CNN (150, 200 and 250 mg/kg, respectively). The rats were sacrificed 1 d or 25 d later. Neither 2- nor 4-CNN had any effect on testicular histopathology (at 1 d) or testes weight and daily sperm production (DSP, at 25 d). In contrast, 1 d after exposure to 3-CNN, signs of early degenerative change were observed in the testes including condensed nuclei and cytoplasmic vacuolation in pachytene spermatocytes of stages VII-X, loosening and disorganization of spermatid layers and Sertoli cell cytoplasmic vacuolation in stages X-XIV and chromatid margination in steps 1 and 2 spermatids. By 25 d after treatment, testis weights and DSP were both significantly lower in 3-CNN exposed males than in controls (1.9 ± 0.2 g and 6.8 ± 2.2 million/g testis per d, respectively, vs. 2.6 ± 0.1 g and 17.8 ± 1.5 million/g testis per d in controls). Thus, 3-CNN but not 2- or 4-CNN is a testicular toxicant in rats under these exposure conditions. Similar isomeric specificity is seen in both CNN and DNB, i.e., only the meta isomer of each is a testicular toxicant after a single dose.

INHALATION TOXICITY OF COBALT SULFATE. J R Bucher, National Toxicology Program, Research Triangle Park, NC, and B J Chou, R A Renne, J R Decker, H A Ragam, Battelle Pacific Northwest Laboratories, Richland, WA.

Male and female B6CF1 mice and F344/N rats (ten per sex and group) were exposed to 0, 0.3, 1.0, 3.0, 10.0 or 30.0 mg/m3 of aerosolized cobalt sulfate by whole body inhalation exposure, 5 days/week for 13 weeks. Two high dose male mice died during the exposure and high dose rats and mice gained less weight than controls. Increases in lung weights (up to 70%) were dose related in both species. Testis weight was decreased by 60% in high dose male mice, where testicular atrophy, mineralization and abnormal sperm were frequently observed. Sperm motility was decreased in the top 3 dose groups of mice. Hematicologic changes were prominent in rats and included polycythemia and decreased platelets. Urinalyses showed dose-related cobalt content, and in male rats there was a dose-related increase in the number of sloughed epithelial cells in urine. Histopathologic findings included inflammatory and regenerative changes throughout the respiratory tract with the larynx appearing most severely affected. Polypoid-shaped nodules of connective tissue covered by stratified squamous epithelium were observed at the base of the epiglottis in 28/40 rats exposed to 10 or 30 mg/m3. Squamous metaplasia of respiratory epithelium at the base of the epiglottis occurred at all chamber concentrations in both species.

CHARACTERIZATION OF NICKEL CHLORIDE-RESISTANT BALB/C-3T3 MOUSE FIBROBLAST CELLS. X W Wang, K J Imura and H Costa, Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY.

Nickel is a toxic and carcinogenic agent that is a contaminant in the environment. We have begun to study the metabolic fate of nickel in mammalian cells by selecting mutant cells that continue to grow and divide in the presence of concentrations of nickel chloride that are toxic to the parent cell line. The phenotype of these resistant cells is being examined by several methods, including cell survival studies, chromosome analysis by Giemsa or C-banding, 53CrCl uptake, and one- and two-dimensional electrophoresis. Preliminary results indicate that the nickel-resistant phenotype is associated with changes in chromosome structure and number and in protein expression. Specifically, increased nickel resistance appears to correlate with an increased number of centric fusions, increased rearrangement of heterochromatin DNA, decreased chromosome number, and increased accumulation of a 44 Kd (PI 47.5) protein. The nickel-resistant phenotype is relatively stable when these cells are grown in the absence of nickel, but the LC50 decreases by one-half after approximately 40 cell generations. These nickel-resistant cells appear to be a useful model for investigating the metabolic response of cells to nickel in the environment. (Supported by Grant No. R013140 from the U.S. EPA)
NICKEL-MAGNESIUM INTERACTION IN SPECIFIC PROTEIN BINDING TO MOUSE SATELLITE DNA. D M Latta, R J Imbra and M Costa. Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY.

Treatment of mouse or Chinese hamster ovary cells with various nickel compounds results in decondensation of heterochromatin (HC); this effect can be reversed by increasing the extracellular level of magnesium (Mg) ions. We have used the band shift assay to study the effects of Ni and Mg on specific protein binding to DNA. Protein extracts from cells were incubated with a mouse satellite DNA (SD) probe, a sequence that comprises the HC of mouse chromosomes, in the absence or presence of metal ions. Complexes of DNA and protein are detected by their migration in non-denaturing polyacrylamide gels. Protein binding to the human metallothionein-IIA (MT) gene promoter was examined for comparison. Ni or Mg alone inhibit protein binding to the SD and the MT probes. When Mg and Ni are added together to the MT probe, the inhibitory effects are additive; in contrast, Mg reverses the Ni-induced inhibition of protein binding to the SD probe. Preliminary results indicate that effects of other metal ions (Ca, Cd, Zn, and Mg), which also reduce protein binding to both the SD and MT probes, are not reversed by the addition of Mg to the reaction. (Supported by Grant No. CA-43070 from NCI and by NIH R12-ES-05423)


Genotoxic effects in human (A549) and rat (L2) type II alveolar epithelial cells exposed to NiCl₂ were measured by quantitating single strand breaks. DNA-protein crosslinks and DNA-DNA crosslinks utilizing the alkaline elution technique. When both cell lines were exposed to 0, 0.13, 0.68, and 1.3 mM NiCl₂ for 24 hr, a dose dependent production of single strand breaks occurred. Based on intracellular nickel, A549 cells were more sensitive than L2 cells. When converted to rad equivalents, a nickel intracellular concentration of 50 μM resulted in single strand break frequencies of 85 and 120 rad-equivalents in L2 and A549 cells, respectively. When both cell lines were treated with proteinase K to break any DNA-protein crosslinks, no difference in the elution rate occurred. Thus, nickel does not produce DNA-protein crosslinks as part of its genotoxic mechanism. Alkaline elution assays for DNA-DNA interstrand crosslinks were carried out by further treating nickel exposed and control cells with a high dose of radiation and proteinase K to eliminate the effects of single strand breaks and DNA-protein crosslinks. Comparison of the slow eluting portions of the control and nickel exposed curves showed no change in slope between the two. Thus, no DNA-DNA interstrand crosslinks result from nickel exposure. (Supported by NIH Grants RR01693, ES07031 and CA14236.)


The increased lung cancer risk of cadmium smelter workers might reflect cadmium exposure, arsenic exposure, or cigarette smoking. Cohort mortality analysis of 597 white males employed over six months to six years in cadmium smelter production area during 1940-69 and a within cohort case-control study of lung cancer mortality prior to 1984 was conducted to assess the contribution of these factors. Cumulative cadmium exposure estimates had been developed by company personnel from interviews and company records for 45% of the cohort. Plant process history indicated three periods of potential arsenic exposure based on arsenic concentration of the feedstock--prior to 1926, very high: 1926-39, high: 1940-69, moderate to low. Based on 25 cases and 75 matched controls, the relative case-to-control cadmium exposure (mg·year/m³) was 1.0 (range 0.9-1.1). The relative cigarette smoking exposure (pack·year/employee with known smoking history) was 2.5 (range 1.6-8.3). SMRs for lung cancer by period of hire were 492 (hired prior to 1926), 285 (hired 1926-39), and 88 (hired 1940-69). The lung cancer risk appears to reflect arsenic exposure rather than cadmium exposure and to be confounded by cigarette smoking.

A MODULATING ROLE FOR THE TESTES IN THE RESPONSE OF THE ADULT MALE RAT TO CADMIUM. M Mautino and J U Bell. Department of Physiological Sciences, University of Florida, Gainesville, FL.

Testicular toxicity has been reported to exist in rodents following exposure to cadmium. This study was designed to determine if there is a modulating role for the testes in the hepatic and renal response to this heavy metal. Adult male rats were castrated, then treated with testosterone (1 mg/day) or vehicle. Twenty-five days after surgery, a single iv dose of cadmium chloride (0.5 or 1.0 mg Cd/kg body weight) was administered and the animals were killed 72 hr later. A sham-surgery control group was also included which did not receive testosterone but was treated with cadmium. In liver and kidney, castration had no effect on the concentrations of either cadmium or metallothionein. In serum, castration led to an exaggerated increase in both copper concentration and ceruloplasmin activity and a decrease in zinc concentration following the high cadmium dose. These responses were attenuated by testosterone. In a similar fashion, the high dose of cadmium caused a fall in serum alaesterase activity in castrated rats, which was not observed in the intact animal. It too was reversed by testosterone. Cholinesterase activity was decreased in serum by castration, yet appeared to be unaffected by cadmium. The decrease was partially reversed by testosterone. Thus, the testes do appear to play a modulating role in the response of the male rat to cadmium.
To determine if cadmium (Cd) exposure causes increased bone loss in ovariectomized mice, we labeled bones of female CFI mice with $^{45}$Ca. 4 w after labeling, mice were either ovariectomized (OV) to stimulate human menopause or sham-operated (SO). 2 w after OV, half the OV and half the SO mice were given dietary Cd at 50 ppm; remaining mice received no Cd. $^{45}$Ca excretion in urine and feces (measured as $^{45}$Ca release from bone) increased immediately after Cd, and the increase in OV mice (26±4%) was clearly greater than in SO mice (6±3%). To determine if added Cd increases resorption in bones in culture, we exposed $^{45}$Ca-prelabeled fetal rat limb bones (FRLB) to 0.01 or 1 μM Cd. 0.01 μM Cd (estimated level in serum of 50 ppm Cd mice) produced a 68±4% release of $^{45}$Ca from the FRLBs as compared to a 27±2% release in control bones. In contrast, Cd at 1 μM had no significant effect alone and inhibited parathyroid hormone-induced bone resorption. In smokers, blood Cd is 0.01-0.03 μM, in the range of levels in both our studies. Blood Cd in nonsmokers is 10-fold less (0.002 μM). Our results suggest that Cd in cigarette smoke may be a cause of the increased incidence of postmenopausal osteoporosis in women who smoke and that Cd may enhance bone mineral loss by a direct action on bone. Work supported by U.S. DOE under contract No. W-31-109-ENG-38.

EFFECT OF CADMIUM TREATMENT IN VIVO ON GLUCOSE PRODUCTION FROM HEPATOCYTES. R R Bell, J L Early, V K Nonvinakere, and Z Mallory. Florida A&M University, College of Pharmacy, Tallahassee, FL 32307. Sponsor: R C Schnell

Cadmium (Cd) is an environmental contaminant which produces toxic effects following long and short-term exposure. The objective of this study was to determine the effect of Cd on glucose production from hepatocytes after Cd treatment in vivo. Male Sprague Dawley derived rats (180-360g) received sodium acetate (1.23 mg/kg) or Cd (0.84 mg/kg) as cadmium acetate intraperitoneally at 30, 60, 90, 120, 150, and 180 min. prior to isolation of hepatocytes. Hepatocytes were isolated using a revised method of Berry and Friend (1966). The average viability of isolated hepatocytes determined by trypan blue exclusion test was 80%. Glucose production from hepatocytes incubated with or without sodium lactate (10 mM) was measured using a glucose analyzer. Cd pretreatment 180 min. prior to isolation of hepatocytes significantly increased glucose output from hepatocytes ($p < 0.05$) incubated with sodium lactate. Results from this study tend to indicate that Cd promotes gluconeogenesis at 180 min. post-treatment. (Supported by NIH RR08110/RCMI G12 RR03620-02).

EFFECT OF CADMIUM ON EPITHELIAL TRANSPORT SYSTEMS IN WINTER FLONDER: STUDIES WITH BRUSH BORDER MEMBRANE VESICLES. C Bevan, R KH Kinne, E Kinne-Saffran, and EC Poulkes. U. Cincinnati, Col. Med., Dept. Environ. Health, Cincinnati, OH; Max-Planck-Institut fur Systemphysiologie, Dortmund, FRG; and MOIBL, Salisbury Cove, ME.

Chronic exposure to Cd can result in impairment of amino acid reabsorption in the proximal tubule of the kidney. Using isolated brush border membrane vesicles from the kidney of the winter flounder, we have studied the effect of Cd on L-alanine transport. Pretreatment of vesicles with 0.1 μM Cd resulted in time-dependent inhibition of L-alanine transport in the presence of a NaCl (but not KCl) gradient. Inhibition was due to a direct interaction with the transport system and not a change in driving force for alanine transport, since Cd did not affect Na-D-glucose cotransport. Cd uptake itself is time- and temperature-dependent, and results in considerable binding to internal sites. Inhibition of alanine transport could not be correlated to binding of Cd at either side of the membrane. Furthermore, inhibition continued to increase even at time points when Cd uptake was at equilibrium. When EDTA was added to Cd-exposed vesicles, inhibition of alanine transport was not reversed. These results suggest that Cd inhibits Na-L-alanine cotransport by binding to the alanine transport system at buried sites in the membrane. (Supported by DFG grant KI 333/1-1 and NIHES grant 1 P30 ES03828)

THE EFFECT OF SODIUM ON THE ACCUMULATION OF CADMIUM BY SINUSOIDAL HEPATIC PLASMA MEMBRANE VESICLES. R R Eastman and L M Frazier. Johns Hopkins University, Baltimore, MD.

The effect of Na$^+$ on the transport of Cd$^{2+}$ across hepatic plasma membranes was investigated using plasma membrane vesicles. Sinusoidal hepatic plasma membrane vesicles were isolated from Male Wistar rats (175-200g) according to the method developed by Bartles et al. (Journal Cell Biology 100:1126-1138, 1985). Accumulation of Cd$^{2+}$ by the vesicles was measured using a rapid filtration method. For the uptake experiments, the sinusoidal plasma membrane vesicles were incubated in media containing 0.035μM Cd$^{109}$ (1.25mCi/μg), 0.25M sucrose, 100mM KCl, 0.5mM MgCl$$_2$$, 5mM Tris-HCl (pH7.4) for 20 min at 37°C. To determine the effect of Na$^+$ on Cd$^{2+}$ accumulation by the vesicles, KCl in the incubation media was replaced by NaCl in a stepwise manner. It was found that as Na$^+$ replaced K$^+$ in the incubation media, the total accumulation of Cd$^{2+}$ by the vesicles increased. When K$^+$ was completely replaced by Na$^+$, Cd$^{2+}$ accumulation increased 2.2 fold. Both nonspecific and specific uptake of Cd$^{2+}$ by the vesicles was increased in the presence of Na$^+$. 
We have shown that CdCl₂ causes MT disassembly in cultured cells. Inhibition of MT assembly in vitro by Ca²⁺ is enhanced by calmodulin (CaM), when microtubule-associated proteins (MAPs) are present. Cd²⁺ has been shown to inhibit MT assembly in vitro by binding to sulphydryl groups in tubulin. However, Cd²⁺ also binds to the Ca²⁺-binding domains of CaM as effectively as Ca²⁺, apparently because the ionic radius of Cd²⁺ (0.97 nm) is close to that of Ca²⁺ (1.17 nm). To determine the inhibitory effect of Cd²⁺ on the assembly of bovine brain MT protein, containing tubulin and MAPs, in the presence and absence of CaM. MT assembly was monitored by measuring the increase in turbidity at 350 nm following the addition of GTP. MT assembly was inhibited by micromolar concentrations of Ca²⁺ or Cd²⁺ alone. As expected, CaM enhanced the inhibitory effect of Ca²⁺. Similarly, the inhibitory effect of Cd²⁺ was enhanced by CaM. Compound 48/80, a potent CaM inhibitor, reversed the CaM enhancement of Cd²⁺-induced inhibition of MT assembly. These results demonstrate that when CaM is available, Cd²⁺ can inhibit MT assembly by binding to and activating CaM, and suggest that tubulin sulphydryl groups may be a primary target of Cd²⁺ binding in vivo.

Cadmium-induced alterations in pulmonary antioxidant enzymes and metal levels. P K Bennett and J S Jamall. Toxicology Program, St. John's University, NY.

Weanling, Sprague-Dawley rats were fed a diet containing 0.5 ppm selenium (Se) with either 0, 10 ppm, or 50 ppm copper (Cu) for 7 weeks. There were two sets of Cd-treated rats. One set (Cd-OP) received 5 mg Cd via osmotic minipumps (Alzet 2002). The second set (Cd-D) received 50 ppm Cd admixed with their feed. Catalase (CAT) activity was increased by 23% and 32% in the rats treated with dietary Cd and fed the diets supplemented with 10 ppm and 50 ppm Cu, respectively. Cd-OP treated rats exhibited a 27% reduction in CAT only in the 10 ppm Cu-supplemented group. Superoxide dismutase (SOD) activity was increased by increasing dietary Cu but was unaffected by either Cd treatment. Glutathione peroxidase (GSH-Px) was also unaffected by the Cd treatments. Tissue peroxidation, measured by thiobarbituric acid reactive substances, was also unaffected by Cd. Increasing dietary Cu resulted in marked reductions in Cd levels in Cd-OP treated rats but increased Cd accumulation in rats treated with dietary Cd. Dietary Cd treatment resulted in up to 48% reductions in lung Se compared to untreated controls. In contrast, Cd-OP exhibited increased Se levels in their lungs. This effect was most pronounced (up to 47%) in rats fed the diet supplemented with 0 Cu. Thus, the effects of dietary and parenteral Cd treatments elicit very different responses in the rat lung.


Oxygen radical generation by activated alveolar macrophages (AM) and other inflammatory cells may be a mechanism of damage to the lung following cadmium chloride exposure. To investigate this possibility, Fisher 344 rats were intra-tracheally instilled with 10 μg Cd as CdCl₂. Numbers of lavaged neutrophils peaked 1 to 2 days later, while numbers of lavaged macrophages and mononuclear cells peaked after 3 to 4 days. Despite the increased potential for superoxide (SO) production in neutrophils as compared to macrophages, SO production in both resting and phorbol ester (PMA)-stimulated lavaged cells of Cd exposed rats was significantly depressed. This depression was noted as early as 1 hr after Cd exposure and was most pronounced 1 to 2 days later. SO production did not reach control levels even after 5 days. Since Cd may have induced free radical production, AMs from control unstirred rats were exposed to 0, 1, 10, or 100 μM Cd with the SO detection system. PMA-stimulated SO production was unaffected, but resting production showed a dose-dependent depression to 36% of control levels with 100 μM Cd. The results indicate that SO production is a mechanism of damage to the lung following acute (CdCl₂) exposure. Instead, the results further show the inhibition of oxidative metabolism by ionic Cd.


There is a continuing search for better chelating agents for the mobilization and excretion of cadmium. N-(2,3-dimercaptopropyl)phosphonic acid (DMPA), DMSA and DMSA are water soluble analogs of BAL and are chelators for As, Hg or Pb poisoning. The 10 day urinary excretion of 109-cadmium is increased four fold and the fecal excretion two fold when a twice daily sc injection of 0.20 nmol DMPO/kg is given to young male rats for 10 days beginning three days after the administration of 109-cadmium (1 mg/kg). The biliary excretion of Cd increases 20 fold when rats receive 0.20 nmol DMPO iv three days after the administration of cadmium-109 (1 mg/kg). DMPS, DMSA, EDTA, DDC or GSH did not increase biliary excretion of 109-Cd. DMPS also increases the biliary excretion of Cd when given iv 10 min after the iv administration of 109-Cd. The increase in biliary Cd is not due to an increase in the rate of bile flow since the latter increases only 15%. Since DMPS increases biliary GSH, it would appear that DMPS has more than one mode of action as far as its antitodal properties and differs in this respect from DMSA. (Supported in part by ES03356 and OH02185)

Previous studies from this laboratory have indicated that while CdCl₂ affects Na⁺-pump by binding to ligands that are protected ATP, CH₃HgCl binds to some non-specific aiiosteric sites. In the present study the relative accessibility of CdCl₂ and CH₃HgCl to the vital SH groups which are sensitive to NEM and DTNB were determined. The reaction of DTNB with Na⁺-K⁺-ATPase was prevented by previous blocking of SH groups with CdCl₂. DTNB failed to reverse NEM inhibition of CdCl₂ inhibited enzyme. Although DTNB can protect the enzyme against CH₃HgCl as well as DTNB inhibition, the protection by ATP was evident only against DTNB but not against CH₃HgCl suggesting that CH₃HgCl reacts with an additional vital SH group than DTNB. Further, protection by DTNB was evident only against CH₃HgCl but not against NEM inhibited enzyme. However, ATP can partially protect against NEM but not against CH₃HgCl inhibited enzyme. All effects of CH₃HgCl and CdCl₂ on DTNB and NEM inhibited enzyme suggest that while CdCl₂ binds to vital SH groups reacting with both DTNB and NEM, CH₃HgCl appears to bind to some additional vital SH groups apart from those sensitive to DTNB and NEM.

EFFECTS OF SUBCHRONIC EXPOSURE TO ARSINE ON IMMUNE FUNCTION AND HISTOARCHITECTURE. G J Rosenthal, M M Fort, D R Germolec, M F Ackermann, P Blair, K R Lamm, and M R Luster. NIEHS, NIH, Research Triangle Park, NC.

Arsine is a potent hemolytic agent primarily used in the semiconductor industry. We examined the effects of arsine inhalation on immunological parameters in female B6C3F1 mice. Animals exposed for 14 days to 0.5, 2.5 or 5.0 ppm arsine demonstrated a dose dependent decrease in natural killer cell and cytotoxic T lymphocyte function. Macrophage function was not affected at any concentration as measured by tumor cell cytostasis nor were numbers of peritoneal exudate cells recovered following arsine inhalation. Arsine induced marked changes in splenic cellular populations. Lymphocyte percentages fell significantly in all arsine exposed groups from 83.4% in air controls to 45.6%, 54.3% and 73.6% in 5, 2.5, and 0.5 ppm arsine, respectively. Expansion of the splenic erythrocyte population indicated that arsine induced splenic erythropoiesis. Splenic T cell percentages were significantly depressed at all concentrations of arsine while B cells were depressed only at the highest concentration. On a per spleen cell basis, arsine resulted in an impaired ability to respond to suboptimal concentrations of Con A while B cell proliferation in response to LPS remained unaltered. However, on a per lymphocyte basis, the lymphoproliferative data is strongly suggestive of lymphocyte hyperreactivity. Host resistance to challenge with influenza, PYB6 tumor cells, and B16F10 melanoma was unaffected by arsine. However, increased susceptibility to L monocytes and P. yoelli was observed. Taken together, these data show that arsine inhalation can have marked effects on the murine immune system and these effects implicate the T cell as a sensitive target.

EVIDENCE FOR OXIDATIVE DAMAGE TO ERYTHROCYTES IN RATS AND MICE INDUCED BY ARSINE GAS. P C Blair, M Bechtold, M B Thompson, C R Moorman, M P Moorman and B A Fowler. NIEHS, Research Triangle Park, NC.

Male and female B6C3F1 mice and F344 rats, 6 weeks of age, were exposed to arsine gas (6 hours/day, 5 days/week) at concentrations of 0.000, 0.025, 0.500, and 2.500 ppm for 90 days. During the study, whole blood samples were collected at 5, 15, and 90 days. Complete hematological profiles (which included methemoglobin concentrations only at mice at 90 days) were performed at each time point. Decreased erythrocyte counts, hemoglobin concentration and methemoglobin concentrations and increased mean cell corpuscular volumes, erythrocyte indices and reticulocyte counts occurred in animals exposed to 2.500 ppm at all time points and, additionally, in those exposed to 0.500 ppm after 90 days of exposure. Increased concentrations of methemoglobin occurred in male and female mice exposed to 2.500 ppm for 90 days. Examination of blood films revealed marked polychromasia, poikilocytosis and anisocytosis accompanied by a presence of Heinz bodies. Treatment with arsine gas produced a regenerative anemia secondary to hemolysis. Glutathione levels in rat and mouse erythrocytes were reduced markedly after in vivo exposure to arsine gas for 1 hour. Data from in vivo and in vitro studies are consistent with a mechanism of oxidation of the heme iron accompanied by a denaturation of hemoglobin.
ARSEINE (AsH₃) AND GALLIUM ARSENIDE (GaAs)-INDUCED ALTERATIONS IN HEME METABOLISM. W E Bakewell, P L Goering, M P Moorman, and R A Fowler, NIEHS, Research Triangle Park, NC

Arsine gas (AsH₃) is a potent hemolytic agent which is widely used in the manufacture of GaAs semiconductors. Prolonged exposure to AsH₃ results in hemolysis with development of a regenerative anemia. GaAs has also been shown to produce alterations in heme biosynthesis and development of porphyria. These studies examined AsH₃ and GaAs-induced alterations in reduced cell 5-aminolevulinic acid dehydratase (ALAD) in relation to urinary excretion of ALA and 6-porphyrin isomers. Acute intratracheal instillation of GaAs (30-200mg/kg) produced a marked inhibition of ALAD activity with an associated increase in urinary ALA. Increased urinary excretion of the 7 & 8 COOH uroporphyrins and lesser amounts of the 4-COOH coproporphyrin was also observed. In contrast, exposure of rats to AsH₃ (0.5-5.0) ppm for weeks produced an increase-related increase in the activity of ALAD and a modest depression of urinary ALA. A marked increase in the urinary excretion of the 7 & 8 COOH uroporphyrins but not the 4-COOH coproporphyrin was observed. These data suggest that AsH₃ and GaAs produce highly specific alterations in the heme biosynthetic pathway which may lead to the development of biochemical "fingerprints" for early detection of ongoing biological activity associated with exposure to these agents alone or in mixtures.

DEVELOPMENT OF AN IN VITRO SCREEN FOR ARSENIC ANTIDOTES USING PYRUVATE DEHYDROGENASE COMPLEX ENZYME ACTIVITY. D Hобсон, T H Snider, M J Chang, and R L Joiner. Battelle Columbus Division, Columbus, OH. Sponsor: CT Olson.

An in vitro assay to screen candidate antidotes to systemic arsenic poisoning was developed using a commercially-available preparation of pyruvate dehydrogenase complex (PDHC). Inhibition of PDHC activity was determined for three As(III) compounds: sodium arsenite, lewisite (L), and chlorovinylarsenous acid (CVA). L and CVA were, respectively, 25 to 50 times more potent inhibitors of PDHC activity than sodium arsenite. The effectiveness of three candidate antidotal compounds, 2,3-dimercaptopropanol (BAL), 2,3-dimercapto-1-propane-sulfonic acid (DMPS), and meso-2,3-dimercaptosuccinic acid (DMSA), was tested against each PDHC inhibitor. The molar ratios (MM reactivator/MM inhibitor) of BAL, DMPS, or DMSA required for complete reactivation of PDHC activity from 90 percent inhibition by sodium arsenite were 1.82, 2.00, and 2.78 respectively. The respective millimolar ratios of BAL, DMPS, or DMSA required for complete reactivation of PDHC from 50 percent inhibition by L were 1.99, 2.13, and 3.20, and 2.42, 3.18, and 7.25 from 90 percent inhibition by CVA. The relative ranking of PDHC reactivator effectiveness against the three inhibitors tested using the in vitro assay was BAL > DMPS > DMSA.

EFFECT OF ARSENIC ON CARBOHYDRATE METABOLISM AFTER SINGLE OR REPEATED INJECTION IN GUINEA PIGS. F X Reichl, L Slinikac, H Kreppel, B Fichtl, and W Forth. Walther-Staub-Institute for Pharmacology and Toxicology, Munchen, FRG. Sponsor: D A Cory-Slechta

Further experiments were performed to investigate whether the metabolic effects of arsenic after repeated administration may differ from those in acute poisoning (cf. Arch.Pharmacol. 335: R17, 1967). Male guinea pigs were sacrificed after a single dose of As₂O₃ (10 mg/kg s.c.) or after repeated treatment (2.5 mg/kg b.i.d. for 5 days). The liver was removed using a freeze stop technique and the content of glycogen and several glycose intermediates was measured. As compared with untreated controls, both dosing regimens resulted in a decrease of total carbohydrate content which was due mainly to a depletion of glycogen (single dose -24%; repeated -81%). There were, however, distinct differences in the pattern of metabolic changes, e.g., an increase of pyruvate after the single dose but a decrease after repeated dosing of As₂O₃. It is concluded that the metabolic consequences of arsenic poisoning are dependent on the duration of exposure suggesting a possible different mechanism of toxicity in acute and chronic poisoning.

COMPARATIVE TOXICITY AND TISSUE DISTRIBUTION OF ANTIMONY POTASSIUM TARTRATE IN RATS AND MICE DOSED BY DRINKING WATER (DW) OR INTRAVENTRICAL INJECTION (IP). K.P. Dieter, C.V. Jameson (NIH, NIEHS, National Toxicology Program, RTP, NC); J.V. Lodge (Research Triangle Institute, RTP, NC): H. Heitmannck, S.L. Grumbel, A.C. Peters (Battelle Columbus Division, Columbus, OH)

Antimony potassium tartrate (APT) is i.v. to treat schistosomiasis, and was tested by NTP because of its possible association with bladder tumors. Both sexes of F344 rats and B6C3F1 mice were given APT by two different routes: DW doses in rats were 0, 16, 28, 59, 94, and 168 mg/kg and in mice were 0, 50, 98, 174, 273 and 407 mg/kg; IP doses in rats were 0, 1.5, 3, 6, 11 and 22 mg/kg and in mice were 0, 6, 13, 25, 50 and 100 mg/kg. APT was much more toxic by the IP route, causing mortality and histopathological lesions at doses about one order of magnitude below those used by the DW route. In DW studies only one animal died; in IP studies 3 rats and 29 mice died. In DW studies there were no toxic responses in rats; there were body and organ weight decreases, liver and mesenteric lesions in mice. APT given IP in rats and mice caused liver, kidney, and mesenteric lesions. Antimony accumulated, blood, liver, spleen, heart, and kidney of rats given APT in DW (94 mg/kg) or by IP (11 mg/kg), at equivalent levels of 15, 6, 4, 3, and 2 mg/gm. In mice there was no antimony in blood, heart, and kidney; there was 24 mg/gm in liver after 273 mg/kg (DW) or 50 mg/kg (IP), and 5 mg/gm in spleen of mice given the IP dose.
ROLE OF HEPATIC GSH AND RENAL T-GTP IN RENAL UPTAKE OF METHYLMERCURY AND INORGANIC MERCURY IN MOUSE. A. Naganuma, T Tanaka and N. Iimura, Dept. of Public Health, Sch. of Pharmaceutical Sciences, Kitasato University, Minato-ku Tokyo, Japan

The role of hepatic glutathione (GSH) and renal T-glutamyltransferase (T-GTP) in renal uptake of mercurials by using a specific depletor for hepatic GSH, 1,2-dichloro-4-nitrobenzene (DCNB) and T-GTP inhibitor, acivicin. DCNB pretreatment reduced the renal uptake of both methyl and inorganic mercuric Hg and prevented the renal toxicity of inorganic Hg. Furthermore, renal mercury uptake was increased by intravenous coadministration of the mercurials with GSH. These results suggest that the hepatic GSH or GSH released from the liver into the plasma plays a key role in the renal accumulation of mercury compounds. Inhibition of renal T-GTP by acivicin pretreatment reduced renal Hg uptake and increased urinary excretion of Hg and GSH, consequently ameliorating the renal and lethal toxicity of inorganic Hg. These facts indicate that methyl and inorganic mercury are transported to the kidney as Hg-GSH complex and Hg in the complex is incorporated into the kidney by a T-GTP dependent system.

82 BRAIN HALFLIFE OF METHYLMERCURY IN THE MONKEY IS LONGER THAN BLOOD HALFLIFE. D C Rice, Health Protection Branch, Ottawa, Ontario, CANADA.

Estimated halflives of mercury following methylmercury exposure in humans are about 75 days for whole body and about 60 (40-160) days for blood. In its most recent review the World Health Organization (1980) concluded that there was no evidence to suggest that brain halflife differed from whole body halflife. In the present study, female monkeys (M. fascicularis) were dosed for at least 1.5 years with 25 or 50 μg/kg/day of mercury as methylmercuric chloride. Dosing was discontinued, and blood halflife was determined to be approximately 15 days. Approximately 230 days after cessation of dosing, monkeys were sacrificed and regional brain total mercury levels determined. One monkey that died while still being dosed had brain mercury levels three times higher than levels in blood. Theoretical calculations were performed assuming steady state brain:blood ratios of 3, 5 or 10. Brain mercury levels were three orders of magnitude higher than those predicted assuming the halflife in brain to be the same as that in blood. Estimated halflives in brain were between 58 (brain:blood ratio of 3) and 40 (brain:blood ratio of 10) days. In addition, there was a dose-dependent difference in halflives for some brain regions. These data clearly indicate that brain halflife is considerably longer than blood halflife in the monkey under conditions of chronic dosing.

THE EFFECTS OF KETAMINE-XYLAZINE ANESTHESIA ON HEPATIC SULFHYDRYL (SH) DISPOSITION AND BILIARY EXCRETION OF CH3Hg. C A White and C D Kluesenberg, Univ. of Kansas Med. Ctr, Kansas City, KS.

Many metals, including CH3Hg, are excreted into bile as SH (glutathione and its degradation products) complexes. These compounds which affect SH disposition may alter the biliary clearance of metals. We have shown previously that ketaminexylazine (KX, 100:10 mg/kg) anesthesia increases biliary excretion of SHs 2-4 fold when compared to urethane-anesthetized (1.5 g/kg ip) rats. The purpose of the present study was to determine the influence of KX-induced anesthesia on the biliary excretion of CH3Hg and total hepatic SH disposition by quantitating SH composition in the blood entering the liver, venous blood exiting the liver as well as in liver and bile. SHs were separated by reverse-phase HPLC and quantitated using electrochemical detection, while 209Hg(CH3Hg (10 μmol/kg, iv) was measured using gamma scintillation spectrometry. KX increased biliary SH excretion 2.1-fold compared to urethane-anesthetized rats. Although the biliary concentrations of CH3Hg were similar after administration of either anesthetic, KX increased both bile flow (78%) and the cumulative amount (112%) of CH3Hg excreted into bile. While the biliary excretion of SHs was higher in KX-treated rats, the concentration of SHs in liver and the blood entering and leaving the liver were equivalent between the two groups. In conclusion, KX increases the efflux of SHs into bile but not from liver into blood. It appears that the increase in biliary excretion of CH3Hg produced by KX is largely due to the increase in biliary SH excretion. (Supported by USPHS Grants ES-01142, ES-03192 and ES-07079)
When mice are exposed to a single dose of $^{203}$Hg as methylmercury, grain counts of autoradiographs reveal an uneven pattern of distribution in the kidney, but all regions contain significant levels of Hg. In contrast, the inorganic Hg formed over time after MeHg exposure and visualized by silver staining is found exclusively in the cells of the proximal tubules (Rodier and Kates, 1967; Rodier, Kates, and Simons, 1967). The distribution of Hg after a single dose of HgCl$_2$ was investigated by the same methods in the present study. Silver stain again demonstrated Hg only in the apical portions of the cells of proximal tubules and on the luminal surface of the brush border. As in MeHg-exposed kidney, there was evidence of active movement of Hg into kidney cells by pinocytosis and into the lumen by sloughing of cytoplasm. Although the resolution of autoradiography was not adequate to describe the intracellular placement of Hg, it was possible to confirm the exclusive localization of Hg in the proximal tubules. Thus, the kidney seems to respond similarly to inorganic Hg whether its source is exogenous or endogenous. (ES 01247, 01248 and 04428).

While the degenerative processes comprising aging have been extensively described, little research has been directed towards understanding whether such processes may be accelerated by exposure to toxicants like lead (Pb), an environmental contaminant to which exposure occurs over the lifespan. Using a combined cross-sectional/longitudinal study design, groups of young (21 day old), adult (8 mo) and old (16 mo) male Fischer-344 rats were exposed to 0, 250 or 500 ppm Pb acetate in drinking water for 7 mo. Blood Pb (PbB), hematocrit, ZPP and urinary D-ALA measurements were made after 3 & 7 months of exposure; tissue Pb concentrations, organ weights and urinary Pb (PbU), Ca, Fe, Zn and Cu excretion after 7 mo. Results suggested that aged animals were more vulnerable to Pb, and that Pb exposure may accelerate aging: elevations of D-ALA and ZPP were greater, morbidity and mortality were highest, and liver weights and hematocrit declined more rapidly in old-Pb rats. Complex changes in tissue distribution with age were seen. Most notably, a marked reduction in bone and brain Pb levels occurred while other soft tissue Pb levels increased. Interactions of age by dose of Pb were noted in both PbB and PbU. Patterns of lead toxicity may reflect stage of life cycle.

Degenerative changes in kidney function over the life span have been well documented. The kidney is also a well-known target organ for lead, an environmental toxicant to which exposure occurs over the life span. The extent to which age-related renal impairments may be exacerbated by chronic lead exposure was the subject of this study. Using a combined cross-sectional/longitudinal study design, groups of young (21 days), adult (8 mo) and old (16 mo) male rats were exposed to 0, 250 or 500 ppm Pb acetate in drinking water for 7 months and maintained at a body weight of about 230 gm. Kidney function was evaluated after 3 and 7 months of exposure. Characteristic age-related changes in kidney function were observed, including a decline in glomerular filtration rate and urine concentrating ability, albuminuria and enzynuria. Pb modified the progression of age-related changes in certain parameters of kidney function, although no evidence of overt Pb nephropathy was found. Specifically, fractional excretion of alpha 2-microglobulin was increased in old Pb-treated animals. These changes could not be explained by alterations in filtered load, suggesting combined effects of lead and aging on renal handling of glucose and amino acids.

While the degenerative processes comprising aging have been extensively described, little research has been directed towards understanding whether such processes may be accelerated by exposure to toxicants like lead (Pb), an environmental contaminant to which exposure occurs over the lifespan. Using a combined cross-sectional/longitudinal study design, groups of young (21 day old), adult (8 mo) and old (16 mo) male Fischer-344 rats were exposed to 0, 250 or 500 ppm Pb acetate in drinking water for 7 mo. Blood Pb (PbB), hematocrit, ZPP and urinary D-ALA measurements were made after 3 & 7 months of exposure; tissue Pb concentrations, organ weights and urinary Pb (PbU), Ca, Fe, Zn and Cu excretion after 7 mo. Results suggested that aged animals were more vulnerable to Pb, and that Pb exposure may accelerate aging: elevations of D-ALA and ZPP were greater, morbidity and mortality were highest, and liver weights and hematocrit declined more rapidly in old-Pb rats. Complex changes in tissue distribution with age were seen. Most notably, a marked reduction in bone and brain Pb levels occurred while other soft tissue Pb levels increased. Interactions of age by dose of Pb were noted in both PbB and PbU. Patterns of lead toxicity may reflect stage of life cycle.

Few controlled animal studies have been performed to investigate how body burden of lead influences blood lead levels (Pb-B) upon removal from chronic exposure. The specific aim of this project was to assess the effect of exposure duration and animal age during exposure on post-exposure Pb-B. Two groups of weanling male Sprague Dawley rats were exposed to 1000 mg Pb/l drinking water for 31 and 60 days. Post-exposure Pb-Bs were compared to those in a third and a fourth group of male Long Evans rats exposed to 5500 mg Pb/l drinking water for 56 days beginning at 406 days of age and 440 days beginning at weaning. Post-exposure Pb-B curves were fit to two- and three-compartment mammillary models. The three-compartment model was required to fit data for rats beginning exposure at weaning. The two-compartment model adequately fit data for rats beginning exposure in adult life. Estimates of terminal half-lives from the sum-of-exponential compartmental analysis for rats beginning exposure at weaning were unsatisfactory because no linear decay constant was identifiable for the terminal compartment. A power function was necessary to fit the terminal Pb-B data from 1000 mg Pb/l exposure groups. The power function has important implications for developing a physiologically-based pharmacokinetic model for lead.
The influence of vitamin B6 status on lead (Pb) intoxication was investigated in a 2x2 factorial experiment. The role of B6 as a cofactor for several enzymes of the trans-sulfuration (TS) pathway is important in the conversion of dietary methionine to cysteine. Since cysteine is rate-limiting in glutathione (GSH) formation, Pb-induced changes in GSH metabolism may be affected by B6 status.

Male weaning Sprague-Dawley rats were maintained for 5 weeks on either a B6-deficient or -adequate diet (AIN-76A purified rat diet, Research Diets, New Brunswick, NJ) supplemented with 0 or 2000 ppm Pb as Pb acetate (H2O). Both Pb and B6 deficiency caused significant (p<.05) reductions in body weight gain, but no Pb x B6 interaction was evident. Hepatic cysteine concentration and renal γ-glutamyl transpeptidase activity were not significantly affected by either Pb or B6 deficiency. In the liver, Pb had a significant effect on GSH content and glutathione reductase (GSSG-R) activity showed significant influences due to both Pb and B6 deficiency with a significant Pb x B6 interaction. The data indicate that Pb-induced changes in certain aspects of GSH metabolism may be influenced by B6 status but do not clearly show it is due entirely to the role of B6 in cysteine metabolism.

Lead (Pb) has been shown to interfere with cellular calcium homeostasis, altering sizes and flux rates of cellular pools of exchangeable calcium and perturbing calcium-mediated cell processes. The present study examined directly the effect of Pb on the intracellular free calcium ion concentration [Ca2+]i, in rat ROS 17/2.8 cells. ROS 17/2.8 bone cells were attached to collagen-coated microcarriers (Cytodex 3) and loaded with the fluorescent calcium ion indicator, 5',5'-di-fluoro-1,2-bis(o-aminophenox)-ethane N.N',N'-tetra acetic acid (5F-BAPTA). The 19F NMR was observed using a Varian VXR-500 NMR spectrometer operating at a magnetic field of 11.7 Tesla. ROS 17/2.8 cells were maintained in a 10 mm NMR tube at 30°C by superfusion at 2 ml/min with F-12 medium saturated with 95% O2:5% CO2. Treatments were added to the superfusion medium. The basal [Ca2+]i was estimated to be 175±17 nM. Treatment with parathyroid hormone (400 ng/ml) produced a 35% increase in [Ca2+]i. Treatment with Pb (5-50 μM) produced a 25 to 100% increase in [Ca2+]i as well as observable intracellular Pb2+. These observations are the first direct demonstration of intracellular Pb2+ and Pb-induced increases in intracellular free Ca2+.

Reduced neonatal body weight associated with maternal lead exposure has been reported in humans. Skeletal ossification site counts and other morphometric indices of skeletal development are correlated with body weight in young experimental animals and neonatal humans. It was hypothesized that lead exposure will result in retarded skeletal development and reduced body weight in rats. Female Sprague-Dawley weaning rats received 0, 50, 250, 500 or 1000 ppm lead in drinking water for 56 days. Food consumption, water intake, tail length and body weight were measured every 2 to 3 days. The results indicated that lead exposure is associated with decreased tail length growth rate in the 1000 ppm lead treatment group (p=0.0009), and reduced body weight in the 500 and 1000 ppm lead treatment groups (p=0.0436 and p=0.0001, respectively). Food intake rates in the lead treatment groups were not reduced when compared to the food intake rate in the control group. Water consumption was reduced in the 250, 500 and 1000 ppm lead treatment groups relative to the control group water consumption. Studies are underway to examine the effect of maternal lead exposure on fetal and neonatal offspring skeletal ossification. (Project supported by NIOSH 1R01-0032376-01).

Previous work has demonstrated an effect of dietary Pb on several precursors of leukotriene (LT) production including increased hepatic glutathione peroxidase activity and increased triacylglycerol acid (AA 20:4)/linoleate (18:2) ratio. We investigated the possible role of LT's as mediators of Pb toxicity. Chicks were reared with diets supplying varying levels of linoleic acid (~55% of total fatty acids in cottonseed oil) in two 3-week trials, adequate fat (2%) vs. 4% or 8% fat and 0 vs. 2000 ppm Pb as Pb acetate were fed in a 3 x 2 factorial arrangement. The decreased growth rate associated with 2000 ppm Pb was not moderated by fat intake. Serum and liver were analyzed for LT by radio-immuno assay. Average concentration of all treatments was 2.42 ng/ml in serum and 1.59 ng/g in liver. Neither Pb nor fat affected tissue LT concentration due to high variation within treatments. Pb significantly increased hepatic glutathione concentration and 20:4/18:2 ratio in hepatic fatty acids. Despite these increases in precursor concentrations. Pb did not show an effect on LT concentration. Although the results do not support a Pb effect on LT synthesis, such an effect could be masked by increased LT concentrations in other tissues or by increased LT turnover.

INDUCTION AND ACTIVATION OF RAT RENAL EPONIDE HYDROLASE BY LEAD. E Gralchen, B Conway, K Phipps, T. Leonard, Smith Kline & French Laboratories, Swedeland, PA

Treatment of rats with salts of metals such as Hg, Ni, and Pb causes dose-related increases in renal microsomal epoxide hydrolase (EH) activity; however, the increases observed were 2 to 8-fold greater in Sprague-Dawley (SD) rats than in Fischer 344 (F) rats. To further examine the basis of the differential EH response to metals, the enzyme kinetics were studied in kidney microsomes from control (C) rats, or rats treated with 3 daily doses of 300 μmol/kg lead acetate (Pb). Using benzo(a)pyrene-4,5-oxide substrate, sigmoidal velocity curves were observed. When fit to the Hill equation, V/Vmax=[S]n/(K'+[S])n. Vmax was found to be 0.86, 2.5, 1.0, and 20.5 nmol/mg pro/min; K' was 0.23, 0.36, 0.87, and 9.0 μM; and the apparent n was 2.3, 2.2, 2.1 and 1.5. In F C, F Pb, SD C, and SD Pb groups respectively. The increases in Vmax in microsomes from Pb-treated animals suggests that EH is induced in both strains; however, the differences in K' and n between C and Pb SD groups suggest that in SD rats renal EH is also altered by Pb treatment. Purified renal EH preparations had specific activities of 24 (SD C) and 96 (SD Pb) nmol/mg pro/min, consistent with activation of the enzyme. These data suggest that renal EH is both induced and activated by Pb treatment of SD rats.


Two experiments were conducted in which varying levels of lead (up to 2000 ppm as Pb acetate 3H2O) and selenium (up to 40 ppm as Na2SeO3) were fed, either alone or in combination, to chicks from 0 through 18 or 20 days of age. Pb additions depressed growth in a dose-dependent manner without affecting mortality. Se addition at 20 ppm was severely growth inhibitory, also without affecting mortality. The growth inhibition by 20 ppm Se was partially alleviated by feeding it in combination with 2000 ppm Pb, however mortality was increased significantly by the combination. In contrast, 40 ppm Se resulted in almost complete cessation of growth and 85% mortality, while the combination with 2000 ppm Pb partially overcame the growth inhibition and eliminated the excess mortality. When Pb or Se were fed alone, hepatic levels of the fed element were elevated. There were further significant elevations when both elements were fed in combination at identical dietary concentrations as the single element additions. The results suggest that Pb and Se are antagonistic. The nature of the antagonism is such that while Pb partially overcomes growth inhibition by Se, the reverse is not observed.

EFFECT OF LEAD TOXICITY ON INTRACELLULAR CALCIUM HOMEOSTASIS. J.G. Pounds. Brookhaven National Laboratory, Upton, NY.

Cellular Ca++-mediated cell functions are implicated as important targets of lead toxicity in a wide variety of cells, tissues, and organs. Alteration of the temporal and spatial regulation of cytoplasmic free Ca++ levels by lead is the critical event in Pb+-Ca++ interactions. Although many studies report alteration of cellular calcium homeostasis, and thus implicated perturbation of [Ca++]i, this effect has not been verified by direct measurement of [Ca++]i. Experiments were conducted using ion-selective electrode techniques to evaluate the effect of lead on the "set point" for [Ca++]i homeostasis with mitochondria, microsomes, and permeabilized cells. Hepatocytes were isolated from Sprague-Dawley rats, placed in to primary culture, and treated with 0,10,50, or 100 μM lead acetate, and mitochondria, microsomes, and permeabilized cells prepared after 20 hr exposure to lead. Previous studies have shown that at 20 hr, the cells are in steady state with respect to Pb++ and perturbations in cellular 45Ca are observed. The rate of Ca++ clearance, and the steady state level was altered in the mitochondrial and permeabilized cell preparations. These studies indicate that lead toxicity alters the dynamic regulation of Ca++ rather than altering the steady state levels, thus explaining in part, the sensitivity of Ca++-mediated process to lead toxicity. (Supported by NIH ES04040).
Milk can be a significant source of Pb for young mammals, including humans. Certain essential trace elements have previously been shown to be associated with particular milk components, thus increasing bioavailability. Our first goal was to determine the distribution on Pb in cream, casein and whey fractions using $^{209}$Pb as a tracer. In rat milk almost 90% of the Pb was associated with the casein micelles, regardless of: a) whether the milk was labeled in vivo or in vitro; b) whether the milk was fresh or frozen; and c) the added concentration of Pb (up to 75 µg/ml). A virtually identical pattern of Pb distribution was observed with bovine milk. Our second goal was to determine whether Pb remained associated with casein as it traversed the gastrointestinal tract of infant rats. For this purpose, rat pups were gavaged with $^{209}$Pb-labeled rat milk and sacrificed 2 hours later. Differential centrifugation of the homogenized luminal contents showed that in the stomach the Pb was associated primarily with the casein curd. By the time chyme reached the distal small intestine, Pb was predominantly in a fraction that was not precipitable by high-speed centrifugation (thus, not intact casein micelles), but was nondialyzable. We conclude that Pb in milk is protein bound and remains this way as it traverses the stomach and proximal small intestine of the infant rat.

Blood lead level and ε-aminolevulinic acid dehydratase activity were measured in 60 subjects of different occupations. The data obtained were analyzed to evaluate occupation, age and length of occupational exposure. Results demonstrated that blood lead level of lead smelters was 49.68 ± 8.56 µg/dl and that of the battery manufacture workers was 73.04 ± 15.84 µg/dl. The blood lead levels of workers at gasoline stations and the gasoline-mixing company were 27.74 ± 4.57 and 17.17 ± 3.27 µg/dl, respectively. Blood lead level of what was considered to be the control group was 30.51 ± 10.49 µg/dl. Generally, the obtained data demonstrated that ε-aminolevulinic acid dehydratase could be used as a measure of lead occupational exposure. Data also indicated that increasing age and length of occupational exposure lead to high blood lead levels.

The current method to determine the need for chelation in Pb toxic children (blood Pb: 25-55 µg/dl; erythrocyte protoporphyrin >35 µg/dl) is based on the result of the CaNa2EDTA provocative test (PbP). The PbP requires an injection and a quantitative 8-hour urine collection to achieve in young children. In this study, a low energy x-ray generator with a silver anode was used to measure Pb L$_{α}$ XRF from the tibia of 41 untreated Pb-toxic children. With the leg immobilized, partially polarized photons were directed at the anteromedial skin surface of the mid-tibia. Based on spectra from a leg phantom and from seven surgically amputated adult legs, the nominal detection limit was 2 µg Pb/gm of bone, when the skin surface dose reached 1.0 rad. Sixteen minute XRF measurements were compared with PbP results performed one week later. When the XRF was <2 µg Pb/gm of bone, 21 of 25 Pb-toxic children had a negative PbP. When the nominal XRF measurement was >2 µg Pb/gm of bone, this was predictive of the need for chelation in 14 of the 16 XRF-positive Pb-toxic children. These results indicate that L-XRF measurements of cortical bone Pb will be useful to rapidly discover Pb toxicity in large populations of children.

Elevated concentrations of lead (Pb) in drinking water are associated with the use of Pb pipes and Pb solder for interior plumbing and with soft water supplies. The greatest exposure to Pb in drinking water occurs during consumption of "first-draw" tap water that has been in contact with Pb plumbing for more than 6 hr. The health consequence of chronic exposure to Pb in first-draw water, as reflected by increased blood Pb levels (PbB), is estimated for high-risk populations (i.e., children and pregnant women). Assuming background exposure to Pb from air (1 µg/m³), food (19-25 µg/day), water (10 µg/l) and dust (1000 ppm), estimates of background PbB levels in children and adult females living in urban areas are 13 and 8.5 µg/dl, respectively. Consumption of two 4 oz. glasses of first-draw water per day at concentrations of 50, 100, 200 and 500 µg/l is estimated to increase background PbB levels in children by 10, 25, 50 and 140%, corresponding to PbB levels of 14, 16, 20 and 31 µg/dl, respectively. The current US EPA (1985) PbB threshold level of concern for Pb exposure by high-risk populations is 15 µg/dl. Thus, exposure to Pb in first-draw water may significantly elevate PbB levels.
LEAD EXPOSURE IN AN OUTDOOR FIRING RANGE. RK Tripathi, PC Sharertz, GC Llewellyn, AW Armstrong, AS Phillips, and SL Remsny. VA Dept. of Health, VA Dept. of Labor and Industry, and VA State Police Academy, Richmond, VA.

Health hazards from lead (Pb) exposures in poorly ventilated indoor firing ranges are well documented. However, possible concern about over-exposure to Pb in an outdoor firing range initiated an industrial hygiene and medical survey. Substantial over-exposures to airborne Pb were found among personnel during firing of non-jacketed bullets. The mean lead levels (ug/m^3, 6-hr TWA) in personal breathing zone samples and area samples were 122, and 68, respectively. Twelve (57%) of 21 personal breathing zone samples and 8 (44%) of 18 area samples exceeded the OSHA standard of 50 ug/m^3. Blood lead levels (PbHb) were found to increase substantially in all individuals after firing non-jacketed bullets, but none of the values exceeded the OSHA standard of 40 ug/dL.

Test of a totally copper-jacketed bullet resulted in low levels of Pb in both personal breathing zone (6 ug/m^3) and area samples (9 ug/m^3). Analysis of airborne copper revealed levels in both personal breathing zone (3 ug/m^3) and area samples (0.8,ug/m^3) well below the OSHA standard of 100 ug/m^3. No substantial changes in PbHb were found in individuals after firing totally copper-jacketed bullets. We conclude that a potential health hazard due to Pb exposure existed at the range, and that the use of a totally copper-jacketed bullet reduced this exposure.


DMSA has been used in the treatment of acute and chronic exposure to lead, mercury and arsenic compounds. Virtually nothing is known about its biotransformation. Urine from fasted normal young men (N=6) given 0.05 mmol DMSA/kg po was collected at various times over a 14 hr period. Urine samples, before and after electrolytic reduction treatment, were analyzed for DMSA and its biotransformants by bromobiminate derivatisation, HPLC separation and fluorescence detection. Metabolites were isolated by anion-cation exchange extraction and TLC. By 14 hr after administration, 18±2±1 SE of the administered DMSA was excreted in the urine as altered DMSA (88% of the total DMSA found in the urine) and only 2.53±0.40 SE was excreted as unaltered DMSA (12% of the total DMSA found). The urinary excretion of altered cysteine versus altered DMSA at 1,2,4,6,9 and 14 hr after administration of DMSA had a correlation coefficient of 0.952 and p<0.003. Approximately 90% of the altered DMSA is a mixed disulfide with cysteine (2 cysteines to 1 DMSA). DMSA disulfide is a minor metabolite. The DMSA-CYS mixed disulfide indicates a thiol-disulfide interchange between DMSA and cysteine. The formation of a water soluble DMSA-CYS mixed disulfide suggests the use of DMSA as potential treatment for cystinuria. (NIHES MOB30356).


N-(2,3-dimercaptopropyl)phthalamic acid (DMPA), DMAP and DMSA are antioxidants for poisoning by As, Hg or Pb. After iv injection of male rats with 0.20 mmol DMPA/kg, the concentration of GSH in bile increased 100% within 10 mins. This is not due to an increase in bile flow since bile volume increases only 27% during this time period. The amounts of GSH, GSSG, unaltered and altered DMPA in the bile have been determined by the bromobiminate assay for dithiols. The increases of DMSA and GSH in the bile coincide. DMSA injection, however, does not increase biliary GSH or GSSG and DMSA is not detected in the bile. When rats are challenged with HgCl2 or CdCl2 and treated with DMPA, the increase of mercury and cadmium excretion in the bile may be due not only to the chelation action of DMPA but also to its activity in increasing the GSH content of the bile. (Supported in part by ES03356 and OM02185).


DMSA is becoming an important chelating agent for therapeutic use in humans. Information about its physico-chemical properties has been very limited even though such knowledge could be helpful in fully understanding its pharmacological properties and potential. The acid dissociation constants, complex formation constants and metal complexing properties of the ligands, meso-dimercaptosuccinic acid (DMSA) and the dimethyl ester of DMSA, CH3OOC-CH(SH)-CH(SH)-COOCH3 2+ have been investigated. DMSA reacts with Pb2+ or Cd 2+ to form chelates consisting of five membered rings that have oxygen and sulfur as the donor atoms. In contrast, the five membered rings formed with DMSA and Hg2+ or Ni2+ have two sulfur atoms as the donors. The acid dissociation constants of the dimethyl ester are comparable with the values that have been obtained for the Ni2+ chelate of DMSA. (Supported in part by ES03356 and OM02185).
105 THE EFFECTS OF 5-SI0-D-GLUCOSE, N-2-MERCAPTO-GLYCINE, AND OTHER SULFHYDRYLs ON THE BINDING OF MERCURY IN BRAIN AND OTHER TISSUES OF MICE. K Amoako-Abahio. Department of Pharmacodynamics, University of Oklahoma College of Pharmacy, Oklahoma City, OK. Sponsor: J A Rieger.

The affinity of methyl mercury radicals for sulfhydryl compounds is often greater than that for proteins. To examine this effect, several sulfhydryl compounds, including N-2-mercapto-glycine and 5-thio-D-glucose, were injected into mice that had been given methyl mercury chloride per os 72 hours earlier. Blood, brain, liver and kidney samples of homogenates were analyzed for concentration of free methyl mercury by gas chromatography with an electron capture detector. N-2-mercapto-glycine and 5-thio-D-glucose were much more effective in releasing methyl mercury from binding sites in mouse brain and liver than were any of the other six compounds. The levels of total methyl mercury chloride were lowest in tissue samples of mice injected with 2,3-dimercapto-succinic acid. The results of the study indicate there is considerable variation in the ability of sulfhydryl compounds to release methyl mercury from the bound state.


Metals have been shown to alter calmodulin (CaM) activity in various tissues. The present studies were conducted to investigate the effect of aluminum (Al), cadmium (Cd), lead (Pb), manganese (Mn), mercury (Hg) and vanadium (V) on CaM regulated Ca^{2+} ATPase activity in Rhesus Monkey Brain (RMB). Cerebral cortex of RMB was dissected and homogenized in 10% sucrose buffer and synaptosomes were prepared. Ca^{2+} ATPase activity was determined by inorganic phosphate method. Different concentrations of metal ions were incubated with RMB fractions containing endogenous CaM and without CaM. The results show that no metal ion inhibited the basal enzyme but showed a profound inhibition on total Ca^{2+} ATPase containing CaM. The order of potency of metals was Cu > Cd > Pb > Mn > Al > V. Exogenous addition of CaM restored the metal inhibited enzyme activity to near normal level. The basal enzyme when reconstituted with CaM showed sensitivity to metal ions. These data indicate that the metal ions alter CaM activity in RMB. (D.D. was supported by UNDP/TORKEN Program).

106 pH AND CITRATE EFFECTS ON BIOAVAILABILITY OF ALUMINUM (Al) FROM DRINKING WATER (DW). E Fulton, S Jaw, and E Jeffery. U. of Illinois, Urbana, IL.

Present in our DW as hydroxide or sulfate, Al is limited by solubility to 2.5 mg/L at pH 7.0. Al given at 100 mg/kg rat/day only accumulates in bone or brain if citrate (C) is co-administered (Slanina et al. Ed.Chem.Toxic. 22:391,1984). We determined if Al, in doses found in DW, accumulates in rats, when C at neutral or acid pH is co-administered, or at acid pH in the absence of C. Al, as (OH)_3 or Cl-, was given ad lib. in DW to male SD rats at 0, 0.1, 2.0, or 100 mg/L, in 4 mM acetate pH 3.2(A), 4 mM C pH 2.6, 4 mM C pH 7.0(C) or distilled water pH 7.0 (W). Growth rates did not differ between groups. Rats given 100 mg Al/L drank significantly less. Thus the DW dose range was approx. 0, 0.01, 0.2, or 5.5 mg Al/kg rat/day. After 10 weeks, rats were killed and tissues wet-ashed in nitric acid for determination of Al by graphite furnace AA. Al in tibia, brain, and liver of W rats were 8.57 ± 0.94, 5.25 ± 0.88, and 5.57 ± 0.01 µg/gw, respectively. In rats receiving 100 mg Al/L tibia Al levels were 9.83 ± 1.76, 10.87 ± 2.38, and 11.84 ± 1.08 µg/gw in W, A, and C rats respectively. Thus, mean tibia Al was increased in all 100 mg Al/L rats, but the increase was not significant. No consistent increases were observed in other tissues. Results suggest that Al, up to 40x that found in DW, does not accumulate significantly in tissue, regardless of acidity or citrate. Supported by Water Resource Center, USGS.

108 MULTIELEMENTAL ANALYSIS OF THE HEPATOPANCREAS OF SELENIUM-EXPOSED SUNFISH. E M B Sorensen, T L Bauer^, and M G Krause^ College of Pharmacy, Department of Pharmacology and Toxicology, Nuclear Engineering Teaching Laboratory, Department of Mechanical Engineering, University of Texas, Austin, TX.

The concentrations of zinc (Zn), iron (Fe), mercury (Hg), and selenium (Se) were determined in the livers (i.e. hepatopancreas) of redear sunfish (Lepomis microlophus) from Martin Lake (Se-contaminated) and a reference Lake (Lake Tyler). Selenium concentrations increased four-fold (p<0.001) and Zn levels increased 1.3 times (p<0.001). The level of Fe decreased from 450 ppm in livers of Lake Tyler fish to 240 ppm in Martin Lake fish (p<0.001). Martin Lake fish form a distinct population of fish compared with the Lake Tyler fish when compared on a multielemental basis. The average Se-contaminated sunfish from Martin Lake accumulated 8 ppm Se, 30 ppm Zn, and 240 ppm Fe. Whereas, the reference average sunfish from Lake Tyler accumulated 2 ppm Se, 20 ppm Zn, and 450 ppm Fe. Therefore, despite the passage of nine years since the initial discharges of Se-contaminated particulate wastes (i.e. fly ash, scrubber sludge, and bottom ash) into Martin Lake and despite the reduction in the discharge of selenium, hepatic levels of a number of elements were altered compared to that of reference sunfish.
To simulate environmental exposures of *Lepomis microlophus* (redear sunfish) to selenium at cooling reservoirs at electric generating stations, a laboratory exposure of this same species was conducted. Young adults were exposed for 7 or 14 days to sodium selenate at 100, 45, 10, 1, or 0 ppm selenium. Liver (i.e. hepatopancreas) was prepared for optical and electron microscopy. The mesonephros (i.e. kidney), heart, gills, ovaries, testes, and spleen were prepared for optical microscopy. Livers showed focal necrosis, vacuolation, fatty infiltration, and architectural disorganization. Kidneys showed proliferative glomerulonephritis, periglomerular fibrosis, and degeneration of proximal convoluted tubule cells. The severity of these alterations increased with exposure to higher concentrations of selenium. Other organs showed minimal histopathological alternations. These changes were consistent with those observed following environmental exposures of this or congenic species to selenium in Martin Lake and Belews Lake.

Sulfide is a deadly industrial toxin to which many workers are constantly at risk. At present, treatment is symptomatic using sodium nitrite and oxygen but this is controversial and there is no generally accepted treatment. The protective action of pyruvic acid against sulfide lethality was examined in male CD-1 mice (Charles River) of two different weights (22-30 g and 33-45 g). The lethality curve of sodium sulfide (i.p.) was significantly different between the two groups of mice with the larger more resistant to sulfide. Pyruvic acid (1 g/kg, i.p.), 15 min prior to sulfide, shifted the lethality curves for both groups of mice to the right with a protection factor of 1.8 for each group. This protection factor is the same as that for nitrites. Pyruvic acid also increases the effectiveness of the current therapy for sulfide poisoning, sodium nitrite. The time course of pyruvic acid protection was also examined. Pyruvic acid (1 g/kg, i.p.) reduced the lethality of sulfide (37.5 mg/kg, i.p.) from 100% to 5% at 15 min, 40% at 20 min, and 50% at 30 min. This data suggests that pyruvic acid may be a safe and effective new antidote against sulfide poisoning, both prophylactically and antidotally. (Supported by SM01 RR02303.)

Increased susceptibility to acetaminophen (APAP)-induced nephrotoxicity in 12-mo old male Sprague-Dawley (SD) rats is due, in part, to an age-dependent reduction in APAP volume of distribution. To define more clearly the role of pharmacokinetics, APAP nephrotoxicity was evaluated under conditions in which blood APAP concentrations were equivalent in 3- and 12-mo old male SD rats. APAP was administered iv at 750 mg/kg in 3-mo old and 500 mg/kg in 12-mo old rats. Despite equivalent blood APAP concentrations, only 12-mo old rats manifested nephrotoxicity 24 h following APAP administration. To assess the role of age-dependent differences in APAP metabolism, APAP metabolism via deacetylation or glucuronidation was determined in vitro in 3- and 12-mo old rats. Deacetylation of APAP to p-aminophenol by renal cortical cytosol in vitro did not differ with age. In vitro glucuronidation of APAP by 3- and 12-mo old liver and kidney microsomes was not different. These data indicate that differences in nephrotoxicity persist under conditions in which blood APAP concentrations are equivalent in 3- and 12-mo old rats. However, differences in APAP deacetylation or glucuronidation cannot account for enhanced nephrotoxicity in 12-mo old rats.


Congenic yellow A/VM-A/a (C3H x VY) F-1 hybrid mice, known to have differential responsiveness to hepatic tumors, were used to test the effects of the obese A/VM-A phenotype on susceptibility to acetaminophen-induced hepatotoxicity. Since acetaminophen hepatotoxicity is known to correlate with the covalent binding of acetaminophen to protein as, 3-(cytochrome P-450)-acetaminophen, an immunochromatography technique was used to quantify this adduct as a function of dose and genotype. Serum alanine and aspartate aminotransferase (ALT and AST) levels were measured as indices of hepatotoxicity. Mice fasted overnight were dosed by intraperitoneal injection of 0, 50, 150, 250, 350, or 450 mg/kg acetaminophen. Four hours after dosing, yellow mice receiving toxic doses of acetaminophen had elevated ALT and AST levels and elevated serum acetaminophen adduct levels as compared to similarly treated agouti mice. In non-fasted mice, resistance to acetaminophen toxicity was increased regardless of genotype, but agouti mice were more susceptible than obese with greater elevation of serum ALT and AST. These data show differential susceptibility to acetaminophen toxicity as a function of the obese A/VM-A phenotype and nutritional status.

ACETAMINOPHEN HEPATOTOXICITY IN OBSESE Zucker RATS: MECHANISM OF RESISTANCE. I Chaudhary, P J McNamara, R A Blouin. Graduate Center for Toxicology, University of Kentucky, Lexington, KY. Sponsor: L Robertson.

The hepatic cytochrome P-450 system in obese Zucker rats is not inducible by phenobarbital (PB) and these rats are more resistant to acetaminophen overdose after PB-treatment. The purpose of this study was to assess the influence of PB on glutathione transferase (GST) and cytosolic glutathione (GS). PB was administered orally to lean (34.7 mg/kg/12 hr) and obese (20.4 mg/kg/12 hr) rats for 5 days. The livers of 9 male control and induced lean and obese rats were homogenized and the final supernatant after 105,000 g was assayed for GST and y-GCS activities. PB treatment caused statistically significant increase in liver protein (mg/g protein) in lean control vs treated (74.12 ± 5.54 vs 80.58±4.82) rats, but no change was observed in obese control vs treated (80.39 ± 5.12 vs 83.42±7.54) animals. PB-induced GST activity (umole/mg protein) in lean treated vs control (1.77±0.7 vs 0.92±0.8) and obese treated vs control (1.51±0.7 vs 0.49±0.7). There was no increase in the y-GCS and GS level in PB treated lean and obese animals compared to controls. Results from this study showed that resistance of obese Zucker rats to acetaminophen could in part be due to the preferential induction of GST over cytochrome P-450.

In Vitro Metabolism and the Age-dependency of Acetaminophen (APAP)-induced Hepatotoxicity in CD-1 Mice. J T Brady, W F Beierschmitt, D S Wyand, E A Khairallah and S D Cohen. Univ. of Connecticut, Toxicology Program, Storrs, CT

Two and 3 month (N) old fasted (18 hr), male, CD-1 mice were given APAP (600 mg/kg, po) or 50% propylene glycol vehicle. Plasma sorbitol dehydrogenase (SDH) activity was measured and liver histopathology assessed 12 hr after dosing. Three N old, APAP-treated mice had significantly greater (7 fold) plasma SDH activities compared to 2 M old, APAP-treated mice. Verification of damage was supported by liver histopathologic evaluations. These data suggest that 3 M old mice were more susceptible than 2 M old mice to APAP hepatotoxicity. In vitro, hepatic cytochrome p-450 levels, aniline hydroxylase, p-nitroanisole O-demethylase, glutathione (GS)-5-transferase (GST) activities and APAP-GSH adduct formation were measured in treated 2 and 3 M old mice and no differences were detected. These data suggest that factors other than age-related differences in APAP bioactivation or GST-mediated detoxification of the APAP reactive metabolite were responsible for the difference in susceptibility of 2 and 3 M old mice to APAP-induced liver damage. Supported in part by NHLBI grant # GM31460 and ES07163 and a Stauffer Chemical Company Fellowship in Toxicology to JTB.)
Three month (M) but not 2 M old male CD-1 mice are susceptible to APAP (600 mg/kg, po) hepatotoxicity. APAP metabolism and covalent binding were studied in vivo to determine if such changes correlated with toxicity. Fasted 2 and 3 M old mice were given APAP (600 mg/kg, po) and killed at selected times for assessment of APAP metabolism. APAP concentrations in both plasma and liver, as well as hepatic glutathione depletion were similar through 4 hours after dosing for the 2 and 3 M old mice. Similar amounts of APAP and its glucuronide, sulfate, cysteine and mercapturate conjugates were excreted by 2 and 3 M old mice 24 hours post-dosing. Covalent binding of APAP reactive metabolite to whole homogenate was similar between ages at 4 hours. Immunohistochemical analysis revealed that at 4 hours after APAP, there were age-related differences in the specific proteins bound by APAP, in liver cytosolic and microsomal fractions. The age dependency of APAP hepatotoxicity in CD-1 mice is not related to metabolism differences but may result from differences in the specificity and outcome of selected protein arylation.

(Supported in part by NIH grants GM-31460 and ES 07163 and a Stauffer Chemical Company Fellowship in Toxicology to JTB.)

Administration of APAP (600 mg/kg, po) to fasted male CD-1 mice results in damage to the liver, kidney and lung. An affinity purified antibody for covalently bound APAP was used to identify APAP-protein adducts among electrophoretically resolved proteins from these target organs. Microsomal and cytosolic fractions were prepared from liver, kidney and lung 4 hours after APAP (600 mg/kg, po) and were resolved on 10% SDS PAGE transblotted to nitrocellulose and analyzed immunohistochemically. In the microsomal fraction, APAP was predominantly bound to proteins corresponding to approximately 44 kD in liver, and 58 kD in liver and lung. No binding was detected in kidney microsomes. APAP-bound proteins corresponding to 33, 44, and 58 kD were detected in cytosol from both liver and kidney, and only 58 kD in lung cytosol. Thus, in all three tissues, APAP binding to proteins was not random but highly selective. As we have suggested for liver, this selective binding may also be mechanistically involved in APAP-induced kidney and lung toxicity. Distinct differences in protein arylation among the three tissues may provide mechanistic insight into APAP target organ toxicity. (Supported in part by NIH grants # GM 31460 and ES 07163.)

The covalent binding of APAP to proteins has been implicated in eliciting hepatotoxicity. We have produced an affinity purified antibody which detects electrophoretically resolved APAP-protein adducts (Biochem. Pharm. 36:1193, 1987). Using this immunoassay, we recently reported that proteins of 44 and 58 kD are the major targets in livers of mice treated with 600 mg/kg of APAP. By 2-D PAGE / Western blotting, the 44 and 58 kD proteins contain clusters of 3 and 4 moieties with isoelectric points of 7.0 to 7.1 and 6.4 to 6.6, respectively. Although by NEM fluorography both clusters exhibited high protein sulfhydryl content (SH), other proteins which contained a high FSH content did not bind APAP. Analysis of liver extracts derivatized directly with NAFQI resulted in a binding profile different from that observed in vivo with decreased relative binding to the major adducts. These data indicate that the selectivity and extent of APAP arylation in vivo may depend upon the intact cell structure, the protein thiol affinity, and the cellular localization of the proteins. (NIH GM-31460).

To evaluate the mechanistic importance of covalent binding in acetaminophen (APAP) induced hepatotoxicity, we have compared the effects of 2,6-DMA to those observed with APAP. In cultured mouse hepatocytes 10 nM APAP is cytotoxic and, by immunohistochemical analysis, can be shown to bind to a few specific proteins. By contrast, at equimolar concentrations 2,6-DMA is not cytotoxic yet radiometrically binds 30% as much as does APAP. The differences in toxicity between these compounds may reflect the differences in the specificity of the targetted proteins. Because the anti-APAP antibody exhibited no anti-2,6-DMA activity, an antibody specific for 2,6-DMA was constructed by covalently linking 2,6-DMA via a p-aminobenzoic acid linker to keyhole limpet hemocyanin. Following immunization with this antigen, an antibody specific for 2,6-DMA were purified using a 2,6-DMA affinity Sepharose 6B column. Using 2,6-DMA covalently bound to BSA, we have demonstrated that the anti-2,6-DMA antibody can detect protein-bound 2,6-DMA by solid phase ELISA and Western blotting. This new antibody will enable the comparison of the reactivity of 2,6-DMA to that of APAP and may help elucidate the "critical" versus "non-critical" nature of the protein targets. (NIH GM-31460).

Inhibition of both glutamate- and succinate-supported mitochondrial respiration is associated with acetaminophen (APAP) hepatotoxicity in vivo. APAP, in vitro, rapidly inhibits mitochondrial respiration with glutamate but not succinate as substrate, suggesting a direct effect at or prior to Complex I of the respiratory chain. To test this, the effect of APAP on respiration of mitochondria isolated from control male CD-1 mice was studied polarographically with alpha-keto-glutarate and beta-hydroxybutyrate as additional Complex I substrates. As with glutamate, state 3 respiration and respiratory control were decreased from 10 to 90% in a dose dependent manner by 2.5 to 20 mM APAP. In contrast to in vivo findings, no covalent bonding was detected in mitochondria exposed to APAP in vitro. Washing APAP from inhibited mitochondria resulted in complete return to control respiratory rates. This indicates that APAP's in vitro action differs from that which predominates in vivo and may result from a direct and reversible interference with electron transfer at Complex I. 

(Supported in part by NIH grant GM 31460 and ES 07163) 

122 EFFECT OF PREGNENOLONE-16α-CARBONITRILE (PCN) ON ACETAMINOPHEN-INDUCED HEPATOXICITY IN HAMSTERS. C Madhu and C D Klaassen, Univ. of Kansas Med. Ctr, Kansas City, KS. 

Excessive dosages of acetaminophen (AA) are known to produce acute liver toxicity in both humans and laboratory animals. Hamsters are especially sensitive to the hepatotoxic effects of AA. In the present study, hamsters pretreated with PCN (75 mg/kg, i.p., daily for 4 days) were given a single dose of AA (300-1200 mg/kg, ip) and liver function determined at 24 hr. Serum activities of alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) as well as histopathology were used as indices of hepatotoxicity. PCN pretreatment decreased AA-induced mortality. PCN dramatically decreased ALT (93-97%) and SDH (63-98%) activities relative to control values from hamsters treated with AA alone and remarkably decreased hepatic centrilobular necrosis produced by AA. To investigate the mechanism of this protective effect, the biliary and urinary excretion of AA metabolites were measured for 1 hr after administration of AA (150 mg/kg, ip) in bile duct-cannulated hamsters anesthetized with pentobarbital. PCN pretreatment resulted in increased urinary and biliary excretion of AA-glucuronide and decreased biliary excretion of AA-glutathione. Microsomes from PCN-pretreated hamsters produced less reactive benzoquinoneimine intermediate than controls, as determined by the formation of AA-GSH. In conclusion, PCN has a dramatic protective effect against AA-induced hepatotoxicity. The mechanism of this protection appears to be due to decreased formation of the reactive metabolite by the cytochrome P-450 pathway, and an increased detoxication by enhanced glucuronidation of AA. 

(Supported by USPHS Grant ES-03192) 


The oxidation of acetaminophen (APAP) by P-450 results in the formation of reactive intermediates which are involved in APAP toxicity. Current data indicates that the 2-electron oxidation product, N-acetyl-p-benzoquinone imine (NAPQI), both binds to and oxidizes cellular sulfhydryl groups. To better understand the mechanisms of APAP oxidation by P-450, reaction mixtures containing microsomal P-450, cumene hydroperoxide and APAP were evaluated for APAP oxidation products. It was shown that P-450 catalyzed APAP to NAPQI which reacted with GSH to form 1-(glutathionyl)p-N-acetyl-p-benzoquinone imine to give APAP polymerization products. However, in reaction mixtures containing NADPH, the APAP polymerization products were not detected. This was possibly due to rapid reduction of APAP free radicals by NADPH. These results indicate that P-450 catalyzes both the 1- and 2- electron oxidation of APAP. 


Acetaminophen (APAP) undergoes metabolic activation by mixed function oxidases to the highly reactive N-acetyl-p-benzoquinone imine (NAPQI). NAPQI readily forms thioether conjugates with both hepatic glutathione and cysteinyl residues of liver proteins. It is speculated to undergo hydrolysis to p-benzoquinone. In mice treated with 14C-APAP [200 mg/kg, i.p.], we have identified S-(2,5-dihydroxyphenyl)-cysteine and S-(2,5-dihydroxyphenyl)-N-acetyl-cysteine [the cysteine and mercapturate adducts, respectively, of hydroquinone (HQ)] as urinary excretory products. Adducts were isolated from 24hr urine by HPLC fractionation and converted to a common derivative, Q-D-tris-acetyl-3-thio-HQ, which was characterized by GC-MS. Quantification of urinary HQ-thiol adducts by HPLC indicated that they accounted for 6.3% of urinary APAP-thiol conjugates. In hemoglobin hydrolysates from 14C-APAP-treated mice, HQ-cysteine was similarly identified as a component of the covalently bound 14C-residues. These findings provide strong indirect evidence that p-benzoquinone is indeed formed in vivo as an additional reactive metabolite of APAP. 

Supported by NIH grant DK 30699.
Cysteine is required for the synthesis of cosubstrates for two pathways of acetaminophen (ACET) metabolism: PAPS for sulfation and GSH for detoxification of the reactive metabolite (RM). Decreased cysteine may reduce PAPS and/or GSH and thereby reduce clearance/or detoxification leading to potentiation of liver injury. Conversely, limitation of sulfur amino acids may result in depression of protein synthesis and hepatic cytochrome P450, and hence in decrease in RM formation and in liver injury. To determine whether the potentiating effects exceeded the protective effects, rats were fed casein- or casein-free diets for three weeks containing various levels of methionine (1.2, 2.4, and 7.2 mmol/kg/day) as the sole source of sulfur in the diet. Sulfur deficiency (SD) was assessed by urinary inorganic sulfate levels. SD retarded growth and suppressed hepatic GSH levels. SD decreased ACET sulfation capacity, whereas ACET RM formation was increased. The predicted potentiation of ACET liver injury in these animals was confirmed by histologic studies which showed increase in both incidence and severity of ACET hepatic necrosis in SD animals. These observations raise the possibility that nutritional inadequacy of sulfur amino acids in man may similarly enhance susceptibility to ACET liver injury. (Supported by GM 36687).

Treatment of rats with AA results in accumulation of MP in centrilobular regions of the liver. To determine if the RBCs were nonspecifically activated, we examined their cytototoxic activity towards normal and transplanted KC. MP were isolated from livers of control (RKC) or AA treated rats (1.2 g/kg, 24 hr) by collagenase/pronase perfusion. Using a 3H-Tdr release assay, both RKC and AA-MP were found to be cytototoxic towards N1830 hepatoma cells in an effector(T) dependent manner, but only after 48-72 hr coincubation. AA-MP were 2-3 times more cytototoxic than were RKC. AA-MP also displayed cytotoxicity towards normal HC as determined by trypan blue dye exclusion. After 24 hr incubation with AA-MP at a 1:10 E:T ratio, HC viability decreased by 10-20%. Cytotoxicity towards HC was stimulated (20%) by factors released from HC (H-MAP) treated with 100 uM AA. RKC were not cytotoxic towards HC unless they were coincubated with AA and H-MAP. These data support our model that AA causes activation of MP in the liver and that these cells may contribute to AA hepatotoxicity. Supported by NIH GM34310.

The correlation between the formation of acetaminophen-protein adducts and the development of hepatotoxicity has previously been shown using a radiolabeled assay. An immunochemical assay was used to quantitate acetaminophen bound to cysteinyl groups on protein. Serum alanine and aspartate aminotransferase (ALT and AST) levels were measured as indicators of hepatotoxicity. ALT and AST levels did not increase following doses of 50, 100, and 200 mg/kg; however, at doses of 300, 400, and 500 mg/kg, levels were significantly increased. Binding to liver proteins was only detected following doses of 300, 400, and 500 mg/kg. Protein adducts in serum were detected and showed a dose response similar to serum aminotransferases. A time course following a dose of 400 mg/kg indicated that liver protein adducts peaked at two hours after dosing, whereas the appearance of serum adducts was delayed concomitant with the decline of liver adducts and the increase in ALT and AST levels. Serum adducts reached maximum by sulfation and at six to twelve hours after dosing. Collectively, these data suggest that adducts were of hepatic origin or that binding to hepatic and serum proteins occurs simultaneously. It is postulated that serum adducts may be used as a biomarker to study acetaminophen toxicity in humans.

ON THE PREDICTION OF CHEMICAL TOXICITY IN VITRO: BIOTRANSFORMATION OF ACETAMINOPHEN (APAP) AND 7-OH-ACETYLAMINOFURORENE (7-OH-AAF). C Harris, K L Stark and M R Jochum, Department of Pharmacology, University of Washington, Seattle, WA.

7-OH-AAF, a putative detoxication product of the carcinogen 2-acetylaminofluorene, was previously shown to be embritotoxic to rat embryos grown in vitro without an exogenous bioactivation system. Investigations into the role of bioactivation of embritotoxic agents led us to propose that exposure to a chemically similar N-acetylaminophenol, APAP, could also result in the same form of embritotoxicity. Our comparisons showed, for the first time, that 7-OH-AAF and APAP elicited virtually identical forms of dysmophogenesis in cultured rat embryos. Doses of APAP and 7-OH-AAF at concentrations of 0.50 and 0.25 mM, respectively, resulted in a 50% incidence of open neural tube (ONT) on gestational day 11. Addition of an exogenous bioactivation system resulted in increased toxicity for both compounds as indicated by severe hyperplasia and necrosis in the caudal and cephalic regions, but no clear changes in the incidence of ONT could be detected. A similar form of dysmophogenesis was observed when conceptuses were cultured with lower concentrations of p-aminophenol and 7-OH-AAF, indicating that deacetylation activity may modify the toxic response. Pretreatment of embryos in vitro with the P450-1A1/2 inducer, 3-methylcholanthrene, produced no significant changes in the incidence of ONT of either APAP or 7-OH-AAF. Conceptuses cultured in a CO2 atmosphere also resulted in no changes in ONT incidence or severity. This showed a possible lack of P450 involvement in the bioactivation step. Bioactivation via the cyclooxygenase pathway was also considered unlikely due to the lack of protection following incubation with inhibitory concentrations of indomethacin. Evidence now suggests that peroxisitive metabolism may be involved in the conversion of 7-OH-AAF and APAP to reactive chemical intermediates which are capable of eliciting dysmophogenesis in rat embryos in vitro. Supported by NIH grants ES-04041 and ES-04342.
Metabolic acidosis and coma have recently been associated with severe overdoses of acetaminophen (AA) in humans in the absence of overt liver damage. AA (2-25mM) and its nonhepatotoxic isomer, N-acetylhydroxycacetanilide (3HAA), have been shown to inhibit NAD-linked respiration in rat liver mitochondria and cause an increase in glucose and lactate output from the isolated perfused rat liver. Both isomers of AA cause profound alterations in serum glucose and lactate levels in vivo. Following administration of either isomer (1 g/kg ip in saline), serum lactate increased from 33.0 ± 2.8 mg/dl (mean ± SEM; n=6) to 84.0 ± 5.4 mg/dl one hour after AA and to 78.8 ± 4.5 mg/dl one hour after 3HAA. Serum glucose increased from 189.2 ± 4.1 mg/dl to 461.0 ± 35.1 mg/dl one hour after AA and to 392.6 ± 9.0 mg/dl one hour after 3HAA. Glycerogen levels in the livers of AA and 3HAA treated rats one hour post-dosing were also diminished. Serum AA and 3HAA concentrations one hour after treatment were 5.4 ± 0.4 mM and 5.9 ± 0.5 mM, respectively. Similar concentrations of acetaminophen have been reported following overdoses in humans. These studies suggest that AA and 3HAA cause toxicity by inhibiting NAD-linked mitochondrial respiration. Supported by AA5848.

Antidotes known to prevent acetaminophen-induced hepatotoxicity were tested for their ability to protect against acetaminophen-induced renal failure. Male Sprague-Dawley rats were treated with acetaminophen (0.5-1.5 g/kg p.o.), liver and kidney damage were monitored by measuring plasma aminotransferase and sorbitol dehydrogenase, urea, creatinine as well as urinary enzyme excretion of α-glutamyltranspeptidase and N-acetyl-B-glucosaminidase over 4 days. Methionine (0.5 g/kg p.o.) partially reduced acetaminophen hepatotoxicity, but had no effect on the nephrotoxicity; a lower dose of methionine (0.15 g/kg p.o.) even enhanced the nephrotoxic response to acetaminophen. With N-acetylcysteine (1.0 g/kg p.o.) a safe hepatoprotective was seen, kidney damage, however, was only partially reduced. Only N-decarbamylacetaminophen (200 mg/kg p.o.) was capable of totally preventing acetaminophen-induced hepato- and nephrotoxicity. It is concluded, that antidotes known as precursors of GSH-synthesis (methionine, N-acetylcysteine) are ineffective in protecting against acetaminophen nephrotoxicity. Inhibition of microsomal bioactivation of acetaminophen by diethyldithiocarbamate resulted in a complete protection against hepato- and nephrotoxicity.

When acetaminophen (AA) (2-25 mM) is infused into isolated perfused livers from rats acutely treated with ethanol (E) (4g/kg for 1-4 hrs), the rate of hepatic O₂ uptake was inhibited in two steps - the rapid inhibition complete within 2-3 minutes (19 ± 1.3% basal respiration inhibited) (mean ± SEM; n=5) and the slow inhibition proceeding at a rate of 79±11% basal respiration inhibited per hr (n=8). The slow inhibition was (1) negligible in livers from control fed rats, (2) unaffected by metyrapone (5 mM), (3) accompanied by lactate dehydrogenase leakage, (4) blocked by 10 mM mannitol, (5) dependent on extracellular Ca²⁺, and (6) abolished by hypophysectomy and thyroidectomy. Since (1) the granulocyte content of the liver increased by 3 fold upon pretreating rats with E for 4 hrs, (2) an incubation of human neutrophils with N-acetaminophen caused covalent binding of AA metabolite (likely N-acetyl-p-benzoquinonimine (NAPQI)), and (3) synthesized NAPQI irreversibly inhibited O₂ uptake by isolated mitochondria, we postulate that the AA-induced slow inhibition of hepatic respiration is in part due to NAPQI formed in granulocytes which then enter hepatocytes to covalently inhibit mitochondrial enzymes. Supported by AA5848.

APAP is primarily excreted in the urine as sulfate and glucuronide conjugates. Following overdoses, a reactive, electrophilic metabolite of APAP causes liver GSH depletion with subsequent centrilobular necrosis. Continued availability of hepatic GSH is, therefore, considered a key factor in attenuating hepatotoxicity.

Groups of adult, male SW mice were gavaged with (1) APAP alone, (ii) APAP + Ascorbyl Palmitate (AP), or (iii) APAP + Ascorbyl stearate (AS), at a dose of 600 mg/kg of each drug. Hepatic GSH levels (15 min - 2 hr) and sulfobromophthalein (BSP) excretion were monitored for all groups. GSH levels were significantly decreased in all groups compared to vehicle controls. However, starting 4 hrs post-dosing, the levels in AP and AS treated groups recovered and reached control values in 12 hours. At 30 min post-dosing, APAP alone caused significant BSP retention; plasma BSP levels in the vehicle, AP and AS treated animals were undetectable. Since BSP is known to be excreted as a GSH conjugate, we suggest that ascorbyl palmitate and ascorbyl stearate provide protection against APAP-hepatotoxicity by increasing GSH turnover.

ASCORBIC ACID ESTERS PROTECT AGAINST ACETAMINO-
PHEN (APAP) HEPATOTOXICITY IN MICE: POSSIBLE ROLE
IN GLUTATHIONE (GSH) REGENERATION. A K Mitra
and V C Ravikumar. Division of Pharmacology &
Toxicology, School of Pharmacy, Northeast Louisi-
ana University, Monroe, LA.
Oltipraz (OTP; 5-(2-pyrazinyl)-4-methyl-1, 2-di-thiol-3-thione, 2.0 mmole/kg, po), 48 hr prior to acetaminophen (AAP; 2.6 mmole/kg, ip), decreases AAP hepatotoxicity. These studies were performed to determine whether OTP altered AAP metabolism. Urinary recoveries of AAP+AAP metabolites were similar (80%). However, more AAP was found as the glucuronide (GLUC) and less AAP was recovered as glutathione (GSH)-derived conjugates in OTP-treated hamsters. The calculated UDGRS synthetic rate, liver UDPGA content and UDP-glucuronyl transferase activity, as well as, the formation rate constant (kf) for GLUC were markedly increased by OTP. OTP treatment decreased in vivo binding of AAP to liver protein, concomitant with decreases in kf for GSH-derived metabolites and AAP reactive fraction (kf GSH/R). Markedly increased rate and extent of AAP glucuronidation, which enhances AAP metabolism, may be a mechanism of OTP hepatoprotection. (Supported by a Sandz Research Institute Fellowship [MHD] and a Burroughs-Wellcome Toxicology Award [RSD]).

Cumarin (1,2-benzpyrone) is known to produce liver damage in the rat but controversy exists as to whether this is due to coumarin per se or to a coumarin metabolite(s). We have compared the toxicity of coumarin and dihydrocoumarin (DHC), which lacks the 3,4-double bond, both in vivo and in primary hepatocyte cultures from male Sprague-Dawley rats. The administration of coumarin (125 mg/kg, i.p.), but not DHC (127 and 254 mg/kg), markedly reduced hepatic reduced glutathione (GSH) levels after 2 hr and produced centrilobular hepatic necrosis and elevated serum enzyme activities after 24 hr. In hepatocyte cultures coumarin, but not DHC, produced a dose dependent inhibition of protein synthesis and depletion of GSH levels. GSH depletion was not due to GSSG formation or to leakage from the cells. Coumarin-induced toxicity was reduced by the cytochrome P-450 inhibitors methapyrone and ellipitone but enhanced in GSH depleted (diethyl maleate treated) hepatocytes. These studies demonstrate a good correlation between the in vivo and in vitro effects of coumarin and DHC and are consistent with the hypothesis that a coumarin 3,4-epoxide intermediate is responsible for coumarin-induced hepatotoxicity in the rat. (Supported by the UK Ministry of Agriculture, Fisheries and Food)

Our previous studies indicated that hepatic glutathione was not central to the protection provided by oltipraz (OTP; 5-(2-pyrazinyl)-4-methyl-1, 2-dithiol-3-thione) in acetaminophen (AAP)-induced hepatotoxicity. These studies were designed to determine if AAP (2.6 mmol/kg, ip) disposition was altered by prior (48 hr) OTP (2.0 mmol/kg, po) treatment. OTP did not influence AAP absorption, as shown by similar PCONEIN-generated ka (0.2 min-1), Tmax (16 min) and Cmax (2200 um) estimates. AAP was eliminated more rapidly by OTP-treated hamsters. Decreased plasma T1/2 (49%) and AUC (46%) were associated with corresponding increases in the elimination rate constant (87%) and systemic clearance (70%) in hamsters receiving OTP. During AAP elimination, plasma OTP concentrations were low (10 um) thus a direct interaction is unlikely. These data indicate that OTP single treatment may protect against AAP hepatotoxicity by enhancing overall AAP elimination. (Supported by a Sandz Research Institute Fellowship [MHD] and a Burroughs-Wellcome Toxicology Award [RSD]).

 Allylamine is toxic to the cardiovascular system causing aortic, valvular and myocardial lesions. Acute toxicity is believed to involve metabolism of allylamine to highly reactive acrolein. Comparative toxicity of allylamine and acrolein was evaluated in cardiac fibroblasts and myocytes, which were obtained from neonatal rat hearts by a differential plating technique. Allylamine and acrolein were added directly to serum supplemented culture media (MI99). Toxicity was assessed by measuring lactic dehydrogenase (LDH) release, ATP levels, and spontaneous beating activity. Beating activity was arrested by 0.05 mM acrolein and 0.1 mM allylamine. Acrolein at a concentration of 0.05 mM was equally toxic to myocytes and fibroblasts reducing ATP levels and causing marked leakage of LDH 4 hrs after treatment. In contrast, 10 mM allylamine was required to reduce ATP levels and cause leakage of LDH in fibroblasts, whereas a comparable response was produced in myocytes by 0.1 mM allylamine. Semicarbazide, a benzylamine oxidase inhibitor, protected myocytes from allylamine toxicity, whereas clorgyline, a monoamine oxidase inhibitor, was ineffective. Semicarbazide was ineffective against acrolein toxicity. The findings support the hypothesis that the toxicity of allylamine in cultured myocytes is dependent on its metabolism to acrolein.
Biocatalytic characterization of hepatic cytochrome P-450 system in obese Zucker rat model has shown that phenobarbital (PB) induced cytochrome P-450 is present at the basal level but is not inducible in order to further characterize the above phenotype at a molecular level, hepatic mRNA levels expressed in both obese and lean Zucker rats, treated (experimental) with PB and untreated (control) were studied. Induction of cytochrome P-450 was carried out by orally administering PB to both lean (34.7 mg/kg/12 hr) and obese (20.5 mg/kg/12 hr) animals. Hepatic total cellular RNA was isolated, and examined by Northern analysis using radiolabelled B and E c-DNA probes. B and E c-DNA probes produced a faint signal in control rats which shows that basal level mRNA for the B and E form are present. Both probes produced a stronger signals in PB-induced animals. However, the difference in induction signal was 5-7 fold greater in the lean animals. The results indicate that the genetic defects in the obese Zucker rats results in the decrease in the responsiveness of cytochrome P-450 response to PB treatment.

2,3,7,8-TCDD and HCB elicit several common biologic and toxic effects including the induction of hepatic microsomal aryl hydrocarbon hydroxylase (AHH). Treatment of rats with 2,3,7,8-TCDD, HCB and 2,3,7,8-TCDD plus HCB resulted in some minor non-additive changes in the induction of hepatic and extrahepatic AHH which could not be linked to HCB-mediated changes in organ Ah receptor levels. However, the most dramatic interactive effects of HCB (400 umol/kg) and 2,3,7,8-TCDD (10 or 30 ug/kg) were observed with two Ah receptor-mediated toxic effects, namely body weight loss and thymic atrophy. HCB synergistically exacerbated both body weight loss and thymic atrophy caused by 2,3,7,8-TCDD whereas HCB administered alone at doses as high as 3000 umol/kg did not cause any significant body weight loss or thymic atrophy in the rats. (Supported by the Natural Sciences and Engineering Research Council of Canada.)
A CANTHARIDIN BINDING SITE IN MOUSE LIVER CYTOSOL: CORRELATION OF BINDING AFFINITY AND ACUTE TOXICITY. M. J. Graziozio, A. L. Waterhouse, and J. E.Casida. Pesticide Chemistry and Toxicology Laboratory, Dept. of Entomological Sciences, U of California, Berkeley, CA.

Cantharidin is a potent natural vesicant isolated from blister beetles. To investigate its mechanism of mammalian toxicity, [3H]cantharidin (14 Ci/mmol; >98% radiochemical purity) was synthesized and assayed as a radioligand using mouse liver as the model target organ. [3H]Cantharidin was shown to interact in a specific and saturable manner with a binding site in the cytosolic fraction with apparent Kd and Bmax values of 30 nM and 1.8 pmol/mg protein, respectively. There was no appreciable binding to any of the membrane fractions. The potency of cantharidic acid (the dicarboxylate form of the anhydride) and 20 other structurally related compounds, including the widely used herbicide endothall, as inhibitors of [3H]cantharidin binding in vitro correlated extremely well with their acute toxicity to mice (r=0.962). Furthermore, [3H]cantharidin binding to the cytosol of mice pretreated with unlabeled cantharidin was inhibited in a dose-related manner-related manner. The results of this study strongly suggest that the mechanism of cantharidin's toxicity involves interaction with a cytosolic binding site. (Supported in part by NIH Grant PO1 ES00049).


2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been shown to antagonize certain estrogenic effects, such as estrogen-induced increase in uterine weight and tumor growth in estrogen-responsive tissues. TCDD down-regulates the estrogen receptor. Recent studies indicate marked changes in specific mouse liver cytosolic proteins. 30 day old C57B/6 female mice were given 0, 20, or 400 ng estradiol a.c. in corn oil, daily for 14 days. On days 7, 9, and 13 they also received 0, 5 or 10 ug/kg TCDD in corn oil, by gavage. After sacrifice on day 15, cytosol was prepared from uteri and livers pooled from each group. Cytosolic proteins were separated on one-dimensional SDS-10% polyacrylamide gels and stained with Comassie blue. Multiple bands of 20-200 kd MW were found. A prominent band of Mr 65 kd was found in uterine cytosol from all groups. This MW is near that of the 68 kd estrogen receptor. This band appeared reduced in intensity in mice receiving TCDD but not estrogen. The dioxin effect was less apparent in estrogen-treated mice. Further studies are in progress to identify this protein. Dioxin increased liver/body weight ratios, which were not altered by estradiol, and uterine/body weight ratios were increased by estrogen exposure.


Our objective is to test the hypothesis that alterations in gene expression might serve as an indicator of chemically-induced hepatotoxicity. Decreased serum albumin gene expression was used as an indicator of liver dysfunction because it is expressed in normal hepatocytes. Actin gene expression was chosen as an internal control; actin is produced at a constant rate in all cells. Bromobenzene (BrB) was administered to phenobarbital-induced male Sprague-Dawley rats at doses of 1.0, 2.5 or 5.0 mmoles/kg; they were sacrificed 24 or 48 hr after BrB. Histopathology and serum alanine aminotransferase (ALT) levels were assessed, and RNA was isolated from hepatic cells. Levels of albumin and actin mRNA were measured by slot-blot analysis using a 32P-labeled cDNA for each gene. The higher doses of BrB resulted in hepatic necrosis and increased ALT with proportional decreases in albumin mRNA. The low dose of BrB did not result in necrosis or changes in ALT; there did appear to be decreases in albumin mRNA. These results indicate that monitoring serum gene expression might serve as a useful adjunct to traditional methods of assessing hepatotoxicity.
GENE EXPRESSION EFFECTS OF TOXIC AGENTS: USE OF 2-D ELECTROPHORESIS TO MONITOR CHANGES IN THE EXPRESSION OF HUNDREDS OF LIVER PROTEINS FOLLOWING EXPOSURE OF MICE TO A VARIETY OF XENOBIOTICS. N L Anderson, F A Gierke**, and N G Anderson*, Large Scale Biology Corp.(**), Rockville, MD and Lake Forest College(***), Lake Forest, IL.

Recently developed techniques based on computer analysis of two-dimensional electrophoretic protein patterns allow accurate measurement of the abundance of hundreds of individual proteins in tissue samples. Many toxic agents cause characteristic changes in the amounts of specific proteins as determined by this technique, offering a relatively comprehensive picture of intoxication effects at the molecular level. Drawing on appropriate computer databases, an analysis of these changes can provide insight into mechanisms and sites of action, as well as allowing classification of chemical effects. Compounds investigated in this study include carbon tetrachloride, cycloheximide, Aroclor 1254, phenobarbital, ibuprofen, ethanol, indomethacin, ethylene glycol, aspartame, clofibrate, and alloxan. Expansion of much studies to a range of organs and the construction of a larger database of reference compounds effects may allow a systematic and quantitative general approach to gene expression toxicology in rodents.

SYNERGISTIC EFFECT OF ETHANOL AND AMITRIPTYLLINE (AMI) ON Na,K-ATPase ACTIVITY OF SYNAPTIC PLASMA MEMBRANES. N A Carfagno1 and B D Muhoberac2, Department of Pharmacology/Toxicology, Indiana University School of Medicine1 and Department of Chemistry2, Indiana University-Purdue University at Indianapolis, IN Sponsor: R E Forney, Sr. 1

The effects of simultaneous exposure of synaptic plasma membranes (SPMs) to ethanol and other CNS-active drugs may be important in understanding the apparent potentiating of respiratory depression and psychomotor impairment induced by combinations of such drugs. In this study Na,K-ATPase (NKA) and Mg-ATPase activities of rat SPMs were measured after acute, in vitro exposure to ethanol (100 & 200 mM) and AMI (6.5 & 9.01 mM) both separately and in combination. Three of the four ethanol/AMI combinations studied showed a statistically significant (p<0.01) difference between the mathematical sum of ethanol- and AMI-induced NKA inhibition measured separately and the experimentally measured effect of the two drugs in combination. For example, ethanol (100 mM) inhibited activity 6.42% ± 0.53 and AMI (9.01 mM) 4.46% ± 1.28 which gives a mathematical sum of 47.8% ± 1.80, whereas the experimentally measured combined effect was 60.77% ± 0.72 inhibition. In contrast, an additive effect was observed with Mg-ATPase at the same concentrations of ethanol and AMI. These data show an acute, in vitro synergistic inhibition of NKA by ethanol and AMI which may be a basis for enhanced CNS impairment. (Supported by NIAAA AA06935)

ETHANOL POTENTIATION OF THE HEPATOTOXIC RESPONSE TO ACUTE COCAINE ADMINISTRATION IN MICE. C S Beyer and D E Petersen, Hepatobiliary Research Center, Molecular and Environmental Toxicology Program, and School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO

Cocaine has been documented to be a potent hepatotoxic in mice. The theorized toxic metabolite of cocaine is thought to be produced by a multistep metabolic pathway beginning with N-demethylation by P-450. Ethanol, because of its P-450 induction capabilities with chronic ingestion and its probable concomitant social use is an obvious choice for interaction studies. Male and female C57 mice were fed an ethanol-containing liquid diet for 6 days. On day 5 of the treatment, mice were injected ip with 50 mg/Kg cocaine and serum glutamic-pyruvic transaminase (SGPT) levels were determined 24 hours later. Of the mice receiving both the ethanol pretreatment and acute cocaine, males showed a five-fold increase in SGPT levels over naive animals receiving the same dose and females showed a twenty-fold increase over naive animals receiving the same dose. In both sexes, cocaine N-demethylase activity was increased 1.8 fold over control animals, thus indicating an inductive effect of chronic ethanol. This evidence indicates that in mice, chronic alcohol ingestion can potentiate the hepatotoxic response to an acute dose of cocaine. (Grant Support NIH AM4914)

α TOCOPHERYL SUCCINATE AS A UNIQUE AND POTENT CYTOPROTECTIVE AGENT. M W Fariss. Environmental Toxicology, Department of Pathology, Medical College of Virginia/ Virginia Commonwealth University, Richmond, VA

We previously demonstrated, using 1 mM free extracellular Ca²⁺ (physiological), that α-tocopherol succinate (α-TS) protects hepatocytes from the toxic effects of chemicals. To determine if the cytoprotective ability of α-TS is related to the release of α-tocopherol (α-T) we compared the protective capacity of α-T and α-TS in rat hepatocytes exposed to a variety of toxic insults. With each insult investigated (ethyl methanesulfonate, ionophore A23187, cadmium and 95% O₂) α-TS was found to be a more potent protective agent than α-T. For example, complete protection against ethyl methanesulfonate-induced toxicity was observed with 2 to 250 μM α-TS whereas 250 μM α-T offered little protection (30%). Cellular α-T was found only in protected cells (2 to 250 μM α-TS) at concentrations ranging from 0.1 to 7.6 nmole/10⁶ cells. The concentration of α-T in protected hepatocytes (α-TS, 2 μM) however was 200 times lower than that observed in unprotected hepatocytes (α-T, 250 μM). It is concluded that cytoprotection observed after α-TS administration does not result from the cellular accumulation of α-T but rather from the presence of the intact α-TS molecule itself appears to be the protective agent.
Intracellular α-Tocopherol Content and Chemical Toxicity in Hepatocytes. M S Sandy, D Di Monte and M T Smith. School of Public Health, University of California, Berkeley, CA.

The α-tocopherol (α-T) content of isolated rat hepatocytes exposed to several types of chemical injury was measured by HPLC-EC. Cellular α-T was depleted following exposure of BCMU-treated hepatocytes to cytotoxic doses of the reduct cycling compound diquat. This loss of α-T could be prevented by addition of the antioxidant N,N'-diphenyl-p-phenylenediamine, although diquat-induced loss of soluble and protein thiols still occurred, as did cell death. Addition of dihydrotiothreitol not only prevented this loss of α-T, but also protected against diquat-induced soluble and protein thiol loss, and cytotoxicity. In contrast, exposure of hepatocytes to cytotoxic doses of either menadione, MPTP, or MPF did not deplete cellular α-T. Although peroxidation of cellular lipids occurs following exposure to diquat, none was observed in cells exposed to either menadione, MPTP, or MPF. On the other hand, a peroxidizing, but non-cytotoxic dose of ADP-Fe²⁺ rapidly decreased cellular α-T levels. These data suggest that cellular α-T loss is neither a prerequisite for, nor a necessary consequence of toxicity. Furthermore, although α-T protects against the oxidation of cellular lipids, the maintenance of hepatocyte α-T content does not prevent the oxidation of soluble and protein thiols.


We investigated the effects of linoleic acid (18:2) and linoleic acid hydroperoxide (18:2-OOH) on membrane structure and function of vascular endothelial cells (EC). Confluent porcine pulmonary artery (PA), coronary artery (CA), and aortic (AO) EC were treated with 15μM each 18:2 or 18:2-OOH. After 2-4 hr incubation at 37°C, membrane fluidity was monitored by measuring fluorescence anisotropy (r₀) of two membrane probes, DPH and TMA-DPH. Insulin receptor binding was measured using 125I-insulin. Exposure of CA and PA EC to 18:2 for 2 hr caused significant reductions in r₀ for DPH (p < 0.001) and for TMA-DPH (p < 0.02). However, for AO EC it required a longer (4 hr) exposure to cause identical changes in r₀ for both DPH and TMA-DPH. Exposure to 18:2-OOH for 2 hr also caused significant reductions in r₀ for DPH in CA and PA (p < 0.001) EC and in AO (p < 0.02) EC. With more prolonged exposure (4 hr), the decreases in r₀ were proportionately greater in all cell types. In addition, specific insulin binding was increased (p < 0.05) in all cell types exposed to 18:2. These results demonstrate that fatty acids and their peroxides alter membrane physical state and receptor-ligand binding of EC from a various vascular beds (supported in part by ADA-FL).

Comparative Toxicity of UI(2-Ethylhexyl), PHTHALATE (DEHP) and UI-N-UNCTYL PHTHALATE (DOP). A R DeAngelo, J Cticmanec, L P McMillan, and P A Wersoning. U.S. Environmental Protection Agency Health Effects Research Laboratory, Cincinnati, OH.

Toxic responses of male F344 rats to DEHP and DOP were measured to gain insight into DOP's ability and DEHP's inability to promote the outgrowth of liver enzyme-altered foci. Groups of animals received DEHP (0.1, 0.5 and 2.0%) or DOP (0.5 and 1.0%) in the diets for 11 weeks. DEHP, but not DOP, increased liver weight and palmitoyl CoA oxidase activity. DOP did produce marked hepatic changes such as hepatocellular cytomegaly, increased mitotic activity, vacuolization, chronic inflammation, and necrosis. DEHP was only slightly effective at the high dose. The other pathological change seen was severe testicular tubular degeneration in the 2.0% DEHP group. Serum enzyme alterations reflected the liver pathology observed in the DOP treated animals. These studies suggest that DOP might increase the outgrowth of altered liver cells through a regenerative response. (Abstract does not necessarily reflect EPA policy).

Comparative Effects of Bis-(BETA-CHLOROETHYL) SULFIDE (BCES) ON THE DNA OF BASAL AND DIFFERENTIATED KERATINO CYTES R Scavarelli, F L Vaughan and I A Bernstein. Toxicology Program. Dept. Env. Ind. Health, Univ. of Michigan, Ann Arbor, MI.

Isolated rat epidermis and stratified rat keratinocyte cultures were exposed topically to BCES for 0.5 h with concentrations ranging from 0.01 to 50 nmoles/cm². After exposure, the cells from each source were harvested and separated into basal and differentiated cell populations. The formation of DNA single strand breaks was measured in both populations by the nucleosome velocity sedimentation assay. The level of DNA alkylation in the two cell types was also measured by first exposing rat epidermis and keratinocyte cultures to "C-BCES for 30 min. Concentrations ranged from 5 to 50 nmoles/cm². DNA from the cell populations obtained from each source was then isolated using a Csat density gradient. These studies demonstrated that the basal cells are more adversely affected by BCES than differentiated cells as indicated by more single strand breaks and higher DNA alkylation levels resulting in the former after similar exposure.
Previous studies in our laboratory demonstrated that after a topical exposure of a stratified keratinocyte culture system to BCES, the DNA synthesis of the culture was inhibited in the concentration range of 0.01 to 500 nmoles/cm². This investigation was initiated to determine if this resulted from the inhibition of semi-conservative DNA synthesis. A method that distinguishes between semi-conservative DNA synthesis and repair synthesis was used which involved labeling the cells with 5-bromodeoxyuridine and separating the newly synthesized heavy DNA from the total DNA in a CsCl density gradient. From these studies it has been determined that semi-conservative DNA synthesis was indeed inhibited in the concentration range of 1-10 nmoles/cm² up to 6 h after a 30 min exposure to BCES. These results indicate that sulfur mustard prevents cells from undergoing division which could seriously affect tissue integrity.

The pyrrolizidine alkaloid senecionine produced an increase in cytosolic free Ca²⁺ concentration in isolated hepatocytes which correlated with an increase in cellular toxicity. The cytotoxicity was greater in the absence of extracellular Ca²⁺ than in its presence, suggesting that alterations in intracellular Ca²⁺ distribution, and not an influx of extracellular Ca²⁺, was responsible for the senecionine-induced hepatotoxicity.

154 STUDIES ON THE EFFECT OF NONENOTOXIC DRINKING WATER CONTAMINANTS ON LIVER DNA SYNTHESIS AND ON THE ACTIVATION OF UNCOGENES IN DIFFERENT MOUSE STRAINS. T V Reddy, A E DeAngelo, H A Perelis, F B Daniel, J C Kanda, R G Stuntz, U S EPA, ELF, Cincinnati, OH, ERAT, Cincinnati, OH and University of Missouri, Columbia, MO

Strains of mice with different susceptibilities for spontaneous tumors (C3H > BALB/c > C58/1C/6) were implanted (S.C.) with osmotic mini pumps filled with [H³] thymidine and then intubated daily for 5 days with nongenotoxic drinking water contaminants ( perchloroethylene 7 mmol/kg, trichloroethylene 18 mmol/kg, trichloroacetic acid 2 mmol/kg, and chloroform 1.5 mmol/kg) and phenobarbital as positive control (20 mmol/kg). On day seven the mice were sacrificed and liver DNA specific activity and mitotic index were determined. All test chemicals and positive control exerted a stimulatory effect on DNA synthesis as compared to corn oil control. However, the differences between the strains were not significant. Hence no positive correlation was obtained between DNA synthesis and spontaneous tumor susceptibility in mouse strains. We are currently investigating the levels of expression of mRNA for proto-oncogenes C-Ha-ras, C-Ki-ras, C-myc, C-fos, raf, and Src in liver and their response to chemical treatment in an attempt to implicate any differences in the pattern of oncogenes to the differences in susceptibility of mouse strains to spontaneous tumors (Abstract does not necessarily reflect EPA policy).

155 THE EFFECT OF THE PYRROLIZIDINE ALKALOID SENECTION AND THE ALKENALS TRANS-4-0H-2-HEXENAL AND TRANS-2-HEXENAL ON INTRACELLULAR CALCIUM COMPARTMENTATION IN ISOLATED HEPATOCYTES. D S Griffin and H J Segall. WV/Pharmacology and Toxicology, University of California, Davis, CA

The pyrrolizidine alkaloid senecionine produced an increase in cytosolic free Ca²⁺ concentration in isolated hepatocytes which correlated with an increase in cellular toxicity. The cytotoxicity was greater in the absence of extracellular Ca²⁺ than in its presence, suggesting that alterations in intracellular Ca²⁺ distribution, and not an influx of extracellular Ca²⁺, was responsible for the senecionine-induced hepatotoxicity. The effect of senecionine on the sequestration of Ca²⁺ in mitochondrial and extramitochondrial compartments was examined in isolated hepatocytes: as well as the effects of trans-4-OH-2-hexenal, a microsomal metabolite of senecionine, and a related alkenal, trans-2-hexenal. Each of the test compounds elicited a decrease in the available extramitochondrial Ca²⁺ stores which was inhibited by pretreatment with dithiothreitol. Senecionine and t-4HH decreased the level of Ca²⁺ sequestered in the mitochondrial compartment of hepatocytes. The presence of a pyridine nucleotide reducing agent, B-hydroxybutyrate, inhibited this reduction. These results suggest that senecionine and t-4HH inhibit the sequestration of Ca²⁺ in extramitochondrial and mitochondrial compartments possibly by inactivating free sulfhydryl groups and oxidizing pyridine nucleotides.


The oral administration of DOTC to young rats induces marked thymic atrophy. Previous experiments conducted at BIBRA have demonstrated that this characteristic lesion is associated at an early stage with both a suppression in spontaneous in vitro thymocyte proliferation and concomitant loss of MRC OX 19 positive, mature medullary thymocytes. Since recent evidence suggests that interleukin-2 (IL-2) plays a central role in the proliferation seen in the active thymus, studies were conducted to evaluate whether DOTC exerts its anti-proliferative effects by compromising IL-2 production. Using an mRNA cytoplasmic dot blot technique, the examination of thymocyte lysates from treated and control animals revealed that DOTC markedly down-regulates, and at high doses abolishes, the normal expression of the IL-2 gene. The housekeeper gene β-actin is however unaffected by its action thus demonstrating the selective effect of DOTC reported by the UK Ministry of Agriculture, Fisheries and Food).
157 EFFECTS OF QUINOLONES ON PROTEOGLYCANS (PGs) IN ADULT AND JUVENILE CANINE CARTILAGE. M J Palmski, P D Williams, D A Laska, J S Bean. Bristol-Myers, Syracuse, NY

Quinolones induce lesions in cartilage from joints of juvenile dogs. Since PGs are necessary for cartilage integrity and function, the effects of quinolones on cartilage PG metabolism were studied. Dose dependent inhibition of PG synthesis was observed in adult canine knee cartilage slices cultured for 48 h in media containing 3S and either ciprofloxacin, norfloxacin, oxolinic acid or nalidixic acid with IC50 values of 80, 85, 230 and 290 µM, respectively. PG degradation, S-PG release into the culture medium, was increased only with norfloxacin and ciprofloxacin (200% of controls at 200 µg/ml). Degradation of total PGs, measured by dimethylle blue, was not increased. Samples from juvenile dogs (18 wks) responded similarly to adult cartilage after norfloxacin exposure with respect to inhibition of PG synthesis. PG degradation was not increased. Adult and juvenile dogs were dosed orally with norfloxacin (100 mg/kg x 7 days) and after sacrifice cartilage slices were cultured with 3S for 48 h. PG synthesis was reduced to a similar extent in adult and juvenile cartilage (30% of controls), while degradation of total PGs was increased only in juvenile cartilage (28% of controls). These results suggest that quinolone effects on PG metabolism may be important in the pathogenesis of articular lesions.

158 Bacillus thuringiensis israelensis CYTOLYTIC TOXIN: INTERACTION WITH CELL MEMBRANE. E Chow, L Shi, S S Gill. Division of Toxicology and Physiology, University of California, Riverside, CA

Bacillus thuringiensis israelensis (BTI) is an effective agent for the control of disease vectors. This bacterium produces parasporal crystalline inclusions, containing 28, 65, and 130kDa protein toxins, which are selectively toxic to mosquito and blackfly larvae. Upon alkaline solubilization the 28kDa toxin is hydrolyzed to a 25kDa toxin by proteases. The interaction of the 25kDa toxin of BTI with cell membrane lipids was investigated utilizing radiolabeled toxin, monoclonal antibodies and sucrose density gradient centrifugation. Once bound, it is not possible to remove the toxin even after extensive washing. The membrane bound toxin is, however, recognized by most of the twenty six monoclonal antibodies (9 IgG, 17 IgM) tested. All of these monoclonal antibodies can decrease the cytotoxicity of the 25kDa toxin. None of the monoclonal antibodies, however, significantly inhibits the binding of the radiolabeled toxin. Analysis of the cell membrane bound toxin by sucrose density gradient reveals aggregation of the toxin at the cell surface with an approximate molecular weight of several hundred thousand kDa. These and additional data indicate the probable existence of three different domains in the toxin molecule for binding, cell lysis, and toxin aggregation.

159 PURIFICATION AND CHARACTERIZATION OF AN EPOXIDE HYDROLASE FROM THE MITOCHONDRIAL/PEROXISOMAL FRACTION OF MOUSE LIVER. S S Gill and C Chang. Division of Toxicology and Physiology, University of California, Riverside, CA

Epoxy hydrolase activity has been reported to occur in most subcellular fractions of mouse liver. The epoxide hydrolases in the microsomal and cytosolic fractions have been purified and characterized, however, the nature of the epoxide hydrolase(s) in the crude mitochondrial/peroxisomal fraction is not known. Therefore the epoxide hydrolase from this fraction was purified. Crude 12,000g pellet from mouse liver, free from cytosolic contamination, was sonicated to obtain a 105,000g soluble fraction containing 80% of the original activity. Epoxy hydrolase in this fraction was purified, using trans-stilbene oxide (TSO) as substrate, by a combination of affinity and hydroxyapatite chromatography. The purified mitochondrial/peroxisomal epoxide hydrolase had a native molecular weight of 56kDa, a molecular weight of 55kDa by SDS-PAGE, and a pi of 5.5. The purified enzyme was observed to be immunologically similar to the cytosolic epoxide hydrolase. The kinetics of hydrolysis of TSO were also similar. The purified mitochondrial/peroxisomal enzyme thus appears to be very similar, if not identical, to the cytosolic epoxide hydrolase. The basis for the subcellular localization of this epoxide hydrolase in the mitochondrial/peroxisomal fraction is not known and is currently under investigation.


Anatoxin-a(s) [antx-a(s)], produced by the freshwater cyanobacterium (blue-green alga) Anabaena flos-aquae NRC-525-17, has been shown to be an anticholinesterase with chemical/functional characteristics similar to the organophosphate anticholinesterases (k: IIP 0.03 um-1 min-1, antx-a(s) 1.36 um-1 min-1). The symptoms of antx-a(s) intoxication include salivation, lacrimation, respiratory distress and muscle fasciculations which suggest direct nicotinic and muscarinic receptor activity along with its anticholinesterase activity. Isolated frog rectus abdominis muscle and the isolated, denervated guinea pig ileum were used to test for nicotinic and muscarinic activity, respectively. Acetylcholine (ACH, 0.01 to 100 uM) was used to generate a standard curve. Antx-a(s) (4 uM) was tested and showed a slowly rising spontaneous contraction in both the frog rectus abdominis and the guinea pig ileum (DPF at 1 uM has been shown to produce a similar response in the guinea pig ileum). Extensive washing brought the muscles back to resting levels and addition of 1 uM ACH showed an increase in twitch height above that elicited by ACH alone.
Previous studies have demonstrated that incubation of isolated rat hepatocytes in Ca²⁺ free media results in malondialdehyde (MDA) production and cell injury (LDH and K⁺ leakage). We have further investigated this phenomenon and compared it to oxidative cell injury induced by paraquat and 1,3-bis(2-chloroethyl)-1-nitrosourea (PQ/BCNU) treatment. Vitamin E (VE) and desferrioxamine (Desf) prevented MDA formation and LDH leakage in both systems. Ruthenium red (RR) and La³⁺, which block Ca²⁺ translocation through the mitochondrial unipor, prevented MDA formation, GSH and protein-GH loss, VE loss, and LDH leakage induced by Ca²⁺ omission, but had no effect on cell damage resulting from PQ/BCNU treatment. Ca²⁺ omission promoted a marked loss of mitochondrial transmembrane potential (ΔΨ) which was prevented by RR, ESTA, VE and Desf. In contrast, PQ/BCNU exposure had little effect on ΔΨ. Treatment with the protonophore CCCP resulted in no LDH leakage or MDA formation, but caused a complete loss of ΔΨ which was not prevented by VE or RR. These studies indicate that in the absence of extracellular Ca²⁺ mitochondrial Ca²⁺ cycling contributes to the observed oxidative stress and resultant loss of cell viability. (Supported by USPHS ES05422 and ES01978)

A mixture of 25 chemicals, frequently found in groundwater, including some heavy metals, aromatic hydrocarbons and halogenated solvents was formulated in water for toxicologic studies (see Yang et al., this issue). Male F344 rats were treated with the mixture at two levels, both near environmentally detected concentrations, for 14 days via drinking water. Body weight and relative liver weights were decreased in the high dose group concomitant with decreased water intake of about 50% compared to controls. The ADP-stimulated succinate oxidation rate in isolated hepatic mitochondria was inhibited by 33% in the high dose group and 19% in the low dose group. Liver microsomal cytochrome P-450 and aryl hydrocarbon hydroxylase (AHH) showed positive trends of induction. AHH activity was significantly higher (60%) in the high dose group than the control group. Clinical chemistry showed little change. The results of this study show that a chemical mixture, at very low concentrations of its component chemicals, could inhibit ADP phosphorylation in hepatic mitochondria and induce microsomal AHH. Comparative in vivo (gavage) and in vitro studies on this mixture are being performed.

Paraquat (PQ) normally induces superoxide dismutase (SOD) and catalase in cells. This demonstrates that superoxide & hydrogen peroxide are involved in PQ toxicity. The ultimate target in cell death has not been conclusively identified although lipid/DNA damage, and alterations in SMP and PQP is thought to be all been suggested. We attempted to identify the cellular target in paraquat toxicity by breeding a PQ-resistant cell line (PQR) (resistant to >80µM/3 days) containing low SOD and catalase activity as compared to the parent line, and studying how such a cell defends itself. The resistance is not due to alterations in PQ uptake. Increases in glutathione peroxidase, transferase, and reductase were found suggesting that peroxidative damage is important in cell death from PQ. The major PQ reducing enzyme NADPH-cytochrome C reductase was unaltered. The alterations in anti-oxidant enzymes were not due to mutations as activity was restored to normal upon removal from PQ. We are currently studying isoenzyme alterations in GS transferase and other important enzymes. We are also altering the SOD concentration of the PQR line by using a pSVMeo-pSV(Cu/Zn-SOD)CDNA vector to see if increased SOD activity will either decrease or further increase PQ resistance.
Acetylcholinesterase (AChE) activity in living cells rapidly recovers from inhibition by organophosphorus esters (OPS) by synthesis of new enzyme. The rate of OP-inhibited AChE and its effect on apparent AChE synthesis was examined using 2-pralidoxime (2-PAM) to reactivate OP-inhibited AChE in quail embryo skeletal muscle cultures. Ten day embryo muscle cells were grown for 10 days in 10% horse serum, 2% embryo extract and 80% Eagles MEM. Cultures were treated with 0.1 uM paraxson for 10 minutes and reappearance of AChE activity in cells and medium was studied after various times, treatments and 10mM 2-PAM. The increase in newly synthesized AChE and the decrease in reactivatable OP-inhibited AChE over time were such that the total AChE of the cell remained relatively constant for more than 72 hours. Release of newly synthesized reactivable AChE into the medium was consistent with the idea that AChE moves in an orderly sequence from the inside to the outside of the cell. Supported by NIH ES-00202.

The toxicological endpoints of acetaminophen (AA) can be grouped into 5 categories: (I) Enzymes (a) Covalent binding of AA metabolite to proteins catalyzed by horseradish peroxidase; (b) Covalent binding catalyzed by prostaglandin synthetase, (II) Subcellular organelles (a) Covalent binding catalyzed by microsomes; (b) Inhibition of oxidative phosphorylation, (III) Cells (a) Enzyme leakage from hepatocytes; (b) Covalent binding catalyzed by pholoster-activated neutrophils; (c) Activation of superoxide anion production in Kupfer cells, (IV) Organs (a) Lactate production in the liver; (b) Coma in the brain), and (V) Organisms (Potentiating effect of acetic alcohol on acetaminophen hepatotoxicity mediated by the pituitary and thyroid glands). Experimental evidence to support Categories IIA, IIB, IIIA, and V have been obtained recently in our laboratories. Although certain toxicological endpoints observed at higher levels of organization can be linked to those manifested at lower levels (II and III), some toxic effects of AA are not directly related to toxic manifestations at lower levels (IIIC and V); certain toxicological endpoints emerge as a result of the increased complexity of the test system, reminiscent of “emergent properties” in physics and chemistry. Supported by AAAS45.

Homogenizing medium makes a big difference in the measurement of the effect of CCl₄ on subcellular calcium transport. V. Prakash and A. Agarwal. Toxicology Research and Training Center, John Jay College of CUNY, New York, NY. Sponsor: H. M. Mendelsohn

Ethanol-induced microencephaly and inhibition of phosphoinositide metabolism. Lucio G. Costa and Walter Balduini, Dept. of Environmental Health, Univ. of Washington, Seattle, WA 98195

The pattern of muscarinic receptor (MR)-stimulated phosphoinositide (PI) metabolism during postnatal development has a striking resemblance with the curve of rat brain growth spurt (JPET, 241: 421, 1987). Therefore, the enhanced hydrolysis of membrane PIs by cholinergic agonists during this period may have a relevant role in cell proliferation and differentiation. We have investigated whether exposure of rat pups to ethanol (EtOH) during the brain growth spurt would alter MR-stimulated PI metabolism in cerebral cortex. Female Long-Evans rats were administered 4 kg EtOH by gastric intubation from postnatal day 4 to day 10. This treatment caused microencephaly but did not have any effect on the pups' body weight as compared to an equally-handled, sucrose-fed group of animals. MR-stimulated PI metabolism was measured in cerebral cortex slices of control and EtOH-treated rats at days 7, 12, 20, and 45 of age and was significantly reduced in EtOH-exposed animals only on day 7. A similar treatment in adult rats did not cause alteration in any of the biochemical parameters measured, despite similar blood EtOH concentrations. These results confirm that the developing brain is particularly sensitive to the effects of EtOH, and suggest that the PI system coupled to MR could represent a likely biochemical target for its toxicity. (Supp. by ADAQ, U. of Nash, and the Dana Foundation.)
ETHYLENE DIBROMIDE (EDB): MULTIGENERATIONAL NEUROTOXIC EFFECTS OF MATERNAL EXPOSURE. L. L. Hsu, M. S. Legator, and P. M. Adams. Dept. of K.M.C. and PMCH, UTMB, Galveston, TX, Dept. of Psychiatry, UTHSC, Dallas, TX Sponsor: G. S. Anvari.

In this study, we assessed the male mediated EDB effects on the brain chemistry of F1 progeny. Young male Fisher 364 rats were given EDB (10 mg/kg) daily with corn oil or 1.25 or 5 mg/kg of EDB/kg, for 5 days. After 7 days, the EDB and corn oil treated males were crossed with untreated virgin females. Young adult male F1 progeny were crossed with their respective litter mates. Brain regions of F1 progeny at 90 days old, including cerebellum (CB), corpus striatum (CS), frontal cortex (FC), hippocampus (HIPP) and thalamus (HY) were assayed for AChE, AChE and GAD. Results indicated: (1) paternal exposure to 1.25 mg EDB increased ChAT in CS and FC of both male and female, it increased ChAT in only male CS and had no effects on ChAT in HY or HIPP; it decreased AChE in all male (but not female) brain regions, and increased AChE in the only male FC. (2) paternal exposure to 5 mg EDB increased ChAT in the male CS but decreased ChAT in the female CS and HIPP, and did not change ChAT in the male brain; it markedly increased (114%) the AChE in the female CS but decreased (40%) AChE in female HIPP and did not affect the male brain. AChE and ChAT were increased in both the male (88%) and female (62%) CS but had no effect on GAD in other brain regions. (Supported by NIOSH Grant No. OH91245)


2.5-Hexanediol (HD) produces a central-peripheral distal axonopathy which is characterized by multi-local swellings of distal axons. The present study examined the possibility that alterations in the phosphorylation of axonal proteins and phospholipids are involved in the neurotoxic mechanism of HD. Male Sprague-Dawley rats (250-300 gm) were divided into groups (n=6) based on the daily treatment to be received: HD (400 mg/kg), 1.6-Hexanediol (414 mg/kg) or saline (3 ml/kg). Groups of animals were injected with chemicals or saline according to one of the following treatment schedules: 7, 15 or 24 days. In a separate experiment rats were treated with HD, hexanediol or saline for 24 days at which time treatments were discontinued for 17 days. At the appropriate time animals were sacrificed by decapitation and both sciatic nerves removed. Nerves were desheathed and incubated for 2 hrs in oxygenated Krebs buffer containing 334 orthophosphate. Nerves were cut into proximal and distal halves and incorporation of radioactivity was assayed by liquid scintillation spectrometry. Morphological analysis (axonal diameter) was conducted to correlate biochemical changes with nerve damage. Results showed that in proximal nerve segments, HD-treated animals exhibited reversible, time-related selective increases in insoluble incorporation into the 160K (unidentified) and 55K (tubulin) protein bands, while in distal segments changes were not evident. Radiolabel incorporation into myelin proteins P1, P2 and P0 as well as into phospholipids revealed inconsistent changes. Morphological analysis showed HD intoxicated animals (24 day treatment) to have significant increases in fiber diameter both proximally and distally (66% and 70%, respectively) when compared to controls. These results suggest that alterations in phosphorylation of proteins might play a role in HD-induced nerve damage. (Supported by NIH grants ESO 3830 and DK 30577).

EFFECTS OF NEUROFILAMENTOUS AXONOPATHY-PRODUCING NEUROTOXICANTS UPON FAST ANTEROGRADE TRANSPORT. D W Sickles. Medical College of Georgia, Augusta, GA.

We hypothesize that neurofilamentous axonopathy-producing toxicants which also produce nerve degeneration adversely affect fast anterograde transport of radiolabeled protein in rat sciatic nerve after injection of 3H-leucine into the DRG were determined. Acrylamide (ACR; 50-100mg/kg) produced a 9.2-20.9% decrease in rate and 42.7-51.4% decrease in quantity of protein transport (Sickles and Pearson, Toxicologist 7:132,1987). 2.5-Hexanediol (2.5-HD; 4-8 mmoles/kg) and 3,4-dimethyl-2,5-HD (0.25-0.5 mmoles/kg) caused a 16.8-22.9% and 15-35% decrease in rate, respectively; the total protein transport was decreased 49.7-61.6% and 46.8-69.6%. The incorporation of 3H-leucine was not decreased by ACR, 2.5-HD or DMED. DPN (0.1%), which causes a neurofilamentous axonopathy without nerve degeneration, and methylene-bis-ACR (108.5 mg/kg) had no significant effect upon rate or quantity of protein transport. This is the first report of a correlation between nerve degeneration and primary decreases in quantity of protein transport caused by single doses of these neurotoxicants. Supported by National Institute of Occupational Safety and Health, #OH 02020.

MICROSOMAL ATPASE ACTIVITY FOLLOWING LONG-TERM EXPOSURE TO 2,5-HEDANEONE. J K Pearson and D W Sickles. Medical College of Georgia, Augusta, GA.

2,5-Hexanediol (HD), and 3.4-Dimethyl-2,5-Hexanediol (DMED) are neurotoxicants known to produce a neurofilamentous axonopathy form of neuropathy by an unknown mechanism. It has been proposed that inadequate supply and transport of vital macromolecules to the distal axon is responsible for the nerve degeneration. We have hypothesized that gamma-diketones produce these changes through interference with the microtubules. It has been demonstrated that HD and DMED affect both the rate and the quantity of radiolabeled protein transported in rat sciatic nerve (Sickles, Toxicologist 8, 1988). The current study tests the possibility that HD compromises the energy utilizing protein (ATPase) associated with axonal transport, which has been demonstrated to be a Ca/Mg-dependent ATPase associated with the microtubules found in the microsomal fraction. This ATPase activity of the microsomal fraction of rat sciatic nerve, following 3 and 4 week exposure to HD (4 mm/kg daily), showed no significant differences between control and experimental nerves. We conclude that HD does not affect transport via inhibition of the transport ATPase. Supported in part by National Institute of Occupational Safety and Health #OH-02020.
THE DECREASE IN AXONAL TRANSPORT OF PROTEINS IN THE RAT OPTIC SYSTEM PRODUCED BY XYLENE INHALATION IS REVERSED BY ETHANOL CONSUMPTION. S Padilla, C N. Pope*, and D P. Lyrleýty*, U.S. EPA, NRTHomework Services, RTP, NC.

Because of the possible metabolic interaction between ethanol and xylene, coupled with the knowledge that human exposure to solvents are often complicated by ethanol ingestion, we investigated the effect of ethanol on the p-xylene-induced decrease in axonal transport in the rat optic system previously reported by our laboratory. Long-Evans, hooded, male, rats were divided randomly into groups (n=8/group): no ethanol and no xylene (Group A); ethanol (10%) in drinking water 6 days prior to and during inhalation exposure but no xylene (Group B); no ethanol, but xylene (inhalation, 1600 ppm, 5 hr/d, 5 d/week, for 8 exposure days) (Group C); or both ethanol and xylene exposure (Group D). Ethanol alone produced no decrease in the axonal transport of [35S]methionine-labeled proteins, i.e., there were no significant differences in radiolabeling of the retinal ganglion cell projections in groups A and B. There was, however, 34% decrease in axonal transport of proteins in the xylene-exposed animals (Group C), while the axonal transport in the xylene-exposed animals receiving ethanol (Group D) equaled that measured in the ethanol controls (Group B). These data suggest that the substantial reduction in protein transport seen in xylene-treated animals was reversed by sub-chronic ethanol consumption. (*Supported by NRC Research Associateship.)

ISOLATED RAT RETINAL MITOCHONDRIAL RESPIRATION: IN VITRO AND IN VIVO LEAD STUDIES. D A Fox, C J Medrano and S D Rubinstein. University of Houston, College of Optometry, Houston, TX.

Developmental Pb exposure produces long-term rod selective degeneration. Although Pb has multiple sites and proposed mechanisms of action, a direct inhibitory effect of Pb on mitochondrial respiration is suggested to be a fundamental and common underlying mechanism accounting for neuronal necrosis. The inhibition of energy metabolism by Pb in isolated whole rat retina (SOT, 1987) supports this idea. However, to determine if Pb inhibits retinal mitochondrial respiration we developed an isolation methodology since, to our knowledge, there were none. Based on the brain preparation of Clark and Nicklas (JBC, 1970) we isolated retinal mitochondria with good respiratory control ratios (mean OCRs: 6.0), State 3 and 4 respiratory rates (mean S3: 31.5 and mean S4: 5.2 mM O2/μg/min) and ADP/O ratios (mean: 3.1) using the NAD-linked substrates glutamate and malate. Further characterization will use enzymatic assays, substrates and RM. In vitro Pb (10-300 μM; incubated for 5 min) produced large dose-dependent decreases in OCR, S3 and ADP/O. S4 is increased at low Pb and decreased at high Pb. In vivo Pb produces large decreases in OCR, S3, S4, ADP/O and more labile mitochondria. These results demonstrate a direct inhibitory effect of Pb on retinal mitochondria and suggest that this effect may contribute to the Pb-induced retinal degeneration. Supported by ES 03183 and BY 07088.

EFFECTS OF LEAD AND POTASSIUM ON RETINAL ATPases. S D Rubinstein and D A Fox. University of Houston, College of Optometry, Houston, TX.

In vitro and in vivo lead exposure produce an inhibition of non-stimulated respiration (NSR) and an enhancement of 50 mM potassium-stimulated respiration (KSR) in isolated whole rat retina (SOT, 1987). Our recent experiments show that: (1) ouabain produces an inhibition of KSR and blocks the enhanced KSR produced by Pb and (2) 30% of retinal metabolism is due to Na-K ATPase activity. These results suggest that Pb may alter retinal metabolism via changes in Na-K ATPase. Studies examined the effects of Pb (10-500 μM) and 50 mM KCl on total, ouabain insensitive (basic) and ouabain sensitive (Na-K) ATPases in rat retinal homogenates using Winkler and Rileys procedures (1977). In adult hooded rat retina, basic and Na-K ATPase comprise 53% and 47% of the total ATPase activity. A two min pre-incubation of the homogenete with Pb produces dose-response inhibition of total and Na-K ATPase, with Na-K exhibiting a greater inhibition at all Pb concentrations. Ion-substitution studies reveal that KCl decreases Na-K activity (-8%). In contrast, pre-incubation with Pb (100 or 250 μM) prior to KCl increases the activity of total ATPase (10%) and Na-K ATPase (40-70%). These results are similar to those seen in the isolated whole retina and suggest that Pb alters retinal metabolism via a yet to be determined interaction with Na-K ATPase. Supported by ES 03183, BY 07088, Sigma Xi and Beta Sigma Kappa.

INCREASES IN FREE INTRACELLULAR Ca2+ ACCOMPANY EXPOSURE OF NEUROHYBRIDOMA CELLS TO THE INSECTICIDE, LINDANE. R M Joy, W W Burns and L G Stark. Depts. of Pharmacology/Toxicology and Physiological Sciences, School of Veterinary Medicine, and Dept. of Pharmacology, School of Medicine, University of California, Davis, CA.

Exposure to the gamma-isomer of hexachlorocyclohexane (lindane) enhances neuronal excitability and convulsions. A role for increased intracellular Ca2+ in these effects has been proposed. We report here that cultured neurohybridoma cells exhibit dose-dependent elevations in free intracellular Ca2+ upon exposure to lindane. Free intracellular Ca2+ was measured using the INDO-1 method. Ca2+ levels were determined after exposure of cells to various concentrations of lindane for periods ranging from 5-45 minutes. Control Ca2+ levels were 234 ± 4.8 nanomolar (X ± SF, N = 48). Exposures to lindane (10-9 - 4x10-6 M) resulted in a dose-dependent increase in free Ca2+ that ranged from 150-220 nanomolar. A time-response study using 10-4 M lindane indicated that the maximal effect had occurred by 5 minutes, the shortest time period tested. Free Ca2+ remained elevated above basal levels throughout the remaining 40 minutes examined.
Electrophysiological studies employing amphibian neuromuscular preparations have shown that Hg in vitro increases both spontaneous and evoked transmitter release. These Hg effects are similar to those produced by the NKA inhibitor ouabain. A number of reports have shown that brain NKA is sensitive to inhibition by Hg. The present study examines the role of NKA in mediating the effects of Hg on neurotransmitter release. Purified rat striatal synaptosomes were either: (a) preloaded with 3H-dopamine (DA), superfused, and release examined, or (b) lysed, and membranes exposed to Hg and NKA activity measured. Hg (3 μM) causes an increase in spontaneous DA release from intact synaptosomes that is insensitive to changes in extracellular Ca2+ levels. Lowering the Na+ concentration (replaced by choline chloride or sucrose) in the superfusing buffer attenuates the Hg effect. Addition of ouabain to Ca2+-free superfusing buffer causes an increase in spontaneous DA release but does not inhibit the Hg effect. Specific activity of NKA in lysed synaptosomal membranes is sensitive to Hg inhibition (IC50=160nM). These data suggest that Hg inhibition of NKA activity may, in part mediate Hg-induced increases in DA release. (Supported by ES-03992.)


Methylmercury (MeHg) in vitro has been shown to increase spontaneous acetylcholine (ACh) release from presynaptic motor terminals. This effect has been attributed to an increase in the intraneuronal ionized calcium [Ca]i that apparently results from a MeHg-induced increase in mitochondrial Ca efflux (Atchison, 1987). In the present study, MeHg was shown to produce a concentration-dependent (0.5-5μM) increase in the spontaneous release of several CNS transmitter substrates (ACh, dopamine, GABA) from superfused rat brain synaptosomes. However, MeHg (under similar exposure conditions) did not produce a corresponding increase in 45Ca efflux from superfused synaptosomes preloaded with 45Ca. An increase in synaptosomal 45Ca efflux would be expected if MeHg was increasing intraneuronal [Ca]i. These results suggest that if the MeHg-induced increase in spontaneous transmitter release results from an increase in intraneuronal [Ca]i, then this [Ca]i is not being extruded from the nerve terminal, due to either increased intraneuronal buffering of the [Ca]i, or the simultaneous inhibition by MeHg of [Ca]i extrusion mechanisms (e.g. Ca-Mg-ATPase). Alternatively, MeHg may be increasing transmitter release by mechanisms not involving an increase in intraneuronal [Ca]i. (Supported by ES-03992.)

**EFFECTS OF MERCURIC CHLORIDE (Hg) ON SPONTANEOUS TRANSMITTER RELEASE AND Na+,K+-ATPase (NKA) IN SYNAPTOSOMES.** M F Hare and D Minnema, Dept. Environ. Hth., U. Cinti., Cinti., OH. Sponsor: E J O'Flaherty.

It should be noted that the use of the term 'in vitro' does not necessarily imply that the results obtained in vitro are directly applicable to in vivo situations. The use of isolated tissue preparations allows for the manipulation and control of variables that are not possible in vivo. This can facilitate the elucidation of mechanisms and the development of therapeutic strategies. However, the results presented here should be interpreted with caution, as they are subject to the limitations and assumptions inherent in in vitro studies.

**MANGANESE TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER IN THE RAT.** L E Kerper, M Aschner, J D Obourn, and T W Clarkson, Environmental Health Sciences Center, School of Medicine and Dentistry, University of Rochester, Rochester, NY.

The mechanism of Mn transport across the rat blood-brain barrier was investigated in the rat. Radiolabelled carrier free 54Mn was injected into the rat common carotid artery in a Ringer's buffer solution at pH 7.55. Fifteen seconds after injection rats were decapitated and brains were removed and counted by means of gamma scintillation spectrometry. Injection with [U-14C]-sucrose, an inert polar substance which does not penetrate the blood-brain barrier, was used to determine the residual radioactivity in the cerebrovascular space. The transport of 54Mn across the blood-brain barrier appears to be a rate-limited process. [U-14C]-sucrose and 3H-methionine uptake does not change with increased concentrations of injected 54Mn up to 0.55 x 10^-6 M, suggesting that the blood-brain barrier remains functionally intact at these concentrations. Calcium and magnesium do not appear to inhibit 54Mn transport. 54Mn transport does appear to be inhibitable by iron-dextran venous infusion in a dose related manner.

Supported by NIH grants: ES-01247, ES-01248 and ES-07026.

Administration of colchicine into the hippocampus has been reported to produce a reduction in [3H]-quinuclidinyl benzilate [QNB] binding and an increase in choline acetyltransferase (CHAT) activity. The muscarinic cholinergic receptors are known to be linked to the inositolphosphate system inside the cell. To study the time-dependent effect of colchicine on agonist-stimulated turnover of inositolphosphates, colchicine was injected into the dentate gyrus of the hippocampus. The rats were sacrificed 1 wk, 3 wk, or 12 wk post treatment. The hippocampi were removed and sliced. Carbachol, a cholinergic receptor agonist, was used to study the stimulated turnover of inositolphosphates. Colchicine was found to alter the normal pattern of inositolphosphate turnover as early as 1 week after administration. The degree of carbachol-induced stimulation of inositolphosphates was also found to be lower in colchicine-treated rats at the same time periods. Thus, colchicine appears to alter the signal transduction process in the rat hippocampus. These changes may be associated with the compensatory changes associated with the damaged hippocampus.


The neurotoxic effects of aluminium and its association with dementia is a subject of great interest. Aluminium plays a role in dialysis encephalopathy and is found in the senile plaques of Alzheimer's Disease. Its effects may be mediated through the cholinergic system, this being confirmed in rat brain synaptosomes where high affinity choline uptake (ChU) and glucose metabolism were inhibited. We have now exposed foetal rat brain reaggregate cultures from 9 DIV for 96 h to 0.1 mM and 0.01 mM AlCl3 and measured choline acetyltransferase activity (CHAT) at different time-points. Exposure for 48 h produced no effect on CHAT but after 72 and 96 h exposure persistent 30-40% losses of CHAT activity (p<0.01) were found. (Control CHAT activities ranged between 40-50 pmol/min/mg protein). These data are consistent with chronic AlCl3 exposure producing a cholinergic functional deficit or neuronal loss. The IC50 for inhibition of synaptosomal CHU is 0.5mM although 0.1mM AlCl3 as used here depresses glucose metabolism, and carbachol-stimulated inositol phosphate release. The degree of selectivity of this lesion and extent of neuronal loss will be delineated, e.g. by measuring neurofilament protein.


We have recently shown that at low concentrations in vitro (12.5 µM) ECMU produces cholinergic lesions in rat brain reaggregate cultures in a serum-supplemented (S-) medium. Injured or denervated brain secretes neurotrophic factors (NTF's) in an age-dependent manner and NTF's such as TRH and NGF stimulate cholinergic regeneration. We have now tested ECMU in serum-free (S-) reaggregates to examine the production and action of NTF's. Foetal rat brain reaggregates were prepared in S- or S+ medium. ECMU was added to the cultures at 9 DIV (12.5 µM). ECMU (12.5 µM) caused a rapid, HC3 sensitive loss of CHAT activity in S+ reaggregates (38%, 2h). In contrast, S- reaggregates showed no immediate loss of CHAT activity out a 40-50% loss appeared by 48h. Although this was also less than that in S+ reaggregates it may correspond to the HC3 insensitive phase representing cholinergic neuronal loss. In both reaggregate types NGF produced increased CHAT activity with more marked effects in S+ (45%) than in S- medium (20-25% increase). TRH had no effect. Despite a residual CHAT pool in the treated cultures, neither NTF reversed the neurone loss.

184 IN VITRO METHODS FOR ASSESSING NEUROTOXICITY. G C Siek & J K Marquis. Dept. of Pharmacology & Experimental Therapeutics, Boston University School of Medicine, Boston, MA.

In vitro cell culture represents a new approach for screening and mechanistic studies of suspected neurotoxicants. The effects of several drugs and toxicants have been observed in neural cell cultures at concentrations similar to those found in the brain after exposure of animals to doses which cause effects in vivo. Four types of neuronal cell cultures can be used in neurotoxicity studies: organotypic cultures, disperse primary cultures, reaggregated cultures, and cell lines. These are compared and evaluated for routine screening assays and test protocols. Our work on PC12 and NG108-15 cell lines, as well as on primary cultures of rat dorsal root ganglion, is used to provide examples for methods development. Although the specific mechanisms of neurotoxicity are not thoroughly defined, endpoints which can be detected in cell cultures must be identified in order to develop standardized study protocols. Proposed neural-specific endpoints include: neurite outgrowth, neurotoxic esterase activity, morphological counts/scores, immunological markers, electrophysiological parameters, and neurotransmitter-related endpoints. Non-neural endpoints, such as protein synthesis, lipid peroxide levels, and cytotoxicity, should be evaluated in parallel.
Pyridostigmine (PYR), a reversible inhibitor of acetylcholinesterase (AChE) is used to prevent intoxication with irreversible AChE inhibitors. To evaluate the monkey as a model for human response to PYR, the pharmacokinetics of oral PYR (0.29, 0.57, 1.14 mg/kg) and its relationship to the time course of AChE inhibition (in red blood cells, RBC) were determined and compared with human data. The gastrointestinal absorption of PYR was erratic, resulting in multiple concentration maxima. The half-life of PYR in plasma was 2-3 hr (means per dose group). Maximal plasma concentrations of 14 to 45 mg/ml occurred ~1 hr after drug administration and increased proportionally with dose. Maximum inhibition of RBC AChE was 30 to 55% and occurred ~16 min after peak plasma concentrations. Pharmacokinetic-pharmacodynamic modeling showed that this delay is consistent with the slow reversibility of AChE inhibition, or with a slow diffusion of PYR to the enzyme site. Comparison with existing human data showed that the absorption and disposition of PYR are similar in humans and rhesus monkeys, and that 50% AChE inhibition can be achieved at lower plasma concentrations than used clinically in humans. (Supported by USAMMDA, Contract No. DAMD-73-C-3129)

MDMA, an amphetamine derivative, has previously been reported to reduce brain 5-HT concentrations and produce serotonergic nerve terminal pathology up to 4 months after 8 successive doses (40 mg/kg po, BID) in the rat. To determine the sensitivity of the nonhuman primate to MDMA, a total of 9 rhesus monkeys were dosed with either vehicle, 5 or 10 mg/kg MDMA (n=3) by gastric intubation twice per day for 4 days. Home cage spontaneous behavior assessed with an activity check-list was not significantly altered by MDMA dosing. Neither body weights nor follow-up spontaneous behavior assessments 3 days after the last dose were altered by treatment. One month after the last MDMA dose, the monkeys were overdosed with pentobarbital and their brains removed, quickly dissected into frontal cortex (FC), caudate nucleus (CN), hippocampus (H) and brain stem (BS) and frozen on dry ice. HPLC/EC analysis of 5-HT and 5-HIAA revealed a significant, dose-related 8 reduction from control for these monoamines: 5-HT (FC) 82, 91%; (CN) 17, 30%; (H) 74, 83%; (BS) 22, 25% and 5-HIAA (FC) 39, 92%; (CN) 23, 45%; (H) 63, 80%; (BS) 20, 30% for 5 and 10 mg/kg po, respectively. These results indicate that the monkey may be more sensitive than the rat to the persistent serotonergic neurotoxicity of MDMA.

TRIADIMIFON INDUCES STEREOTYPED BEHAVIOR AND ALTERS BIgenic AMINE ACTIVITY IN RATS. G D Walker, M H Lewis, K C Crofant, and R B Mulin. University of North Carolina, Curriculum in Toxicology and Biological Sciences Research Center, Chapel Hill, NC, and Environmental Protection Agency, Research Triangle Park, NC.

Triadimifon, 1-[4-(chlorophenyl)-3,3-dimethyl-1-(1H,2,4-triazol-1-yl)-2-butanone], is a triazole fungicide licensed for use on fruits and grains. Because initial behavioral investigations showed that triadimifon induces hyperactivity in mice and rats, the present study examined its behavioral effects using a computer-supported observational method designed to quantify changes in multiple behavioral topographies. Additionally, neurochemical effects were assessed by quantifying monoamine concentrations in terminal fields of nigrostriatal and mesolimbic dopaminergic pathways. Triadimifon was administered i.p. (0, 50, 100, or 200 mg/kg) in corn oil (2 ml/kg) four hours prior to behavioral assessment to female Sprague-Dawley rats (n=10). Each animal was observed for a one minute period every five minutes for one hour. Triadimifon administration resulted in a highly significant dose-dependent increase in stereotyped behavior. At the highest dose, triadimifon induced intense gnawing, head weaving, backward locomotion, and circling. At lower doses, less intense stereotyped behaviors including gnawing, licking, rearing, and locomotion were observed. Immediately after behavioral testing, rats were sacrificed, and striata and olfactory tubercles removed. Concentrations of dopamine (DA), and its acidic metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), as well as serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), were determined by HPLC with electrochemical detection. Significant dose-related increases in HVA and 5-HIAA were observed in the absence of changes in DOPAC in both brain regions. No significant changes in 5-HT, and an decrease in DA at only the highest dose, were detected.

A mouse adrenergic neural tumor cell line (N2 AB-1) was used as an in vitro model to investigate the toxicity of 1-methyl-4-phenylpyridinium iodide (MPP⁺), the active metabolite of 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). Cells were differentiated by PGE₁/dbcAMP to induce a neuronal phenotype. Both mitotic and differentiated cells were exposed to a moderate concentration of MPP⁺ (34 μM) for 24 to 48 hours. Differentiated cells were less sensitive to the toxic effects of MPP⁺ than mitotic cells, as determined by morphologic observation. MPP⁺ toxicity manifested itself morphologically by increased cytoplasmic inclusions (or vesicles), cytoplasmic blebbing and cell death, noted as an increased number of floating cells and debris. Gangliosides (GA), a neurite promoting factor in the CNS, have been shown to protect neurons in vivo from MPP⁺ toxicity. Cells were treated with 200μg/ml mixed GA. GA induced neurite formation in both mitotic and previously differentiated cells. Mitotic cells appeared to be significantly protected from MPP⁺ toxicity with 24 hour GA pretreatment; while differentiated cells showed slight morphologic protection during the same time period. Tubulin, a structural protein in neurites, was observed for disturbances by MPP⁺; none were seen until the cells were near death. Tetanus toxin immunofluorescence confirmed GA interaction with the cell surface. Neurotransmitter storage and uptake in N2AB-1 cells was confirmed by glyoxylic acid induced histofluorescence.

SPECIES DIFFERENCES IN THE SUBSTRATE AND INHIBITOR SPECIFICITY OF BRAIN ACETYLCHOLINESTERASE. J R Kemp and K B Wallace. Dept. of Pharmacol., Univ. of Minnesota, Duluth, MN.

Rodent brain acetylcholinesterase (ACHE) is more sensitive to in vitro inhibition by diethyl-p-nitrophenyl phosphate than are fish preparations. This investigation assessed differences in the relative size of the catalytic site in rat and rainbow trout detergent-solubilized brain ACHE by examining the kinetics of hydrolysis for thiocholine substrates (acetyl-, propionyl-, and butyryl-) and the inhibitor potencies for a series of dialkyl-p-nitrophenyl phosphates (dimethyl-, diethyl-, dipropyl-). The Michaelis-Menten constants (Kₘ) for rats were determined to be: acetyl (78.9 ± 4.7 μM), propionyl (83.3 ± 4.2 μM) and butyryl (337.8 ± 40.5 μM). The corresponding values for trout were: 202.0 ± 54.1 μM, 2078.5 ± 203.2 μM and not detectable for the butyryl-analog. The inhibitor data revealed similar trends and species differences: the IC₅₀ for dimethyl- and diethyl- were comparable for rats whereas in trout the IC₅₀ increased progressively with increasing methylene substitutions. These data are consistent with the exclusion of large substrates and inhibitors by the relatively small catalytic site of fish ACHE and may have important implications regarding the comparative toxicity of anticholinesterase agents of varying molecular size. (Supported in part by U.S. EPA CR-810983 and by a grant-in-aid from the Univ. of Minn. Graduate School.)


Endocrine mechanisms associated with fescue summer toxicity in steers (n=6, each treatment) grazed on endophyte infected versus endophyte free fescue have been studied. Reduced prolactin (9.28 vs. 30.49 ng/ml) and average daily gains (-0.15 vs. +0.57 lbs) have been correlated (P<0.05, t=15 wks) with elevated anterior pituitary dihydroxyphenylacetic acid (108 ± 59 ng/g) and 5-hydroxyindoleacetic acids (265 ± 148 ng/g) (major metabolites of dopamine, DA, and serotonin, 5HT, respectively). In addition, diurnal pineal 5-hydroxytryptophan (5HTP, precursor to 5HT) was elevated (502 vs 280 ng/g, n=4) while the 5HT/5HTP ratio was depressed (0.589 vs 0.879). No significant differences were observed in the neurochemicals measured in the hypothalamus. The results suggest a possible pituitary-pineal (induced) involvement in the seasonal fescue summer toxicity observed in steers grazed on endophyte infected fescue.

EFFECT OF CYANIDE ON BRAIN ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION. C F Egan, J L Borowitz and B K Ardelt. Dept. of Pharmacology & Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN.

Accompanying inhibition of cytochrome oxidase, CN initiates a series of intracellular events resulting in cellular injury. CN alters neuronal Ca²⁺ homeostasis resulting in accumulation of cytosolic Ca²⁺ and subsequent peroxidation of lipids via a Ca²⁺-activated xanthine oxidase mediated process. In the present study, the status of the brain antioxidant enzymes in mice intoxicated with KCN was correlated with lipid peroxidation. Thirty min after the administration of KCN (7 mg/kg, sc), activity of brain superoxide dismutase (SOD), catalase (CA), glutathione peroxidase (GP) and glutathione reductase (GR) were significantly reduced (percent inhibition: SOD 15%, GA 44%, GP 23%, CR 38%). Reduction of enzyme activity was accompanied by lipid peroxidation of brain membranes as measured by the conjugated diene method. These intracellular responses may partially account for the neurotoxicity of CN. (Supported in part by PHS grant ES04140).
**PYRETHROID INSECTICIDES ALTER MEMBRANE POTENTIAL IN FISH AND RAT BRAIN SYNAPTOSOMES.**


Pyrethroid insecticides modify the ionic permeability of nerve membranes producing distinctive poisoning syndromes in insects, fish and mammals. Fish are more sensitive than mammals to pyrethroids. The aim of this investigation was to compare the actions of the pyrethroid, deltamethrin (DM), on membrane potential in trout and rat brain synaptosomes. Accumulation of $^3$H-tetraphenylphosphonium (TPP) was used to estimate membrane potential.

In trout synaptosomes, DM decreased TPP accumulation in contrast to its actions in rat synaptosomes where an increase in TPP accumulation was observed. These data indicate that DM depolarized fish synaptosomes and hyperpolarized rat synaptosomes. The hyperpolarizing effect of DM in rat synaptosomes was reversed by the addition of the sodium channel activator, veratridine (Vtd), resulting in a decrease in TPP accumulation greater than that observed with Vtd alone.

Fish brain synaptosomes were more sensitive to the depolarizing actions of DM and aconitine than rat brain synaptosomes suggesting a species difference in sodium channel function. The mechanism of the unusual hyperpolarizing action of DM on rat synaptosomes is under investigation. (ES 01985, NSF-OCB 8713027)

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**ALTERED PHOSPHORYLATION OF PHOSPHOLIPIDS IN HEN SCIATIC NERVE BY TRI-N-CRESTYL PHOSPHATE: POSSIBLE ROLE IN ORGANOPHOSPHORUS-INDUCED DELAYED NEUROPATHY (OPIDN).**


Some organophosphorus compounds can induce a delayed progressive degeneration of certain long tracts in the central and peripheral nervous systems (OPIDN). We examined the effects of TOCP (590mg/kg, p.o.) on in vitro labeling of hen sciatic nerve phospholipids either 2 or 7 days after administration. Nerve segments were incubated for 30 or 60 min in oxygenated physiological buffer containing 20 μCi [32P]phosphoric acid and lipids extracted. Extracts were separated by TLC, lipids identified by autoradiography and counted by liquid scintillation. There was a tendency for all lipids to be phosphorylated less in the TOCP-treated nerve with a significant reduction (p<0.025) at 7 d post-TOCP after 60 min incubation. When individual lipids were examined at either 2 or 7 days post-TOCP, phosphorylhydroisotol (PI) labeling appeared to be reduced at 60 min but similar to controls on 60 min; apparently, the rate of labeling of PI was increased in the TOCP-treated nerve. Because no change was seen in total phospholipid concentration in the nerves after TOCP treatment, changes in phosphorylation may indicate altered turnover of phospholipids.

The observed changes in lipid phosphorylation following OP exposure are early events and may be involved in the pathogenesis of OPIDN. (*Supported by NRC Research Associate*)

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**EFFECT OF TRIORTHOCRESYL PHOSPHATE (TOCP) ON MEMBRANE BOUND ATPases IN HEN BRAIN AND SPINAL CORD.**

J A Wiser, H R Beech Jr., and R B Foreman. Indiana University School of Medicine, Indianapolis, IN.

The mechanism for organophosphorus-induced delayed neuropathy (OPIDN) is unknown; but inhibition of neuropathy target esterase (NTE) has been correlated with the development of OPIDN. Previously we reported that NTE activity was positively associated with Ca$^{2+}$/K$^+$-ATPase (CKA) in canine cardiac membrane vesicles (MV) and subfractions. Therefore we undertook to evaluate NTE, CKA, and Na$^+$/K$^+$-ATPase (NKA) activities in MV from nervous tissue, taken from brain and spinal cord of adult white leghorn hens dosed orally with TOCP (750mg/kg) or corn oil and sacrificed at 24 and 72 hours later. NTE activity was significantly decreased in brain and spinal cord at 24 and 72 hours (p<0.01). NKA remained unchanged from control values at both times and in both tissue preparations. CKA was found to be significantly increased (p<0.05) in brain preparations 24 hours after dosing, but remained unchanged at control values by 72 hours. Spinal cord CKA activities were unchanged from control at both time points. Although statistically significant, the small change in CKA activity may not be of pathologic significance. These data suggest that alterations of NAK and CKA may not be involved in the initial development of OPIDN.

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**EFFECT OF DIALLOXY PHOSPHOROFUORIDATE (DFP) ON AXONAL TRANSPORT IN THE CAT.**

C D Carrington, D N Lapadula, and M B Abou-Donia. Duke University Medical Center, Durham, NC.

Experiments were performed to assess the role of axonal transport in organophosphorus compound-induced delayed neuropathy (OPIDN). Cats were dosed with 5.0 mg/kg DFP, s.c. which resulted in over 80% inhibition of neuropathy target esterase in brain and produced a mild to severe hindlimb ataxia. Proteins were labeled by injecting 35S-methionine into both L7 dorsal root ganglia. In one experiment, the sciatic nerve was doubly ligated 24 hours after injection. After an additional 24 hours, accumulation was measured between proximal and distal to the ligated area. An increase of about 100% was observed on both sides. No significant difference was observed between control and treated animals. The retrograde accumulation was too variable to be indicative. In a second experiment, the radioactivity per mg tissue was measured in 9 mm segments along the entire sciatic nerve and the tibial and peroneal branches (19-25 cm). In control animals and animals treated 4 days prior with DFP, the labeling decreased along the sciatic nerve and then increased in the branches. Animals treated 7 (n=6) and 14 (n=7) days prior exhibited a similar pattern except that there was little or no increase at the distal end. The dfpg/mg in the distal nerve was significantly less (about 50%) in the 7 and 14 day animals when compared to the proximal or middle portions of the nerve. Supported by NIOSH grant OHO 2003.
BRAIN REGIONAL SPECIFICITY OF GUANOSINE 3',5'-NOROPHOSPHATE (cGMP) RESPONSE TO DIISOPROPYLFLUOROPHOSPHATE (DFP) ADMINISTRATION. L. Davenport, G. Gianoutsos, and S. Cohen. Toxicology Program, University of Connecticut, Storrs, CT.

Organophosphate cholinesterase (ChE) inhibitors, such as DFP, induce toxicity through an increase in synaptic concentrations of acetylcholine subsequent to inhibition of ChE. cGMP has been implicated as a second messenger following muscarinic receptor stimulation. The role of cGMP in DFP toxicity was investigated in male SD rats administered 2 mg/kg DFP sc. At selected times after DFP administration, animals were sacrificed for determination of cGMP and ChE levels in the brainstem (BS), cerebellum (CE), and cortex (CX). ChE was inhibited by approximately 60% by 15 minutes post dose in all brain regions and further declined to greater than 90% by 1 hour, the time when overt signs of poisoning were first observed. cGMP levels exhibited selective regional sensitivity to DFP. BS cGMP levels were elevated threefold thirty minutes after DFP and remained elevated at 2 hours. No effect was observed in the CE or CX at these times. These results suggest that cGMP accumulation following ChE inhibition is brain region specific. (Supported in part by the Center for Biochemical Toxicology and the UCAN Research Foundation.)

ENZYME INHIBITION IN CHICKS INJECTED WITH DESBROMOLEPTOPHOS AT TWO PERIODS DURING INCUBATION. M. Farage-Ellavar and B. N. Francis. University of Illinois, Urbana IL.

Organophosphorus esters (OP's) inhibit acetylcholinesterase (AChE), causing acute neurotoxicity. Certain OP's are also associated with organophosphate induced delayed neuropathy or OPIDN, which is correlated with the inhibition of neuropathic target esterase (NTE). Some OP's also cause teratogenic effects if administered to chick embryos early in incubation.

We previously showed that desbromoleptophos (DBL), injected on day 15 of incubation, causes NTE inhibition until day 10, AChE inhibition until day 4, and affects the growth of the chicks for at least 6 weeks posthatching. To discriminate between teratogenic and neurotoxic effects of DBL on chick development, we compared NTE and AChE inhibition when DBL was injected on days 3 and 15 of incubation. After treatment on either day, NTE inhibition remained >80%, and AChE inhibition >70%; at least until day 2 after hatchling. Both treatments resulted in 50% mortality in the chicks; the weight of surviving chicks was not decreased. Wry neck, crossed beak and other overt teratogenic effects were not seen, but treated chicks were unable to stand and had curled toes. Some were paralyzed at hatching and even 6 days after hatching. We conclude that DBL teratogenesis is a functional, rather than a structural, teratogen.


To estimate the potential of small doses of the Sarin-I and Sarin-II to cause delayed neuropathy, 400, 200, 50, and 0 ug/kg (Sarin-I) and 281, 141, 70, and 0 ug/kg (Sarin-II) by gavage were compared with 510 mg/kg tri-e-cresyl phosphate (TOCP), in 14-15 month-old SPF white Leghorn hens (4/dose). Protectant atropine (100 mg/kg). The NTE activity 24 h after dosing was determined in brain, spinal cord, and lymphocytes and in plasma and brain for cholinesterase (ChE) and carboxylesterase. TOCP inhibited the 3 tissue NTE's 60%. Sarin-I showed no NTE effect statistically. Sarin-II showed an effect only in lymphocytes. Sarin-I inhibited ChE and carboxylesterase in plasma (55% and 47%, respectively) and in brain (46% and 16%). Sarin-II inhibited ChE and carboxylesterase in plasma (66% and 56%) and in brain (65% and 23%). Using 60% inhibition of brain NTE to predict delayed neuropathy, Sarin-I 6 II were biochemically predicted not to cause delayed neuropathy at nonlethal doses. (Supported by US Army Medical Research & Development Command, contract DA-2402768).

DELAYED NEUROPATHY OF METHYL-CYANOENPHOS, 0-METHYL-0-(4-CYANOPHENYL) PHENYLPHOSPHONOTHIOATE, IN CHICKEN. S. A. Soliman, K. A. Osman, N. S. Ahmed, K. S. El-Gendy, and M. E. El-Shennawy. Laboratory of Environmental Chemistry and Toxicology, Faculties of Agriculture and Medicine, Alexandria University, Alexandria, Egypt.

The delayed neurotoxic effect of the organophosphorus ester, methyl-cyanophosphonophosphate, was studied in the chicken. Hens were orally treated by the compound either once, or with subsequent daily doses. Some of the control and treated hens were sacrificed 24 hrs after the last treatment and their brain neuropathy target esterase (NTE) was measured. The remaining hens were used for clinical and histological examinations. Results demonstrated that methyl-cyanophosphonophosphate at a single dose of 200 mg/Kg was able to produce delayed neuropathy in hens. This compound was also able to produce such effect in chickens given 25 mg/Kg/day for 13 days. The effect was demonstrated by the standard clinical and histological changes and manifested by inhibition of NTE activity. However, methyl-cyanophosphonophosphate did not produce neurotoxic effect in chickens treated daily at 1 mg/Kg for 180 days.
203 INVESTIGATIONS INTO THE ROLE OF BIOTRANSFORMATION IN THE COVALENT BINDING OF 1,2,3-TRICHLOROPROpane (TCP) TO HEPATIC PROTEIN AND DNA. GL Weber and IG Sipes, Dept. of Pharmacology and Toxicology, College of Pharmacy, Univ. of Arizona, Tucson, AZ.

Trichloropropane (TCP) is used as an intermediate in the manufacturing of pesticides and polysulfide rubbers. TCP requires rat liver S-9 to be mutagenic. In a National Toxicology Program chronic bioassay, TCP produced gastrointestinal tract and liver tumors in the rat and mouse. We have undertaken preliminary investigations into the role of biotransformation in TCP induced tumor formation. Male Fischer 344 rats (n = 6) were administered 30 mg/kg 14C-TCP (100 μCi/kg) intraperitoneally and killed 4 hours later. The extent of covalent binding to hepatic protein and DNA was 418 ± 19 pmol/mg and 244 ± 29 pmol/mg, respectively (mean ± SE). Incubations with hepatic cytosolic and microsomal fractions were performed to determine their respective roles in the covalent binding of TCP to macromolecules. Glutathione decreased protein binding and increased the formation of aqueous soluble 14C-TCP equivalents in cytosolic incubations. However, in vitro cytosolic and microsomal incubations of TCP with DNA failed to show any significant covalent binding under the conditions used. At this point the in vivo binding suggests that TCP is genotoxic, however the role of biotransformation in TCP induced genotoxicity is still unclear. (Supported by NIEHS NO1-ES-3-5031.)

204 COVALENT INTERACTION OF A REDUCTIVELY ACTIVATED 5-NITROIMIDAZOLE WITH DNA. GL Kedderis, LS Argenbright, and GT Miwa, Merck Sharp & Dohme Research Laboratories, Rahway, NJ.

The interaction of reductively activated 5-nitroimidazole drugs with DNA is believed to be the mechanism of the antiparasitic properties of these agents. However, the nature of the interaction of the reactive intermediate with DNA is controversial, and nitroimidazole-DNA adducts have not been characterized. Therefore, we have investigated the interaction of reductively activated ronidazole [(1-methyl-5-nitroimidazole-2-yl)-methylcarbamate] with DNA in vitro. After anaerobic reduction of radiolabelled ronidazole (50 μM) with dithionite in the presence of calf thymus DNA (1 mg/ml), the DNA was recovered by ethanol precipitation, extensively washed, and the DNA concentration and radioactivity were determined spectrally. The anaerobic covalent binding of [14C]ronidazole to DNA was maximal at 2 moles of dithionite per mole of drug, indicating that 4 electron reduction of the nitroimidazole to a hydroxylamine is required for covalent binding. Characterisation of the ronidazole-DNA adduct with variously radiolabelled drug molecules demonstrated the presence of the 1-[14C]methyl, 4,5-[14C], and 2-[14C]methylene labels in the adduct while the 4[14C] and 2-[14C]carbamoyl labels were not present. This labelling pattern is the same as for the enzyme-catalyzed covalent binding of ronidazole to protein sulfhydryl groups. These results demonstrate the formation of a ring-intact ronidazole-DNA adduct after anaerobic reduction of the nitro group to a hydroxylamine.

DBCP was found to induce DNA damage in liver cells at low concentrations (1-10 μM), as measured by alkaline elution. At higher concentrations (0.5-2.5 mM), DBCP was metabolized to products that were mutagenic to Salmonella typhimurium TA100 co-incubated with the liver cells. At these concentrations a marked depletion of cellular GSH was seen and at 2.5 mM DBCP was cytotoxic. Precipitation of the liver cells with diethylmaleate (DEM), reduced DBCP-induced DNA damage. In contrast, DEM lead to increases in DBCP-induced bacterial mutagenicity and cellular toxicity. Perdeuterated (D)-DBCP induced less DNA damage in the liver cells than DBCP. A greater reduction than this was seen on mutagenicity with D,-DBCP compared to DBCP, whereas the two compounds were equally cytotoxic. The cytotoxic effect was inhibited by ascorbate. Apparently different mechanisms are involved in DNA damage, bacterial mutagenicity and DOT cytotoxicity induced by DBCP in vivo. We suggest that: 1) Both oxidation and formation of an episulfonium ion are involved in cellular DNA damage; 2) Oxidation leads to formation of products mutagenic to the bacteria, whereas 3) the cytotoxicity induced by DBCP in the liver cells is caused by oxidative damage following GSH depletion.


Spin trapping has made the detection of highly reactive free radicals in biological systems possible by converting non-persistent radicals to persistent ones. We have used this technique to trap free radicals which are generated in specific organs of intact animals which had been subjected to toxic substances. The method can determine in which subcellular organelles the free radicals were formed. Both the intensity and duration of free radical formation can be assessed by this procedure. It has been demonstrated that highly reactive free radicals (-CCl3) are produced in the endoplasmic reticulum of animals exposed to CCl4 vapors. Other studies have demonstrated that chronic consumption of alcohol results in the production of carbon-centered lipid radicals in the liver and heart of rats. The production of these radicals continues for at least 4 days after administration of ethanol is stopped, suggesting that alcohol initiates a chain reaction (probably lipid peroxidation) which persists for a significant period of time. In vivo spin trapping has also been used in our laboratory to demonstrate that exposure to paraquat and other related pyridyl diradical compounds also produces reactive radicals in the lung, liver, heart and spleen of intact animals. Supported by NIH Grant GM 36512.

CALCULATIONS ON THE REACTIVITY OF ACRYLATE ANION WITH BIOLOGICAL NUCLEOPHILES. C H Reynolds and C B Frederick Rohm and Haas Co., Spring House, PA.

A recent paper has concluded that the ionized form of acrylic acid, acrylate anion, may form Michael adducts with biological nucleophiles based upon a 40 day in vitro reaction at pH 7.0 (Segel et al., Chem. - Biol. Interact. 61, 189-197, 1987). Since the reaction of a negatively-charged species (acrylate anion) with a nucleophile appeared contrary to conventional reaction theory, the pathway for the addition of two representative nucleophiles (methyamine and imidazole) was explored with the semi-empirical quantum model, AM1. The results indicate that there is no viable reaction pathway for the addition of acrylate anion to the nucleophiles. An alternative route for the formation of the Michael products in vitro via the non-ionized form of acrylic acid was explored and found to be theoretically possible. The alternative route is plausible, but is considered to be insignificant in vivo based upon the rapid metabolism and excretion of acrylic acid (excretion half-life of 1-8 hrs after oral dosing).

PROTECTIVE EFFECT OF DILTIAZEM AGAINST COCAINE INDUCED HEPATOTOXICITY AND HEPATIC LIPID PEROXIDATION. K A Suares and S Bhonsle. Dept. of Pharmacology, Chicago College of Osteopathic Medicine, Chicago, Il.

Intracellular accumulation of calcium (Ca) has been postulated to be important in hepatotoxic induction. We decided to determine whether influx of Ca was a feature of cocaine (C) hepatic injury and whether damage could be altered by diltiazem (D).

C was administered (70 mg/kg) to phenobarbital induced, female ICR mice. D (20 mg/kg) or saline was administered 1 hr prior to and 6 hrs after C. Mice were sacrificed at various time intervals after C for determination of hepatic glutathione (GSH), malondialdehyde (MDA) and Ca levels or serum ALT, AST and ICDH. C produced a dramatic increase compared to controls, in MDA (>5x), ALT (>100x), AST (>15x), and ICDH (>50x) measured 24 hrs after C. The elevation in hepatic Ca (2x) was far lower than the rise (6x) reported for CHCl3 and CCl4 in rats. D pretreatment produced virtually complete protection against C induced hepatic injury, the decline in hepatic GSH (at 4 and 6 hrs) and the elevation in total Ca content (at 10 and 24 hrs). Because the C induced rise in hepatic Ca was far less than reported for other hepatotoxins, it may be a consequence rather than a cause of cocaine induced hepatotoxicity. Supported by a grant from CCOM.
STUDIES ON DAPSONE-N-HYDROXYLAMINE (DDS-NOH) INDUCED MORPHOLOGICAL CHANGES IN RAT ERYTHROCYTES. RA Budinsky, JV Sinnamon, V Price, and DJ Jollow, Dept's Pharmacol & Anatomy, Med U SC, Chas., SC. Previous studies have indicated that the hemolytic activity of dapsone in rats results from the action of its N-hydroxymetabolites (DDS-NOH and monoacetyl DDS-NOH) on the red cells. Incubation of rat red cells in vitro with hemotoxic concentrations of DDS-NOH have been observed to induce striking transformation in cell morphology DDS-NOH treated red cells develop echinocyte morphology (types I, II, and III) with significant numbers of severely deformed cells designated as "jade-shaped". The changes were observed under light microscopy, SEM and TEM. Few, if any, Heinz bodies were seen under TEM. Phenylhydrazine used as a positive control induced a spherocytic morphology with numerous Heinz bodies. Quantitation of the morphological transformation using a computer/microscope (IBAS) indicated an EC50 of about 120µM, which is similar to those of GSH (depletion and commitment) to splenic sequestration 1^1Cr-Tg0, 180µM. Time studies indicated linearity with a maximal response occurring by 20 min. This response was significantly faster than was commitment of the cells to sequestration (1^1Cr-Tg0). Cysteamine prevented but did not reverse the shape changes. It is suggested that the morphological transformations reflects intracellular changes important in the premature sequestration of the cell by the spleen. (Supported by HL30038).

OXIDATION OF CATECHOL BY HORSERADISH PEROXIDASE AND HUMAN LEUKOCYTE PEROXIDASE. REACTIONS OF o-BENZOSEMIDUINONE (BSQ) AND o-BENZOQUINONE (BQ). V V Subrahmanyan, A Sadler and D Ross. Molecular and Environmental Toxicology Program, School of Pharmacy, University of Colorado, Boulder, CO.

Hydrogen peroxide dependent oxidation of catechol by horseradish peroxidase and peroxidases present in human leukocytes resulted in BQ production, which was characterized as its brothophenol adduct. BQ-glutathione conjugates were formed during peroxidatic oxidation of catechol in the presence of glutathione (GSH). As much as 80% of catechol removed during peroxidatic oxidation could be recovered as monoo and di-GSH conjugates of BQ. GSH had no inhibitory effect on the removal of catechol during peroxidatic oxidation. In the presence of Mg2+ or Zn2+, which slow the rate of BSQ disproportionation, GSH was found to inhibit catechol removal. This suggests that in the absence of stabilizing metal, reduction of BSQ by GSH cannot compete with other rapid reactions of the radical such as disproportionation. No interaction of BSQ with oxygen could be detected even in the presence of stabilizing metals or superoxide dismutase which inhibits the reverse reaction of the SQ + O2 → Q + O2 equilibrium. These data show that generation of thiol conjugates of BQ can be used as probes of peroxidatic oxidation of catechol. Supported by ES04112.

BUTYLATED-HYDROXYTOLUENE (BHT) INDUCED INCREASES IN NAD(P)H-QUINONE-REDUCTASE (QR) ACTIVITY IN MOUSE LUNG AND LUNG CELLS. D Siegel, A Malkinson and D Ross. Molecular and Environmental Toxicology Program, School of Pharmacy, University of Colorado, Boulder, CO.

BHT, a widely used food additive, can modulate the toxic activity of several xenobiotics and the pulmonary toxicity of BHT itself is reduced after repeated BHT administration. BHT forms a quinone during metabolism and its protective actions may be related to increased activity of QR, a protective enzyme against quinone toxicity. More quinone is formed from BHT in lung microsomes isolated from A/J than from C57 mice so QR activity in these two strains was examined. Lung QR activity was increased in both strains by treatment of mice with BHT and was observed as early as 6h post-BHT (500mg/Kg ip) in C57 mice. Dose-response data indicated that QR activity in A/J mice could be increased by BHT (100mg/Kg ip) whereas higher doses were needed in C57 mice. Clara cells and lavaged macrophages isolated from mouse lung and a Clara cell line had little basal or BHT-induced QR activity whereas alveolar type II cell lines exhibited high QR activity. These data show that QR activity may be localized to certain cell types and increased activity play a role in BHT-mediated protection against lung toxicity. Supported by UROP, Univ. Colorado and the American Institute for Cancer Research.

EVALUATION OF 3-METHYL-2-BENZOTHIAZOLINONE HYDRAZONE HYDROCHLORIDE (MBTH) FOR ACUTE TOXICITY, PRIMARY IRRITATION, AND MUTAGENICITY. R C Myers, R S Siegalski, and W Mallansy. Bushy Run Research Center, Union Carbide Corporation, Export, PA.

MBTH, used extensively in analytical laboratories, was evaluated for potential handling hazards. It had moderately high acute oral toxicity in both the rabbit (LD50 of 177 and 268 mg/kg for males and females, respectively) and the rat (LD50 of 308 and 149 mg/kg). By 24-hr occluded cutaneous contact, MBTH had low acute toxicity in the rat (LD50 > 16 mg/kg), but was more toxic in the rabbit (16 mg/kg killed 1/5 males; LD50 = 12.3 mg/kg in females). Cutaneous doses > 4 mg/kg and oral doses > 125 mg/kg elicited convulsions in the rat. No cutaneous inflammation was evident from 4 hr of occluded contact with 0.5 g of moistened MBTH; the 24-hr test produced irritation, edema, and instances of necrosis. MBTH caused mild to moderate eye irritation depending on the amount dosed. No adverse effects were apparent among male or female rats exposed to 6 hr in an inhalation chamber containing solid MBTH (equilibrated overnight). MBTH was mutagenic in the Ames assay, particularly in the absence of metabolic activation. These studies demonstrate a need for skin and eye protection, as well as avoidance of ingestion, during handling of MBTH. Copyright © 1987 Union Carbide Corporation.
In most animal species, toxicity to dibenz-p-dioxin is observed when 3 of the 4 lateral positions are chlorine substituted and at least one other position remains non-chlorinated. The purpose of our studies was to determine if a similar structure-activity relationship (SAR) would be observed in a developing fish embryo. Individual embryos were exposed immediately post-fertilization to nominal concentrations of dioxin congener under static conditions. Embryos were staged daily and checked for visible lesions and death. The 2,3,7,8-tetrachloro congener was the most toxic to the developing medaka with 100% lethality and lesions occurring at the 1 ng/L level. The major lesions were hemorrhage and pericardial edema, resulting in the collapse of the yolk sphere. No visible lesions were observed prior to the formation of the embryonic liver rudiment. The 1,2,3,4,7,8-hexachloro congener caused a similar sequence of lesions with an LC50 of 230 ng/L (std. error 107 ng/L) and EC50 for lesions of 35 ng/L (std. error 19 ng/L). At 10 µg/L, the 2,3-dichloro and octachloro congeners were not toxic. Based on these findings, a similar SAR is observed in the medaka embryo to that found in mammals and birds. (USGS 28322)

We examined the effects of butyl 2-chloroethyl sulfide (BCS), a potent vesicant analog of bis(2-chloroethyl) sulfide (sulfur mustard) on groups of athymic nude mice (n=4), weighing 30-35 g, subcutaneously with 5 ul of neat BCS. After 1, 24 and 48 hrs, we sacrificed the mice along with an untreated control group, and analyzed the brains for biochemical markers of oxidative injury. Compared to controls, the activity of glutathione (GSH) peroxidase increased 75%, P<0.05 at 24 hrs, and GSH S-transferase 27%, P<0.05 at 48 hrs. GSH content was markedly lower (25 and 20%) after 1 and 24 hrs. Concomitant with decreased GSH, lipid peroxidation increased almost 3-fold. We conclude that BCS administered subcutaneously affects mouse brain via an oxidative mechanism, possibly reflecting the initial injury phase by vesicants. These observations may contribute to the understanding of the molecular mechanism of vesicant toxicity and could offer a new approach for treatment and protection.
COMPARISON OF UPTAKE AND DISTRIBUTION OF DIETHYL-NITROSAMINE (DENA) IN ORYZIAS LATIPES AND PINEPHALES PROMELAS. T. L. Holaday, P. E. Davis, and K. Hinton. West Virginia University Medical Center, Morgantown, WV. *School of Vet. Medicine, University of California-Davis.

O. latipes, Japanese medaka, and P. promelas, fathead minnow, are small laboratory fishes of similar body size and culture requirements. Exposure to DENA caused tumor formation in O. latipes but not P. promelas. Uptake and distribution of 14C-DENA was compared in these two species. Two week old fry of both species were exposed to 10, 100 or 200 ppm 14C-DENA for 3, 6, 12, 24, or 48 hr. Following exposure, fish were rinsed and radioactivity determined in whole fish or visceral mass (liver, kidney, intestine and gonads). A dose dependent uptake of radioactivity into whole fish and visceral mass of both species was seen. The time course of incorporation and disappearance of radioactivity in the visceral mass differed in the two species. The peak uptake into the visceral mass occurred at 12 hr in P. promelas and 24 hr in O. latipes. Disappearance, presumably by elimination occurred earlier in P. promelas. O. latipes also had higher tissue concentration of radioactivity than P. promelas. Therefore, it appears that differences in tumor production by these two species may be related to availability of the carcinogen.

(Supported by USGS Grant No. 1408800161052).

A MODEL SYSTEM FOR STUDYING THE INTESTINAL ABSORPTION OF A HEPATOTOXIN FROM BLUE-GREEN ALGAE. A. M. Dahlem, L. A. S. Hassan, S. P. Brandon, W. N. Carmichael, and Y. B. Beasley. Department of Veterinary Biosciences, Urbana, IL, and Department of Biological Sciences, Wright State University, Dayton, OH.

Rats were used to evaluate a model system for studying the hepatotoxicity caused by microcystin-A, a cyclic peptide toxin produced by the cyanobacterium, Microcystis aeruginosa, and for evaluating the therapeutic potential of cholestyramine resin (CTR). Female rats were assigned to one of two groups and treated with ether toxin (5 mg/kg) or an equivalent volume of saline vehicle instilled into the lumen of an in situ isolated ileal loop. Male rats were dosed with toxin as described above and then were dosed with either CTR (50 mg/rat) or an equivalent volume of vehicle. The surviving animals in both studies were killed six hours postdosing and hepatotoxicity was assessed by change in liver weight as a percent of whole body weight. In all groups given toxin alone, there was a significant (p < 0.05) increase in liver weight. Liver weights of the toxin plus CTR treated rats were similar to those in vehicle-treated rats. When the toxin was administered into a similarly isolated jejunal loop, liver weight was significantly (p < 0.05) less than that found when an equivalent dose was administered into the ileal loop. These results indicate that a site specificity exists for intestinal absorption, and that CTR produces a reduction in harmful effects.


Examination of similarities and differences between chemically-induced toxicity in teleost and mammalian model systems could lead to better understanding of the mechanisms underlying toxic action. The present studies examined metabolism using the rainbow trout and the Sprague-Dawley rat as models. The trout's susceptibility to carbon tetrachloride (CCL4)-induced lipid peroxidation (LP) was also investigated. In metabolism studies utilizing liver microsomal and cytosolic preparations, kinetic constants for glucuronyl transferase and sulfotransferase were obtained with 1-naphthol as substrate. The metabolic capacity (Vmax) and affinity (Km) of the trout conjugative enzymes were markedly lower than those of the rat. For example, the Vmax for the trout and the rat glucuronyl transferases were 0.77 and 32 nmol/min/mg protein, respectively. Similarly, the teleost Vmax for 1-naphthol sulfation was approx. 1/2 that for the rat. Further studies investigated CCL4-induced LP in fish and rat liver microsomes. LP was measured by HPLC analysis of the thioarbitruric acid adduct of malondialdehyde (MDA). CCL4 induced a concentration dependent increase in the amount of MDA formed in both species. Further studies are underway to examine LP and metabolism in freshly isolated trout and rat hepatocytes, and to evaluate the in vivo sensitivity of the trout to CCL4 hepatotoxicity.

IN VITRO GLUCOSE AND SULFATE CONJUGATION OF 4-METHYL UMELIFERONE (4-MeU) BY THE SPINY LOBSTER (PANULIRUS ARGUS). J. D. Schell and M. O. James. C.V. Whitney Laboratory and Dept. Medicinal Chemistry, Univ. of Florida, St. Augustine, FL.

The ability of various tissue fractions of the spiny lobster to catalyze conjugation reactions was studied using the fluorescent molecule, 4-MeU as the acceptor substrate. The hepatopancreas (HP) exhibited UDP-glucosyltransferase (UDPT) activity and used UDP-glucose as a co-factor. The activity was localized in the microsomal fraction. HP microsomes had no UDP-glucuronyl transferase activity with UDP-glucuronic acid as a co-factor. Under assay conditions of 25 μM 4-MeU, 2.6 mM UDP-glucose, pH 7.6 and 300C, UDP-glucosyl transferase activity (mean ± SD, n=17) was 0.77±0.61 pmol/min/mg protein. HP cytosol had no sulfotransferase activity and completely inhibited the sulfotransferase activity of fish liver cytosol. The cytosolic fraction of the green gland (GG) contained sulfotransferase activity. This enzyme had a rate of 63.4 ± 49.3 (n=4) pmol/min/mg protein at the optimal pH of 7.0. GG contained no detectable UDPT activity with 4-MeU in any subcellular fraction. The role of these enzymes as in vivo detoxification pathways in xenobiotic metabolism is currently being investigated. Supported by USPHS CA44297.

Chlorinated diphenyl ethers are demonstrated environmental contaminants in the Great Lakes region. One of the most predominant congeners found in Lake Ontario fish is 2,2',4,4',5-pentachlorodiphenyl ether (PCDE). But little information is available on its toxicity or metabolism. The present study was carried out as part of an ongoing program on chlorodiphenyl ethers and provides information on the tissue distribution and metabolism of this isomer in the rat. PCDE was distributed in all tissues examined with the highest concentrations being found in fat followed by skin, liver, kidney and muscle. Most of the radioactivity found in the tissues was due to the unchanged PCDE. The decay profile of PCDE in the blood was fitted to a four compartmental pharmacokinetic model (4 exponential terms) and the last exponential term had a half-life of 5.6 days. A total of 58% and 1.3% of the administered dose was excreted in feces and urine respectively in seven days. More than 64% of the fecal radioactivity was due to the unchanged PCDE, while the hydroxylated PCDE accounted for 23% of the excreted radioactivity.

EFFECT OF METHOD AND DURATION OF EXPOSURE OF PIPERONYL BUTOXIDE ON THE HEPATIC MONOOXYGENASE ACTIVITY OF RAINBOW TROUT. D A Erickson, M L Haasch, and J J Lech. Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI.

The induction of hepatic monooxygenase (MO) activity in fish by agents other than polycyclic aromatic hydrocarbons is not well documented. This study characterizes the induction of hepatic MO in rainbow trout using piperonyl butoxide (PBO). When trout were exposed by bath to PBO over 21 days, hepatic MO activity increased to maximal levels in 15 days. Following transfer to clean water, trout (induced by PBO by bath for 14 days) had hepatic MO activity that was half the fully induced level after 9 days. When trout were exposed to PBO by i.p. injection, hepatic MO activity increased to relatively low maximal levels and rapidly returned to control levels. When levels of P450 mRNA were measured over time following i.p. injection of either β-naphthoflavone (BNF) (100mg/kg) or PBO (450 mg/kg) the P450 mRNA reached maximal levels at 18 hrs. and 48 hrs., respectively. These data indicate that induction of hepatic MO activity in trout by PBO is affected by the type and/or duration of exposure and that the genetic regulation of induction of hepatic MO activity by PBO and BNF may be mechanistically different. (Supported by NIEHS grant ES 01080)


The immune defense mechanisms of fish are closely related and similarly competent to that of mammals. Because of this, interest in the immune response of fish as a model for higher vertebrates in toxicological studies, is increasing. Investigations to establish normal baseline criteria for different components of the immune response of fish are needed prior to toxicological studies. Macrophages are a pivotal cell in the immune response and host defense of fish and mammals. In this study we have examined the morphology and in vitro phagocytic activity of unexposed rainbow trout peritoneal macrophages and compared these results with those of rabbit alveolar cells (RAM). Results showed that trout and rabbit macrophages were similar in size, shape and nuclear configuration and both were esterase positive. To determine the phagocytic index (PI) and capacity (PC), cells were incubated with latex beads for 30, 60 and 90 minutes using culture conditions appropriate for the species. Though cells from both species take up particles in a similar manner (i.e., by membrane engulfment) the PI and PC for trout cells were low compared to RAM. These results provide comparative information needed to evaluate the usefulness of fish macrophages to serve as a model for higher vertebrates in immunotoxicological studies. Supported by NIEHS Ctr. Grant ES00260.

COMPARATIVE INDUCTION OF HEPATIC CYTOCHROME P450 mRNA AND CATALYTIC ACTIVITY IN VARIOUS SPECIES: STUDIES USING A COMPLEMENTARY DNA PROBE. M L Haasch, P Wajksnora, J J Lech. Medical College of Wisconsin and University of Wisconsin-Milwaukee, Milwaukee, WI.

Comparative induction in rainbow trout (Salmo gairdneri), brook trout (Salvelinus fontinalis), painted turtle (Chrysemys picta) and rat was examined by determination of levels of P450 mRNA and corresponding catalytic activities. Levels of P450 mRNA with determined utilizing pP450-3'- 3′, a 3′-specific, 1.5 kb complementary DNA (cDNA) clone derived from 3-methylcholanthrene-induced P450 mRNA of rainbow trout (gift of D M Nebert, Bethesda). Animals were treated with beta-naphthoflavone (β-NF, 100 mg/Kg, i.p., 24 hrs.) Rainbow trout, brook trout and rat were significantly induced (25-, 76-, and 16-fold, respectively) with respect to ethoxyresorufin-O-deethylation (EROD). In addition rat was significantly induced (4.4- and 1.4-fold) with respect to ethoxycoumarin-O-deethylation (ECOD) and total cytochrome P450. All of the species examined possessed mRNAs which were recognized by pP450-3' with rainbow trout and brook trout having an increase in an mRNA of about 2.5 kb (8.3- and 6.8-fold, respectively). These data support previous findings and provide evidence for the usefulness of pP450-3' for examining induction and P450 gene regulation in many different aquatic species. (Supported by ES 01080).
A VIBRATING ELECTRODE STUDY OF EXTRACELLULAR MEMBRANE CURRENTS FROM ACETABULARIA EXPOSED TO TRIBUTYL Tin METHOXIDE. S B Baumann, Northrop Services, Inc. Research Triangle Park, NC. Sponsor: K T Kitchin

Tributyltin (TBT) is used as an anti fouling agent in marine paints, and it appears in sea water samples from many harbors. The large-celled, marine algae, Acetabularia, has been studied previously with a vibrating electrode system that measures slowly varying ionic currents near the cell exterior (Bowles and Allen, in Ionic Cur rents in Development, 1986). In this study stable, baseline measurements were taken for one hour at 10 points around the rhizoid and stem regions. Within 5 minutes of treatment with 5 nanomolar (Zppt) TBT, currents decreased from an average of 22 uA/cm² to 4 uA/cm². Although current densities recovered to about 1/3 of original levels after 5 hours of TBT exposure, both current magnitude and polarity showed great disturbance from the normal pattern. Since TBT is one of many toxic compounds that affects cell membrane permeability, this study demonstrates that a vibrating electrode system can be used as a sensitive bioassay for membrane toxicity.

WATER QUALITY CRITERIA FOR HEXACHLOROETHANE. P S Hovatter, K A Davidson, and R H Ross, Oak Ridge National Laboratory*, Oak Ridge, TN. Sponsor: P Y Lu.

Hexachloroethane is a USEPA designated priority pollutant, which has been detected in drinking water, groundwater, surface water, and wastewater effluents. It is primarily used by the military as a constituent of white smoke grenades and markers. Based on the existing data concerning the environmental fate, aquatic toxicity, bioaccumulation, and mammalian toxicity of hexachloroethane, water quality criteria for the protection of aquatic life and its uses and of human health are calculated using USEPA guidelines. The aquatic criteria consists of two values, a criterion maximum concentration based on acute toxicity data and a criterion continuous concentration based on chronic toxicity and bioaccumulation data. The human health criterion is derived from carcinogenicity data based on concentrations of the chemical related to risks of 10⁻², 10⁻³, and 10⁻⁴ per chronic toxicity data based on acceptable daily intake.


The health and environmental effects of eight structural categories of organosilanes have been reviewed under the Chemical Hazard Information Profile program of the USEPA. Organosilanes are bifunctional (able to react with both organic and inorganic substrates) and many are used in industry as coupling agents or surface modifiers. Structure-related trends in toxicity were not observed for any of these organosilanes. Most organosilanes are generally of low to moderate toxicity in humans and animals, but some have elicited serious chemical-specific effects using experimental systems. For example, methacryloxypropyltrimethoxysilane, administered acutely or subchronically as an aerosol, caused an unusual form of granulomatous laryngitis in rats; trimethyl[2-(7-oxa-hydropent-3-yl)ethy][silane (TMOS) was carcinogenic in C3H/HeJ male mice; and others were positive for genotoxicity. Limited data indicate that these organosilanes are of low acute toxicity in aquatic species.


TSCA INTERAGENCY TESTING COMMITTEE (ITC). E K Weisburger, National Cancer Institute, Bethesda, MD.

The ITC recommends chemicals to the EPA for priority testing consideration. All chemical substances regulated under the Toxic Substances Control Act (TSCA) are subject to ITC review. The ITC consists of statutory members from 12 agencies specified in TSCA and representatives from seven agencies invited to participate. The ITC has developed a process to screen large numbers of chemicals to select a few for detailed review. The process involves evaluation of exposure and biological effects potentials and current consumption values to score and rank chemicals. Since 1977, more than 25,000 chemicals have been rated, approximately 7,000 have been scored for exposure and/or biological effects and over 1,000 have been selected for detailed review. The ITC has issued 21 Reports to the EPA Administrator recommending over 400 chemicals for priority testing considerations. The status of all chemicals scored, reviewed and recommended by the ITC is maintained on a computerized tracking system. On-line database searching capabilities, which are available to all interested parties, will be demonstrated during the session. Interested parties are also encouraged to nominate chemicals to the ITC for its consideration.
HAZARD COMMUNICATION: THE CASE FOR CATEGORY 4 "CANCER INFORMATION. J E Betso, R J Kociba. The Dow Chemical Company, Midland, MI.

Today's demands to provide scientific information to a broad spectrum of the public suggest changes in the way chemical labeling is conducted. It is now appropriate to provide supplemental information beyond a simple warning—that is, enumeration of a broader range of possible adverse effects that may be associated with overexposure. With regard to cancer hazard warnings, it is fairly easy to identify known human carcinogens and even confirmed animal carcinogens—ones without dispute among professionals. The new ANSI (American National Standards Institute, Inc.) guidelines have recommended that cancer warnings be limited to known human and confirmed animal carcinogens. It seems appropriate, however, to extend cancer label statements to include information about certain animal tests which were indicative of some degree of carcinogenic activity, but which are not believed to be relevant to human risk when consideration is given to all of the scientific data. Information concerning carcinogenicity test results and their relevance to humans can be added to label text but need not be in the form of a warning. Examples are given of chemicals for which certain positive cancer test data exist, but which do not deserve full precautionary labeling.

AN INTERACTIVE ROLE FOR TOXICOLOGISTS IN COMMUNITY RISK MANAGEMENT. J S Heath and J Pfeensteden-Kadon, Cornell University, Ithaca, NY.

People misunderstand toxicological information, and hence risk management options, not because they are incapable of reasoned thinking and sound judgement; rather because adequate, relevant toxicological risk information has not been provided. Case studies of a multidisciplinary research project on environmental chemicals and community risk management illustrate how lack of involvement of toxicologists either directly or through an integrated risk management team resulted in misunderstanding and mismanagement. Underestimating of toxicologic risk information is prerequisite to constructive participation in state, federal and corporate risk management efforts. In the absence of toxicologists, this information is provided by engineers and others not necessarily qualified to interpret or even convey information pertaining to the basic concepts of toxicology and toxicologic risk. We will focus on the many ways that toxicologists could participate meaningfully in a community's effort to understand and manage environmental risk situations: interpreting analytical results, epidemiologic data and potential health effects; explaining routes of entry, biotransformation, etc.; identifying relevant laws and regulations. Toxicology, as a profession, has failed to participate meaningfully in risk communication at the local level. We propose a more active role.


Responding to the public's need for accurate and reliable information about environmental and occupational health risks, UMDNJ-Robert Wood Johnson Medical School has developed EOHIP as a model program to provide information and services to the general public, lay and professional employees, small industry, schools, and physicians. The program produces a variety of educational materials on specific risk issues. An Advisory Committee guides the program with representation from the diverse sectors involved in these environmental and occupational health issues, including labor, industry, government, media, public interest organizations, and academia.

This presentation will review the goals of the program and discuss materials and services available to date. The "Healthy Environment—Healthy Me" school curriculum on environmental and occupational health and a videotape series on "Teaching Our Children About Hazardous Substances" will be highlighted. EOHIP is designed to be a model program here in New Jersey that can be replicated in other states.

PESTICIDE INFORMATION PROFILES. A M Beale and A L Craigswell. Environmental Toxicology Extension, University of California, Davis, CA.

A series of 20 informational pamphlets were developed as part of a project in conjunction with Cornell University, Oregon State and Michigan State for the EPA. These pamphlets were created to address the needs of professional/agricultural or home applicators of a variety of commonly used herbicides and pesticides. Information regarding the effect of the chemical on humans as well as target and nontarget species of wildlife and plants; Environmental issues such as accumulation/magnification and ground water contamination; physical/chemical properties of the chemical and standard accepted uses was garnered from a variety of sources. This information was then distilled into an easy to read format, standardized between the participating institutions, and readied for future distribution on an as requested basis.
Public perception about the risks of exposure to chemicals in the environment, and recent legislation have prompted the need for educational programs to address public concern on these issues. Two slide tape programs were developed to present background information and practical management tools for target audiences in rural and urban areas of tenth to twelfth grade education levels.

One objective of the project is to promote a wider perspective on the role of chemicals in our daily lives, and just what risk they present to us. A second objective is to present information in an easily digestible format to assist county staff in answering questions about environmental risk and hazardous wastes.

The SOT Committee on Public Communications has recognized a growing need for a comprehensive computerized registry of educational and informational resources in toxicology and related topics which could be utilized by members of the SOT and other interested professionals to assist in teaching and public communications efforts. To meet this need, the Committee has established the Toxicology Resource Information Service (TRIS), containing information about audiovisuals (films, slides, videotapes); publications (books, monographs, brochures); computer software (computer-assisted teaching, analysis and utility programs); and teaching materials (syllabi, course/curricula outlines) which have been acquired or developed by members of the SOT and other professional groups. The TRIS currently holds over 300 specific entries, and a prototype version of the TRIS will be displayed during the special session on Communicating Basic Concepts in Toxicology at the 1988 annual meeting of the SOT. All items submitted to the TRIS up to that time will be included in the display. It is anticipated that, when brought to fruition, the TRIS will constitute a resource of major importance to members of the SOT in enhancing their individual teaching and public communications efforts in toxicology and related topics.

At the present time, occupational/environmental health requests for information constitute up to 10% of the calls received by PCCs. Health professionals are responsible for the great majority of these inquiries, currently. The volume of requests to PCCs regarding occupational/environmental health issues is expected to escalate due to the recent right to know laws. At present, most PCCs answer these inquiries using a background in acute toxicology. The objective of the present research has been to assess resource utilization and staff training needs of PCCs on a national scale. Ninety-eight PCCs participated in this survey. The results determined that most PCCs underutilize government agencies primarily using the PSLI system to answer occupational/environmental requests. Education about right to know laws, hazardous spills, reproductive and cancer risks were regarded as very important needs by PCCs. Most PCCs desired 40 hours/week of occupational/environmental health training. Future research will demonstrate how the development of efficient resource materials and training programs can aid PCCs to implement a unique occupational/environmental health program for the public.

The Center for Environmental Toxicology has operated an inquiry-response system for over five years. Center staff have responded to about one thousand inquiries involving a variety of environmental toxicology issues. Inquiries have been received from a diverse audience including public agencies, Cooperative Extension Service agents, industry, the media, and private citizens. Each inquiry has been utilized as a means to public education since time has been taken to explore the situation fully with each individual and to impart information appropriate to the issue involved in the inquiry. In addition, the distribution and nature of inquiries have proven very useful in identifying issues which require more general education. Yearly summaries of the data reveal a number of trends including an increased interest in groundwater contamination, pesticide toxicity, and testing of environmental samples and a decreased interest in chlorinated hydrocarbons. A currently emerging issue is indoor air. Recognition of these trends has led to the development of publications and presentations to address issues of importance. Examples of these materials will be part of this presentation.
SUMMARY OF LITERATURE REVIEW ON METALS IN HUMAN URINE AS A BIOLOGICAL INDICATOR OF EXPOSURE.


Potential worker exposure to metals in fumes or dusts by ingestion, skin contact, or inhalation can take place during industrial processes using metals and alloys. One useful approach to monitor such exposure is through the assay of the metals in the urine of the exposed workers. However, exposure data to obtain average baseline urine levels of nonexposed (background) and exposed (occupational and medical treatment) populations are scattered or scarcely available. A comprehensive literature search of 20 metals excreted in human urine was performed on TOXLINE and MEDLINE.

From the limited information available we were able to compile the following data for (1) nonexposed population: uranium, thorium, iridium, tantalum, and zirconium, ≤0.1 μg/L; platinum, bismuth, ≤1 μg/L; lead and mercury, ≤10 μg/L; (2) occupationally exposed population: thallium, platinum, and zirconium, ≤2 μg/L; mercury, ≤50 μg/L; lead, uranium, and selenium, ≤100 μg/L; titanium, ≤3000 μg/L; and (3) population under medical treatment: gold and bismuth, ≤20 μg/L; mercury, ≤100 μg/L; and platinum, ≤20,000 μg/L.

*Lawrence Livermore National Laboratory, Livermore, CA.

IMMUNOTOXICITY AND RISK ASSESSMENT OF CONTAMINANTS IN DRINKING WATER. S Sriharan, Selma University, Selma, AL and Z V Ohanian, Office of Drinking Water, EPA, Washington, D.C.

The process of risk assessment is being recognized as essential to every organized human society. The Safe Drinking Water Act Amendments of 1986 require the U.S. EPA to promulgate national drinking water regulations which specify risk of adverse human health effects from exposure to environmental contaminants. Quantitative risk assessment usually involves performing two separate evaluations: (1) a hazard assessment to determine potential toxicity, and (2) an exposure assessment. These separate assessments are combined to derive an estimate of risk to the exposed human population.

This paper evaluates the incorporation of immunotoxicity data into EPA's health research aimed at improving risk assessment methodologies. The EPA's Office of Drinking Water (ODW) has developed Health Advisories (HAS) that describe the concentrations of contaminants in drinking water at which adverse effects would not be anticipated to occur following 1-day, 10-day, longer term or lifetime exposure. Studies carried on tetrachloro-p-dioxin (TCDD), a potent immunosuppressant, are discussed here for risk assessment and deriving ten-day Health Advisory number.

HEALTH EFFECTS ASSOCIATED WITH BROMINE AND BROMINE COMPOUNDS. F M Martin, Oak Ridge National Laboratory*, Oak Ridge, TN. Sponsor: P Y Lu.

The purpose of the overview is to determine whether or not evidence exists which suggests that bromine and its compounds exert effects on human health at concentrations commonly encountered by the general public under ambient air exposure conditions. Acute and chronic health effects are addressed, including systemic toxicity, genotoxicity, carcinogenicity, and reproductive and developmental effects. Other topics discussed are chemical and physical properties, uses, production, sources, environmental fate, ambient levels, pharmacokinetics, and regulations and standards. Bromine is known to be highly toxic via oral and inhalation routes. Chronic exposure leads to fatigue, tremors, delirium, and schizophrenia in man. Several compounds, such as methyl bromide, ethylene dibromide, and dibromochloromethane, have given positive results for genotoxicity, and some like dibromochloropropane, a banned pesticide, have shown carcinogenic effects and/or adverse effects on reproduction. The ambient concentrations reported for bromine have been below the OSHA maximum time-weighted average of 0.1 ppm. *Operated by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400.

HAZARD EVALUATION OF AFLATOXIN IN FOOD. P F Berteu and A M Fan. Hazard Evaluation Section, Calif Dept Health Services, Berkeley, CA.

This study evaluates the basis of the action level for aflatoxin B1 in peanuts and the comparative health hazards associated with aflatoxin ingestion and the fungicides used to control molds, such as Aspergillus flavus, which produce aflatoxin. The action level of 20 ppb was established in 1969, based on the analytical detection limit, which, combined with toxicological data and the decision to permit peanuts and peanut products to remain on the market, serves as the basis for the Food and Drug Administration's regulatory action. Aflatoxin induces liver cancer in many species of experimental animals, but epidemiological data are difficult to interpret. Liver cancer is relatively rare in the United States particularly in the rural Southeast where peanuts are grown. In developing countries such as Mozambique where liver cancer is more common, infection with hepatitis B virus is a compounding factor in individuals also exposed to aflatoxin. Health hazards associated with fungicides, which are limited in their effectiveness on Aspergillus are evaluated. Many of these compounds are still in the testing stage.

The U.S. EPA is developing a centralized health risk information system in response to the need for easy access to risk assessment and risk management data by U.S. EPA personnel and the general public. The U.S. EPA's IRIS is an electronic database of the U.S. EPA's risk assessment and regulatory information on chemical substances. Each chemical file contains up to five sections: reference doses (RFDs) for systemic toxicity (non-carcinogenic effects); risk estimates for carcinogenicity; drinking water health advisories; regulatory actions; and supplementary information. Each health hazard assessment contained in the first two sections is a consensus of one of two intra-agency review groups of EPA scientists: the Reference Dose Workgroup or the Carcinogen Assessment Review Workgroup. These sections each provide a description of the basis for the hazard assessment and a discussion of the uncertainties in that assessment in a summary format (2-5 pages). IRIS will be made available to the public in early 1988. Future plans include the development of an interactive mainframe and/or PC version of the database.

MITOCHONDRIAL INHIBITION BY CATIONIC RHODAMINES AS A POSSIBLE TERATOGENICITY MECHANISM. S. Ranganathan and R. D. Hood. Biology Department, The University of Alabama, Tuscaloosa, AL.

Teratogenic cationic rhodamine (Rh) dyes, Rh 123 and 6G, accumulate in mitochondria, interfering with energy metabolism. We investigated the possibility that rhodamines affect embryonic mitochondria. On gestation day (g.d.) 12 (plug = g.d. 1), mitochondria were isolated from embryos of mice treated on g.d. 7-10 (with 15 mg/kg/day Rh 123 or 0.5 mg/kg/day Rh 6G) or left untreated. Effects were assessed by polarographic measurement of oxygen use. Control mitochondrial O2 consumption increased from 51 to 180 ngatomeg/min after addition of 100 nM ADP, with succinate as substrate. Values for mitochondria from Rh 123-treated embryos were 62 and 135 ngatomeg. Similar values were obtained following in vitro Rh 123 exposure of mitochondria from untreated embryos (70 and 130 ngatomeg/min with 5 μg Rh 123/mg protein). Much lower amounts of Rh 6G caused similar results in vivo and in vitro. Association of rhodamines with embryonic mitochondria was assessed by spectrophotometric measurement. Mitochondrial rhodamine content was 4-5 times greater under energized than nonenergized conditions. These results support the possibility that interference with oxidative phosphorylation is a mechanism for the developmental toxicity of cationic rhodamines.

Supported by PHS Grant NRSC 1751-08.


Male and female rats were neonatally exposed to Aroclor 1254 (100 or 300 µmol/kg) and the effects of this PCB mixture on neonatal imprinting of benzo[a]pyrene hydroxylases were determined in 120-day-old animals. Aroclor 1254 inducibility with respect to the rate of formation of the 9,10-, 4,5-, and 7,8-dihydrodiol, quinone and 9-hydroxybenzo[a]pyrene metabolites was significantly decreased in both male and female rats exposed neonatally to Aroclor 1254. Only neonatorally implanted published showed any differences in constitutive benzo[a]pyrene hydroxylases and those were decreased formation of the 4,5-dihydrodiol metabolite and increased 3-hydroxyl activity. The most significant effects of Aroclor 1254 on the neonatal imprinting process were observed at a dose of 300 µmol/kg, which is considerably higher than the potential environmental exposure to these compounds via lactation. (Supported by the Texas Agricultural Experiment Station.)

pκA VALUE DETERMINES RETINOID EMBRYOTOXICITY IN VITRO. C. E. Steele, R. Marlow, J. Turton and R. M. Hicks. SK&F Research Ltd., Welwyn, U.K. and Middlesex Hospital Medical School, London, U.K. Sponsor: G. B. Leslie. To determine what factor(s) controls intrinsic retinoid embryotoxicity (i.e., independent of the effects of maternal pharmacokinetics) we have cultured rat embryos for 48 hr in serum containing one of ten retinoids at concentrations ranging from 0.5-400 µg/ml.

Trans-retinoic acid, 13-cis-retinoic acid and etretinate caused abnormal development in the concentration range 0.5-1 µg/ml. Six retinamides (NPR, 13cisNPR, QH2NPR, 4HP, 5B and TAZR) were less toxic and adverse developmental effects were only evident at concentrations of 50 or 100 µg/ml. Etretinate, the parent compound of etretinate, was without effect at 10 µg/ml. In this culture system, TAZ and 13-cisRA caused abnormal development at very low concentrations but in contrast, ETR was non-toxic at 100 µg/ml. Therefore these findings indicate that in vivo, maternal pharmacokinetics, and bioactivation in particular, play a major role in inducing abnormal development. Cis/trans isomerization was not a major determinant of toxicity. However, there appeared to be a relationship between abnormal development and the actual or estimated pκA values of the 10 retinoids tested.
POLYCHLORINATED DIBENZOFURAN PCB CONGENERS WHICH ANTAGONIZE THE TERATOGENIC EFFECTS OF 2,3,7,8-­ 
TETRACHLORODIBENZO-p-DIOXIN (TCDD) IN C57BL/6 
MICE. L BIEGEL, J M HAEKE, S SAFE, K MAYURA 
and T D PHILLIPS, Department of Veterinary 
Physiology and Pharmacology and Department of 
Veterinary Public Health, College of Veterinary 
Medicine, Texas A&M University, College Station, 
TX.

Previous work in our laboratory has demonstrated that the commercial PCB mixture, Aroclor 1254 (750 umol/kg), protects C57BL/6 mice against 2,3,7,8-TCDD-induced cleft palate. Several PCB compounds present in Aroclor 1254 exhibited low but measureable affinity for the Ah receptor protein but did not elicit 2,3,7,8-TCDD-mediated responses. Two of these congeners, namely 2,2',4,4',5,5'-hexa- and 
2,2',5,5'-tetrachlorobiphenyl, at dose levels of 750 umol/kg also antagonized the teratogenic effects of 2,3,7,8-TCDD. Administration of [3H]-2,3,7,8-TCDD to C57BL/6 mice showed that there was a rapid uptake of radiolabel into the fetal palate. However, cotreatment with [3H]- 
2,3,7,8-TCDD plus 2,2',4,4',5,5'-hexachlorobiphenyl resulted in decreased uptake of radio-
label ito the fetal palate tissue. The significance of these interactive effects on the mecha-
nism of PCB-mediated antagonism will be discussed. (Supported by the National 
Institutes of Health.)

THE ROLE OF ALTERATION IN THE DISTRIBUTION OF 
SECAI ONIC ACID D IN THE ANTITERATOGENIC EFFECT 
OF DMSO. H M R ELDER and C S REDDY. Depart-
ment of Veterinary Biomedical Sciences, University 
of Missouri-Columbia, MO.

DMSO is known to antagonize teratogenic effects of secalonic acid D (SAD) in mice. To establish optimum protective dose of DMSO, pregnant CD-1 mice were treated, ip, with 30 mg/kg of SAD in NaHCO3, with 0, 10, 20 or 30% DMSO on day 12 of gestation. Data indicate that 10 and 20% DMSO afford a dose-related protection against SAD-
induced cleft palate whereas 30% DMSO enhanced SAD-induced fetal resorptions with no reduction in the incidence of cleft palate. Distribution and elimination of [C]SAD was studied in fetal and maternal tissues from pregnant mice at 24 
and 48 hr after exposure to 30 mg/kg of [C]SAD, ip, in NaHCO3 (control) or with 20% DMSO. 
Maternal exposure to DMSO: (A) significantly reduced (42 to 75%) radioactivity in fetal heads 
and bodies; in placenta; and in maternal tissues including GI tract, uterus, salivary gland, bone 
and tongue; (B) significantly increased (222X) the radioactivity in maternal liver; and (C) 
significantly reduced (44 to 99%) fecal and urinary elimination of radioactivity; compared 
to control. These results suggest that the antiteratogenic effect of DMSO against SAD may 
be mediated by increased SAD (or its metabo-
lites) retention by maternal liver leading to 
both reduced SAD uptake by the fetus and reduced elimination in urine and feces. Supported by 
NIH grant HD07107.

SECRETION OF HIGH CONCENTRATIONS OF CIMETIDINE 
INTO RAT MILK DURING LACTATION. L A DOSTAL, 
R W WEAVER, and B A SCHWETZ, National 
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Research Triangle Park, NC 27709.

High milk/plasma ratios (4-12) for cimetidine 
(pK 7.1) have been reported in humans after in-
egestion. To investigate the excretion of cimet-
idine into milk and its effect on lactating rats 
and their pups, rats were given daily oral doses of cimetidine on Days 13-16 of lactation. Ma-
ter nal exposure to cimetidine (18 or 80 mg/kg/ 
day) had no effect on milk protein, lipid, or 
water content, nor on total mammary DNA or RNA 
content. However, a small increase in milk lact-
tose was found at 180 mg/kg/day, and the RNA/DNA 
ratio was increased at both doses. There was no 
effect on growth, testis weight, analgenital dis-
tance, or hepatic alanineaminopeptidase N-demethylase 
activity in the pups. Four hrs after the last 
dose of 18 mg/kg/day, plasma and milk cimetidine 
levels were 0.77 ± 0.16 and 16.7 ± 1.8 μg/ml 
(Mean ± SE, n = 6), respectively; at 180 mg/kg/day, 
cimetidine levels were 3.6 ± 0.4 and 113 ± 9 
μg/ml in plasma and milk. In vitro, [3H]cimetid-
ine is mainly distributed in whey (69%) and 
casein (28%) with only 18% protein binding 
in whey. Protein binding was 60% in skim milk 
and 45% in plasma. Thus, milk/plasma ratios of 22-
31 in rats cannot be explained by ionic equilib-
rium or protein binding in the milk. The possi-
ibility of active uptake of [3H]cimetidine into the 
membranes gland is under investigation.

2,5-HETE ROXANEDINE-INDUCED TESTICULAR INJURY 
AND MICRO TUBULE ALTERATION. K BOEKELHEIDE. 
Brown University, Providence, RI.

We have proposed alterations in Sertioli cell microtubule assembly as the basic mechanism of 2,5-hexanedione-induced testicular injury, an hypothesis supported by the differential testicular toxicity and microtubule effects of two γ-diketone 
congeners (TAP 84, 370-382 and 383-396) and the early onset of altered assembly kinetics during 2,5-hexanedione in-
toxication (TAP, two articles in press). Two new experimental approaches further strengthen this association. Charles River CD rats (220 g) were intoxicated with 131 ± 2 mmol/kg 
2,5-hexanedione delivered in the drinking water at dose rates of 
6.1, 3.8 and 1.9 mmol/kg/day over 21, 35 and 69 days, 
respectively. The extent of testicular injury, as determined by testis weights and histopathology, correlated with the dose rate 
and not total dose. The extent of biochemical abnormality in 
testis pynocyte content and microtubule assembly kinetics, 
determined in rats intoxicated at the various dose rates for 3 
weeks, was also a function of dose rate. In a morphometric 
study, the closest inter-microtubule distance was measured for cytoplasmic Sertioli cell microtubules in stage VII seminiferous 
tubes. When compared with controls, rats intoxicated for 3 
weeks with 15% 2,5-hexanedione had significantly decreas-
ked numbers of microtubules with intermediate inter-microtubule 
distances (25 - 50 nm), with the majority of microtubules 
closer than 25 nm. This morphometric result is consistent with the in vivo induction by 2,5-hexanedione of an increased 
number of shorter microtubules, a known in vitro morphologic 
effect of 2,5-hexanedione tubulin treatment.
ASSOCIATION OF SPERM, REPRODUCTIVE ORGAN WEIGHT AND VAGINAL CYTOLGY (SMVCE) DATA WITH FERTILITY OF SWISS (CD-1) MICE. R E Morrissey, J C Lamb IV*, B A Schuetz, J L League, and R W Morris. NTP/NIHIS, Research Triangle Park, NC; *US EPA, Washington, DC; and *ASA, Research Triangle Park, NC.

To evaluate the effectiveness of the SMVCE as a screen, changes in SMVCE endpoints were compared with results of 25 continuous breeding reproduction studies in which crossover mating trials were conducted to determine the affected sex. Historical control data were used to calculate statistical power of each endpoint. For male reproductive toxicants, sperm motility was the endpoint most frequently decreased (90%) followed by epididymis and testis weights. Among studies with no detectable change in male breeding performance, no change in epididymal weight was most frequently observed (87%), followed by lack of an effect on testis weight. Endpoints providing the best overall association with breeding data and the greatest statistical power were epididymis and testis weights, sperm motility, and cauda weight; sperm concentration and percentage abnormal sperm were not as well associated with breeding data. A change in female cycle length was associated with an adverse effect on female breeding performance. Results with this group of 25 chemicals suggest that testis and epididymis weights and sperm motility have a high association with changes in fertility.

THE SEMINAL VESICLE AS A TARGET ORGAN OF TOXICITY. R E Bagdon, C J Molloy and J D Laskin, Joint Graduate Program in Toxicology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

The seminal vesicle is sensitive to hormonal (androgens, estrogens) and chemical (gossypol, hexachlorobenzene, pesticides) stimuli. It has a tubular mucosal structure and is lined with a discontinuous layer of basal cells and a layer of columnar epithelial cells containing secretory granules. We have examined alterations in the seminal vesicles of mice following vitamin A deficiency. Male CF-1 mice, maintained on a vitamin A deficient diet from birth, developed abnormalities including body wasting and xerophthalmia within 16 weeks. Severe testicular degeneration was evident and morphological examination revealed that the seminal vesicles were thickened, enlarged and fibrous. Numerous keratin containing cysts were apparent. Microscopic examination revealed that the normal luminal epithelium was replaced by a squamous metaplasia with histological features resembling epidermis. Our data suggests that vitamin A is required for normal seminal vesicle development. Furthermore, alterations in retinoid metabolism and the seminal vesicle toxins may in part underlie organ specific toxicity in this tissue.


Losulazine hydrochloride, a guanethidin compound, is an effective hypotensive agent in experimental animals and man. Reproductive and developmental studies in rats, revealed interrupted estrous cycles in females, decreased fertility in males, and delayed development in offspring of treated dams. This presentation will discuss the pathologic changes in the accessory sex glands of rats treated with losulazine, and associated ejaculatory disturbances. Rat were treated with 2 to 32 mg/kg/day of losulazine for up to 1 year for pathologic evaluation of the reproductive organs; reversibility was determined in rats treated with losulazine for 24 weeks followed by a 20 week drug withdrawal period. Ejaculatory performance was evaluated by mating male rats (treated with 4 or 16 mg/kg/day for 4 weeks followed by a recovery period of up to 11 weeks) to untreated females. Losulazine at 4 or more mg/kg/day resulted in reversible exudative prostatitis, resolving ampullary gland sperm granulomas, and nonreversible seminal impaction. There were reversible decreases in ejaculatory sperm count and seminal plug weights in treated rats. The pathologic lesions in the accessory sex glands of rats treated with losulazine were consistent with pharmacologically-mediated ejaculatory stasis.


As cancer survival rates increase, there is an escalating concern about the adverse effects of chemotherapeutic agents on male fertility. Agents affect proliferation, metabolism and synthetic processes, however, there is only sparse information as to initial insult to the testis. The present study using plastic embedment, employed four chemotherapeutic agents (actinomycin-D, A-D; doxorubicin, DX; cisplatinum, C-P; 5-fluorouracil, 5-F) to determine the initial site affected. Morphological patterns of response were found that were highly characteristic for each agent and invariably stage-specific. For example, certain spermatagonia (A-D, C-P, DX), preleptotene (A-D, DX) and pachytene spermatocytes (A-D) were targeted in a stage-specific manner; the chromatoid body was often abnormal (A-D, C-P); an unusual asynchrony of the spermatogenic cycle was accompanied by meiotic metaphase arrest (5-F); spermatid and Sertoli nuclear changes (A-D), nuclear vacuolation (C-P) and failed release of sperm (DX) were noted. Although agents show some shared responses, many sites of injury differ, suggesting that strategies may be developed to minimize testicular damage.
DEVELOPMENTAL TOXICITY OF BROPIRIMINE. T A Marks, D L Black, S M Poppe and R D Terry, The Upjohn Company, Kalamazoo, MI.

Bropirimine is an immunomodulator and interferon inducer with antiviral and antitumor activities. During routine Segment II Teratology Studies, oral administration of bropirimine to pregnant rats resulted in pronounced developmental toxicity at doses (200 and 400 mg/kg) which also were maternally toxic (Teratology 35: 30A, 1987). In subsequent rat studies, maternal toxicity always was observed at the doses which caused developmental toxicity, even when bropirimine was given only once. Bropirimine administration to pregnant rats, resulted in a 4-8% decrease in weight within 24 hours of a single dosage (200 or 400 mg/kg), while control rats given vehicle alone gained weight. Bropirimine did not cause maternal lethality at the doses employed. In order to determine the period of in utero development which were most susceptible to its effects, bropirimine was given once between days 3 and 19 of gestation. Except for day 6 and days 12-17 of gestation, such treatment led to significant decreases in the survival of the offspring within 72 hours of exposure. The results of these studies have provided us with a mechanism involved in the developmental toxicity of bropirimine. Hopefully future studies will enable us to better understand why in utero exposure to bropirimine can be lethal whereas the maternal animal is able to survive the effects of this drug.


2,3,4,6 - tetrachlorophenol (TCP) was evaluated for subchronic toxicity and teratogenicity in Sprague-Dawley rats. Groups of rats (30/sex/dose) were administered 0.25, 100 or 200 mg/kg/day TCP by gavage for 30 days. At 200 mg/kg/day there was a significant decrease in the male body weight; liver and kidney weights were significantly higher than controls in males and females at sacrifice. Also, centrilobular hypertrophy was observed in 15/30 males and 6/30 females and a number of clinical pathology parameters were significantly changed at this dose. At 100 mg/kg/day dose liver and relative liver weights were significantly elevated in both males and females. In females, both absolute kidney and relative kidney weights were elevated; centrilobular hypertrophy was seen in 12 males and one female. At 25 mg/kg/day there were no significant adverse effects. In the teratology study, rats administered 0, 25, 100 or 200 mg/kg/day TCP by gavage daily on day 6-15 of gestation showed a statistically significant reduced maternal weight gain at the highest dose; no significant maternal effects were seen at the two lower doses. No adverse developmental effects were observed at any of the doses tested.


Octabromodiphenylxide and Pentabromodiphenylxide are chemically similar materials used in the manufacturing of flame retardants. Pregnant rats (25/group) were given (gavage) Octabromodiphenylxide at doses of 0 (corn oil), 2.5, 10 or 25 mg/kg/day or Pentabromodiphenylxide at doses of 0 (corn oil), 10, 100 or 200 mg/kg/day on days 6 to 15 of gestation. The dams were sacrificed on day 20 of gestation, and the fetuses were examined for external, visceral and skeletal alterations. Octabromodiphenylxide produced developmental toxicity (resorption and decreased fetal weight) at doses of 10 and 25 mg/kg/day, resulting in a developmental NOEL of 2.5 mg/kg/day. Maternal toxicity (decreased maternal body weight) occurred only in a pilot study at doses of 100 mg/kg/day or greater. Pentabromodiphenylxide at 200 mg/kg/day produced a slight decrease in the average fetal body weight and maternal toxicity (decreased maternal body weight gain). Maternal toxicity (decreased body weight gain) was also produced at 100 mg/kg/day. Based on these data Octabromodiphenylxide was a unique hazard to the conceptus with an A/D ratio (adult/developmental) of 10, while Pentabromodiphenylxide was shown not to be a unique hazard (A/D ratio of 0.5).

CYTOXICITY MEASUREMENTS WITH PRIMARY CULTURES OF CRYOPRESERVED (CP) RAT HEPATOCYTES. K S Santone, D C Melder and G Powis. Mayo Clinic, Department of Pharmacology, Rochester, MN.

We have previously reported a technique for bulk CP of rat hepatocytes that maintains functional viability. Presently we have performed experiments to assess the usefulness of primary cultures of CP hepatocytes for cytoxicity measurements. 2 x 10⁶ viable fresh and CP hepatocytes were cultured for 24 hr before exposure for 2 hr to various concentrations of either chlorpromazine (CPZ), cadmium chloride (Cd) or menadione (MND). Lactate dehydrogenase (LDH) leakage and intracellular reduced glutathione (GSH) concentration were chosen as indices of hepatocyte damage. The results listed below are the drug concentrations (µM) to produce a 50% release of total LDH or a 50% decrease in GSH (mean ± S.E., n = 3 separate preparations. The following LDH results were obtained, fresh vs. CP: CPZ 215 ± 30 vs. 235 ± 20, Cd 272 ± 23 vs. 200 ± 5 and MND 44 ± 8 vs. 24 ± 7. GSH results fresh vs. CP were: CPZ 235 ± 8 vs. 200 ± 8, Cd 213 ± 7 vs. 242 ± 19 and MND 21 ± 3 vs. 22 ± 2. The above results show that CPZ, Cd and MND toxicity in CP hepatocytes correlates well with the toxicity seen in fresh hepatocytes. These results indicate that CP hepatocytes may be a useful model for determining xenobiotic toxicity. Supported by NIEHS Contract ES55110 and The John Hopkins Center for Alternatives to Animal Testing.
Rat hepatocytes were cryopreserved using a number of procedures and the viability, attachment, and metabolic activity of the cryopreserved cells were compared. Several cryopreservation agents (DMSO, glycerol, FVP, dextrans) and combinations of these agents were tested. Other variables tested included the freezing rate and the concentration of DMSO in the freezing medium. The greatest recovery of viable attached cells was obtained using DMSO at concentrations of 10% or higher, and a freezing rate of c. 1°C/minute. Varying serum concentration in the freezing medium did not affect cryopreservation results. Using this procedure, the recovery of viable hepatocytes was 70%. Metabolic endpoints used to evaluate cryopreserved cells included activation of pro-mutagens in the CHO/HGPRT gene mutation assay, ethoxycoumarin-O-deethylase activity, p-chloromethylamine demethylease activity, and urea synthesis. Each of these endpoints remainedunchanged following cryopreservation. In addition, peroxisome proliferation at a level similar to that found in freshly isolated hepatocytes was observed in cryopreserved hepatocytes following treatment with Wyeth 14,643. Similar results were obtained with hepatocytes isolated from two human livers.

ROLE OF THE 4S BINDING PROTEIN IN THE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE IN THE RAT. M. H. Harris, S. Kaspe and S. Safe, Departments of Veterinary Physiology and Pharmacology and Biochemistry and Biophysics, Texas A&M University, College Station, TX

A survey of several rat strains demonstrated that the levels of the hepatic cytosolic 4S binding protein (using [3H]-benzo(a)pyrene as the radioligand) were highly variable. The concentrations of this binding protein in Sprague Dawley (Harlan, -4S), Sprague Dawley (Hascos, +4S), Fischer 344, Wistar, and Lewis rat hepatic cytosol were 0, 298 ± 57, 0, 244 ± 21, and 216 ± 48 fmol/mg cytosolic protein, respectively. Dose-response induction of hepatic microsomal aroly hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 3-methylcholanthrene (MC) in Sprague Dawley (+4S) and Sprague Dawley (-4S) rat strains gave comparable ED50 values for AHH induction. Studies with these inducers and other polynuclear aromatic hydrocarbons with affinity for the 4S binding protein suggest that this protein plays a minimal or antagonist role in the regulation of AHH induction in the rat.

(Submitted by the Texas Agricultural Experiment Station and the National Institutes of Health.)

EFFECTS OF NITROUS OXIDE AND BODY WEIGHT ON THE GUINEA PIG MODEL OF HALOTHANE HEPATOTOXICITY. TC Lind and AJ GandeTfi, Department of Anesthesiology, University of Arizona, Tucson, AZ.

Halothane (H), is often administered concurrently with N₂O. Since N₂O can exacerbate liver injury, this combination of anesthetics was evaluated in a guinea pig model of halothane hepatotoxicity. Male and female strain 13 guinea pigs (300-1000 g) were exposed to 1% H, 40% N₂O for 4 hr with or without 60% N₂O. The addition of N₂O affected neither plasma concentration of H metabolites nor the degree of hepatic injury (ALT and histopathology). Animal weight was a factor with larger (572-970 g) animals of both sexes demonstrating significantly greater elevations in 48 hr ALT and incidences of centrilobular necrosis vs smaller (318-565 g) animals (ALT=134 ± 74 units/ml vs 27 ± 7; necrosis=17/24 vs 0/17, respectively). There were no significant differences between large and small animals in levels of plasma metabolites of H. Following 0.1 ml/kg CC1 (ip), larger guinea pigs also developed significantly greater elevations in ALT over those in smaller animals. Further studies will be required to elucidate factors involved in this variation in hepatotoxic response in guinea pigs of different sizes. (NIM AM165715).
HEPATIC ENERGY STATUS DURING CCL\textsubscript{4} TOXICITY IN RATS PRETREATED WITH CHLORDEcone, MIREX AND PHENOBARBITAL. M S Prasad, R, U M Joshi and H M Mehendale. Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS.

Previous studies indicated perturbation of Ca\textsuperscript{2+} homeostasis and decreased hepatic ATP concentration in chlorodecone (CD) + CCL\textsubscript{4} hepatotoxicity. Since these phenomena are indicative of perturbed energy status of liver, studies were designed to test this possibility. Hepatic ATP levels were determined after CCL\textsubscript{4} (0.1 ml/kg) administration to rats on diets with or without CD (10 ppm) for 15 days. Rats pretreated with phenobarbital (PB, 225 ppm) and mirex (M, 10 ppm) were selected as positive and negative controls. There were no significant differences in hepatic ATP or Mg\textsuperscript{2+}-ATPase in CD, M or PB treated rats. CCL\textsubscript{4} alone did not alter hepatic ATP in control rats. CCL\textsubscript{4} administration to CD pretreated rats significantly decreased the hepatic ATP as early as 1-hr and this decrease was progressive with time. Mg\textsuperscript{2+}-ATPase was significantly decreased after 6 hr after CCL\textsubscript{4}, administration. This was not observed in M or PB pretreated rats. These results indicate that severely compromised energy status of liver after CCL\textsubscript{4} administration to CD, but not PB or M pretreated rats leads to the disturbance of Ca\textsuperscript{2+}-homeostasis and of cell division. (Supported by EPA R-81072 and CRS814053010.)

MECHANISM-BASED TOXICITY OF HMG-CoA REDUCTASE INHIBITORS (HRI's) IN RABBITS. D Kornbrust, C P Peter and J S MacDonald. Merck Sharp & Dohme Research Laboratories, West Point, PA

HRI's have been shown to be highly effective in lowering serum cholesterol in animals and man, and thus represent a promising approach to the treatment and prevention of cardiovascular disease. During preclinical safety evaluations of several HRI's, oral doses which were tolerated in chronic studies by dogs, rats and mice were found to be lethal to rabbits. Subacute studies. Postmortem findings in rabbits consisted of centrilobular hepatic necrosis frequently accompanied by renal tubular necrosis. These lesions did not occur in rats, mice, dogs or monkeys which received comparable or higher doses for much longer periods. Qualitatively similar effects in rabbits were produced by (5) structurally diverse HRI's, including MK-0803 (lovastatin), MK-733 and CS-514, but not by a pharmacologically inactive isomer. The liver and kidney damage were preceded by marked anorexia and body weight loss. Forced-feeding greatly reduced the toxicity (of MK-733), thus demonstrating the potentiating role of decreased food intake. The liver and kidney damage induced by MK-733 and MK-0803 were completely prevented by coadministration of mevalonic acid, the product of the inhibited HMG-CoA reductase enzyme. These findings indicate that the unique susceptibility of rabbits to HRI's is due to extreme sensitivity to the pharmacological action of these compounds.


2-Methylfuran (2-MF) was administered by inhalation (6 hours/day for 14 consecutive days) to randomized Sprague-Dawley rats (wt. range: 180-201 g for females; 270-299 g for males) divided into low-level (80 ppm), high-level (160 ppm) and control groups (15 rats/sex/group). Twenty hours after the last exposure, 10 rats/sex/group were necropsied and the remaining 5 rats/sex/group were kept for a 12-week recovery study. Hypertrophy of hepatocytes was observed in both high-level females (10/10) and males (9/10). Hyperplasia of the spleenic white pulp (1/10 females and 7/10 males) and thymus atrophy (1/10 females and 3/10 males) were also observed in the same group. In addition to hypertrophy of hepatocytes observed in low-level females (2/10) and males (1/10), proliferation of bile duct was observed in low-level males (9/10). After a 12-week rest period, morphological changes in the bile duct were still observed in both high-level females (2/5) and males (2/5). (Sponsored In part by Canadian Panel on Energy Research and Development.)

Because inhalation of 1600 ppm p-xylene for 3 days appeared to enhance the hepatotoxicity of orally administered carbon tetrachloride (CCl₄) in rats, we conducted experiments to determine if a single exposure to p-xylene could potentiate CCl₄ hepatotoxicity. Male F344 rats were exposed to 0 or to 1600 ppm p-xylene by inhalation for 6 hours, gavaged 48 hours later with 0 or with 0.075 ml CCl₄/kg, and killed 24 hours later for assessment of hepatotoxicity. Exposure to CCl₄ only, but not p-xylene only, increased serum alanine aminotransferase and aspartate aminotransferase activity, increased the liver-to-body-weight ratio, and caused mild centrilobular vacuolar degeneration as well as minimal centrilobular necrosis. Combined exposure (p-xylene plus CCl₄) did not result in increased hepatotoxicity relative to CCl₄ only. These results indicated that a single 6-hour exposure to 1600 ppm p-xylene was not sufficient to potentiate the hepatotoxicity of CCl₄. The induction of hepatic cytochrome P450 at the time of CCl₄ dosing, observed after 3 exposures to p-xylene, suggests a possible mechanism of the p-xylene mediated potentiation. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

Responses of the Lung to Inhaled Carbon Black and Inhaled Diluted Diesel Exhaust. R. F. Henderson, R. K. Wolff, J. L. Maunderly, and R. Q. McCallan, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Inhalation of diesel exhaust by rats causes an inflammatory response in the lung at lung burdens of soot >1.5 mg/g lung. We hypothesized that this response was due to the particle load in the lung and not to the gases or adsorbed organic components associated with the soot. To test this hypothesis, F344 rats were exposed 7 hr/day, 5 days/wk for 12 wk to diesel exhaust or carbon black at 3.5 or 10 mg particles/m³. The inflammatory response of the lung was measured at the end of the exposure by analysis of bronchoalveolar lavage fluid (BALF) for macrophages, neutrophils (PMNs), lactate dehydrogenase, β-glucuronidase, acid proteases and protein. Lung burdens achieved by the low and high level exposures were (X ± SD) 0.94 ± 0.37 and 2.8 ± 0.6 mg/g lung for carbon black and 0.99 ± 0.40 and 2.5 ± 0.5 mg/g for diesel soot. A similar response was caused by both types of particles. There was no indication of a pulmonary inflammation at the lower dose of either particle. At the higher dose, there was an increase of PMNs and acid protease activity in BALF, indicating a mild inflammatory response to both particles. The data indicate that the inflammatory response to diesel exhaust is due mainly to the accumulation of carbonaceous particles in the lung. (Research supported by U.S. DOE/ONR under Contract No. DE-AC04-76EV01013.)


SK&F 93944, a competitive histamine H₁-receptor antagonist, produced appreciable, but intermittent elevations in the plasma activity of alanine aminotransferase (ALT) and glutamate dehydrogenase (GLDH) when administered chronically to dogs but not rats. The objective of this study was to assess the potential of SK&F 93944 to produce acute hepatocellular dysfunction in 3 species: rats, dog, and monkey. Male Wistar rats, beagle dogs, and cynomolgus monkeys received iv infusions of SK&F 93944 at rates ranging from 5 to 35 mg/kg/hr for 24 hrs. Hepatocellular dysfunction was assessed by monitoring serum concentration of bilirubin (BIL), and the activity of ALT, GLDH, and alkaline phosphatase (ALK) at 0 (control), 12, 24, and 48 hour from the start of the infusion. The results, at large dosages, indicated that: (1) SK&F 93944 could produce hepatocellular dysfunction in the dog; and (2) the dog was more susceptible to SK&F 93944-induced hepatic dysfunction than the monkey: the rat was the least susceptible species.

Pulmonary Effects of Combined Inhalation Exposures of Rats to Oil Shale Dust and Diesel Exhaust. J. A. Pickrell, E. B. Barr, A. F. Eidson, R. F. Henderson, J. R. Harkema, and J. L. Maunderly, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Because diesel powered vehicles may be used to mine oil shale, workers may be exposed to combined aerosols of oil shale dusts (S) and diesel emissions (D). We studied potential interactions of D (carbonaceous particles) and S (mineral particles) in rats exposed for 7 hr/day, 5 days/wk for up to 30 mo to raw oil retorted S at 5 mg/m³, 0 D at 3.5 mg soot/m³, to combinations of S and D, or to air as controls. Histopathology demonstrated more chronic inflammation, parenchymal epithelial proliferation, and focal fibrosis in D-than in S-exposed groups. Bronchoalveolar lavage (BAL) measurements suggested progressive mild inflammation, cytoxicity, and alveolar phagocytosis. BAL, collageneous peptides were increased between 18 and 30 months of exposure, indicating persistent remodelling. Increased lung collagen accompanied histologic evidence of fibrosis. There was a mild restrictive impairment of respiratory function. Effects were more severe in D- than in S-exposed rats, and most severe in those exposed to both S and D interact in an approximately additive (independent) fashion to produce lung disease. (Research supported by the U.S. DOE Office of Health and Environmental Research under Contract No. DE-AC04-76EV01013.)
SUBCHRONIC INHALATION TOXICITY OF NICKEL OXIDE TO RATS AND MICE. C H Hobbs, J M Benson, D G Burt, Y S Cheng, J K Dunnick, A F Eldson, P J Haley, and J A Pickrell. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; *NIH/ES/NTP.  

Occupational exposure to nickel oxide may occur during nickel refining. This study evaluated the subchronic inhalation toxicity of nickel oxide (calcined at 1350°C) to F344/N rats and B6C3F1 mice exposed to 0, 0.6, 1.2, 2.5, 5, and 10 mg NiO/m³ 6 hr/day, 5 day/week for 13 weeks. There were no significant exposure concentration related effects on clinical signs or mortality. Weight gain was depressed in mice but not rats exposed to 10 mg/m³. Lesions in rat lung included inflammation in rats exposed to 2.5 mg/m³ and greater and alveolar macrophage hyperplasia and perivascular lymphocytic infiltrates in rats exposed to 1.2 mg/m³ or greater. Similar lung lesions occurred in mice with inflammation occurring in mice exposed to 10 mg/m³ and alveolar macrophage hyperplasia and perivascular lymphocytic infiltrates occurring in mice exposed to 2.5 mg/m³ or greater. Biochemical endpoints in bronchoalveolar lavage fluid indicated an inflammatory response in lungs of both species, with the lung being affected to a greater extent than mice. Results suggest that exposure of rodents to NiO at concentrations near the current TLV produces minor lesions in the respiratory tract. (Research conducted under IAA YO1-ES-30108 between U.S. DOE Contract No. DE-AC04-76EV01013 and NIH/ES/NTP.)

SUBCHRONIC INHALATION TOXICITY OF NICKEL SULFATE TO RATS AND MICE. J M Benson, D G Burt, Y S Cheng, J K Dunnick, F Eldson, P Hahn, C H Hobbs, and J A Pickrell. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; *NIH/ES/NTP, RTP, NC  

Occupational exposure to nickel sulfate (NiSO₄) may occur during nickel smelting and electroplating. This study evaluated the subchronic inhalation toxicity of NiSO₄ to F344/N rats and B6C3F1 mice exposed to 0, 0.12, 0.25, 0.50, 1.0, and 2.0 mg Ni/m³ as NiSO₄•6H₂O hr/day, 5 day/week for 13 weeks. Lesions in rat lung included necrotizing pneumonia and degeneration of the bronchiolar epithelium. Lesions in mouse lung included nodular chronic inflammation with focal fibrosis, chronic active pneumonia, and alveolar macrophage hyperplasia. Atrophy of the olfactory epithelium of the nose occurred in rats and mice. Biochemical and cytologic endpoints evaluated in bronchoalveolar lavage fluid indicated the presence of inflammation in lungs of both species. The incidence and histopathological changes and the magnitude of changes in biochemical parameters were related to exposure concentration in both species but were greater in rats than mice. Results suggest that inhalation exposure of rodents to NiSO₄ at concentrations near the TLV produces substantial lesions in the respiratory tract. (Research conducted under IAA YO1-ES-30108 between U.S. DOE Contract No. DE-AC04-76EV01013 and NIH/ES/NTP.)

A SUBCHRONIC INHALATION STUDY OF A SPECIAL TEST TONER IN RATS. R Klipper, U Mohr, S Takenaka, O Creutzzenberg, R Mermelstein, and H Muhle. 1 Corporate Env. Health & Safety, Xerox Corp., Rochester, NY; 2 Fraunhofer Inst. For Toxicology, Hannover, FRG; 3 University of Rochester, Rochester, NY  

A subchronic inhalation study, using SPF, F-344 rats, was conducted for 6 hr/day, 5 day/week for 13 weeks. The special test toner was enriched about 10-fold, respirably (ACGIH), relative to product. Nominal exposure levels were 0, 1.0, 4.0, 16.0 and 64 mg/m³ (0.35, 1.4, 5.6 and 22.4 mg/m³, respirable). The exposures were well-tolerated, with no unscheduled deaths. Significant findings were restricted to the high exposure groups.

The MTD (pulmonary) was determined relative to a defined Maximum Functionally Tolerated Dose (MFTD). Lung and brain measurements of toner and a spike of iron oxide (Fe-59) were essentially unchanged from controls. At 16 mg/m³, slight effects were observed. At 64 mg/m³, no appreciable toner clearance occurred after 60 day exposure. The MFTD was deemed to be exceeded at 64 mg/m³ and 16 mg/m³ was chosen for the chronic study high concentration.

LONG-TERM INHALATION STUDY OF TEST TONER IN RATS. R Mermelstein, U Mohr, W Koch, D Dasenbrock, R Klipper, J Mackenzie, F Horrow, and H Muhle. 1 Corporate Env. Health & Safety, Xerox Corp., Rochester, NY; 2 Fraunhofer Inst. For Toxicology, Hannover, FRG; 3 University of Rochester, Rochester, NY  

SPF, F-344 rats were exposed 6 hr/day 5 day/week for up to 24-month to a special test toner at 0, 1, 4, and 16 mg m⁻³ TiO₂ at 3 mg m⁻³ TiO₂ and SiO₂ at 1 mg m⁻³ by the inhalation route. The test toner material was enriched in fine particles, such that the respirable aerosol concentrations (ACGIH) corresponded to 0, 0.35, 1.4 and 5.6 mg m⁻³. The design of the investigation, which included periodic measurement of lung retention, collagen determination, bronchoalveolar lavage, alveolar clearance and histopathological examinations will be discussed in detail. Body weight development, food consumption, and clinical chemistry as well as animal health and survival were satisfactory. Mortality rates and causes of death were independent of treatment and in accordance with published values. The relative lung weights of both the toner high and SiO₂ exposed groups increased during the study, such that compared to concurrent controls, they reached 117% and 173% after 15 months and 144% and 235% after 24 months of exposure respectively. Both the Maximum Functionally Tolerated Dose (MFTD) and Maximum Tolerated Dose (MTD) were exceeded at the toner high exposure level within the study.

Male and female F-344 rats were exposed 6 hrs/day 5 days/week for up to 24-months to a special test toner at 0, 1, 4, and 16 mg m⁻³, TiO₂ at 5 mg m⁻³, SiO₂ at 1 mg m⁻³ by the inhalation route under SPF conditions. The dynamics of alveolar clearance and pulmonary retention were measured after 3, 9, 15, 21, and 24 months exposure.

The quantity of test toner retained in the lungs increased with exposure level and duration. At the toner high exposure level, the retained quantity of material in the lungs was significantly higher than the results projected from either low or middle exposure levels. Iron oxide (Fe-59) and polystyrene latex (Sr-85) periodically inhaled by the nose-only route were used to measure alveolar clearance. Clearance of both tracers was substantially retarded in the toner high and SiO₂ exposure groups, while only polystyrene retained was retarded in the middle toner group. The excessive quantity of retained toner and retarded clearance in the toner high exposure groups are indicative of lung overloading.

BRONCHOALVEOLAR LAVAGE FLUID (BALF) ANALYSIS FOLLOWING ALUMINUM OXIDE (Al₂O₃) AND TITANIUM DIOXIDE (TiO₂) ADMINISTRATION. M A Perkins, R C Lindenschmidt, K E Driscoll, J K Maurer, and J M Higgins. Procter & Gamble, Miami Valley Laboratories, Cincinnati, OH.

This study characterized the time and dose-related changes in selected biochemical and cellular endpoints following intratracheal instillation of the fibrogenic silica dusts, Al₂O₃, TiO₂, P₃₄₄ rats were intratracheally instilled with 0, 1.0, or 5.0 mg/100 gm body weight of the test materials. Groups of 5 animals/treatment were sacrificed 1, 7, 14, 28, and 63 days post instillation, and the changes in biochemical and cellular components of BALF determined. Both dusts elicited similar responses. At the initial time point the dose-related increases in BALF levels of lactate dehydrogenase, beta-glucuronidase, n-acetyl-glucosaminidase, and numbers of neutrophils and lymphocytes represented the most sensitive indicators of response. Importantly, however, the changes in these endpoints were transient. At the low dose most parameters returned to baseline levels by day 14, while changes at the higher dose level were decreasing, but persisted beyond day 28. The magnitude and temporal nature of this response to these silica dust types is in marked contrast to the persistent alterations seen in these same parameters following administration of silica, a known fibrogenic agent.

DIFFERENTIAL RESPONSES IN RATS FOLLOWING ACUTE INHALATION OF NUISANCE DUSTS. W Hartsky and DB Sellek. Du Pont-Haskell Lab., Newark, DE.

Nuisance dusts are generally defined as particulates which when inhaled produce few adverse toxic effects. We sought to compare the lung responses to 2 nuisance dusts, zinc oxide (ZnO) and titanium dioxide (TiO₂) with a known fibrogenic dust, i.e., alpha quartz silica dust (Si). CD rats were exposed to aerosol of either Si, ZnO, or TiO₂ for 6 hrs at 100 mg/m³. Time course studies were carried out on cells and lavage fluid (BAL) from groups of sham and dust-exposed rats. LD₅₀, alkaline phosphatase (AP), and protein were measured in concentrated BAL fluid. Our results showed that inhalation of Si or ZnO resulted in a PMN inflammatory response. ZnO-induced inflammation was resolved within 8 days, while Si changes persisted. In contrast, no increases in PMN or cell numbers were observed at any time after TiO₂ exposure. In addition, acute and permanent lung cytotoxic and permeability changes, respectively, were manifested by increases in LD₅₀, AP, and protein (P < 0.05) in ZnO and Si-exposed rats. No significant differences were seen in these parameters following exposures to TiO₂. Our results suggest that all nuisance dusts are not alike; brief exposures to ZnO produced acute pulmonary changes while TiO₂ produced no adverse effects.
PARTICLE-MACROPHAGE RELATIONSHIPS DURING THE CLEARANCE OF PARTICLES FROM THE ALVEOLAR MACROPHAGE COMPARTMENT. B E Lehert, K E Toews, Y E Valdez, and R J Sebring. Los Alamos National Laboratory, Los Alamos, NM.

Little experimental attention has been given to examining the relationship(s) between particles retained in the alveolar space compartment and the lung's population of alveolar macrophages (AM) during alveolar clearance. In this study, we quantitatively characterized the distributions of particles among lavageable AM over a 30 day period after the acute intratracheal instillation of ~ 3 mg of 1.9 μm dia polystyrene microspheres into the lungs of rats. Information obtained for particles retained in the lavageable AM compartment and particle-AM distribution data were collectively examined using a simple, first order kinetic model for AM removal from the lung. The results of our analyses suggest that a volume load of particles in a macrophage up to at least ~ 450 μm³ does not inhibit the mobilization of macrophages from the alveolar compartment. Additionally, the kinetic analyses of the particle-macrophage distributions suggest that macrophages that replenish those AM that are translocated from the lung on a continual basis during alveolar clearance are not and/or do not remain particle-free in the alveolus. This latter observation can be explained by: 1) the influx of particle-bearing macrophages into the alveolus, or 2) the in situ proliferation of particle-bearing AM, or 3) the release of particles by AM and the subsequent phagocytosis of the particles by newly arrived cells, or 4) a combination of these possibilities.

LIVER CYTOSOLIC METABOLISM OF TRANS,TRANS-MUCONALDEHYDE TO TRANS,TRANS-MUCONIC ACID. T A Kirley, B D Goldstein, and G Witz. Joint Graduate Program in Toxicology, UMNJ–Robert Wood Johnson Medical School/Rutgers University, Piscataway, NJ.

Trans,trans-muconaldehyde (MUC) has been shown to be a hepatotoxic metabolite of benzene in mice. In the present study we examined the in vitro metabolism of MUC by mice and rat liver cytosol to trans,trans-muconic acid (MA), a urinary metabolite to mucosal rats treated with benzene. MUC incubated with liver cytosol from male DBA/2 mice and Sprague-Dawley rats in the presence of NAD+ and pyrazole, an alcohol dehydrogenase inhibitor, resulted in NADH production as monitored at 340 nm, suggesting the formation of MA. In mouse liver cytosol metabolism of MUC gave a biphasic Lineweaver-Burke plot, with Km's of 0.07 and 0.01 mM and corresponding Vmax values of 13 and 10 mmol min⁻¹ mg⁻¹. A Km of 3.3 mM and Vmax of 80 mmol min⁻¹ mg⁻¹ was obtained for metabolism of MUC by rat cytosol. HPLC analysis of ether extracts of the cytosolic metabolism mixtures indicated the formation of MA by showing a peak at the retention time of authentic MA. Omission of MUC, NAD+, or use of boiled cytosol resulted in no peak on the HPLC chromatogram corresponding to MA. Preliminary data indicate that intraperitoneal injection of 2 mg/kg MUC into mice results in the formation of MA as detected by HPLC analysis of urine extracts. The data suggest that MUC is metabolized to MA by cytosolic aldehyde dehydrogenases. Supported by NIH grant # ES02558.

SHORT-TERM INHALATION EXPOSURE TO BENZENE PRODUCES MYELODYSPLASTIC SYNDROME AND LEUKEMIA IN C57BL/6 MICE. H P Cathro, W S Stillman, W H Steinhausen and R D Irvine, CIIT, Research Triangle Park, NC.

Benzene (BZ) is an established human leukaemogen with chronic exposure known to result in myelodysplastic syndrome (MDS), myelogenous leukemia and lymphoma. However, little is known about its mechanism of action. Historically, studies on the mechanism of BZ toxicity have been hampered by the inability to reliably reproduce MDS in experimental animals following benzene exposure. The present study was undertaken to characterize the regimen-dose dependence of BZ myelotoxicity in male C57BL/6 mice and was not designed to quantitate tumor incidence. Nevertheless, MDS was encountered in 12 of 20 mice exposed 100 or 300 ppm (3-h/da, 5d/ww) for 12 weeks and held post-exposure for an additional 6 mo. Lesions constituted a continuous spectrum ranging from inversion of the lymphoid/myeloid cell ratio in peripheral blood and moderate myeloid hyperplasia in spleen to marked myelodysplasia, preleukemia and leukemia. Neoplastic lesions included T cell lymphoma and early myeloid leukemias as defined by histopathology, surface marker analysis and growth requirements in culture. These findings suggest, for BZ, regimen as well as dose may be important considerations for modeling carcinogenesis.

METABOLISM OF 3H-BENZENE IN F344/N RATS AND B6C3F1 MICE: SPECIES AND DOSE EFFECT. P.J Sabourin, L S Binbaum, G Lucier and R F Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; *NIEMS, Research Triangle Park, NC.

Benzene causes aplastic anemia and leukemia in humans. Studies by the NTP indicate that B6C3F1 mice were more susceptible than F344 rats to benzene toxicity. We have initiated studies to determine the effect of dose on the metabolism of benzene in the two species. Water-soluble benzene metabolites were measured in blood, urine, liver, lung and bone marrow during and following a 6 hr exposure to 5, 50 and 600 ppm 3H-benzene. The area under the curve (AUC) of metabolite concentration vs. time was determined. In both rats and mice, the AUC of hydroquinone glucuronide (HOG) and muconic acid (MU) in blood, lung and liver was not linear with vapor concentrations above 30 ppm. Between 80 ppm and 600 ppm metabolism shifted to formation of phenylglucuronide and pre-phenylmercapturic acid. In all tissues analyzed, mice had a higher concentration of HOG and MU, markers for pathways of metabolism leading to the toxic benzene metabolites, benzoquinone and muconaldehyde. In rats, HOG was barely or not detectable. These results may, in part, explain why mice are more susceptible to the toxic effects of benzene than rats. (Research supported by NIEMS through IAA ES-20092 under U.S. DOE Contract No. DE-AC04-76EV01013.)
The relationships between DNA single-strand breaks (SSBs) and micromolecule formation induced by benzene and its metabolites were investigated. The micronucleated polychromatic erythrocytes in bone marrow cells of male mice were markedly increased at 48 h after a single oral administration of benzene. SSBs detected by alkaline elution assay in bone marrow cells were markedly increased at 36 h after the administration of benzene. In addition, the time-dependent response of SSBs was similar to that of the micronucleus formation. In the micronucleus test of the benzene metabolites, phenol and hydroquinone induced micronuclei but catechol, hydroxyhydroquinone, 1,4-benzoquinone and 4,4′-biphenol did not. In vitro studies using the Chinese hamster cells, hydroxyhydroquinone induced SSBs in the alkaline elution assay, and hydroquinone, catechol and mucous acid induced metaphase arrest in the metaphase/analphiase analysis. Moreover, hydroquinone, catechol, hydroxyhydroquinone and 4,4′-biphenol exhibited clastogenicity in the cultured Chinese hamster cells. These results suggest that the induction of micronuclei by benzene follows after SSBs, and the several metabolites play an important role to induce micronuclei.

Benzene (B) affects hematopoietic progenitor cells leading to bone marrow depression (BMD) and genotoxic effects such as micronucleus formation. Progenitor cell survival and differentiation is inhibited by prostaglandins produced by macrophages. Administration of 800 mg/kg 8 ip to DBA/2 mice caused BMD and a significant increase in marrow PGE level (radioimmunoassay) that was inhibited by indomethacin (1). Phenol, the major metabolite of B is converted in macrophages by the endoperoxidase activity of prostaglandin synthase (PS) to species that covalently bind to protein and DNA. This suggests a role for PS in BMD and genotoxicity. The ability of PS inhibitors to prevent BMD and genotoxicity was tested. Administration of R (200-1000 mg/kg) ip to DBA/2 or C57BI/6 mice caused a dose-dependent decrease in nucleated cells which was prevented by I (2 mg/kg), aspirin (50 mg/kg) or meclofenamate (4 mg/kg). B (400 mg/kg) caused a 50% decrease in cellularity and a 3 to 5-fold increase in micronucleus formation in peripheral blood polychromatic erythrocytes both of which were prevented by the coadministration of I. PS maybe involved in causing the myelo- and genotoxic effects from B. Supported by NIEHS grant ES 03724.
EFFECTS OF BENZENE METABOLITES IN COMBINATION ON FE UPTAKE INTO ERTHROCYTES IN MICE. E. Dimitriadi, R. L. Guy, P. Hu, K. R. Cooper, and R. Snyder. Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

Eastmond et al. (Toxicologist 7,4, 1987) suggested a toxicological interaction among benzene (B) metabolites. Using the ferrokinetic method of Lee et al. (Environ. Health Persp. 39:29, 1981) we have shown that the reduction in $^{37}$Fe uptake produced by B is a measure of B-induced experimental anemia. A similar procedure was used to evaluate the effects of mixtures of B metabolites on red cell production in mice. Phenol (P), catechol (C) and hydroquinone (H) were administered ip at doses between 25 and 100 mg/kg 3 times over a 36 hr period. $^{37}$Fe was administered iv 36 hrs later after which 24 hr uptake was measured. Using this protocol P produced no decreases in $^{37}$Fe uptake; a dose related decrease was observed with H. Mixtures of P+H or P+C produced decreases which were greater than predicted by the effects of the compounds given alone. No decreases were observed when the mixtures were given at the same time as the $^{37}$Fe indicating there was no effect on hemoglobin synthesis. The results suggest that B-induced decreases in red cell production may be the result of a cooperative effect of B metabolites. (Supported by ES02931)

HYDROQUINONE INHIBITS MACROPHAGE REGULATION OF STROMAL CELL DEPENDENT B-LYMPHOPOIESIS. A. King, K. Landreth, and D. Wiera. Deps. of Pharmacology/Toxicology and Microbiology and Immunology, West Virginia University Medical Center, Morgantown, WV.

Previous investigations have shown that benzene metabolite, hydroquinone (HQ), inhibits B cell production by preventing the maturation of pre-B cells. Our data indicated that HQ altered the production of interleukin-4 (IL-4) by stromal cells necessary for B-lymphopoiesis. The purpose of this study was to identify the stromal cell population affected following HQ exposure. A cloned bone marrow stromal cell line (SCL-173) was developed which produced IL-4 activity. HQ exposure of SCL-173 cells did not alter IL-4 production at any dose tested. Addition of untreated macrophages to HQ treated marrow cells restored pre-B cell maturation to IgM expression. However, addition of HQ treated macrophages was without effect. Further analysis suggests that HQ inhibits macrophage production of IL-1 which, in turn, induced production of IL-4 by stromal cells. In summary, HQ affects the ability of the bone marrow macrophages to regulate stromal cell production of IL-4 which is apparently required to sustain B cell formation. Supported by grants from the EPA R813986, and NIH AI-23950, ES-04808, and an SOT fellowship sponsored by Hoffman La Roche.

ACTIVATION OF BONE MARROW MACROPHAGES (MP) AND PMN FOLLOWING BENZENE TREATMENT OF MICE. L. MacEachern, R. Snyder, and D. Laskin. Rutgers Univ., Piscataway, NJ.

Flow cytometry/cell sorting was used to study the mechanism underlying benzene induced bone marrow (BM) toxicity. Analysis of BM cells from male Balb/c mice revealed two distinct subpopulations differing in size and density. Differential staining of sorted cells showed that the larger population was PMN and the smaller, mononuclear cells consisting of MP and immature blast cells. Treatment of mice with benzene (1 ml/kg s.c., 3 d), resulted in a 45-50% decrease in the total number of cells recovered from the BM. However, the proportion of MP and PMN was not altered. Using dichlorofluorescein, we analyzed the production of $H_2O_2$ by BM MP and PMN. We found that phagocytes from benzene treated mice produced an average of 25% more $H_2O_2$ following phorbol myristate acetate stimulation than did cells from control animals. In addition, using the calcium probe, Indo-1, benzene treatment was found to induce the appearance of a unique subpopulation of phagocytes in the BM that displayed elevated levels of intracellular calcium. These results suggest that MP and PMN in the BM are cellular targets for benzene toxicity. Supported by NIH Grants ES02931 and GM34310.

BONE MARROW STROMAL MACROPHAGE PRODUCTION OF INTERLEUKIN-1 ACTIVITY IS ALTERED BY BENZENE METABOLITES. D. J. Thomas, D. Wiera. Dep. of Pharmacology and Toxicology, West Virginia University Medical Center, Morgantown, WV.

Previous investigations have shown that benzene metabolites alter bone marrow stromal cell function. To determine which stromal cell population was most sensitive to benzene metabolites, we assayed the effect of hydroquinone (HQ), benzoquinone (BQ), phenol (PH), and catechol (CT) on independent populations of bone-marrow derived stromal macrophages and fibroblasts. Fibroblast cell function was expressed as the capacity of fibroblast conditioned media to stimulate the formation of granulocyte/macrophage colonies in agar. The level of lipopolysaccharide inducible interleukin-1 (IL-1) secreted by macrophage was used as an index of macrophage function. IL-1 regulates fibroblast dependent myelopoiesis. Macrophages or fibroblasts were cultured with different concentrations of 10^-7 M of metabolite for 24 or 48 hrs, and conditioned media assayed for activity. Metabolites did not effect fibroblast function except at concentrations of 10^-4 M. In contrast, macrophage production of IL-1 activity was markedly inhibited by HQ and BQ and stimulated by CT and PH. These results indicate that benzene can exert its myelotoxic action by selectively influencing stromal macrophage production of IL-1. (NIH ES04808)
.activation of phenol and hydroquinone to covalently binding metabolites by mouse macrophage lysates. M J Schlosser and G F Kalf Department of Biochemistry and Molecular Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA.

Benzene(B)-induced bone marrow (BM) hypoplasia is due in part to its metabolites phenol (P) and hydroquinone (HQ) which are metabolized in BM to reactive intermediates via peroxidases. A prostaglandin H synthase(PHS)-peroxidase is likely since B-induced myelotoxicity is prevented by inhibitors of PHS activity. Macrophages, a major BM cell-type, contain relatively large amounts of PHS-peroxidase and are known to metabolize P. Incubations of either C-P or C-HQ with lysates of macrophages collected from mouse peritonium resulted in a H,0_, dose, and time-dependent metabolic activation, as indicated by covalent binding (CVB) of labeled metabolites to albumin. Production of metabolites from P (0.5mM) was inhibited by aminooxacetone, ascorbic acid and B-M HQ (52%), whereas binding from HQ (0.5mM) was increased by 5mM P (17%). Glutathione (GSH), a nucleophile and PHS-peroxidase substrate, inhibited CVB from both P and HQ in a dose-dependent manner. CVB of H-GSH (0.5mM) was increased by 5mM P (36%); however, GSH binding was decreased by 5mM HQ (11%), suggesting the presence of a GSH-conjugate. These results suggest that P and HQ are metabolized by a macrophage peroxidase to CVB intermediates. (NIHES grant ES03724).


The timecourse of triethylthine (TET) induced effects on hypothalamic brain-stimulation reward (BSR) was examined. Male hooded rats were trained in a threshold procedure that daily determined the shortest stimulus duration supporting discrete-trial leverpress responding for BSR. After a stable baseline threshold duration was obtained, 3 mg/kg TET-Br was administered by i.p. injection. Threshold duration was elevated 1 hr postexposure but returned to baseline by 24 hr postexposure. A second increase in threshold occurred approximately 5 days postexposure, returning to baseline by approximately 4 weeks postexposure. The later increase probably reflects the myelotoxic effects of TET, whereas the early increase in threshold may reflect a rapid suppression of synaptic transmission like that observed following acute TET exposure in the in vitro hippocampal slice (See for Neurosci, Abstr 13: 695, 1987). To evaluate the latter hypothesis, neurochemical analysis of the timecourse of TET effects will be presented that may help explain the frequently observed dissociation in timecourse of behavioral and anatonical consequences of TET exposure.

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TMT is a potent limbic-system neurotoxin with known effects on cognitive function in adult animals. The present study examined the effect of neonatal TMT administration on cognitive development. Long-Evans rat pups received an i.p. injection of either TMT (6 mg/kg in 10 ul/g saline) or saline vehicle on postnatal days 10 (PND 10). These pups were then trained and tested for retention on an aversive olfactory discrimination task (Kucharski and Spear, Develop Psychobiol, 1984, 17, 465-479). Training took place on either PND 12 or PND 18 and testing took place either immediately or after 24 hrs or 7 days. Saline pretreated animals showed the typical, age related emergence of long-term retention at the 7-day retention interval: i.e., 18-day-olds showed good retention of the discrimination after 7 days whereas 12-day-olds did not. TMT pretreatment prevented this age-related increase in retention capacity. A second experiment revealed a similar pattern of results when TMT injection followed original training. These findings indicate that neonatal exposure to TMT compounds can impair cognitive development, and illustrate the value of olfactory conditioning as an animal model for assessing neurotoxic effects on the ontogeny of learning and memory.

The acute effects of Bis(tri-n-butyltin)oxide on motor activity: oral versus inhalation exposure. K M Crofton, M E Biteshe, L P Sheets, V M Boncak, R F Dean, and L B Reiter. Neurotoxicology Division, US EPA, RTP, NC and Northrop Services, Inc, Environmental Services, RTP, NC

Bis(tri-n-butyltin)oxide (BTTO) is an organotin compound used as aocide in marine paint. The purpose of the present study was to determine and compare the effects of oral and inhalation exposure to BTTO on neurobehavioral function using motor activity (MA). Dosage-effect functions were determined in male Long Evans hooded rats (n-8/group). Acute oral (0-150 mg/kg BTTO) or nose-only inhalation exposure (0-11 ppm BTTO as vapor for 2 hr) was followed (1 hr or 1/2 hr, respectively) by activity measurement for 1 hr in figure-eight mazes. Oral exposure produced a dosagedependent decrease in MA (ED50 ~20 mg/kg). Inhalation exposure reduced MA in a concentration-dependent manner (ED50 ~10 ppm). The relationship between a behaviorally effective dosage and lethality differed with route of exposure. Inhalation exposure produced respiratory distress (at > 9 ppm) and mortality (LD50 ~11 ppm). Oral exposure to BTTO decreased activity at 1/10 the oral LD50, whereas inhalation exposure decreased activity only at dosages that produced respiratory distress or lethality. These findings suggest differences in the toxicity of BTTO following oral and inhalation exposure.
ANALYSIS OF THE COGNITIVE IMPAIRMENT INDUCED IN RATS BY TRIMETHYLLIN: AUTOMAINTAINED REVERSAL LEARNING. D New*, P J Bushnell, and D D Dunn*. Neurotoxicology Division, US EPA, RTP, NC, and *Northrop Services, Inc. - Environmental Sciences, RTP, NC.

Trimethyltin (TMT) is a potent neurotoxicant known to disrupt learning and memory. It thus provides a positive control treatment for characterizing cognitive dysfunction in test animals. Eight male hooded Long-Evans rats, maintained at 350±10 g body weight, were presented two retractable bars for 19" (CSs) per trial on independent TTT (45°) schedules. A food pellet (UCS) was delivered upon retraction of one of the bars (hot CS), but not the other (cold CS). Presses on both bars (CSs) were counted but had no programmed consequences; thus one pellet was delivered per trial regardless of the rat's behavior. At asymptote, ho SC exceeded 10/trial, while cold CS approached 0/trial. When the positions of the hot and cold CSs were reversed (R), CSs shifted to the new hot bar. Successive reversals (2/week) occurred at faster rates: by R12, reversal was complete within one 100-trial session. Injection of TMT (7 mg/kg, iv) to half the animals after 544 reduced hot CS rates for 1 week, after which rates were normal. Thereafter, ratios of hot CS to total CSs were normal when no delays were used. With delays of 2 to 4 sec, treated animals reversed more slowly than controls. These results indicate that TMT increases rats' sensitivity to temporal factors.


The conditioned flavor-avoidance paradigm was used to determine the behavioral toxicity of acutely administered cadmium and the interaction of cadmium with the heavy-metal chelators dimercaptopropanosuccinic acid (DMPSA) and dimercaptoethyl (BAL). Rats received cadmium (ip) either alone or in combination with BAL or DMSA (ac) 20 min after consuming saccharin. Twenty-two hrs later they were given concurrent access to saccharin and water, and saccharin preferences were recorded. Rats receiving cadmium displayed significant reductions (compared to controls) in saccharin preference (i.e., conditioned flavor aversions). BAL and DMSA were also capable of producing conditioned flavor aversions when given alone. Rats receiving cadmium in combination with either BAL or DMSA displayed significant attenuations of conditioned flavor aversions when compared to the flavor aversions of rats receiving cadmium alone. Chelator-induced attenuation of cadmium-induced flavor-avoidance conditioning was not obtained when BAL or DMSA administration was delayed by 4 hrs. These results extend earlier findings of an attenuation of lead- but not thallium-induced flavor-avoidance conditioning by these heavy-metal complexing agents.
MANGANESE ADMINISTRATION INDUCES PERSISTING EFFECTS ON EFFORTFUL BEHAVIORAL RESPONSES.
R Weiss and M C Newland, Environmental Health Sciences Center, Univ of Rochester School of Medicine and Dentistry, Rochester NY.

High doses of manganese (Mn) produce signs of basal ganglia dysfunction in primates. To characterize the effects of lower doses, three Cebus monkeys were trained to execute a rowing-type motion through a 10 cm displacement against a 4 kg force. Performance was maintained by a multiple fixed ratio-fixed interval (multi FR FI) schedule of reinforcement. Two monkeys received saline, followed by two doses of 5 mg/kg Mn, iv. at intervals of at least 4 weeks. The third monkey received a total of 30 mg/kg in four doses at least one week apart. The high-dose monkey exhibited a persistent movement tremor, but no other sign of toxicity. In all monkeys, Mn increased the number of incomplete responses (those that did not extend to the full 10 cm) by 10- to 100-fold. This effect appeared a few days after the last dose, persisting up to several weeks, and undulating in cycles of several weeks duration. Schedule patterning remained intact in the two low-dose animals, but vigorous FR-like responding began to appear in the FI performance of the high-dose monkey several months after the last dose. (Supported by ES-01247, ES-01248, and AA-01588.)

CHOLINESTERASE INHIBITION AND NEUROBEHAVIORAL EFFECTS IN PARAOXON-TREATED LONG-EVANS RATS.

To determine the effects of the organophosphate paraaxon on neurobehavior using a battery of neurobehavioral tests, 24 male 400-600 g Sprague-Dawley Long-Evans rats were by intraperitoneal injection administered in groups of eight 200 or 400 µg of paraaxon/kg b.w. or a control solution. Brain, plasma, and erythrocytes were assayed for cholinesterase (ChE) activity at the end of the experiment. Brain ChE was inhibited 57% and 19% with 400 µg/kg and 200 µg/kg paraaxon, respectively. Neurobehavioral endpoints included; Figure 8 (FB) maze activity (counts/5 min), Accelerating Rotarod (AR) (sec on rod), Grip Strength (GS) (kg), and Startle Reflex (SR) (amplitude, voltages, and latency in seconds). No significant differences were detected in GS or AR performance between toxicant and control groups of rats. Audio and tactile SR amplitudes were increased with 200 µg/kg of paraaxon and decreased with 400 µg/kg of paraaxon. Initial exploratory activity and activity during the remainder of the 60 min FB session were decreased in paraaxon treated rats. Overall, paraaxon affected the sensory and exploratory behavior paradigms, but did not affect neuromuscular or balancing performance at these treatment levels. (Supported in part - US Army.)


Previous work from this lab showed that Type I pyrethroids (T-I) increase and Type II pyrethroids (T-II) decrease ASR amplitude in adult rats. We have recently found age-related differences in their effects: T-I do not alter and T-II decrease ASR amplitude in preweanling rats. These differences could reflect separate mechanisms; a DDT-like effect of T-I on axonal sodium channels and a picrotoxin (PTX)-like effect of T-II on the GABA receptor complex. The present study examined the effects of DDT and PTX on the ASR during development to compare with the effects of T-I and T-II. Long Evans preweanling (ages 17 and 21 days) and adult (70 day old) rats (n=12/group) were tested 3 hr after DDT (po in corn oil) or immediately after PTX (ip in saline). The results confirm reports that DDT (25 mg/kg) increases and PTX (1-2 mg/kg) decreases the ASR in adult rats. In preweanling rats, DDT did not alter ASR amplitude even at much higher dosages (125 mg/kg), whereas, PTX decreased the ASR at the same dosages used in adults. Age-related similarities between DDT and T-I support a sodium channel mechanism for T-I to increase the ASR and indicate that critical development occurs after postnatal day 21. The results are consistent with a PTX-like mechanism for the effects of T-II on the ASR. (Supported by NRC Research Associateship.)

ASSESSMENT OF MEMORY DEFICITS FOLLOWING REPEATED ORGANOPHOSPHATE EXPOSURE. R Hafsele, D Columbus, and Z Anmao. The Johns Hopkins University, Baltimore, MD.

To assess changes in memory and development of tolerance following repeated exposure to organophosphate anticholinesterases, and to investigate a possible mechanism for the development of tolerance, rats were trained in a serial reversal protocol. Each day, a rat was required to remember the correct response learned the day before, and then learn a new correct response. After rats achieved stable performance they were divided into two groups. One group received disopropylfluorophosphate (DFP), injected i.p, 1 mg/kg on the first day and 0.5 mg/kg every 3rd day thereafter. The other group received saline injections. To determine whether DFP-tolerant rats would be more sensitive than normal rats to stress on the cholinergic system, rats were also injected with scopolamine, a muscarinic receptor blocker, 20 min. prior to testing on some days. A given dose of scopolamine produced greater impairment in DFP-tolerant rats than in control rats, even if the DFP-tolerant rats were performing normally when no scopolamine was present. This demonstrates that, although there may be no obvious impairment following repeated exposure to organophosphate anticholinesterases, a tolerant system is not the same as a normal system and may not be able to react normally when challenged. (Supported by NIHS 5732 ES 07149.)

The effect of acute sub-lethal and antidoted/lethal doses of paraoxon were studied on the retention of avoidance behavior and concurrently on 3H-QNB (quinuclidinyl benzilate) binding in the corpus striatum. Adult male rats were trained to avoid a shock in response to a buzzer in a shuttle box. Following stabilization, rats were injected i.p. with the toxinant and/or antidote (atropine). Their performance was monitored at 1 and 2 days after treatment. On day 2, membranes from the corpus striatum were obtained for 3H-QNB binding studies. About 90% inhibition of brain acetylcholinesterase was observed on the day of injection with both doses of paraoxon, with still about 50% inhibition remaining on day 2. There were some decreases in avoidance percentages and increases in escape percentages on day 1, with recovery by day 2. Avoidance latencies were unaltered and inconsistent effects on escape latencies were seen on day 1. There were no statistical differences in affinities or densities of muscarinic receptors. Despite the extensive and persistent inhibition caused by an acute exposure to a high dose of paraoxon, relatively minor effects were observed in performance and no effects on receptors. (Supported by EPA R-811292).

Reversibility and Tolerance to Tricresyl Phosphate-Induced Neurotoxic Effects in F344 Rats. G B Freeman, R Irwin, R Trejo, M Heftmancik, M Ryan, and A C Peters. Battelle Columbus Division, Columbus, OH and NIEHS, Research Triangle Park, NC.

After 13 weeks of dosing with tricresyl phosphate (CAS No. 1330-78-5) in feed, hindlimb grip strength decreased in male rats (600 and 300 ppm), but not in female rats. Serum cholinesterase was reduced significantly relative to control in the 600 (-27%, 45%) and 300 (-18%, 35%) ppm male and female dose groups, respectively. Animals in the 600 ppm dose groups were fed tricresyl phosphate-dosed feed through week 23, after which they were maintained on blank (undosed) feed. At the 39-week interim termination, all clinical pathology, organ weight and hindlimb grip strength data for the 600 ppm male and female dose groups were statistically similar to those of controls. Therefore, all previously seen compound-related effects were apparently reversed when 23 weeks of exposure to tricresyl phosphate in feed at a concentration of 600 ppm was followed by 16 weeks of undosed feed. Hindlimb grip strength scores of male rats in the 300 ppm group that remained on the tricresyl phosphate were no longer different from control animals after 39 weeks, although serum cholinesterase was still reduced significantly relative to control (-19%). This suggests a development of tolerance to the neurobehavioral effects of tricresyl phosphate in the diet as well. (Supported by Contract No. 501-ES-45050 from NTP).

The Interaction of Carbon Monoxide with Ethanol, Chlomprazine, Pentobarbitol or Diphenylamine on Fixed-Ratio Performance in the Mouse. J S Knisely, D C Reese, R L Balster, and L J Thomas. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA.

The behavioral effects of various dosage combinations of CO with ethanol (EtOH), chlomprazine (CPZ), pentobarbitol (PB) or diphenylamine (DPA) were investigated in mice trained to lever press under a fixed-ratio 32 schedule of water reinforcement. Following a dose-response curve determination for each drug, animals were then administered intraperitoneal CO (3.75, 7.5, 15 and 30 ml/kg) alone and in combination with the ED50 of EtOH (1.1 g/kg), CPZ (2.2 mg/kg), PB (23 mg/kg) or DPA (1.4 mg/kg). Only the highest dose of CO (30 ml/kg), when given alone, produced significant decreases in rates of responding in some animals. However, when CO was given in combination with EtOH, a significant interaction was observed at doses of CO as low as 7.5 ml/kg (associated EtOH levels of <20%). Also, response-rate suppression following 30 ml/kg CO was enhanced when administered with CPZ, and DPA. When PB was given with CO, no significant interaction was obtained. (Supported by grants ES-03809 and ES-07087).

Proconvulsive Activity of Quinolone Antibiotics in an Animal Model. P D Williams and R Helton, Lilly Research Laboratories, Toxicology Division, Greenfield, IN.

The side effect profile of quinolone antibiotics in man includes CNS disturbances such as dizziness, vertigo, and convulsions. Although the convulsive liability has been suggested to involve interaction with GABA receptors in the CNS, no animal model has been described to evaluate or confirm the mechanism of this effect. The proconvulsive behavior of the quinolones, nalidixic (NAL) and oxolinic (OXO) acid, were examined in male CF mice utilizing pentylenetetrazol, picrotoxin, strychnine and electroshock as convulsants. Following single oral doses of 10, 30, 100 mg/kg NAL or OXO, the threshold for pentylenetetrazol, picrotoxin, and strychnine-induced convulsions was not altered; however, electroshock-induced seizures were potentiated in a dose-dependent fashion. OXO and NAL (100 mg/kg) produced a 60 and 50% incidence of convulsions, respectively, at an ED10 dose of electroshock (6.9 mA). These results suggest that 1) the mechanism of convulsive liability of quinolones may not involve GABA antagonism (as tested by picrotoxin) but may involve pathways related to electroshock seizure activity and 2) the electroshock model may provide a useful tool for evaluating the convulsive liability of new quinolone antibiotics.
Zacopride: A Promising Radiation Antiemetic.

Zacopride (p.o., 0.3 mg/kg) was tested in monkeys (mean weight 3.5 kg) for its ability to block radiation-induced emesis and to assess behavioral toxicity. In the emesis study, monkeys were tested after placebo- or zacopride-only, and after placebo- or zacopride-radiation (N=6/group). Zacopride or placebo were given 15 min before 8 Gy whole-body, gamma radiation. No emetic effects were noted after placebo- or zacopride-only. After radiation, 70 emetic episodes occurred in 5 of 6 monkeys in the placebo/radiation condition compared to 1 episode after zacopride/radiation (p < 0.01). Also, 353 retching episodes occurred in the 6 radiation-only monkeys compared to 175 episode in 4 of 6 zacopride/radiation monkeys (p < 0.01). In the toxicity study, monkeys performed a visual discrimination task (VDT). Maximum response time was 0.7 sec to discriminate between a circle and square (correct), randomly presented every 10 sec. Monkeys (N=6) were tested for a 3 hr in control-, placebo-, and zacopride-only conditions. Monkeys maintained performance above 95% correct in all behavioral conditions. These results suggest that zacopride significantly inhibits radiation-induced retching and vomiting, and that it is not behaviorally toxic, which may have important operational and clinical implications.

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Cognitive deficits in humans accompany the motor dysfunction, tardive dyskinesia (TD), which results from chronic neuroleptic administration. It is not clear if the cognitive deficits result from neuroleptic exposure or if preexisting neurological damage which causes cognitive deficits predisposes patients to TD. We injected male and female albino rats monthly with haloperidol or fluphenazine decanoate in doses equivalent to (0.5, 1, 2 and 4 mg/kg/day). After 16 months the rats were tested for exploration in an 8-arm radial maze. Both haloperidol (p<.025) and fluphenazine (p<.001) caused decreases in the distribution of choices as measured by entries to repeat. Response speed was slowed by haloperidol (p<.005), but fluphenazine only had a marginal effect (p<.10). The decrease in choice distribution demonstrates that long term neuroleptic administration does affect cognitive function. In humans chronic neuroleptic exposure may induce not only motor disorders but cognitive dysfunction as well. (Supported by Swedish MRC grant # 4546).

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BEHAVIORAL IMPAIRMENT IN THE RAT AFTER COLCHICINE LESIONS OF THE NUCLEUS BASALIS. W R Mundy and H A Tilson. NIEHS, Res. Tri. Park, NC.

Neuronal loss in the nucleus basalis of Meynert (NBM) has been consistently associated with memory impairment. In the present study rats received bilateral injections of colchicine (2.0 μg/site) into the NBM and examined for changes in three models of learning and memory. Retention of a step-through passive avoidance task was examined 28 days after surgery. Rats with NBM lesions had decreased step-through latencies compared to controls. Training began in a Morris water maze on the next day. NBM lesions had no effect on escape latencies during water maze acquisition. After water maze training, rats were food-deprived to 85% original body wt. and trained on an autoshape task, in which they learned to associate lever presentation with food pellet delivery. NBM-lesioned rats initially made a greater number of responses (lever touch) compared to controls, but both groups reached criterion in the same number of trials. NBM-lesioned rats also made a greater number of responses during the intertrial interval. At the completion of autoshape training rats were given free access to food and tested for retention of the water maze task. There was no difference in retention between lesioned and control rats. These data indicate that NBM lesions do not affect acquisition but selectively impair retention of a nontemporal reference memory task.
THE DIRECT APPLICATION OF NMDA TO THE HIPPOCAMPUS PRODUCES MEMORY DEFICITS IN RATS. B C Rogers, W R Mundy, P Pediatakis, and H A Tilson. Toxicology Curriculum, University of North Carolina, Chapel Hill, NC 27514 and NIHES, Research Triangle Park, NC 27709.

Reductions in cortical and hippocampal N-methyl-D-aspartate (NMDA) receptors have been reported in patients with senile dementia of the Alzheimer's type (SDAT) raising the possibility that glutaminergic hyperactivity in these areas may contribute to the progression of this disease. We report that direct application of NMDA (1,2, or 4 mg) to the frontal cortex has no effect on overall motor activity, water maze acquisition, or cholineacetytransferase (CHAT) activity despite producing reductions in cortical width at the site of application. The effects of NMDA on the hippocampus were studied by injecting rats bilaterally with either artificial CSF, 2,5, 5.0, 10.0, or 20 ug/site NMDA. Intrahtppocampal NMDA damaged pyramidal and to a lesser extent granule cells. NMDA produced a dose-related increase in motor activity each week of testing. In addition, NMDA produced selective dose-related elevations in hippocampal CHAT activity. Animals receiving 10 ug/site injections were also significantly impaired in water maze acquisition. These results suggest that NMDA may be useful in studying hippocampal glutaminergic dysfunction which may be associated with SDAT. (B.C.R. was funded by ES07126).


The aversive and immunosuppressive effects of cyclophosphamide (CY), an unconditioned stimulus (UCS), were paired with a novel saccharine drinking solution (SACC), a conditioned stimulus (CS), in Balb/c mice. The objective was to determine the temporal relationship between exposure to the CS and immunization with sheep red blood cells (SRBCs), a T-dependent antigen, and Type III pneumococcal polysaccharide (S3), a T-independent antigen, on subsequent antibody responses. Responses to either CS or UCS occurred on days -2, 0, +2, or +4 relative to immunization. Antibody responses were measured six days after immunization. A strong association between the CS and UCS developed producing flavor aversions evidenced by decreased SACC consumption on the second presentation relative to the first, or to unpaired controls. CY consistently suppressed both types of antibody responses. CS presentation (i.e., SACC) had no effect on anti-S3 antibody response. However, the anti-SRBC response was significantly depressed following CS exposure. Exposure to the CS only on days -4, or +2 relative to immunization resulted in anti-SRBC suppression. These results support the premise that conditioned immune suppression is specific for T-dependent antigens and CS presentation relative to immunization is an important factor in eliciting this response.

PERINATAL IMMUNOTOXICITY OF BENZENE. D Wierda, W J Leubke and R J Smialowicz. Dept. Pharmacology and Toxicology, West Virginia University Medical Center, Morgantown, WV and U S EPA, Research Triangle Park, NC.

Development of the immune system involves a precise sequence of steps which begins early in gestation. In mammals, the fetal liver is a major site of lymphopoiesis during embryonic development. We initiated studies to examine whether benzene exposure of mouse fetuses in utero compromises the development of fetal B lymphopoiesis. Pregnant Balb/c dams were injected i.p. with 200 mg/kg benzene twice a day beginning on day 12.5 of gestation. Fetal livers were obtained on day 19.5 on gestation and a dispersed suspension of hematopoietic cells was planted into liquid culture. Maturation of B lymphocyte precursors into pre-B cells and B cells was analysed phenotypically over a two day culture period by immuno-fluorescence. Fetal liver cultures from benzene exposed dams contained 30-50% fewer pre-B and B cells than did control cultures. Separate cultures of adherent, fetal liver, stromal cells established from corresponding fetuses contained 50% fewer cells. These results indicate that benzene does not impair fetal B cell maturation at discrete stages of B cell development but instead causes a nonselective reduction in all fetal cells involved in B cell development. (Supported by EPA Coop Agreement CR815583; and does not represent EPA policy)
A large volume of information has been accumulated in rodents on the effects of various xenobiotics on the immune system. This has provided valuable information regarding dose, kinetics, and target cell populations affected. To gain insight into the relevancy of these observations to man, a study is underway to determine the influence of 5 immunotoxicants of rodents on human lymphocyte function. Lymphocyte function is assessed by 2 assays: mixed lymphocyte response (MLR) and quantitation of IgM and IgG by an ELISA following stimulation of B lymphocytes with pokeweed mitogen. In each assay, PB are preincubated with xenobiotics at 4-5 concentrations and then cultured in vitro. The chemicals under study include: TCDD, PCB, DMBA, BaP, and 2-AAP. Preliminary data for the MLR, have provided approximate ED 10 0 (TCDD), 10 0 (PCB), 1.4 µg (BaP), and 2 µg (DMBA). 2-AAP did not alter the MLR prior to or following metabolic activation with a liver microsome preparation (S9) at any of the concentrations studied (0.2-100 µM). Additional functional assays are currently being evaluated. These studies should improve risk assessment for human populations regarding relevant dose and better define pertinent biomarkers for immune dysfunction following xenobiotic exposure.

MODULATION OF PHA-INDUCED T-CELL CALCIUM MOBILIZATION BY DMBA T A Thompson, R H Fincher, and S W Burchiel, University of New Mexico College of Pharmacy, Albuquerque, NM 87111

The purpose of these studies was to evaluate the effect of 7,12-dimethylbenz(a)anthracene (DMBA) on calcium mobilization in murine and human T-cell lines exposed to phytohemagglutinin (PHA). We have previously shown that DMBA inhibits T-cell proliferation induced by PHA. DMBA may inhibit PHA-induced calcium mobilization that is necessary for T-cell activation. In these studies, Indo-1 was used to analyze calcium mobilization in a murine (WEHI-7) and a human (Jurkat) T-cell line. A FACS Analyzer was used to determine the fluorescence ratio of Indo-1 in its calcium-bound and calcium-free states. The kinetic changes that occurred in the fluorescence ratio (violet to blue) was monitored for up to 10 minutes following the addition of 50 µg/ml PHA to WEHI-7 or Jurkat. Exposure of WEHI-7 and Jurkat to 10 µM DMBA for 5 hrs resulted in a decrease in calcium mobilization induced by PHA. A 20 hr exposure of Jurkat to DMBA demonstrated a dose-dependent decrease in calcium mobilization at 1, 4 and 10 µM. These studies suggest that one effect of DMBA is to modulate activation of T-cells by altering calcium mobilization.

THE DISRUPTION OF LYMPHOCYTE TRANSMEMBRANE SIGNALLING BY XENOBIOTICS. LM Thurmond, RV House, JH Dean. CIIT, RTP, NC.

Early events in lymphocyte activation include the interaction of mitogen or antigen with membrane receptors, followed by release of Ca 2+ from endoplasmic reticulum, activation of protein kinase C, and turnover of membrane phospholipids. Exogenous induction of calcium flux using ionomycin and kinase activation using phorbol myristate acetate (PMA) bypasses normal signalling events. Experiments have been designed to identify the stages of lymphocyte activation affected by exposure to the model PAH immunosuppressant 7,12-dimethylbenz[a]anthracene (DMBA). Proliferation of DMBA-exposed lymphocytes to concanavalin A was suppressed in the presence of PMA and Ionomycin, as was the production of IL 2. IL 2 receptor function was impaired by a two-fold decrease in affinity for I-IL 2. Measurements of intracellular Ca 2+ using the fluorescent probe Quin 2 show that DMBA-exposed lymphocytes have three-fold higher basal Ca 2+ concentrations, but are less responsive to Ionomycin-induced Ca 2+ flux. Estimations of membrane phosphoinositol lipid turnover show altered accumulation of it-inositol phosphates in DMBA-exposed cells. These data suggest that normal signal transduction across lymphocyte plasma membranes is impaired by DMBA. Thus, the immune system may be vulnerable to adverse effects of hydrophobic xenobiotics due to the dependence of the immunoregulatory network on membrane-mediated activation events.

EFFECTS OF 2-ACETYLAMINOFLUORENE ON IN VITRO IMMUNE RESPONSES IN MURINE SPLENOCYTIES. K H Yong, D H Kim, T Kawabata*, and M P Holanapple*, Korea Advanced Institute of Science and Technology, Seoul, Korea and *Medical College of Virginia, Richmond, VA.

The immunosuppressive effects of 2-acetylaminofluorene (AAF) were investigated in female BALB/c mouse splenocytes. Direct addition of AAF into spleen cell suspensions resulted in a dose-related suppression of the in vitro antibody responses to LPS and SRBC. AAF also produced a dose-related suppression of the proliferative responses to LPS and Con A. Time course study showed that AAF produced the maximum suppression of the lymphoproliferative responses when was added to the culture along with mitogen at the initiation of cultures. To determine the role of metabolic activation of AAF for the immunosuppressive effects, mouse splenocytes were cocultured with rat hepatocytes for 4 hr along with AAF. AAF did not produce the suppression of the in vitro antibody response to LPS in this coculture system. Meanwhile, AAF induced a dose-related unscheduled DNA synthesis in spleen cells only if activated by hepatocytes in the splenocytes-hepatocyte coculture system. These results suggested that the reactive metabolites of AAF which mediates its mutagenicity are not essential for its immunosuppressive activity. (Supported by the Korea Science and Engineering Foundation Research Grant).
An impurity in malathion, 0,0,5-trimethyl phosphorothioate (OOS-TPM), was previously shown to be immunosuppressive. The immune cell type which induced immune suppression caused by OOS-TPM at 24 hrs after administration was found to be splenic macrophages. Further characterization of macrophages from OOS-TPM treated mice indicated that OOS-TPM led to macrophage differentiation. In this study, these initial studies were continued and extended to examine the effects of OOS-TPM on splenic and peritoneal macrophages at various times following exposure. Administration of OOS-TPM increased the size heterogeneity of cell volume, phagocytic capability and respiratory burst activity of splenic and peritoneal macrophages. However, by day 7 splenic and peritoneal macrophages from treated animals had frequency histograms, phagocytic capability and respiratory burst activity similar to control. These data would suggest that macrophages not previously exposed to inflammatory stimuli migrated to the spleen and peritoneum of treated animals. This migration may allow the restoration of the ability of splenocytes from treated animals to generate an immune response. Supported by NIMH Grant ES09437.
320 INHIBITION OF HUMAN SERUM COMPLEMENT (C3)
ACTIVITY BY DITROXYFURYLFLUOROPHOSPHATE (DFF) AND SELECTED ANTIOXIDASE INSECTICIDES.
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Activation of the human C3 system, a major line of defense against infections, requires the participation of serum esterases. The insecticides carbaryl (Ca), carbosulfan (CS), dichlorvos (DDVP), and parathion (Ps) inhibit serum esterases including C3E. The present study evaluated potencies of the latter to inhibit C3-mediated lysis of sheep red cells (SRBC), by a panel of 6 normal, human sera. Test chemicals were added to diluted sera, just prior to incorporation into C3 reaction mixtures. Potencies to inhibit C3 and serum cholinesterase (CHE) were compared to potencies of DFF, a potent serum esterase inhibitor. At 0.5 to 30 mU, Ca, CS, DDVP, and DFF produced a dose-dependent inhibition of lysis, while Ps was not inhibitory. On a molar basis, Ca was 3 times more potent than DFF, and inhibited lysis 15-25% at 1.0 and 3.0 mU, respectively. Ca, DDVP and DFF were equipotent. Mean T50s for inhibition of CHE by DFF, Ps, DDVP, CS, and Ca were 1.0x10^-6 M, 2.0x10^-6 M, 1.0x10^-6 M, 3.3x10^-6 M, and 1.0x10^-5 M, respectively. Potencies of insecticides to inhibit CHE did not predict absolute or relative potencies to inhibit human, C3 activity. Supported by NAFIA, ES/USDA.

322 SUPPRESSION OF HUMORAL IMMUNITY BY MONO-NITROTOLUENES (A STRUCTURAL ACTIVITY STUDY).
H H Lye, J A McCoy, K L White, Jr, and A E Munson. Deps. of Pharmacology and Toxicology, and Biostatistics, Medical College of VAI/VCU, Richmond, VA.

Mononitrotoluenes are intermediates in the manufacture of a variety of products, from explosives to pharmaceuticals. It has been estimated that over 8000 occupational exposures occur annually. The effects of toluene and three mononitrotoluene isomers on humoral immunity were evaluated. Female B6C3F1 mice were exposed by oral gavage to toluene, 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), or 4-nitrotoluene (4-NT) for 14 days. Doses for 3-NT and 4-NT were 200, 400, and 600 mg/kg/day. Toluene and 2-NT were administered at 600 mg/kg/day. The IgM antibody forming cell response to the T-dependent antigen, sheep erythrocytes, was suppressed in a dose-dependent manner. The greatest suppression observed was 38% and 61% for 3-NT and 4-NT, respectively. Toluene and 2-NT did not alter the IgG response. The IgG antibody forming cell response to sheep erythrocytes was not suppressed by any of the chemicals. A slight trend toward a decreased lymphocyte proliferation response was observed only with 4-NT. Host resistance assays to bacterial challenges revealed that susceptibility to Streptococcus pneumoniae was not altered, while susceptibility to Listeria monocytogenes was enhanced when animals were exposed to the nitrotoluenes. These results suggest that the immunosuppression observed with the nitrotoluene compounds is related to both the presence and position of the nitrate moiety. (Supported by NIEHS contract ES 55594).

321 THE IMMUNOMODULATORY ROLE OF [ALA] METHIONINE ENKEPHALONAMIDE IN INHIBITION THE TUMOR EXPRESSION OF PYB6 TREATED MICE.
B Srivastava, L E Sikorski, A E Munson, S E Loveless. Dept. of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA, and E 1 duPont de Nemours & Co, Inc, Glenolden, PA.

Tumor growth has been shown to be influenced by the endogenous opioid system. The objective of the present study was to investigate the immunomodulatory role of the synthetic peptide, [D-ala2]methionine enkephalinamide (AS), in inhibiting tumor growth of the PYB6 fibrosarcoma. Daily intraperitoneal injections of AS (between 0.5 and 1 mg/kg) for 7 days decreased tumor size. The magnitude of the antitumor response by AS was a function of the number of tumor cells transplanted. Pretreatment with the opioid receptor antagonist, naloxone (0.05-4.00 mg/kg), reversed the suppressive effects of AS on tumor growth, suggesting that the pharmacological action of AS may be mediated via the opioid receptor. AS did not directly affect the viability of PYB6 tumor cells, as evaluated by the measurement of cellular ATP levels in AS treated PYB6 cells. Therefore, the antitumor activity of AS acts via an indirect mechanism. Immunological studies indicated that AS selectively enhanced the lymphoproliferative response to the T-cell mitogen, concanavalin A, but not to the B-cell mitogen, lipopolysaccharide. Natural killer cell activity and the number of IgM plaque forming cells in response to the T-dependent antigen, sheep red blood cells, were unaffected after both in vivo and in vitro exposure to AS. (Supported by E 1 duPont de Nemours & Co, Inc.)

323 IMMUNOTOXICITY IN THE RAT: AN IMPROVED MODEL.
J L Bussiere, J H Exxon and G G Wether. Dept. Veterinary Science, University of Idaho, Moscow, ID.

Research in this laboratory during the past several years has been directed to developing a comprehensive panel of assays to assess immunotoxicologic activity of chemicals and drugs. Particular attention has been given to assays that are sensitive, reproducible, economical, simple to perform and representative of the major arms of the immune system. Furthermore, the rat has been utilized as the animal model due to the general familiarity of many toxicologists with this species and because a preponderance of general toxicologic testing is currently performed in the rat. We have previously reported an economical, multiple assay approach to immunotoxicologic testing in the rat (Exxon et al., 1986). Herein we report significant improvements and expansion in that model that increase versatility and better facilitate application to a variety of toxicity testing protocols. These improvements include 1) elimination of Freund's adjuvant from the antigen treatment schedule, 2) use of a single antigen to assess cell-mediated and humoral immune responses, 3) coordination of timing for testing DTH and antibody responses and 4) inclusion of several additional optional assays for different types of immune responses. The improvements in this rat model should increase its attractiveness as an economical and versatile paradigm to accommodate tier 1 and 2 immunotoxicologic assessment.
**JMMUNE MODULATION PRODUCED BY INTRATRACHEAL INSTILLATION OF GALLIUM ARSENIDE.** J A McCay, E E Sikorski, K L White, Jr, and A E Munson. Department of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA

Gallium arsenide (GaAs), used in the semiconductor industry, was administered to female B6C3F1 mice by intratracheal instillation at doses between 50 and 200 mg/kg in a 100 μl volume. The AFC response to SRBC was reduced by day 6 and remained suppressed throughout the 25 day period. Dose response studies on immune functions were conducted on day 15 post i.t. instillation. The spleen IgM and IgG AFC response to SRBC was suppressed dose dependently with i.v. immunization, but the IgM AFC response on the lung associated lymph nodes increased with i.t. immunization. The IgM AFC response of spleen cells to the T-independent antigen, DNP-Ficoll, was not altered. In vitro exposure of spleen cells to GaAs produced a suppression of the AFC response to the T-dependent antigen, SRBC, which was reversed with Interleukin 1. No modulation occurred using the T-independent antigens, DNP-Ficoll and LPS. The DHR response to KLH of mice exposed to GaAs was suppressed dose dependently, to a level of 20% of control. The MLR was suppressed dose dependently, to a level of 45% of control; however, the response to T- and B-cell mitogens was not suppressed. Fc-mediated phagocytosis of peritoneal cells was increased, but phagocytosis of latex beads was decreased. These studies show that GaAs administered by the i.t. route modulates the immune response. (Supported by NIEHS Contract ES 55094).

**ADHERENT AND NON-ADHERENT FRACTIONS OF SPLENOCYTES ARE TARGETS OF BENZ[α]PYRENE [B(α)P] AND 7,12-DIMETHYLBENZANTHRACENE (DMBA) INDUCED SUPPRESSION OF THE HUMORAL IMMUNE RESPONSE.** K L White, Jr., M C Parrott, and T T Kawabata. Deps. of Pharmacology and Toxicology, and Biostatistics, Medical College of VA/VCU, Richmond, VA

B(α)P or DMBA exposure in vivo and in vitro suppresses the T-dependent antibody forming cell (AFC) response to SRBC. Since T- and B-lymphocytes and macrophages are required in the generation of AFCs to SRBC, studies were undertaken to determine which cell type(s) was affected by these polycyclic aromatic hydrocarbons (PAH). Female B6C3F1 mice were administered B(α)P (200 mg/kg) or DMBA (10 mg/kg) s.c. daily for 7 days, and splenocytes were separated into adherent (macrophages; AD) and non-adherent (Sephadex G-10 purified; non-AD) fractions. Combinations of fractions from vehicle (VH; corn oil) or PAH dose groups were exposed to SRBC and evaluated after 5 days of culture. B(α)P and DMBA treatments resulted in a decreased AFC response in unfractionated splenocytes by 80-95%. This suppression was completely attenuated with 2-mercaptoethanol (25 μM) in the cultures. Cultures containing AD cells from PAH treated mice and non-AD cells from VH treated mice or AD cells from VH-treated mice and non-AD cells from PAH exposed mice had significantly decreased responses, as compared to cultures containing AD and non-AD splenocytes from VH treated mice. The greatest suppression was observed when both splenocyte fractions were obtained from PAH treated mice. These results suggest that B(α)P and DMBA suppress the AFC response by affecting cell types present in both non-AD and AD fractions. (Supported by NIH grant ES03434).

**DIFFERENTIAL EFFECTS OF CO-ADMINISTRATION OF AMINOACETONITRILE ON DIMETHYLNITROSAMINE-INDUCED IMMUNOSUPPRESSION AND HEPATOXICITY PRODUCED IN VIVO.** H.G. Haggerty, L.H. Boise, S.D. Jordan, and M.P. Holsapple. Dept. of Pharmacology & Toxicology, Medical College of Virginia/VCU, Richmond, VA

Aminoacetonitrile (AAN) is an inhibitor of the high affinity form of dimethylnitrosamine (DMN) demethylase. The reversal of the immunosuppression and hepatotoxicity induced by the in vivo exposure to DMN, by AAN was investigated. Female B6C3F1 mice were exposed to either 3 or 6 mg/kg of DMN (in saline) i.p. for 7 consecutive days. The animals were also treated (IP) 1 hour prior and 6 hours after the DMN exposure with either 10, 30, 100 or 300 mg/kg AAN or saline. Mice were sensitized with SRBC I.V. on day 8. On day 12 body and organ weights were determined, serum chemistry and histopathology were evaluated, and day 4 IgM antibody response was measured (antibody forming cells were enumerated). Hepatotoxicity caused by DMN, as reflected by an increase in body weight and the production of ascites and a 266.7% increase in the SGPT levels, was reversed by doses of AAN as low as 10 mg/kg. Conversely, doses of AAN as high as 300 mg/kg were unable to reverse the suppression of the AFC response to SRBC produced by DMN. Currently, alterations in the distribution of reactive species to known target organs (liver) and immune IgM (spleen) after subchronic exposure of (methyl-14C)DMN in AAN is being evaluated. (Supported by NIH grant ES03564 and Training grant ES07087).


Erythrocytosis is the central feature of polycythemia, a blood disease with diverse etiologies. Inbred Wistar rats developed a 40% incidence of polycythemia following cephalosporin exposure to methylmercury and ethylmercury. Mean onset was 8 weeks of age, hematocrits exceeded 60%, and death occurred within months. An etiologic algorithm was used to determine the probable underlying cause of the polycythemia. Physiologic or myeloproliferative mechanisms were ruled out, whereas secondary polycythemia was diagnosed based on elevations in red cell mass and serum erythropoietin. A 10mm Hg mean left shift of the O2-RBC dissociation curve, with normal pI and levels of 2,3-diphosphoglycerate, indicated that RBCs or hemoglobin had an abnormally high affinity for O2. To determine if a high-affinity mutant hemoglobin existed, isoelectric focusing in an agarose gel matrix was performed on purified hemoglobin. None of the 5 major hemoglobin bands significantly differed between affected rats and age/sex matched controls. High O2 affinity of the RBCs could still result from a mutated hemoglobin that is electrophoretically silent, or is insufficiently present in the borderline (hematocrits = 56-59%) polycythemic rats tested. Further studies will assess severely polycythemic rats and alternate mechanisms for high O2 affinity.
HEMATOPOIETIC EFFECTS IN FEMALE B6C3F1 MICE EXPOSED TO ARSINE GAS. H L Hong, B A Fowler and G A Boorman. National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC. Sponsor: G A Boorman

Arsine gas is a potent hemolytic agent. Concern about silicoid workers prompted an in-depth study of arsine at NIEHS to determine the hematopoietic effects of prolonged exposure to the tolerated doses of this gas. Female B6C3F1 mice were exposed via inhalation to 0, 0.5, 2.5, and 5 ppm arsine 6 hr/day for 16 days. Body weights in exposed mice were comparable to controls, but a marked, dose-related splenomegaly was observed. Arsine exposure produced significant dose-related decreases in red blood cells, hematocrit and hemoglobin, with increases in white blood cell counts and mean corpuscular volume of red blood cells. Furthermore, erythropoiesis as measured by quantitation of erythroblast precursors in culture revealed a significant reduction of CFU-E/femur cells for all treated groups on day 3 post-exposure and only at the 5 ppm dose group on 24 days postexposure. There was no alteration in bone marrow cellularity and a less significant effect on granulocyte-macrophage progenitors. A 12-week study of arsine at 0, 0.025, 0.5 and 2.5 ppm (6 hr/day) via inhalation showed similar but less effects on hematopoiesis in mice. These effects were seen at 3 postexposure. In conclusion, arsine exposure at well-tolerated doses produces a dose-related stress on the hematopoietic system characterized by a hemolytic anemia.

THIRTEEN WEEK DOSED FEED TOXICITY STUDIES OF m-NITROBENOZOIC ACID IN F344 RATS AND B6C3F1 MICE. K R Abdool, M Ewell, B S Levine, L T Mulligan, R Kovatch, D Raushheck, RTP, NC; 2Department of Pharmacology, Microbiological Associates, Bethesda, MD; 3PAI, Ijamsville, MD.

Ten F344 rats of each sex received 0, 0.063, 0.125, 0.25, 0.5 or 1.0% m-nitrobenzoic acid in their diet for 13 weeks. Similarly, groups of 10 B6C3F1 mice of each sex received 0, 0.125, 0.25, 0.5, 1.0 or 2.0% of the compound in their diet. No rats or mice died during the study. Final body weights of rats at 1% and mice at 0.5% were lower(p<0.05) than those of the controls. Male rats at all dose levels had significantly more heinz bodies than controls. Methemoglobin concentration in males at 1% dose level was significantly increased. Females at 0.5 and 1% dose levels had lower hematocrit values, hemoglobin concentration and red blood cell count. Methemoglobin concentration was increased (p<0.05) in females at 0.25% dose levels. There was a significant decrease in total and cerolunaemoglobin levels of 7-4 in males at 0.5% and 1% dose levels. Hematology and clinical chemistry were not done on mice. The only compound-related histopathological change was testicular atrophy in rats at 1% dose level. No compound-related histopathological changes were observed in mice.

ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY STUDIES OF THE CARDIOTONIC ISOMAZOLE (LY175326) IN RATS AND DOGS. J R Means, G E Sandusky, and D B Meyers. Lilly Research Laboratories, Toxicology Division, Greenfield, IN

Isomazole (LY175326, IMZ) was evaluated for toxicity in a series of acute, subchronic, and chronic studies. Minimum lethal oral doses were 125 and 400 mg/kg in rats and mice, respectively. Dogs and monkeys survived single oral doses of 50 and 100 mg/kg, respectively; however, one of two female dogs died at 100 mg/kg of IMZ. Rats were given IMZ in the diet for three months at an average daily intake of 0, 20, 65, or 198 mg/kg. Prominent effects were increased relative liver weights and a marked fat deposition in the liver in the 198 mg/kg group and periarteritis of mesenteric vessels in both the 65 and 198 mg/kg groups. Similar findings were seen in the one-year study (0, 10, 25.5, and 68 mg/kg) with the hepatic effects occurring at doses as low as 25.5 mg/kg. Beagle dogs given oral doses of 0, 2, 6, or 16 mg/kg/day for one year did not exhibit any effect at a given dose level. Changes in heart rate were observed at two hours post-dose in the 6 mg/kg females and moderate to marked increases occurred in the 16 mg/kg animals of both sexes. Basal heart rate was slightly decreased relative to control in the 16 mg/kg group after one month of treatment. The heart was the target organ with increased relative heart weight (57%) in the 16 mg/kg group and multi-focal myo-cardial fibrosis in the 6 (1/4 females) and the 16 mg/kg (2/4 males, 3/4 females) groups.


SQ 29,852 (SQ) is the first of a new class of ACE inhibitors containing a phosphonic ester function. It is believed to be a highly active antihypertensive agent. Acute studies of SQ in mice and rats revealed that its minimum lethal dose was greater than 8000 mg/kg po and 4000 mg/kg iv for both species. In an iv study, dogs given SQ at daily doses of 2 to 50 mg/kg for 2 weeks showed no significant drug-related effects. Mice given doses of 4 to 100 mg/kg iv for 2 weeks showed no changes, other than a slight decrease in total leukocyte counts at 100 mg/kg. In dogs, SQ given po at 30 mg/kg for 3 months induced no drug-related changes. At doses of 150 and 750 mg/kg, slight increases in serum glucose and magnesium were observed, but no serious adverse effects were noted in dogs at these dose levels. Rats given SQ po at 25 to 3000 mg/kg for 3 months did not show any major adverse effects. Cecal distention was noted grossly in high-dose rats. Histologically, a reduced incidence of renal tubulopathy was noted at 120 mg/kg or greater, and mild juxtaglomerular-cell hyperplasia was seen at 600 and 3000 mg/kg. These renal changes were probably associated with the pharmacologic properties of the drug. SQ was not genotoxic in a battery of mutagenicity assays, with and without metabolic activation. The results of these studies demonstrate that SQ is one of the least toxic ACE inhibitors reported to date.
332 VITAMIN K DEPENDENT TOXICITY OF L163443 SODIUM, A NEW LTD4 ANTAGONIST, IN LABORATORY RATS. R B L van Lier, J P McGrath, and I D Cherry, Toxicology Division, Lilly Research Laboratories, Greenfield, IN.

L163443 sodium, 1-[2-hydroxy-3-propyl]-4-[4-(1H-tetrazol-5-ylmethyl)phenoxy]methyl]phenyl]ethaneone, sodium salt (LY) is a new LTD4 antagonist being developed for the treatment of asthma. Hemorrhage and prolonged clotting times were the principal signs of toxicity observed in rats and rabbits during toxicological testing. These signs were not observed in mice, dogs, or monkeys. Analysis of plasma from affected rats with specific coagulation factor free plasma indicated that at least factors II and VII were reduced suggesting that vitamin K absorption or utilization was affected. The effect of vitamin K on LY toxicity was studied in F344 rats (5 males/group) given diets containing 0, 0.25, 0.3 or 0.4% LY in Purina Certified Rodent Chow (#5002) or in Teklad 4% diet which contained 9 ppm added menadione. The mortality in animals maintained on non-menadione fortified diet was 0, 20, 100, and 100%, respectively, with a mean time to death of 5 days. No mortality was observed with LY in fortified diets. Activated partial thromboplastin times and prothrombin times were both elevated in all surviving animals receiving the non-fortified feed. Coagulation parameters were completely unaffected in animals maintained on fortified feed at these doses. Mean APTT and PT values for animals receiving LY in fortified diets were below those for animals receiving LY in non-fortified diet suggesting that some commercially available rodent chows may contain insufficient quantities of vitamin K to sustain normal clotting function in rats during periods of stress. These data demonstrate that LY toxicity can be modulated by dietary vitamin K.


PR 934-423A (PR) is effective orally to control grand mal seizures in animal models (ED50 for maximal electroshock seizures (mouse, 23 mg/kg, ip, 33 mg/kg, po; rat, 20 mg/kg, po); TDS50 for neural impairment (mouse-inverted screen test, 85 mg/kg, ip, 581 mg/kg, po; rat-plank walk test, 690 mg/kg, po); therapeutic indices in mouse (ip = 3.8, po = 17.6) and rat (po = 34)). In anesthetized dogs, 10 and 30 mg/kg, iv of PR depressed myocardial contractility, cardiac output and heart rate. In hypertensive rats, 100 mg/kg, po did not alter arterial blood pressure. PR exhibited a large volume of distribution (Vd=3.3 L/kg [rats] and 3.8 L/kg [dogs]) and followed a two compartment model with rapid elimination [T1/2=0.36 hour (rats) and 1.12 hour (dogs)]. L50 values were determined for: rat (897 mg/kg, po; 142 mg/kg, ip) and mouse (761 mg/kg, po; 51 mg/kg, iv). Single oral doses of >400 mg/kg produced focal necrosis and hemorrhage of the duodenal epithelium in mice. Daily oral doses of 400 mg/kg for 30 days were lethal in 8 of 20 rats and produced lymphoid atrophy of the thymus, spleen and lymph nodes. Daily oral doses of 60 mg/kg for 90 days were lethal in 2 of 10 beagle dogs. The desirable efficacy/safety ratios and the lack of significant toxicological effects in animals define PR as a promising anticonvulsant now in clinical trials.


CT-953 (N-(2-chloro-6-methylphenyl)-N'-4-pyridinylurea, monohydrochloride) has potential efficacy for the treatment of generalized tonic-clonic and partial seizures in human beings at doses of 5-10 mg/kg. Acute oral toxicity studies in fasted mice and rats revealed 14-day median lethal doses of about 350 mg/kg. Subacute oral toxicity studies were conducted in Beagle dogs for 2 weeks (escalating regimen of 10-220 mg/kg and repeated doses at 10, 40, 80, and 120 mg/kg) and rats for 90 days (30, 100, 300, and 600 mg/kg by gavage). Mortality in the rat study occurred at 600 mg/kg. Reduced food consumption, body weight loss/gain suppression, and CNS depression occurred at high dose levels in both species. Biochemical analyses and pathologic examination revealed the liver, kidney, and stomach to be the target organs at high dose levels. Hepatic changes were mild in both species and were probably related to induction of mixed function oxidase. Renal effects occurred only in dogs and included pyelitis, associated necrosis, and transitional cell erosion. Gastric erosions/ulceration occurred in the noneglandular mucosa of rats, and in the glandular mucosa of dogs. Supported in part by NINCDS Contract N01-NS-5-2358)

335 EXCRETION AND TISSUE DISTRIBUTION OF METHYLPHENIDATE-HCl (MPH) IN RATS AND MICE AFTER A SINGLE ORAL DOSE. CR Duerson, DL Carter, KG Singer, Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

MPH is a central stimulant widely prescribed for treatment of attention deficit disorder in children. Recently, MPH has been shown to cause liver tumors in male mice but not in rats. Our experiments are designed to determine if the toxicity finding can be explained by differences in MPH disposition. Excretion and tissue distribution of total radioactivity were examined in male Fischer F344 rats and male B6C3F1 mice after a single oral dose of 14C-MPH. Rats were treated with 7, 35, or 70 mg/kg, while mice were treated with 2.1 or 19 mg/kg. The treated animals were placed in plastic metabolic chambers and feces and urine were collected separately at 4, 8, 12, and 24 hours. At termination, necropsies were performed, tissues were sampled and analyzed for total radioactivity after oxidation to CO2. Less than 1% of the radioactivity remained in the tissues at 24 hours. Liver showed the highest tissue level of radioactivity. Total radioactivity in feces of rats at 24 hours after 7, 35, or 70 mg/kg MPH was 24.2, 18.9, and 22.7 percent of the dose, respectively. Total radioactivity in urine with 7, 35, and 70 mg/kg MPH was 83.4, 86.2, and 78.4 percent of the dose, respectively. Total radioactivity in feces of mice at 24 hours after 2.1 or 19 mg/kg MPH was 15.7 and 19.7 percent of the dose, respectively. Urinary recovery was 80.5 and 75.0 percent of the dose, respectively. Excretion and tissue distribution of MPH were fairly similar in the rat and B6C3F1 mouse. Supported by NIEHS NO1-ES-3-5031.
TOXICITY OF ELASMINICIN, A POTENTIAL ANTICANCER AGENT. T J Davidson, C L Bregman, R A Buroker, R S Hirth, and H Medisoo, Department of Pathology and Toxicology, Bristol-Myers Company, Syracuse, NY.

Toxicity evaluations of the succinate salt were conducted in mice, rats, and dogs. The LD₅₀ in CD-1 mice was 16.3 mg/kg (N) and 26.0 mg/kg (F). Most deaths occurred within 2-4 days after treatment. When the drug was administered for 5 consecutive days to mice, the LD₅₀ was between 9 and 12 mg/kg/day (M and F). In rats, single doses of 5.5 mg/kg or more resulted in transient bone marrow and lymphocytotoxicity. Systemic toxicity and mortality were noted within 3-4 days after a dose of 16 mg/kg. Following 5 consecutive daily doses of 4 or 8 mg/kg, hematologic and lymphocytotoxicity were apparent within 3-4 days. In the XL and x5 studies, neutrophilic leukocytosis was evident shortly after treatment and surviving males showed testicular atrophy at 30 days. Elasminicin was significantly more toxic in the beagle dog. Single doses of 0.16 mg/kg were lethal within 1-3 days and 5 daily doses of 0.07 mg/kg were lethal 24 hours after the last dose. Hemocytocentrifugation and neutrophilic leukocytosis with a left shift were detected within 24 hours after treatment and a shock or shock-like state preceded deaths in both studies. The single toxic dose in the dog is 0.008 mg/kg based on a transient neutrophilic leukocytosis.


Lipid and vitamin levels in liver, bladder, and serum were evaluated in Sprague-Dawley rats exposed to 7.5% sodium saccharin (NaS) in the diet from conception until sacrifice at 30 days post-birth (dpb). Litter sizes and body weights (BW) at birth were similar in the NaS and control groups. BW of NaS pups were depressed after 4 dpb and were 60% of control at 30 dpb. Food consumption was not affected by NaS treatment in F₁ rats, but it was reduced in weaned NaS-fed F₂ rats. NaS-treated F₂ rats exhibited several changes in lipid and vitamin levels. Serum cholesterol, triglycerides, and vitamin (vit.) E increased by approximately 45%, 100%, and 100%, respectively. Serum high-density lipoprotein, vit. A, and folate decreased by 45-50%. Hepatic concentration of vit. A. A palmitate increased in males only, vit. E increased in females only, and folate decreased in males and females. Total amounts of folate, vit. A, vit. E, and palmitate, and vit. E in the liver decreased with NaS treatment. Cholesterol in bladder epithelium was unchanged. In summary, high doses of NaS from conception through 30 dpb produced profound biochemical changes in the weaning rat. Supported by a grant from the Intl. Life Sciences Institute.


P-2546 is being developed as a feed additive to increase the feed efficiency and rate of weight gain in beef cattle and of viability of tissue residues of P-2546 present in beef liver, modified Gallo-Torres (1977) procedure was used. In the modified procedure rats intubated for bile infusion and collection were free moving with free access to food, water and survived up to 7 to 10 days. The surgically prepared rats were gavaged with a suspension of beef liver spiked with C-14-22546 or liver from beef cattle orally dosed with C-22546. Distribution of radioactivity in all the tissues was monitored. Bioavailability is expressed as the percentage of an oral dose absorbed from the gastrointestinal tract. Rats orally fed with beef liver spiked with C-22546, only 44.8% of P-2546 was bioavailable while P-2546 residues present in liver were less than half or only 19.2% were bioavailable. The modified procedure in free moving rat model, is useful for determining bioavailability of residues or free drug.

EFFECTS OF ASPARTAME ON PENTYLENE-TETRAZOL(PTZ)-INDUCED CONVULSIONS IN CD-1 MICE. P C Jobe, A F Bettendorf, S M Lasley, and J W Dailey. The University of Illinois College of Medicine at Peoria, Peoria, Illinois 61636.

Large doses of aspartame were administered orally to CD-1 mice. For each dose of aspartame, a convulsive dose fifty (CD₅₀) was determined for PTZ at one hour following gavage with 0.5% methylcellulose vehicle. Each animal employed received only one dose of PTZ. The convulsive endpoint was a generalization clonus with loss of righting reflex. Milder forms of response such as facial and forelimb clonus with or without rearing were not assessed. First, CD₅₀'s were determined in fasted male mice weighing 13 to 28 grams each and housed in groups before and after the injection of PTZ. Under these conditions, the CD₅₀ for tests of 3 different doses of aspartame matched with the appropriate vehicle controls were: (1) 1500 mg/kg aspartame CD₅₀ = 63.9 mg/kg (confidence interval - 57.9 - 70.6) versus vehicle CD₅₀ = 54.5 (45.5 - 65.3), (2) 2000 mg/kg aspartame CD₅₀ = 67.1 mg/kg (61.3 - 73.3) versus vehicle CD₅₀ = 58.6 mg/kg (49.3 - 64.9); (3) 2500 mg/kg aspartame CD₅₀ = 59.3 mg/kg (50.1 - 70.7) versus vehicle CD₅₀ = 56.5 mg/kg (49.4 - 64.5). In a second study group mice were fed, rather than fasted, and the individual body weights ranged from 9 to 13 grams. Aspartame was administered in a dose of 1500 mg/kg. The CD₅₀ for this dose was 69.2 mg/kg (61.5 - 77.8), whereas the CD₅₀ for vehicle was 65.5 mg/kg (59.0 - 72.8). According to these data, aspartame does not facilitate PTZ seizures in grouped male CD-1 mice, whereas the doses used produced a significant anticonvulsant effect. (Supported in part by a grant from the NutraSweet Company.)

SC-35311 was found as a minor impurity in a synthetic process of the cardiac antiarrhythmic SC-60230. SC-35311 is a bis-piperidine ring (pKa1=6.2, pKa2=9.6) analogue of disobutamide. Disobutamide induces CCV in vivo and in vitro, which are signs of intracellular drug storage and not of overt toxicity (Human Pathol. Dec. 1987). The three compounds are structurally similar cationic amphiphilic bis-tertiary amines, but differ in basicity of the amine functions. To test whether SC-35311 is vacuologenic, we incubated it with rat urinary bladder carcinoma (RBT CC-8) and rabbit aorta muscle cells at 0, 1, 2, 4, 6, 8 & 10 x 10^{-4} M for 24 hr. We examined cells in situ by light microscopy and the medium for lactic dehydrogenase (LDH). Disobutamide and SC-60230 served as positive and negative controls, respectively, for CCV induction. SC-35311 induced CCV at 4 x 10^{-4} M or higher. There was no morphologic evidence of cell death nor significant release of LDH. We concluded that, similar to disobutamide (Proc. Soc. 184:165-171, 1987), the relative high basicity of the bis-tertiary amine functions of SC-35311 determined induction of CCV, and that the induced vacuolation is not accompanied with overt toxicity.

THE EFFICACY OF SUPERACTIVATED CHARCOAL IN TREATING RATS EXPOSED TO A LETHAL ORAL DOSE OF POTASSIUM CYANIDE. R. J. Lambert, B. L. Kindler, and D. J. Scheaffer. Illinois Animal Poison Information Center, Dept. of Veterinary Biosciences, University of Illinois, Urbana, IL.

Due to the relatively low binding capacity of regular activated charcoal (AC) for potassium cyanide (KCN) in vitro, the use of oral activated charcoal therapy for oral exposure to cyanide compounds is controversial. In this study rats were given a lethal oral dose of ground granular KCN (35 or 40 mg/kg) in a gelatin capsule followed immediately by either 4 g/kg of superactivated charcoal (SAC) in a 20% suspension or a similar volume of deionized water. Signs of cyanide toxicity occurred rapidly, with a mean time to signs of 3.3 and 2.7 min in control animals receiving 35 or 40 mg/kg KCN, respectively. All 26 of the control rats showed signs, and all but 1 in the 35 mg/kg group died within 19 minutes. Only 12 of 26 rats treated with SAC showed signs of KCN toxicity and 8 of those animals died. A regression model for the 23 animals dying indicated that time to onset of signs was correlated with the time to death (time to death = 5.21 + 2.16signs, R = 0.73). Oral exposure of rats to lethal doses of KCN can be effectively treated by immediate administration of superactivated charcoal.

KINETICS AND METABOLISM OF THE RADIOPROTECTIVE AGENT, S-2-(3-METHYLAMINOPROPYLAMINO)ETHYLPHOSPHOROTHIOIC ACID (WR 3689), IN RHEUS MACAQUES. A. Duckpiit, A. L. Healthy, S. P. Johnson, and M. Goldman. Institute for Environmental Health Research, UC Davis, Davis, CA and Walter Reed Army Medical Center, Washington, DC.

WR 3689 is one of several aminothiol which have protective actions against gamma irradiation. These agents also enhance the therapeutic index of several antineoplastic agents. The present studies have examined the kinetics of 14C-WR 3689 in Rhesus Macaques after IV and PO administration of 150 mg/kg. WR 3689 is cleared rapidly from plasma with a t1/2 of 15 min and a Vd of 1600 ml/kg after IV administration. Parent drug could not be detected after PO administration. Radioactivity in the plasma and whole blood after IV 14C-WR 3689 had elimination t1/2's of 10 and 26 hours, respectively. The long t1/2 of radioactive parent and metabolite in comparison with the short t1/2 of parent drug in plasma suggests that metabolites are secreted from the blood. Urinary and fecal excretion were 70 and 10% of the administered label after IV and 37 and 52% after oral administration. Based on whole blood radioactivity, the oral bioavailability was 30%. Four metabolites and the parent compound were isolated from urine; no qualitative differences were noted between the metabolites assessed after PO vs IV administration. Supported by DAMD 17-36-C-6177.
Sulfadimethoxine (SDM) is a sulphonamide drug used to control bacterial infections in the aquaculture industry. Studies of the pharmacokinetics and metabolism of SDM have been undertaken to aid in predicting the uptake and tissue disposition of this drug in catfish and to estimate the accumulation of drug residues in edible flesh. Channel catfish were administered 14C- or 3H-SDM by intravenous (IV) injection or per os (PO) to determine vascular clearance, absorption from the gut, tissue disposition, metabolism and elimination. Vascular clearance of SDM administered IV was rapid (t1/2=0.06; t½=12.8 hr) with a volume of distribution (Vd) of 662 ml/kg. Plasma protein binding of SDM was low (18%) and dose-independent. Na+-SDM administered in food had a bioavailability of 44.7% and distributed rapidly to muscle tissue. Within 3 hr after dosing, 13% of the dose was present in muscle as parent compound. However, elimination of SDM from muscle occurred quickly with a t½ of 14 hr. SDM metabolism in channel catfish was also rapid. Six hr after IV dosing, about one-half the SDM in urine was present as N-acetyl-SDM and greater than 90% of the SDM that accumulated in bile was present as the N-acetyl metabolite. Thus, N-acetylation appears to be the most important transformation pathway leading to elimination of SDM in the channel catfish. Supported by FDA-CVM-00150 and NIEHS Ctr. Grant ES-00260.

SPR is a nonabsorbable, noncaloric lipid-like material which has the potential to be used as a fat replacement in many foods. Studies were designed to evaluate the effect of SPR on the ABS of 3 drugs with a range of partition coefficients (PCs): diazepam (DM), propranolol (PL), and aspirin (AN). Cholesterol (CH) was used as a positive control since previous studies showed that SPR decreases the ABS of this highly lipophilic (high-PC) material. Male SD rats were orally administered a single dose of radiolabeled compound followed by 1 ml of either H2O, corn oil, or SPR. ABS profiles were evaluated by monitoring total radioactivity in plasma, urine, and feces over time, and in selected tissues taken at necropsy. Results indicate that SPR had no effect, relative to H2O, on the ABS profiles of DM, PL, or AN. Corn oil reduced the rate, but not the amount of ABS of all 3 drugs. SPR decreased the ABS of CH as reflected by increased fecal excretion of radio-label. These results indicate that the potential effect of SPR on the ABS of a material can be predicted on the basis of its PC. A comparison of the relative PCs of the 4 materials tested suggests that SPR will not affect the ABS of commonly-prescribed oral pharmaceuticals.


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Theophylline (TPL) is a methylxanthine pharmaceutical agent widely used for the treatment of various respiratory disorders and certain acute cardiovascular conditions. 13-week subchronic toxicity studies of TPL were conducted by gavage and dosed-feed in F344 rats and B6C3F1 mice (10 animals/group). TPL in the feed (0.0, 0.1, 0.2, 0.4%) resulted in no mortality or body weight effects in F344 rats, but did induce polyarthritis (nodosas) of the pancreas (males and females) and an increased severity of polyarthritis (males). Dietary TPL resulted in no mortality in B6C3F1 mice but terminal body weights were significantly decreased in all dosed groups and there was a dose-related increased incidence of hepatocellular glycogen depletion in males and females. Administration of TPL by gavage in corn oil to F344 rats (0.37, 0.5, 0.75, 1.5 mg/kg) resulted in slight mortality and significant body weight effects at the high dose. There was a dose-related increased incidence and severity of mesenteric perivascular inflammation in male and female rats. Gavage administration of TPL to B6C3F1 mice (0.75, 1.5, 3.0 mg/kg) resulted in the early death of all high-dose females and 3/10 high-dose males and decreased terminal body weights in high- and mid-dose males and low-dose females. As in the dosed-feed study, hepatocellular glycogen depletion was observed, although only in females.


Groups (0, 15, 45, 135, 400, and 1200 mg/kg) of ten F344 rats or B6C3F1 mice each received 5 daily doses of Scopolamine Hydrobromide per week for 13 weeks. The vehicle was deionized water. Body weights, food consumptions, and clinical signs were recorded, hematologic analyses performed, and pathology evaluations were conducted. Significant mortality was noted in male (6/10, 400 mg/kg; 6/10, 1200 mg/kg) and female (7/10, 1200 mg/kg) rats. Food impaction of the pharynx appeared to be a primary cause of death. Body weights were depressed in all treatment groups and food consumption was increased in treated rats (400 mg/kg female; 1200 mg/kg male and female). Primary clinical signs involved the eyes and included dilated pupils (all treated animals), red, dry eyes (13/40 rats, 400 and 1200 mg/kg) and lacrimation (15/40 rats, 400 and 1200 mg/kg). Hypoactivity was noted in 8/20 rats and 9/20 mice (1200 mg/kg). The WBC differential shifted toward increased segmented neutrophils/lymphocytes in all treated male rats and female mice and the three highest dose male mice groups. No histopathologic changes were associated with drug treatment.
A potent thymimetic, SKF L-94901, was designed for the treatment of hyperlipidemia and had been demonstrated to be devoid of the adverse cardiac side effects which limit the use of conventional non-selective thymimetics. However, oral administration of this compound to rats revealed several proliferative lesions. SKF L-94901 was given to rats over a period of 30 days at dose levels between 0 and 100 mg/kg/day. A positive control group of 1 mg/kg/day of L-thyroxine (L-T4) was included in an attempt to separate a toxic effect from a thymimetic effect. SKF L-94901 produced treatment-related lesions in three main sites: bone, serosal surfaces and kidney. The bone changes were a focal deposition of new trabecular bone with associated spindle cell proliferation in the medullary cavity. In the kidney there were multi-nucleated epithelial cells in the distal tubules, and in the abdominal cavity there was focal capsular fibrosis primarily on the serosal surfaces of the liver and spleen. The rats which received L-T4 had a low survival due to extensive myocardial damage, and there was a low incidence of lesions similar to those mentioned above. The proliferative lesions were therefore attributed to a thymimetic effect, and the possible association with a local over-production or amplification of growth factors will be considered.

Doxylamine succinate, a commonly used antihistamine, was administered in the feed to male and female Fischer 344 rats at dose levels of 0, 500, 1000, and 2000 ppm. Food consumption, body weights, and signs of clinical toxicity were determined weekly. Gross and microscopic pathology examinations were performed. Weight gain was depressed greater than 10% in the 2000 ppm dose group of females only. Liver weights were elevated in the two highest dose levels for males but no effect was observed in females. Serum enzyme levels of ALAT and SDH were elevated in the 2000 ppm dose level in males. In males, reticulocyte counts were elevated in the 1000 ppm and 2000 ppm dose groups and hemoglobin levels were decreased in the highest dose group. Both sexes exhibited a dose-response effect for cytoplasmic alterations of the acinar cells of the parotid salivary gland. Doxylated fatty change occurred in all treated groups of males and the highest dose group of females. Centrilobular hypertrophy of the liver was observed in the 1000 ppm and 2000 ppm dose groups of females but none was observed in males. These results indicate the need for longer term studies of doxylamine for possible carcinogenicity activity in the rat.

Following eight-week exposures of Sprague-Dawley rats to ethanol (37% caloric substitution), brains were preserved for optical and electron microscopy or impregnated using the Golgi-Cox staining procedure. Dense bodies were observed in processes and excess lipofuscin was observed at the electron microscopy level of ethanol-fed (EF), but not pair-fed (PF) or chow-fed (CF) controls. Morphometric analyses indicated that surface density of the processes of EF rats were significantly different than those of CF rats. With respect to hippocampal areas, PF rats also were statistically different from CF rats. Areal measurements of whole brain, hippocampal space, and ventricular space showed no statistical differences between EF and CF rats. However, differences were evident in the surface density of the processes, whole brain areas and hippocampal areas of PF and EF rats.

Bioavailability of oxalic acid from common foods varies widely. Oxalate availability from a high fiber food—Sugarcane Pulp (SFP)—was compared to spinach and sodium oxalate, tested in nine women using a triplicated 3 by 3 Latin square arrangement with a 2 day control period and 3 test periods consisting of a test day followed by a control day. Test material which provided 120 mg oxalate was consumed at breakfast on test days. Subjects consumed the same diet every day. Intake of test materials was 50 g beet pulp, 25 g spinach, and 182 mg sodium oxalate. Total 24 hr urines were collected and analyzed daily for oxalate. Oxalate excretion was similar following spinach or sodium oxalate ingestion (23.6 and 25.7 mg/day), however, it was higher (P<.05) following sodium oxalate intake than following beet (19.0 mg/day) or control (18.3 mg/day) diets. Oxalate excretion did not differ among control days and was not significantly increased with SFP consumption. Low bioavailability of oxalate from SFP may be attributable to its high ratio of calcium and magnesium to oxalate, its high fiber content or the loss of soluble oxalate during processing.
SPONTANEOUS AND INDUCED ACCUMULATION OF α2GULBIN IN THE KIDNEY CORTEX OF RATS AND MICE.

We show that several strains of male rat that produce α2Gulbin (Fischer-344, Sprague Dawley, Buffalo, and Norwegian Brown) accumulate this protein in the kidney cortex as hyaline droplets. Exposure to decalin increases the accumulation of only α2Gulbin and creates larger hyaline droplets that are nephrotoxic. We also show that the NCI-Black-Reiter male rat which does not synthesize this protein, forms no hyaline droplets, and does not accumulate any protein in its kidney either spontaneously or when exposed to decalin. We expect that this strain will demonstrate no chronic toxicity. Mice produce an α2Gulbin called MUP. Its sequence differs slightly from rat α2Gulbin yet it does not accumulate in the kidney of either sex of mice either spontaneously or after decalin exposure. To determine whether this difference between rats and mice is due to specific characteristics of rat α2Gulbin, both sexes of mice were injected with male rat α2Gulbin at a level consistent with male rat output. Histology shows only very small droplets in the kidneys of the injected mice. Dosing with decalin had no effect on either the histology or the protein content of the tissues. Other factors such as metabolism or renal function must be required to explain the differences between mice and rats.

CONTINUOUS INTRAVENOUS INFUSION STUDIES WITH 2',3'-DIDEOXYADENOSINE (ddA) IN BEAGLE DOGS. J G Page, M E Placke, K A Colling, L E Mezza, G W Wientjes, C K Grieshaber*, and J E Tomaszewski*. Battelle Columbus Division, Columbus, OH and *National Cancer Institute, Bethesda, MD.
/ddA inhibits the cytotoxic effects of human immunodeficiency virus (HIV) in vitro at a minimum inhibitory concentration (MIC) of 10 μM (Mitsuya and Broder, PR, 1991). Dogs rapidly deaminate ddA to 2',3'-dideoxyinosine (ddi). ddA was infused in dogs at 3.13, 31.3, and 93.9 mg ddA/kg/hr. Administration of 31.3 mg/kg/hr for 240 hrs produced a mean plasma level (Cpss) of 30.5 μg/ml (129μM) and few clinical signs of toxicity. Severe GI toxicity occurred in dogs receiving 93.9 mg/kg/hr starting on day 6 of the infusion. In this group, Cpss was 142 μg/ml (males) and 205 μg/ml (females). Infusion was terminated at 215 hrs in this group and 3 of 4 dogs were judged moribund and necropsied over the next two days. Moderate to severe necrotizing inflammation and focal mucosal hyperplasia in the GI tract, bone marrow hypocellularity and marked thymic lymphocyte depletion were detected in most high dose dogs. Dose-related tachycardia and hypotension were also observed. (This work supported by NCI contract No. N01-CM-67669)

QUANTIFICATION OF ETHOXYQUIN IN MOUSE TISSUES; H L Kim, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX. Sponsor: S Safe

Dietary administration of ethoxyquin (EQ), an antioxidant, reduces the carcinogenic effects of a variety of chemicals in rodents. Rapid and nearly complete excretion of orally administered 14C-EQ is reported. However, traces of radioactivity are reportedly remaining in tissues for up to 4 weeks and it has been assumed that the persistent radioactivity is due to the presence of EQ metabolites, though not identified.

The uptake and metabolism of EQ in mice was determined using an HPLC-fluorometric detection method. Mice were given powdered feed containing EQ·HCl, 0.125% or 0.5%, and the EQ content of tissues including liver, kidneys, lung and brain was quantified 1–7 days after the feeding started, and 1–7 days after the feeding terminated. The highest EQ residues were found in the liver; 0.74 and 7.72 μg/g tissue, respectively, 24 hours after consuming feed containing 0.125 and 0.5% EQ·HCl. EQ residues were detected in all tissues 7 days after the feeding terminated; the level ranged from 0.08 μg/g brain to 0.7 μg/g liver. These results suggest that the persistent radioactivity is, in part, due to the presence of EQ. (Supported by the Texas Agricultural Experiment Station.)

SINGLE-DOSE PHARMACOKINETICS AND BIOAVAILABILITY STUDY OF 2',3'-DIDEOXYADENOSINE (ddA) IN BEAGLE DOGS AND RATS. M E Placke, J G Page, K A Colling, G W Wientjes, C K Grieshaber*, and J E Tomaszewski*. Battelle Columbus Division, Columbus, OH and *National Cancer Institute, Bethesda, MD.
/ddA is currently being considered for treatment of AIDS. ddA inhibits DNA synthesis and shows antiviral activity in vitro against human immunodeficiency virus (HIV) at concentrations of 10–200 μM without evidence of cytotoxicity. Pre-clinical studies were conducted to determine the pharmacokinetics and bioavailability of ddA in dogs following IV and oral administration and in rats following IV, SC, and IP administration. ddA was rapidly deaminated to dideoxyinosine (ddI), with suggested dose-dependent kinetics. The half-life in dogs was approximately 61 min following an IV dose 500 mg/kg of ddA. The half-life in rats was 17 min after 175 mg/kg of ddA, IV. Plasma concentrations and clearance rates of ddI were demonstrated to related to ddA doses, suggesting there was a concentration-limited metabolism of ddI. Oral doses of ddA were poorly tolerated in dogs and only partially retained, complicating the determination of bioavailability. When doses were retained, the oral bioavailability in dogs ranged from 28–50%. The bioavailability in rats was calculated to be 48% after SC doses and was greater than 100% after IP doses. (Supported by NCI contract No. N01-CM-67669)
A wide variety of hydrocarbons, including \( \delta \)-limonene (DL), have been shown to induce a specific triad of nephrotoxic events which to date have been unique to the mature male rat. The present study was designed to further evaluate the uniqueness of this lesion by determining the chronic toxicity of DL in a non-rodent species. Five male and five female adult Beagle dogs were gavaged twice daily over a 6-month period with tap water (control) or DL at 0.12 or 1.2 ml/kg body weight/day (100-1,000 mg/kg BW/day). Feed consumption and body weight were unaffected by treatment. The highest dose of DL resulted in slight increases in serum alkaline phosphatase and cholesterol levels in both sexes, and slight decreases in urine specific gravity and pH in females. Linear regression analyses indicated a positive dose-related trend for absolute and relative male and female liver weights, and absolute and relative female kidney weights. There was no microscopic evidence of histopathologic change in the liver and kidneys which would correspond to the organ weight changes. Most importantly, this includes an absence of light-microscopically evident hyaline droplets in renal tubules which is recognized to occur in the kidneys of male rats treated with DL.


Quinine is the main alkaloid and active principle of cinchona bark, a febrifuge used for 350 years. 40% of the world market is now taken up by the food industry, where it is used as a bittering agent in carbonated drinks. In 1980 the scientific committee of the EEC asked UNESA to supply safety data, because few formal toxicity studies had been done. This poster presents the results of 3 month studies in the rat, a rat embryo-toxicity study and a specific study to investigate ototoxicity. The result of these studies led to the setting of an acceptable daily intake of 40mg quinine hydrochloride for an adult. There were no indications of teratogenic effects and sophisticated audiometric techniques showed no indication of interference with auditory function in rats receiving up to 200mg/kg.

THE EFFECT OF TCDD ON RAT HEPATIC VITAMIN A LEVELS, AND RETINOYL-AND P-NITROPHENYL-UDP GLUCURONOSYL TRANSFERASE (UGT) ACTIVITIES. R H Powers, L C Gilbert and S D Aust. Department of Biochemistry, Michigan State University, East Lansing, MI.

A single po dose of TCDD caused a dose dependent depression of hepatic retinyl ester levels in male Sprague Dawley rats by 12 days following treatment. Loss of hepatic retinyl esters in rats treated with > 10 nmol/kg was significantly greater than in untreated rats fed a vitamin A deficient diet. A dose dependent increase in both p-nitrophenol- and retinyl-UDPST activity was observed in the liver microsomes from TCDD-treated rats. p-Nitrophenol-UDPST activity in TCDD treated rats was elevated to a maximum of about 7k that of untreated controls, and was unaffected by feeding rats a vitamin A deficient diet for 12 days. Retinyl-UDPST activity in TCDD treated rats was elevated to a maximum of about 4x that of untreated controls, and was significantly depressed in untreated rats fed a vitamin A deficient diet, when compared to rats fed a complete diet. Micronasal retinol-UDPST activity was not detected. We suggest that elevated rates of formation of retinol-3-glucuronide in the livers of TCDD-treated rats contributes to the depletion of vitamin A reserves. (Supported by NIH Grant ES3585.)

SELECTIVE ENHANCEMENT OF TERATOGENICITY IN MICE BY TCDD AND VITAMIN A (RA). L S Birmbeen, M W Harris, and R E Morrissey. NIEHS, Research Triangle Park, NC.

Many of the signs of TCDD toxicity resemble those seen during RA deficiency. Both TCDD and RA are also well known teratogens. In mice, TCDD causes cleft palate and hydronephrosis at doses where there is no overt fetal or maternal toxicity. A similar situation exists for RA in the induction of cleft palate and skeletal abnormalities. In order to determine if excess RA could overcome the teratogenic effects of TCDD, or vice versa, c57BL/6N mice were treated po on gestation day (gd)10 with 10 ml corn oil/kg containing TCDD (0-18ug/kg), all trans retinoic acid (RA)(0-100 mg/kg) or combinations of the two chemicals. Dams were killed on gd 18, and developmental toxicity assessed. Coadministration of TCDD and RA had no effect on maternal or fetal toxicity beyond what would be expected by either compound alone. The incidence of cleft palate was dramatically enhanced by administration of the two compounds together. No increase in the incidence or severity of hydronephrosis was seen in the combination treatments over that expected by TCDD alone, nor was there any increase in the incidence of pattern of limb bud anomalies in the combination groups over that caused by RA alone. No other soft tissue or skeletal abnormalities were detected. Thus, coadministration of TCDD and RA selectively increases the incidence of cleft palate, a common target tissue for these chemicals, without interacting to affect development in target organs unique to each compound.
THE EFFECT OF 2,3,7,8-TETRAChLOROBENZo-p-DIOXIN (TCDD) ON HEPATIC GLUCURONIDATION OF RETINOIC ACID. P A BANK, K L SALyERS, AND M H ZILE. Dept. of Food Science and Human Nutrition, Michigan State University, E. Lansing, MI. Sponsor: S SIEgle.

TCDD and related compounds cause a decrease in liver vitamin A reserves and an increase in urinary and fecal excretion of vitamin A metabolites. No correlation has been established for TCDD increased UDP-glucuronosyltransferase (UDP-GT) activity toward the substrate p-nitrophenol and decreased hepatic vitamin A concentration. We have investigated the effect of TCDD on hepatic vitamin A metabolism in male Sprague-Dawley rats receiving a single oral dose of 10 ug TCDD/kg. Ten day post-exposure to TCDD there was a 30% reduction in hepatic vitamin A esters as compared to pair-fed controls. The hepatic microsomal UDP-GT activity toward the substrate 3H-all-trans-retinoic acid (RA) was five-fold greater for TCDD-treated rats compared to pair-fed controls. Verification of the in vitro formation of 3H-retinyl glucuronide (RG) was by cochromatography of authentic RG on reverse phase HPLC and by identification of 3H-RA as the hydrolys product after β-glucuronidase treatment. Increased retinoid acid glucuronidation may be a contributing factor to the hepatic depletion of vitamin A following TCDD exposure. Supported by NIH grant ES 05347-03 and by USDA grant 87-3CR-1-2449.

FACTORS INFLUENCING THE INDUCTION OF DNA SINGLE STRAND BREAKS IN RATS BY 2,3,7,8-TETRAChLOROBENZo-p-DIOXIN. Z Z Wahab, S J Stohs, T A Lawson, W J Murray. University of Nebraska Medical Center, Omaha, NE.

TCDD induces hepatic DNA single strand breaks (SSB) in conjunction with lipid peroxidation in rats. The mechanism involved in DNA damage by TCDD is not known. The oral administration of 100 µg TCDD/kg resulted in 1.3-, 4.3-, 7.2- and 7.8-fold increases in DNA elution constants 3, 7, 10 and 14 days post-treatment, respectively. Similar changes occurred in hepatic nuclear lipid peroxidation as determined by the content of thiobarbituric acid reactive substances (TBARS). Treatment of rats with the dithiolthione antioxidant oltipraz (30 mg/kg/day) inhibited the formation of DNA SSB by TCDD. Incubation of hepatic nuclei from control animals with microsomes, mitochondria and cytosol from rats 3, 7, 10 and 14 days after treatment with TCDD resulted in increased DNA elution constants of 1.2-, 1.9-, 2.0- and 2.3-fold, respectively. Mitochondria and microsomes but not cytosol from TCDD treated rats enhanced DNA damage in nuclei from control rats. The addition of the iron chelator desferrioxamine inhibited in vitro DNA damage by microsomes and mitochondria from TCDD treated rats. The addition of Fe²⁺ and Fe³⁺ to nuclei from control animals enhanced DNA damage. Thus, TCDD induced DNA SSB involves iron and the formation of reactive oxygen species and/or free radicals.

THE EFFECT OF 2,3,7,8-TETRAChLOROBENZo-p-DIOXIN (TCDD), 2,3 DICHLOROBENZo-p-DIOXIN (DCDD), AND 2,3,7,8 TRICHLOROBENZO-p-DIOXIN (TrCDD) ON CYTOCHROME P450 GENERATED REACTIVE OXYGEN. D S Brandwene, P C Kahn. Rutgers University, Joint Graduate Program in Toxicology, Piscataway, NJ. Sponsor: G WITZ.

Studies have implicated reactive oxygen as a possible mechanism of toxicity for TCDD. We compared the effects of TCDD, DCDD, and TrCDD on hydrogen peroxide production from TCDD induced rat liver microsomes. Male Sprague-Dawley rats were given a single oral dose of TCDD (25 µg/kg) and sacrificed 3 days later. Microsomes were assayed for hydrogen peroxide production in the presence of TCDD, DCDD, or TrCDD as substrate. Concentrations of 10⁻⁶M-10⁻⁵M were used for all three congeners; hydrogen peroxide was measured at 3, 7, 11, and 15 minutes. There was no significant difference between TCDD, DCDD, and TrCDD treated microsomes in hydrogen peroxide production. TCDD at a dose of 40 µg/kg inhibited glutathione peroxidase activity 39% compared to corn oil controls. These results suggest that a decrease in the metabolism of active oxygen species may play a role in TCDD toxicity.

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TCDD has been shown to downregulate several receptors for estrogen, progestin, androgens, corticosteroids, epidermal growth factor, low density lipids, and thyroxine. Many of the known effects of TCDD can be attributed to the downregulation of estrogen receptors and the compensatory responses. We suggest that downregulation of the estrogen receptor results in an increased synthesis of the natural ligand via feedback regulation. Therefore, both hypo- and hyper-hormonal responses will occur. Species that cannot excrete estrogens well are able to elevate estrogen levels sufficiently to overcome TCDD's toxic effects and will survive. Species that excrete estrogens well are unable to build up sufficient hormone levels and will die from toxic effects of over-synthesis of estrogens and the ensuing hormonal imbalance. Because hormonal systems are closely interregulated, other hormones are probably involved. (Supported by USEPA CR# 812114-01-1).

Atrophy of the gastrointestinal (GI) mucosa that occurs in pair-fed control rats is not observed in TCDD-treated rats (Christian et al., 1986). Our objective was to determine if the GI trophic hormone, gastrin, is involved in the antiatrophy effect of TCDD. TCDD-treated rats (100 μg/kg) were markedly hypergastrinemic 14 days posttreatment whereas pair-fed rats were normogastrinemic. Feed restriction-induced atrophy of both fundic and antral mucosa was observed in pair-fed rats, but not in TCDD-treated animals. Since hypergastrinemia stimulates cellular proliferation and growth of fundic but not antral mucosa, the antiatrophy effect of TCDD on fundic mucosa could in part be due to hypergastrinemia. However, its antiatrophy action on the antral mucosa must occur by another mechanism. Reductions in the antral content and concentration of both gastrin and somatostatin were observed 7 days after treatment in TCDD-treated rats but not in pair-fed controls. The dose of TCDD required to reduce antral levels of gastrin and somatostatin was significantly less than that needed to produce hypergastrinemia. In addition, the TCDD-induced reduction in antral gastrin was not due to a decrease in the number of gastrin-containing cells in the antral mucosa. (Supported by NIH ES01332).

The comparative antiestrogenic activities of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and progesterone on nuclear and cytoplasmic progesterone (PRn and PRc) and estrogen (ERn and ERc) receptor levels in the rat uterus were determined. Treatment of the rats with estradiol (5 μg/kg) and estradiol plus either progesterone (1 mg/animal) or 2,3,7,8-TCDD (80 μg/kg) showed that both progesterone and 2,3,7,8-TCDD significantly antagonized the estradiol-mediated increases in uterine PRn, PRc, ERn, PRc, and PRn; moreover for 2,3,7,8-TCDD, the decreased receptor levels persisted for up to 7 days. In an in vitro rat uterine strip assay system, both 2,3,7,8-TCDD and progesterone act as antiestrogens. In contrast, the antiestrogenic activity of progesterone was inhibited by both protein and RNA synthesis inhibitors whereas the antiestrogenicity of 2,3,7,8-TCDD was inhibited only by the RNA polymerase inhibitor, actinomycin D. These results suggest that the antiestrogenic effects elicited by 2,3,7,8-TCDD and progesterone are expressed through different mechanisms. (Supported by the Texas Agricultural Experiment Station.)

Hypergastrinemia occurs during the later stages of the wasting syndrome in TCDD-treated rats (40-100 μg/kg). Our objective was to determine the mechanism. Serum disappearance of gastrin-17 was similar in hypergastrinemic control and hypergastrinemic TCDD-treated rats suggesting that reduced serum clearance of endogenous gastrins is not involved. Since reduced acidity of stomach contents normally leads to elevated serum gastrins, a TCDD-induced decrease in parietal cell acid secretion was postulated. In support of this hypothesis, TCDD-treated rats that were hypergastrinemic had decreases in gastric secretory volume, gastric acidity and total gastric acid output. Additionally serum gastrin concentrations of these animals were negatively correlated with total gastric acid outputs. The decreased gastric acid secretion was not due to a reduction in fundic mucosa parietal cell mass but appeared due to TCDD inhibiting the ability of endogenous gastrins or exogenously administered pentagastrin from stimulating parietal cell acid secretion. Neither hypergastrinemia nor reduced gastric acid secretion was observed in pair-fed control rats. Thus hypergastrinemia in TCDD-treated rats appears to be due to a decrease in gastric acid secretion resulting from parietal cell dysfunction. (Supported by NIH ES01332).

Histopathology of a usually nonlethal (25 μg/kg) and of a usually lethal (125 μg/kg) single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, ip in 95:5 corn oil:acetone) was studied in male Sprague-Dawley rats and compared to pair-fed as well as to ad libitum-fed controls at 1, 2, 4, 8, 16 and 32 (28 for lethal dose) days after dosing. Pair-feeding itself had numerous effects on the morphology of tissues as compared to ad libitum-fed controls. However, TCDD had dose-dependent differential effects as compared to pair-fed controls on the following tissues and organs: pituitary, pancreas, adrenal, thyroid, testes, thymus, lymphoid tissue of the gut, liver, brown adipose tissue and non-lymphoid tissue of the gut. Tissue-specific histopathology is discussed in the context of known endocrine, immunological and metabolic effects of TCDD. In addition, this study demonstrates the importance of appropriate viz. pair-fed controls also for histopathological studies, when body weight loss is a prominent feature of toxicity as it is the case in many instances. The "normalization" of morphology using pair-fed controls aids in avoiding potential misdiagnosis of toxic effects, which in fact are attributable to reduced feed intake rather than to the toxic agent. (Supported by NIH ES-07079)


De novo fatty acid synthesis (FAS) was determined by the 3H2O method in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated (125 μg/kg), pair-fed and ad libitum-fed rats to examine if this energy-inefficient process plays a role in the wasting away caused by TCDD. Of the 12 tissues and organs examined, liver showed an increased and interscapular brown adipose tissue (IBAT) a decreased FAS in TCDD-treated vs. pair-fed or ad libitum-fed controls. De novo FAS was unaffected in other organs and tissues examined. Increased FAS in liver coincided with increased plasma triiodothyronine (T3) whereas decreased FA synthesis in IBAT paralleled decreased plasma thyroxine (T4) levels. Thyroidectomy decreased FAS in both liver and IBAT. TCDD elicited no response in either of these organs in thyroidectomized (TXD) rats. These findings suggest that changes observed in non-TXD rats are probably secondary effects. Known tissue-specific effects of T3 on liver, and of T4 on IBAT provide a likely explanation for the altered FAS in these organs. It is suggested that this increased FAS in the livers of TCDD-treated rats is responsible for the additional wasting away observable in TCDD-treated vs. pair-fed rats. The partial protection from TCDD toxicity in TXD rats may now be explained as a result of a reduction of an energy-inefficient metabolic pathway, viz. de novo fatty acid synthesis. (Supported by NIH ES-07079)


Bilateral adrenalectomy or adrenal demedullation was performed in male Sprague-Dawley rats by established surgical techniques. Subsequently, the dose-response (mortality and mean time to death) to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was determined in both adrenalectomized (10, 20, 40 μg/kg TCDD ip in 95:5 corn oil:acetone) and demedullated (15, 30, 60 μg/kg) rats. Adrenalectomy resulted in a dramatically increased mortality and a much shorter mean time to death. The estimated LD50 was about 5 times lower than in non-adrenalectomized rats. Conversely, adrenal demedullation had no effect on mortality or mean time to death. It was concluded that factor(s) modulating the acute toxicity of TCDD reside in the adrenal cortex and not in the medulla. Administration of corticosterone (25 μg/ml in drinking water) to adrenalectomized rats restored toxicity of TCDD to "normal" suggesting that this hormone is another key factor (in addition to the thyroid hormones) in the acute toxicity of TCDD. Corticosterone supplementation (25, 50, 100 μg/ml in drinking water) to non-adrenalectomized rats, resulted in no additional beneficial effect indicating that factor(s) other than thyroid hormones and corticosterone are also involved in the acute toxicity of TCDD. (Supported by NIH ES-07079)


Male Sprague-Dawley rats were hypophysectomized (HXD) by an established surgical technique. Similar to adrenalectomy, hypophysectomy aggravated the toxicity (mortality and mean time to death) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 125 μg/kg ip in 95:5 corn oil:acetone) when compared to sham-HXD rats (100% mortality with 9 ± 1 days mean time to death versus 90% mortality with 32 ± 6 days mean time to death, respectively). However, administration of corticosterone (25 μg/ml in drinking water) to HXD rats resulted in an attenuation of the dose-response to a range of TCDD doses (125, 250, 500 μg/kg) much higher than the LD50 in non-HXD rats (about 60 μg/kg TCDD).

Based on the data and those obtained in adrenalectomized rats it is concluded that one or more key factors which are capable of modulating the toxicity of TCDD, reside in the pituitary. (Supported by NIH ES-07079)

The effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 125 μg/kg) was studied on the conversion of 14C-alanine into 14C-glucose in male Sprague-Dawley rats by established procedures (determination of alanine and glucose by enzymatic assays and isolation of 14C-alanine and 14C-glucose from whole blood by column chromatography). TCDD-treated rats converted only 1/2 as much 14C-alanine into 14C-glucose as did their pair-fed or corn oil counterparts indicating reduced gluconeogenesis in TCDD-treated rats. This finding suggested that reduced gluconeogenesis in TCDD-treated rats may contribute to the profound hypoglycemia observed in intoxicated animals. Corticosterone, a key hormone in gluconeogenesis, provides partial protection from TCDD-induced toxicities in normal (HxD) rats. Therefore, the conversion of 14C-alanine into 14C-glucose was also determined in HxD rats dosed with TCDD (125 μg/kg) and given corticosterone (25 μg/ml in drinking water). These rats also converted only 1/2 as much 14C-alanine into 14C-glucose as did their pair-fed counterparts. However, in contrast to non-HxD rats, these rats maintained normal glycogen even at 64 days after dosing with TCDD. It is concluded that TCDD reduces gluconeogenesis in rats but the protective effect of corticosterone in HxD rats is unrelated to gluconeogenesis. (Supported by NIH ES-07079)

TRICHLOROETHYLENE (TCE) AND THE AREA UNDER CURVES (AUC) FOR ITS METABOLITES IN BLOOD. J L Larson and R J Buti. Pharmacology/Toxicology Program, College of Pharmacy, Washington State University, Pullman, WA.

Mice have been shown to be more sensitive than rats to the hepatocarcinogenic effect of TCE. Several studies have demonstrated that the metabolism of TCE is saturated at doses above 1 g/kg in the rat. It has been suggested that this saturation is responsible for the lower sensitivity in rats. The present study more carefully analyzes the blood concentration versus time AUC for TCE and metabolites at doses of 3.0, 0.6, and 0.125 g/kg TCE. Blood samples were collected at 1, 2, 4, 8, 12, 24, 48, and 72 hours following oral dosage of TCE in a 1% Tween 80 vehicle. The AUC for TCE increased by 6.3, and the AUC for the metabolites dichloroacetic acid, trichloroacetic acid and trichloroethanol were increased 1.9, 2.3 and 3.0, respectively. Therefore, the saturating dose of TCE resulted in substantial prolongation of the period of time that appreciable amounts of TCE and metabolites were present in blood. This must be considered when assessing the impact of saturating doses of TCE on carcinogenic response. (This work was funded by U.S. Air Force Grant AFOSR-86-0284)

PHARMACOKINETICS (PK) OF VOLATILE HALOCARBONS: COMPARISON OF SINGLE ORAL BOLUS VS GASTRIC INFUSION OF 1,1,1-TRICHLOROETHANE (TRI). S Muralidhara, R Ramnathan, J M Gallo*, C E Dallas, and J V Bruckner. Deps. of Pharmacol & Toxicol. & Pharmaceutics*, College of Pharmacy, University of Georgia, Athens, GA.

The objective of this study was to assess the influence of different patterns of ingestion on the PK of TRI, a halocarbon-contaminant of water supplies. TRI was given to unanesthetized male S-D rats as an Emulphor emulsion, either orally as a single bolus or infused through a surgically implanted gastric cannula over 2 hr at a dose of 40 mg/kg. Blood samples were collected from an indwelling carotid arterial cannula from 0 to 540 min during and post-infusion. The blood samples were analyzed for TRI using a GC-ECD headspace technique. Cmax, AUC, elimination half-life and other PK parameters were determined and compared for these two oral dosage regimes. Reductions in the AUC and Cmax and increased terminal elimination half-life were observed when TRI was given by continuous gastric infusion. Similar experiments were also performed using a low dose of 6 mg/kg of TRI. These results indicate that the pattern of consumption of contaminated water can significantly influence the PK of TRI, and thereby likely affect the toxic potential of the chemical. (Supported by EPA Cooperative Agreement CR812267 and U.S. Air Force AFOSR 870248)


Physiologically-based pharmacokinetic (PBPK) models have been applied to correct for interspecies and interdose differences when estimating human cancer risk. Differences in uptake, absorption, metabolism, excretion and tissue partitioning may explicitly be taken into account through these models. Model input parameters are derived from various in vitro and in vivo experiments, usually performed on relatively few specimens under conditions different from those of human exposure or the cancer bioassays. Inaccuracies in the input parameters influence the overall accuracy of the model predictions. Monte Carlo simulation is used to evaluate the precision of model predictions resulting from the parameter uncertainties quantified from the published data. PBPK models developed for tetrachloroethylene and vinyl chloride are used as examples. Under the specified model assumptions, these analyses provide a means of quantitatively assessing the reliability of using PBPK models to scale between species and high to low dose, as well as the sensitivity of model predictions to particular parameters. Supported by the State of California, Department of Health Services, Contract 85-87088.
PHARMACOKINETICS (PK) of VOLATILE HALOCARBONS: COMPARISON OF SINGLE ORAL BOLUS VERSUS INFUSION OF TRICHLOROETHYLENE (TCE). R Ramanathan, S Muralidhara, J W Gallo*, C.E. Dallas, and J V Bruckner. Deps. of Pharmacol. & Toxicol. and &Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA.

The objective of this study was to gain a better understanding of the PK of TCE, a common halocarbon contaminant of drinking water. TCE was given to unanesthetized male S-D rats as an aqueous Emulphor emulsion, either orally as a single bolus or infused through a surgically implanted gastric cannula over 2 hr at a dose of 76 mg/kg. Blood samples were collected from an indwelling carotid arterial cannula during and post infusion from 0 to 540 min. The blood samples were analyzed for TCE using a GC-EC head space technique. Cmax, AUC, elimination half-life and other PK parameters for the two patterns of ingestion were determined and contrasted. Significant reductions in the AUC and Cmax and increased terminal elimination half-life were observed when TCE was infused intra-gastrically. Similar experiments were done using a low dose of 8 mg/kg of TCE in order to evaluate the linearity of the PK of the chemical. Our findings indicate that the PK of TCE can be significantly affected by both the route of administration and regimen of dosing employed. (Supported by U.S. EPA Cooperative Agreement CR812267 and U.S. Air Force AFOSR 870248)

DIFFERING TOXICITY AFTER SUBACUTE TRICHLOROETHYLENE (TCE) EXPOSURE IN AQUEOUS AND CORN OIL GAVAGE VEHICLES IN MICE. B A Herrick, M Robinson and L W Condie. USEPA, WRL, Cincinnati, OH

Subacute toxicity of TCE was evaluated in male and female B6C3F1 mice using a corn oil or an aqueous (20% Emulphor emulsion) gavage vehicle. Mice received oral doses of TCE 5 times a week for 4 weeks at 600, 1200 and 2400 mg/kg/day for males and 450, 900 and 1800 mg/kg/day for females. Control mice were dosed with either corn oil or Emulphor. A dose-related increase in lethality occurred in male and female mice receiving TCE in Emulphor but not in corn oil during the first week of treatment. Lethality was consistent with CNS depressant effects of TCE. At sacrifice, body weights were not altered by TCE treatment but liver/body weight ratios were uniformly increased by TCE administered in either vehicle in both sexes. Only male mice treated with TCE in corn oil, however, sustained elevations in serum enzyme levels accompanied by liver histopathology. TCE in corn oil produced inflammation-associated focal necrosis in one-third of the male mice with increasing severity from low to high dose. Lipid accumulation by Oil-Red-O staining was most prevalent in male mice treated with TCE in corn oil. This study indicates that the type of oral gavage vehicle is an important factor in determining the nature of TCE toxicity. (Abstract does not necessarily reflect EPA policy).

A STUDY OF THE JOINT ACTION OF CARBON TETRACHLORIDE (CCL4) AND TRICHLOROETHYLENE (C2HCl3) FOLLOWING SIMULTANEOUS GAVAGE ADMINISTRATION IN THE RAT. R H Granger, T M O'Hara, L W Condie*, and J F Borzellino. Deps. of Pathology and Pharmacology/Toxicology, Medical College of VA, Richmond, VA and *U.S.E.P.A., Cincinnati, OH

The joint action of (CCL4) and (C2HCl3) following simultaneous oral administration has been investigated in the male CD rat. This study, prompted by previous reports of enhanced CCL4 hepatotoxicity following C2HCl3 pretreatment, is one of a series designed to identify and characterize interactions occurring among common drinking water contaminants. Rats with indwelling arterial cannulas were gavaged with mixtures of CCL4 and C2HCl3 at doses of 0, 100, 250 and 400 mg/kg in a 4x4 grid design. Hepatotoxicity was evaluated as a measure of ATR, ALT and SDH plasma enzyme activity at 0, 3, 6, 12, 24, 36, 48 and 72 hours post gavage. Additional blood samples were taken between 0 and 6 hours post gavage for CCL4 and C2HCl3 pharmacokinetic analysis. Time course response data from individual rats were calculated as area-under-the-response-time-curve (AUC). Response data were analyzed for possible interactions using response surface methods (RSM). Statistically significant "greater than additive" interactions between CCL4 and C2HCl3 were observed under these conditions and may be related to the pharmacokinetics of the combination. (Supported by EPA Cooperative Agreement 812558.)

THE SYNERGISTIC HEPATOTOXICITY OF CARBON TETRACHLORIDE AND TRICHLOROETHYLENE IN MALE F 344 RATS. D A McMillan, M Tokars, C Eskelson and JG Sipes. Dept. of Pharmacology and Toxicology, Univ of Arizona, Tucson, AZ

The interactive hepatic effects of carbon tetrachloride (CCL4) and trichloroethylene (TCE), two common drinking water pollutants, were studied using male F 344 rats. The chemicals were administered orally in an aqueous (10% Emulphor) vehicle. Dose-response studies with CCL4 and TCE indicated that a non-toxic dose of TCE (0.25 or 0.50 ml/kg) (as indicated by plasma alanine aminotransferase, ALT, and sorbitol dehydrogenase, SDH, activities) administered simultaneously with a minimally hepatotoxic dose of CCL4 (0.05 ml/kg) produced potentiation of the halogenated hydrocarbon-induced hepatotoxicity. A time-course study using CCL4 (0.05 ml/kg) + TCE (0.5 ml/kg) showed that the binary combination maximally elevated ALT and SDH activities and depressed hepatic reduced glutathione content at 24 hrs after administration. Ethane exhalation, as a measure of lipid peroxidation, was found to be similar for rats receiving CCL4 (0.05 ml/kg), or TCE (0.5 ml/kg) or both chemicals simultaneously. Rats treated with either 14C-CCL4 or 14C-CCL4 + TCE did not differ in the amount of 14C-equivalents expired either as 14CO2 or as exhaled organic compounds. Gas chromatographic analysis of the exhaled organic samples showed that the 14C-CCL4 + TCE group expired more chloroform than the 14C-CCL4 alone group. These data indicate that the synergistic hepatotoxicity of CCL4 + TCE may involve changes in CCL4 metabolism. (Supported by EPA No. CR-812557.)
A STUDY OF THE JOINT HEPATOTOXIC ACTION OF CARBON TETRACHLORIDE (CCL₄) AND CHLOROFORM (CHCl₃) FOLLOWING SIMULTANEOUS GAVAGE ADMINISTRATION IN THE RAT. T M O'Hara, R H Granger, L W Condie* and J F Borzelleca. Deps. of Pharmacology/Toxicology and Pathology, Medical College of VA, Richmond, VA and *U.S.E.P.A., Cincinnati, OH.

The joint action of CCl₄ and CHCl₃ following simultaneous oral administration has been investigated in the chronically arterial-cannulated male CD rat. CCL₄, CHCl₃, or their mixture were administered at doses of 0, 100, 250 and 400 mg/kg in a 5% Emulphor vehicle, completing a 4x4 grid design. Hepatotoxicity was evaluated as a measure of AST, ALT and SDH plasma enzyme activity at 0, 3, 6, 12, 24, 36, 48 and 72 hours post gavage. Additional blood samples were taken between 0 and 6 hours post gavage for CCl₄ and CHCl₃ pharmacokinetic analysis. Response data were analyzed for possible interactions using response surface methods (RSM). Hepatotoxicity increased in a dose-dependent manner for both CCL₄ and CHCl₃ when administered alone. Interactions between CCl₄ and CHCl₃ appeared biphasic with an initial "infra-additive" response at 6 hours followed by a "supra-additive" response at 24, 36 and 48 hours. Data indicate alterations in the pharmacokinetics of CCl₄ when co-administered with CHCl₃.

(Supported by EPA Cooperative Agreement 812558.)

METABOLISM AND DISPOSITION OF LOW DOSE CCL₄ IN PARTIALLY HEPATECTOMIZED, CHLORDEcone PRETREATED RATS. R A Young, F Siddiqui, and H M Mehendale. Univ. Miss. Med. Ctr., Jackson, MS.

Previous work in our lab has indicated suppressed hepatocellular regeneration to be a potential mechanism for the potentiation of CCl₄ hepatotoxicity by chlordecone (CD). Additional studies demonstrated that livers induced into active regeneration by partial hepatectomy (PH) provided protection against this amplified toxicity. Since bioactivation of CCl₄ is necessary for expression of toxic effects, it was imperative to determine if CCl₄ metabolism and disposition were altered in PH rats. SH Hepatectomized (SH) and PH (male Sprague-Dawley, 150-175g) were given dietary CD (10 ppm) for 15 days. On day 15 the rats were surgically manipulated (SH or PH) and on day 16 challenged with a single dose of CCl₄ (100 µl/kg, i.p.) containing 14C-CCl₄ (20µCi). After 6 hours post challenge, there was no difference between the SH and PH rats in recovery of exhaled 14C, or formation of 14CO₂. As anticipated, hepatic content of 14C-CCl₄-derived 14C-per-g-tissue was greater in PH rats. Values for free 14C-CCl₄ and covalently bound 14Cwere similar for livers from SH and PH rats. The data suggest that metabolism and disposition of CCl₄ are not altered by PH, thereby serving as a valid model for studying the CD-CCl₄ interaction in an actively regenerating liver. (Supported by EPA, R-811072 and CR814053010.)

THE INFLUENCE OF STRUCTURAL ANALOGUES OF CARBON TETRACHLORIDE (CCl₄) ON HEPATOCYTE FUNCTIONS IN VITRO. J B Coleman, L W. Condie*, J F Borzelleca and R H Granger. Deps. of Pharmacology/Toxicology, Medical College of VA, Richmond, VA and *U.S.E.P.A., Cincinnati, OH.

Isolated and cultured hepatocytes were incubated (15 min to 18 h) with various concentrations (0.1 to 1.0 mM) of bromotrichloromethane (CBrCl₃), CCl₄, flurtrichloromethane (CFCl₃), and chloroform (CHCl₃). Phospholipase C (PLC) was rapidly activated (translocation) by membrane (phospholipid)-associated chemical metabolites and preceeded chemical-dependent alterations in endoplasmic reticulum (ER), mitochondria and plasma membrane functions. The observed rank order of PLC activation and alterations in cellular functions: CBrCl₃ > CCl₄ > CHCl₃ > CFCl₃, corresponds to the reported hepatotoxicity of these agents in vivo. Therefore, the rapidly activated, PLC-mediated degradation of membrane phospholipids may: 1) represent the event that injures membranes, such as the ER; 2) explain in part the alterations in membrane functions associated with reactive metabolite-mediated hepatocyte injury.

(Supported by NIH AM 31115 and EPA Cooperative Agreement 812558.)

TIME-COURSE OF LIVER INJURY AND 3H-TYTHIDINE INCORPORATION IN CHLORDEcone-POTENTIATED CHC13 HEPATOTOXICITY. K R Purushotham and H M Mehendale, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS.

We have shown that chlordecone (CD)-potentiated CHCl₃ hepatotoxicity leads to increased lethality in male S.W. mice. We intended to study the time-course of such hepatotoxicity and 3H-thymidine (3H-T) incorporation into nuclear DNA in mice fed CD (10 ppm), mirex (M, 10 ppm) or phenobarbital (PB, 225 ppm) diets for 15 days. The mice received 0.1 ml CHCl₃/kg in corn oil daily. Liver damage was assessed by plasma ALT, AST and histology at 4, 12, 24, 36, 48, 72 and 96 hr after CHCl₃. Elevation in plasma enzymes and the hepatocellular damage were seen only in CD pretreated mice. The enzyme elevations were maximum at 24 hr. The centrilobular and midzonal necrosis was seen from 12 hr onwards. The pretreatments did not show any change in hepatic nuclear DNA levels but altered 3H-T incorporation. None of the dietary treatments altered liver regeneration. Among the combination treatments, 3H-T incorporation was maximal and biphasic (36 and 72 hr) with CD. M showed a single peak at 72 hr and PB showed a progressive but least increase. These results indicate the impact of dietary CD + CHCl₃ on the hepatocellular damage and the incorporation of T into nuclear DNA. (Supported by EPA R-811072.)
BIOCHEMICAL EFFECTS OF THREE CARCINOGENIC CHLORINATED METHANES IN RAT LIVER. K T Kitchin and J L Brown. Research Laboratory, US EPA, Research Triangle Park, NC

The mechanism (initiation or promotion) by which these chlorinated methanes cause rodent liver tumors was investigated. Varying oral doses of either carbon tetrachloride, chloroform or methylene chloride were given to adult female rats both 21 and 42 days before sacrifice. Then DNA damage, ornithine decarboxylase activity (ODC), cytochrome P-450 and glutathione content were assayed in liver. DNA damage and ODC induction are considered markers for carcinogenic initiation and promotion, respectively. With or without increased serum alanine aminotransferase (SGPT) activity, carbon tetrachloride increased rat hepatic ODC and decreased cytochrome P-450 content. Chloroform increased hepatic ODC with minimal or no elevation in SGPT activity. Methylene chloride (1275 mg/kg) caused a small but significant amount of hepatic DNA damage. When these three compounds are compared on a toxin or equitoxic (1/5 LD50) basis, the order of potency of inducing hepatic ODC or increasing serum alanine aminotransferase activity was carbon tetrachloride > chloroform > methylene chloride. As the degree of chlorination increases in this series, the compound is more likely to promote, rather than initiate, the carcinogenic process. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
CHIRAL EFFECTS IN THE INDUCTION OF DRUG-METABOLIZING ENZYMES USING SYNTHETIC ATROPISOMERS OF POLYCHLORINATED BIPHENYLS. M. Pfitzmann, A. Mannsrech, and L. W. Robertson. Graduate Center for Toxicology, University of Kentucky, Lexington, KY and Department of Organic Chemistry, University of Regensburg, Regensburg FRG.

Atropisomers of the polychlorinated biphenyls 2,2',3,4,4',6-hexachlorobiphenyl (II) and 2,2',3,3',4,4',6,6'-octachlorobiphenyl (III), stable to racemization under physiological conditions, were administered to immature male Sprague-Dawley rats. The racemic hexachlorobiphenyl (II) was found to be a potent (phenobarbital-type) inducer, whereas (+)-II and (-)-II, administered at 100 µmol/kg, showed clearly differing potencies as inducers with (+)-II enhancing amyloperoxidase N-demethylase, aldrin epoxidase and cytochrome P-450 content more potent than that of the racemic octachlorobiphenyl (III) and its individual enantiomers were only weak phenobarbital-type inducers of cytochrome P-450 and the enantiomers of III were equally (weakly) potent. Separate studies conducted to investigate the differential potencies of the enantiomers of II showed that (+)-II was apparently more rapidly metabolized than its antagonist. Therefore, enantiomericity in disposition rather than in recognition is responsible for the differential potency seen.

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TERATOGENICITY OF 2,3,4,7,8-PENTACHLORODIBENZOFURAN (4-PCDF) IN F344 RATS. L. A. Couture, M. W. Harris, and L. S. Birnbaum. NIEHS, RTP, NC and UNC, Chapel Hill, NC.

PCDFs are ubiquitous environmental contaminants that have also been detected in human tissues. 4-PCDF, similar in chemical structure and toxicity to 2,3,7,8-tetrachlorodibenzofuran, is one of the most toxic members of the class of halogenated aromatics. To evaluate the teratogenicity of 4-PCDF in F344 rats, dams were dosed per gestation day 12 with doses of 0,10,30,100, or 300 µg/kg. All animals were sacrificed on gestation day 20 and maternal and fetal toxicity was assessed. Determination of fetal toxicity involved both soft tissue and skeletal examinations. Maternal weight gain decreased in a dose-related fashion, while the liver:body weight ratio increased and the ratio of thymus:body weight decreased. A steep dose-response curve was observed for fetal mortality reaching 81% at a dose of 300 µg/kg. There was a 100% incidence of cleft palate in all surviving fetuses in the 300 µg/kg group. Soft tissue anomalies observed in the high dose group were a reduction in the size of the lungs and thymus or an absence of the thymus altogether. In conclusion, the spectrum of teratogenic and maternally toxic effects are indicative of a pattern similar to those observed following exposure of murine species to polychlorinated aromatic compounds. Further experiments are currently on-going to verify such a pattern.

A UNIQUE APPROACH TO THE SYNTHESIS OF 2,3,4,5-SUBSTITUTED POLYBROMINATED BIPHENYLS (PBBs): QUANTITATIVE IN FINECONTR F-1 AND FIREMASTER BP-6. G. Kubickiz, E. Oschman, and L. W. Robertson. Graduate Center for Toxicology, University of Kentucky, Lexington, KY and Institute of Toxicology, University of Mainz, Mainz FRG.

Unraveling the toxicity of a complex chemical mixture necessitates the isolation or synthesis of the individual components. From a single starting material, 2,6-dibromo-4-nitroaniline, a synthetic scheme is presented which provides in good yields 4 anilines (3,5-di-, 3,4,5-tri-, 2,3,4,5-tetra- and 2,3,4,5,6-pentabromanilines), as well as the 1,2,3,4-tetrabromobenzene, all useful precursors for the synthesis of polybrominated biphenyls (PBBs). The aryln-arylation coupling of these and other bromoanilines with 1,2,3,4-tetrabromobenzene provides a versatile approach to the synthesis of 2,3,4,5-substituted PBBs. The synthesis and characterization of nine such PBBs will be reported. Aside from the desired coupling product, 1,2,3,4,5,6-octabromobiphenyl was a by-product of each coupling reaction, ranging from less than 2 to 63% of the polybrominated biphenyl products. Capillary gas chromatographic quantitation of the nine synthetic PBBs in FireMaster FF-1 and FireMaster BP-6 will be presented.

IMMUNOTOXICITY OF POLYCHLORINATED DIBENZOFURANS: STRUCTURE-ACTIVITY RELATIONSHIPS AND INTERACTIVE EFFECTS. D. Davis and S. Safe. Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

The dose-response effects of several polychlorinated dibenzofuran congeners on the splenic plaque-forming cell (PFC) response to sheep red blood cells (SRBC) were determined in C57BL/6 mice. The dose for a 50% reduction in PFC's/10^6 viable cells (ED50) for 2,3,7,8-tetrachlorodibenzofuran (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-PeCDF and 1,3,6,8-TCDF were 2.4, 3.0, 14.8, 710 and 35,780 µmol/kg respectively. The relative potencies and structure-activity relationships (SARs) for the PeCDFs were comparable to those observed for other Ah receptor-mediated responses. The interaction of 1,3,6,8-TCDF and 2,3,7,8-TCDD demonstrated that sub-toxic levels of 1,3,6,8-TCDF (6.6 - 49.3 µmol/kg) a weak agonist significantly antagonized the immunotoxicity of the more potent agonist, 2,3,7,8-TCDD (3.7 µmol/kg). The effectiveness of the partial antagonist was dependent on several factors including the dose and the time of administration relative to both the immunotoxin and the SRBC antigen. (Supported by the National Institutes of Health and the United States Environmental Protection Agency.)
TOXICITY OF PERFLUORODECANOCIC ACID (PFDA) IS UNLIKE THAT OF TCDD. D W Brewster, M W Harris and L S Birnbaum, NIEHS, Research Tri. Pk., NC.

PFDA is an industrial surfactant that has been reported to produce similar signs of toxicity as does TCDD upon administration to rats. To determine whether PFDA toxicity is mediated by the Ah locus and to characterize the toxicity of PFDA in the mouse, congenic female C57BL/6J mice differing only at the Ah locus, (Ah/b, b), Ah b/g, and Ah b/d) were administered a single oral dose of PFDA. The wild type (b/b) strain was killed 2, 7, 14, or 30 days after administration of 0, 40, 80, 100, 120, or 160 mg PFDA/kg. The 2 substrains were killed 30 days after dosing with 0, 40, 80, or 160 mg/kg. In all 3 strains the LD50 was estimated to be ~120mg/kg. PFDA produced a 30% loss of body weight, a 2.5 fold increase in liver weight, a decrease in thymus and spleen weights, only a 20% increase in hepatic palmitoyl Co-A oxidase activity, a 70% decrease in hepatic ethoxyresorufin-o-deethylase (EROD) activity and an increase in serum T4 concentrations. Total hepatic lipids were increased at an early time point and at the lowest dose. At later time periods and/or higher doses lipid concentration was decreased ~70% from that of controls. There was little difference in any of these parameters between the 3 strains and the effects were dose and time dependent. These results suggest that the Ah allele is under significant influence in regulating the toxicity of PFDA, that the biochemical response to PFDA is markedly different from that of TCDD, and that the biochemical response to PFDA in the mouse is different from that in the rat.

DIFFERENTIAL EFFECTS OF DIETARY SELENIUM ON GLUTATHIONE-DEPENDENT ENZYME ACTIVITIES IN RATS TREATED WITH PEROXISOME PROLIFERATORS. L C Chen, T Borges, L W Robertson, H P Glauert and C K Chow. Graduate Center for Toxicology and Department of Nutrition & Food Science, University of Kentucky, Lexington, KY.

One-month-old male Sprague-Dawley rats, fed a diet containing either 0.04 or 1.0 ppm Se for 14 days, were treated with either a ciprofibrate (CIP, 0.025 ppm in the diet) or perfluorodecanoic acid (PFDA, 35 mg/kg, single IP) 2 weeks prior to sacrifice. Control animals were pair-fed. Hepatic GSH levels and GSH peroxidase (GSH Px) activity in S9 and cytosol were significantly increased in PFDA-treated rats fed the low Se diet, whereas the activity of GSH transferase (GST) was decreased. In contrast, a significant decrease in GSH Px was found in PFDA-treated rats fed the high Se diet. Likewise, CIP caused a significant decrease in GSH Px activity in the high Se group, whereas GST activity was diminished in both groups. CIP had no significant effect on GSH levels. Serum GSH P x activity was significantly decreased by CIP, but not by PFDA, in both dietary groups. The results suggest that dietary Se modulates GSH levels and related enzymes in rats treated with peroxisome proliferators. The mechanism of these differential effects remains to be elucidated. (Supported by AICR 86866 and NIH CA43719)

TERATOLOGIC EVALUATION OF PERFLUORODECANOCIC ACID (PFDA) IN C57BL/6J MICE. M W Harris and L S Birnbaum, Systemic Toxicology Branch, NIEHS, RTP, NC.

PFDA is a representative of the perfluorinated carboxylic acids used as commercial wetting agents and flame retardants. Signs of PFDA toxicity have been reported to resemble those seen after exposure to TCDD. To determine if PFDA is teratogenic, time mated C57BL/6J mice received PFDA by gavage in corn oil (10 ml/kg) on gd 6-15 or gd 10-13 at levels ranging from 0 to 12.8 mg/kg/day or 0 to 32.0 mg/kg/day respectively. Dam were sacrificed on gd 18 and maternal and fetal toxicity assessed. Fetuses were examined for external malformations, cleft palate as well as other soft tissue or skeletal anomalies. Maternal body weight gain was significantly reduced as a result of PFDA treatment at 6.4 and 12.8 mg/kg/day (gd 6-15) and 16.0 and 32.0 mg/kg/day (gd 10-13). Fetal viability was decreased only in those groups showing reduced maternal weight gains. Fetal body weights were significantly reduced at levels as low as 0.1 mg/kg/day (gd 6-15) and 0.5 mg/kg/day (gd 10-13). No hydrenephrosis, cleft palate or edema were observed nor were any other soft tissue or skeletal anomalies detected. Thus, unlike TCDD, PFDA is not teratogenic in C57BL/6J even at doses which are maternally toxic.

EFFECTS OF CIPROFIBRATE AND PERFLUORODECANOCIC ACID ON LIPID METABOLISM AND GROWTH OF MALE SPRAGUE-DAWLEY RATS FED 2 LEVELS OF SELENIUM. T Borges, L C Chen, L W Robertson, C K Chow, and H P Glauert. Graduate Center for Toxicology and Department of Nutrition & Food Science, University of Kentucky, Lexington, KY.

Rats fed diets containing either 0.04 ppm or 1.0 ppm selenium were treated with the hypolipidemic drug ciprofibrate (CIP, 0.025% in the diet) or the industrial chemical perfluorodecanoic acid (PFDA, 35 mg/kg in corn oil IP). Rats administered either peroxisome proliferator gained less weight than their pair-fed controls. Toxicity of PFDA, particularly apparent in rats fed the low selenium diet, was reflected by lowered food intake, feed efficiency, weight gain and thymus weights as compared to pair-fed controls. These effects were less evident in rats fed the high selenium diet. Peroxosomal beta-oxidation was increased in all CIP-fed rats but was decreased in PFDA-treated animals. Plasma triglycerides and cholesterol were decreased in CIP-fed rats consistent with increased lipid metabolism. Plasma triglycerides but not cholesterol was lowered in PFDA-treated rats. The results suggest that selenium influences PFDA-induced toxicity whereas no effect of selenium was seen in CIP-fed animals. (Supported by AICR 86866 and NIH CA43719)

The ability of DBCP to cause acute renal necrosis was determined by injecting single i.p. doses (170-680 μmol/kg) in male rats, mice, hamsters and guinea pigs. DBCP showed appreciable species differences with respect to induction of proximal tubular necrosis. Substantially less necrosis was found in mice compared to rats, whereas no necrosis was observed in hamsters. To investigate the reason for these differences in nephrotoxic potential, renal distribution of DBCP was studied 1, 3 and 8 hr after a dose of 85 μmol/kg. Furthermore, in both time- and dose-dependent experiments, the ability of DBCP to induce DNA damage in kidney nuclei isolated after single i.p. doses of DBCP was compared in the four species. In order to study the involvement of GSH-mediated metabolism in the renal toxicity of DBCP in rats, mice, hamsters and guinea pigs, bromide release from DBCP with kidney cytosols fortified with 1 mM GSH was performed and correlated with DBCP-induced renal necrosis and DNA damage.

MORPHOMETRIC ANALYSIS AND STRENGTH DETERMINATION OF OSTEOSClerotic BONE RESULTING FROM HEXACHLOROBENZENE (HCB) EXPOSURE. J E Andrews† and W E Donaldson‡. U.S.E.P.A., HERL, RTP, NC; ENCSU, Toxicology Program, Raleigh, NC.

We have shown previously that hexachlorobenzene (HCB) exposure induces hyperparathyroidism and osteosclerosis in rats. Therefore, we investigated the effects of HCB on femur morphometry as well as breaking strength. Fischer 344 rats were dosed 5 days/wk for 15 weeks with 0, 0.1, 0.5, 1.0, 10.0 or 25 mg HCB/kg body weight. Femur weight was significantly increased in the rats receiving 0.1, 1.0 and 25.0 mg HCB/kg body weight whereas density was increased significantly at 1.0, 10, and 25 mg HCB/kg dose levels. Bone strength was also significantly increased at the three higher dose levels. Cross-sectional area of the middle of the femur was used to determine effects on cortical and medullary parameters. Cortical area as well as the proportion of the total area of the bone which the cortex occupied were significantly increased at the three higher dose levels. Medullary cavity area was significantly decreased at the two higher dose levels of HCB. The right femur was significantly predominant to the left femur in weight, volume and density through all dosing regimens. Data from this study indicate that HCB does affect bone development and morphometry as well as strength. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

SUBACUTE AND SUBCHRONIC TOXICOLOGICAL RESPONSES OF RATS AFTER ORAL EXPOSURE TO CHLOROPICRIN (CP). L W Condie, M Robinson, and E A Merrick. USEPA, Health Effects Research Laboratory, Cincinnati, OH.

The toxicology of CP, a chlorination disinfection byproduct, was evaluated by oral gavage in male and female Sprague Dawley rats. Subacute treatment (SA-Tx) of rats was for 10 consecutive days at dose levels of 10, 20, 40, and 80 mg/kg/day. The subchronic 90 day treatment (SC-Tx) exposure levels were 2, 8 and 32 mg/kg/day. Controls were treated with corn oil. SA-Tx with CP showed significant decreases in final body and thymus weights in both sexes. Thymic lymphoid depletion was noted in the male high dose treatment group while increased spleen weight was detected in the highest female treatment group. Examination of gastric mucosa revealed inflammation-associated necrosis with increasing dose. Gastric epithelial hyperplasia was noted at all doses but ulceration only at higher doses of both sexes. SC-Tx with 32 mg/kg of CP produced 60% and 80% lethality in males and females, respectively. Histopathology of survivors showed that the primary affected organ was the stomach. Gastric mucosa was rough, pitted and thickened. While these studies indicate that the stomach was the major target organ of CP toxicity, other observations suggest that the immune system may be adversely altered. (Abstract does not necessarily reflect EPA policy.)

1,3-DICHLOROPROPANONE REDUCES CARDIAC OUTPUT. R D Laurie and T K Wessendorp. USEPA, HERL, Cincinnati, OH. Sponsor: L W Condie.

It has been shown that 1,3-dichloro-2-propanone (DCP) (also known as 1,3-dichloroacetonitrile), when given 2 hours before carbon tetrachloride (CCl₄), inhibits the hepatotoxic effects of CCl₄. Pharmacokinetic studies in our laboratory have shown that the amount of CCl₄ exhaled is decreased when preceded by DCP (19 mg/kg). We have shown that pathophysiological analysis of the stomach and intestines revealed that there did not appear to be enough necrosis to account for the decrease in CCl₄ absorption after animals had been dosed with DCP. The current studies were completed using a radiolabeled microsphere technique. The basis of the procedure involved intra-arterial (right carotid) injection of 0.1 to 0.2 ml of microspheres averaging 15 μm in diameter and labeled with cerium 141. Starting five seconds prior to this injection, a one ml blood sample was taken from the left femoral artery over a period of one minute. The resulting data clearly indicate that cardiac output and the consequent blood flow to individual organs is reduced by DCP. (Abstract does not necessarily reflect EPA policy.)

We examined the mutagenic responses of the tk and hgprt loci in mouse lymphoma (MOLY) cells, of the tk locus in human lymphoblasts (HULY), and of the hgprt locus in CHO cells to determine the relative sensitivity of these targets. Initially, twelve compounds that were positive at the MOLY tk locus were examined at the HULY tk locus. In the absence of S9, there was agreement for all the chemicals. In the presence of S9, there was substantial disagreement. These same compounds were then tested at the CHO hgprt locus. Only three compounds were found to be positive. We then examined eleven compounds that were strongly positive at MOLY tk and were mutagenic at the Salmonella histidine locus or chromosomal aberration assay. These compounds were negative at CHO hgprt. Five of these eleven were also tested at MOLY hgprt and found to be negative. Thus, the tk locus appears to be the most sensitive of the loci, the Salmonella histidine locus of intermediate sensitivity and hgprt the least sensitive of the loci. Species differences do not appear to account for the differences observed.


More than 50% of all carcinogens identified by the NTP/NIH carcinogenesis assay increase the incidence of liver tumors in at least one rodent sex/species. The liver may be the only site or one of several sites of tumor induction. It was expected that both the in vitro and in vivo DNA damage/repair (UDS) assays which were performed in male F344 rat hepatocytes would readily detect liver carcinogens. This proved not to be the case. Only 7 of 22 hepatocarcinogens were detected in the in vitro UDS assay. Of 10 hepatocarcinogens tested, only 4 were detected in the in vivo UDS assay; however, 10 of these did induce scheduled DNA synthesis (SOS), a response indicative of either mitogenic or hepatotoxic activity. All of the hepatocarcinogens detected in both UDS assays were active in one or more other genetic toxicity assays; however, both genotoxic and nongenotoxic liver carcinogens induced in vivo SOS in rodent liver. These results indicate that while some liver tumors are induced by genotoxic mechanisms, in other instances chemicals may induce tumors by nongenotoxic mechanisms such as by chronic induction of hepatic proliferation.
Twenty-three chemicals and chemical combinations have been designated by the International Agency for Research on Cancer as causally associated with cancer in humans. The literature was searched for reports of their activity in the Salmonella mutagenicity assay and for evidence of their ability to induce chromosomal damage in the bone marrow of rodents. The purpose of this study was to determine the extent to which human carcinogens exhibit genetic toxicity in vitro and in vivo and to what extent they can be detected using these two widely employed short-term tests. Nineteen of the 23 carcinogens have been found active in one or both short-term tests. Two others, for which short-term test results are not available, are predicted to be active based on their structures. Asbestos and conjugated estrogens are not mutagenic to Salmonella; asbestos is not likely to induce cytogenetic effects in the bone marrow and the potential activity of conjugated estrogens in the bone marrow is difficult to anticipate. These findings indicate that genetic toxicity is characteristic of the majority of human carcinogens. If the 23 IARC human carcinogens are representative of potential human carcinogens in general, then two short-term tests may serve as an effective primary screen for chemicals that present a carcinogenic hazard.

Pyrolizidine alkaloids (PAs) have been shown to interact with cellular DNA in a number of in vitro assay systems. Further characterization of the mechanism by which PAs interact with cellular DNA may be useful in understanding the carcinogenic and antineoplastic capabilities of these compounds. In this study, we investigated the potential DNA-interstrand (DD) and DNA-protein (DP) cross-linking activity of seven PAs in cultured Madin-Darby bovine kidney epithelial cells (MDBK) using gravity-flow alkaline elution. Cells were cultured two hr with the PA (100, 300 and 500 uM) and rat liver S9 containing a NADPH-generating system. The alkaloids which produced either DD or DP cross-links (ranked from highest to lowest) were: seneciphylline (DP > DD), riddelline (DP > DD), retorsine (DP > DD), senecionine (DP > DD), monocrotaline (DD > DP), heliosupine (DP only), retronecine ( neither DD nor DD). None produced DNA single-strand breaks since the tested PAs differ only in the diester substituent of the pyrrole ring, the characteristics of these groups may be an important determinant in the genotoxicity of pyrolizidine alkaloids. (Supported by ES 03591).

Deleterious effects on health following exposure of greenhouse workers to pesticides has become a concern in recent years. Attempts to assess exposure by measuring the concentration of the compound on clothing or by air monitoring has been inconclusive. Monitoring the excretion pattern of workers is more informative with regard to exposure. In this study, the urine of twelve greenhouse workers, exposed to different spraying regimens was monitored for mutagens, eight hours after exposure to pesticide. These workers served as their own controls following assay of urine samples 72 hours after exposure. Urine was evaluated by both Salmonella typhimurium TA 98 and TA 100 for frameshift and base pair mutations respectively. A 9000 x g (S9) preparation from Aroclor-treated rats was used as the activating system. Five workers demonstrated urinary mutagens following exposure to benlate, orthene, ketwelane and sumithrin. Benlate provides an equivocal response in the Ames mutagenicity assay whereas orthene has been shown to be mutagenic in short term tests. An inverse relation was observed between the use of protective clothing and the absence of mutagens. As excretion of mutagens in urine is indicative of exposure to deleterious genotoxic agents, use of a monitoring system as described in this study is useful in assessing the health effects of pesticides.

Lack of correlation between the debrisoquine polymorphism and aflatoxin B1 (AFB1) genotoxicity. C A McQueen, B M Way and G M Williams. American Health Foundation, Valhalla, NY

Hydroxylation of debrisoquine is under polymorphic genetic control. Two phenotypes, poor metabolizers (PM) and extensive metabolizers (EM) have been identified. Studies in humans and rats have suggested that the debrisoquine polymorphism plays a role in susceptibility to AFB1 toxicity. The present study was undertaken to compare AFB1 genotoxicity in hepatocytes isolated from two rat strains of the EM phenotype, Lewis and F344, and from DA rats, of the EM phenotype. DNA repair, determined by autoradiography, was used as a measure of DNA damage. AFB1 induced DNA repair in hepatocytes from all strains, but quantitative and qualitative differences were observed. At 10^-5M, AFB1 was toxic to hepatocytes from Lewis rats (EM) while maximum repair was observed at 10^-5 to 10^-6M. Maximum repair was observed at 10^-5M with hepatocytes from both F344 (EM) and DA rats (PM). Thus, no correlation was found between phenotype and AFB1 genotoxicity. Nevertheless, the results suggest that Lewis rats should be more susceptible to AFB1 toxicity than DA or F344 rats.
Ascorbic acid and its oxidation products have been implicated as both causative agents and as protective agents in disease processes involving oxidative stress. We are developing in vitro model systems to explore the potential roles for ascorbate mediated "OH formation in causing DNA damage. Two hydroxyl free radical ("OH) generating systems were shown to cause single-stranded nicks in supercoiled pBR322 plasmid DNA. In one system (0.03% hydrogen peroxide plus U.V. light), the amount of single-stranded DNA nicking activity correlated with the accumulation of an adduct of DNA, 8-hydroxydeoxyguanosine (8-OHdG), which is caused by "OH attack on guanosine. In another system, ascorbate and iron(III) caused DNA nicking which was inhibited by known hydroxyl radical scavengers. DNA nicking was temperature dependent according to Arrhenius theory. An initial lag in the rate of DNA nicking was not due to a corresponding lag in "OH production as assessed by measurement of salicylate hydroxylation products in this system. The "OH dependent formation of salicylate hydroxylation products was quantitated by HPLC electrochemical detection. Measurement of 8-OHdG levels, and its correlation with loss of ascorbate that occurs in this system, are in progress. Supported in part by NIH Grant No. CA42854.

Formation of 8-hydroxyguanine within calf thymus DNA has been studied after exposure to UV-H2O2 as a hydroxyl free radical generating system. Using high pressure liquid chromatography with electrochemical detection, we measured the amount of 8-hydroxy-2-deoxyguanosine (8-OHdG) in the enzymatically digested DNA. The 8-OHdG content of UV-exposed DNA increased with increasing H2O2 levels up to 0.03%, but then increased only slightly at higher levels. All hydroxyl free radical scavengers studied (mannitol, ethanol, thiourea and salicylate) caused a decrease in the amount of 8-OHdG formed in DNA exposed to the UV-H2O2 system. Thiourea reacted with 8-OHdG as an integral part of DNA but not with the nucleoside free in solution, to cause a decrease in the amount of the hydroxyl free radical modified guanine present. In contrast to thiourea, however, reduced glutathione did not react with 8-OHdG either as an integral part of DNA or free in solution. Ozone, which has been shown to react with DNA to cause damage, did not initiate formation of 8-OHdG within DNA solubilized in a buffer system. Supported by EPA Proj. No. CR-812710-01-0, and NIH Grants CA42854, NS23307, and ES04296.

A series of 10 low tar cigarettes, yielding from 1 to 10 mg tar, were smoked on an automatic cigarette smoking machine that collected both mainstream (MS, inhaled) and sidestream (SS, between puffs) smoke. The collected MS and SS cigarette smoke condensates were evaluated for mutagenicity by the Ames test and compared to MS and SS condensates from a high tar cigarette. Both MS and SS condensates were dissolved in DMSO and tested, at concentrations of 50-200 µg/plate, with the bacterial strain TA 1538, previously shown to be highly responsive to cigarette smoke condensates. The results for two experiments were averaged. Except for two of the cigarettes, the MS mutagenicities of the low tar cigarette smoke condensates were significantly reduced (5-50%), when compared to the high tar (23 mg) cigarette. Just the opposite result was found for the sidestream smoke condensates. Most of the SS condensates produced more revertants/plate than the SS of the control (high tar) cigarette, indicating greater concentrations of mutagenic compounds (10-30%) in their SS smoke. The results will be discussed in terms of MS:SS distribution of known cigarette smoke mutagens.

Genotoxicity studies of tetrandrine. T Ong, J D Stewart, C H Lu, H-X Jin, and W-Z Wong. Division of Respiratory Disease Studies, NIOSH, Morgantown, WV. Sponsor: V Castrenova

Tetrandrine, a compound isolated from Stephanie tetrandra, has been found to inhibit silicosis in laboratory animals. Results from clinical studies have shown that treatment of patients with tetrandrine significantly reduced pathological symptoms associated with silicosis. Since this compound may be used for the treatment of silicosis, the potential genotoxic and carcinogenic hazards of tetrandrine to the treated patients need to be investigated. The genotoxicity of tetrandrine and the effect of tetrandrine on the genotoxicity of chemicals and complex mixtures have been studied in our laboratory with the Ames Salmonella and the SOS/umu assay systems. Results show that tetrandrine was weakly mutagenic to Salmonella typhimurium TA98 and did not induce SOS response. However, tetrandrine was found to be a potent mutagenesis enhancer. It highly increased (over 100%) the mutagenic activity of benzo[a]pyrene, trimethoprim, 2-aminoanthracene, diesel emission, airborne particles, fried beef, and cigarette smoke. The mechanism for the mutagenesis enhancement is not known. Data from this study seem to indicate that tetrandrine may enhance error prone DNA repair. Further studies with in vivo assay systems need to be performed to evaluate the potential health hazards of tetrandrine.

Quantitation of unscheduled DNA synthesis (UDS) in hepatocytes from rats treated with the peroxisome proliferator Wy-14,643 (Wy). RC Gatlery, T Smith-Oliver, BE Butterworth, and JA Popp, CIIT, RTP, NC. Sponsor: E Gross-Bermudez

The peroxisome proliferator hepatocarcinogens lack genotoxic activity in in vitro assays. The effect of Wy-14,643 treatment with the potent carcinogen Wy on DNA repair was quantitated in F344 rat hepatocytes. Peroxisomal palmitoyl CoA oxidase in isolated cells was elevated 9-fold by day 5 of Wy gavage (50 mg/kg) and 13-fold by day 27 of Wy feeding (0.1%). Cultures of these cells were incubated with [3H]-thymidine and repaired UDS was measured in autoradiographs as net nuclear grains (NG). Neither gavage treatment for 1 to 5 days (NG = -3.8 Wy 5 days vs. -3.8 ctrl) nor feeding for 27 days (NG = -4.6 Wy vs. -4.5 ctrl) induced DNA repair. Wy treatment partly blocked the in vitro repair response to 2-AAF (NG reduced 30%). Cells from Wy gavaged rats (0-5 day) were treated with 0.8 mm 3x) to evaluate the ability of UDS to detect repair which might be induced by peroxisomal metabolism. H2O2 did not induce repair or block 2-AAF-induced repair. Higher doses of H2O2 (1.9-3.6 mm 3x) to cells from a naive rat resulted in ~20% of cells in repair. In summary, the peroxisome proliferator hepatocarcinogen Wy lacked genotoxic activity detectable as UDS in hepatocytes despite peroxisome proliferation due to in vivo treatment.
416 SYNTHESSES OF N-OXIDIZED DERIVATIVES OF 4,4'-METHYLENEDIISOBUTYRIC(2-CHLOROANILINE) (MBOCA) AND THEIR DIRECT MUTAGENICITIES TOWARD S. TYPHIMURIUM TA98 AND TA100. B I Kusilika, S H Chen and W E Brattleon. Dept. of Pharmacology and Toxicology, Michigan State Univ., East Lansing, MI. (Sponsor: W D Atchison)

MBOCA, a commercially important crosslinking agent and an environmental contaminant is an aryline, a class of compounds thought to be activated to proximate carcinogens by metabolic oxidation to the corresponding hydroxyarylines. Consistent with this hypothesis, MBOCA requires rat liver S9 activation to become a mutagen in the S. typhimurium mutagenicity assay. This study tested the hypothesis that MBOCA is activated through N-oxidation. We synthesized N-oxidized derivatives of MBOCA by oxidation with m-chloroperoxybenzoic acid. The N-hydroxy and nitroso metabolites formed by liver microsomal enzyme systems were identical to the chemically synthesized derivatives. The N-hydroxy metabolite showed strong frame shift mutagenicity towards S. typhimurium TA98 as well as strong base pair mutagenicity towards TA100. The nitroso derivative showed a slight positive effect on TA100. The dinitroso compound was not active. A third major metabolite of microsomal oxidations, ortho-hydroxy MBOCA, was also not mutagenic at any of the concentrations tested. These studies show that the N-hydroxy derivative of MBOCA is highly mutagenic and may account for the majority of the mutagenicity of MBOCA seen in the S9-activated bacterial test system. (Supported by the Mich. Dept. of Public Health.)

417 SISTER CHROMATID EXCHANGE IN EPILEPTIC PATIENTS ON ANTICONVULSANT THERAPY. V B Winge, S Schaumann*, V E Barry. University of Minnesota, Minneapolis, MN. * Veterans Administration Medical Center, Neurology Service, Minneapolis, MN

Sister chromatid exchange (SCE) techniques were used to test the mutagenic potential of anticonvulsant drugs in epileptic patients. The following drugs were studied: valproate (n=13), phenytoin (n=17), phenytoin and phenobarbital in combination (n=9). Each patient was matched with a control (n=30) by sex, age and smoking habits. All individuals with exposure to known environmental mutagens or taking other drugs were excluded. No statistically significant differences in SCE level were found between the patient and control groups, indicating a lack of mutagenic potential of the tested anticonvulsants chronically administered (six months or more) within the therapeutic dose range. Supported by the Veterans Administration.

418 A FILTER BINDING ASSAY TO DETECT CHROMIUM-INDUCED DNA-PROTEIN CROSSLINKS IN ISOLATED NUCLEI. T P Croog and M Costa. Institute of Environmental Medicine, New York University Medical Center, Tuxedo, NY.

Exposure to chromium results in DNA-protein crosslinks (DPC) that are persistent and stable. Since this type of lesion may play an important role in chromium carcinogenicity, the mechanism of chromium-induced DPC was investigated using a filter binding assay. Prior to isolation of CHO cell nuclei, DNA and proteins were labelled using 3-H thymidine and 35-S methionine, respectively. Nuclei were suspended in 10 mM HEPES buffer (pH 6.8), and exposed to either UV irradiation, CrCl3, or K2CrO4. Following exposure, nuclei were incubated at 37°C for 20 min in a high salt solution; aliquots were loaded onto nitrocellulose filters and washed with a low salt solution. DNA (3-H) retained on each filter was normalized based on the protein retained (35-S). Exposure of isolated nuclei to UV irradiation resulted in a dose dependent increase in DPC as indicated by the increasing 3-H/35-S ratio. CrCl3 induced DPC in a biphase manner over the concentration range tested (0-200 μM), whereas K2CrO4 only induced DPC at the higher conc. (>100μM). Incubation of K2CrO4 with sodium bisulfite prior to nuclei exposure resulted in DPC similar to that observed with CrCl3 alone. (Supported by Grant No. CA-43070 from the NCI)

419 INDUCTION OF ANEUPLOIDY BY Ni(II) AND Cr(VI) IN A HUMAN/MOUSE HYBRID CELL SYSTEM. K Conway, L S Athwal and M Costa. Inst. of Environmental Medicine, NYU Medical Ctr., Tuxedo, NY and Dept. of Microbiology, NJ Medical School, Newark, NJ.

Increasing evidence indicates that aneuploidy may play an important role in the multistage process of carcinogenesis. In the present study, the ability of the carcinogenic metals, Ni(II) and Cr(VI), to induce aneuploidy was assessed in a cell culture system using a human/mouse somatic cell hybrid containing a single copy of human chromosome 2. The human chromosome present in the mouse cells served as a cytogenetic marker to quantitate abnormal chromosome segregation and could be easily identified by the G-11 staining technique. The frequency of cells containing 0 or 2 human chromosomes in the progeny of metal-treated hybrid cells provided a direct measure of aneuploidy. Preliminary results indicate that 8-cristalline NiS, NiCl2, and K2CrO4 induced aneuploidy in this system in a dose-dependent manner and the highest frequency of aneuploidy observed for all three metal compounds was in the range of 2 to 3-fold above background. Each of these compounds also produced an increase in the number of polyplid cells. MgCl2, a non-carcinogenic metal compound, did not induce aneuploidy or polyploidy in this system. Similar results were obtained when aneuploidy was assayed with primary Chinese hamster embryo cells. (Supported by Grant No. R813140 from the U.S. EPA)
EFFECTS OF AMITRIPTYLINE ON CALCIUM UPTAKE AND HIGH ENERGY PHOSPHATES IN PRIMARY MYOCARDIAL CELL CULTURES. D. Acosta, Y. Park, J. Bradlaw, and AA Welder. University of Texas College of Pharmacy, Austin, TX and Food and Drug Administration, Washington, D.C.

Tricyclic antidepressants (TCAs) are used in the treatment of endogenous mental depression. There are an estimated 3,000 to 10,000 cases of overdosing or poisoning each year with TCAs. Of the TCAs, amitriptyline is the most cardiotoxic, with evidence of arrhythmias and contractile disturbances. In a previous study (J. Toxicol. Environ. Health. 2:137, 1984), we demonstrated that TCAs were directly cytoxic and activated uncoupled cultured myocardial cells. The purpose of this investigation was to determine the direct effects of amitriptyline on $^{42}$Ca$^{++}$ uptake and high energy phosphate stores in primary cultures of rat myocardial cells. Cultures obtained from hearts of 3-5 day old Sprague-Dawley rats were exposed to various concentrations of amitriptyline (1 x $10^{-4}$ and 1 x $10^{-5}$ M) for 4 to 24 hr. Glucose utilization, $^{42}$Ca$^{++}$ uptake, adenosine triphosphate (ATP) and creatine phosphate (CP) levels were evaluated after amitriptyline treatment. Glucose utilization was not affected by either concentration of amitriptyline after 24 hr of treatment. $^{42}$Ca$^{++}$ uptake was depressed after the 4 hr exposure to the highest concentration of amitriptyline tested. ATP levels were also depressed after 4 hr and depleted after 8 and 24 hr exposure to 1 x $10^{-4}$ M amitriptyline. In contrast, CP levels were not affected by the drug. These data suggest that amitriptyline activity of myocardial cells after exposure to amitriptyline may be associated with depletion of high energy phosphates andionic disturbances. (Supported by FDA contract #223-86-2109)

THE PSORALEN RECEPTOR AS A MEDIATOR OF CHEMICAL PHOTOTOXICITY J. D. Laskin, E J Yurkow and M A Gallo, UMDNJ-Robert W. Johnson Medical School, Piscataway, NJ.

Psoralens, or furocoumarins, are potent skin photosensitizing agents. Epidermal cells are known to contain specific high affinity binding sites for the psoralens and we have hypothesized that these binding sites mediate the biological effects of these compounds in the skin. We have characterized the psoralen binding sites from PAM 212 mouse epidermal cells and HeLa cells. We have found that they can be alkylated by $^3$H-8-methoxypsoralen following ultraviolet light (UVA) exposure. Covalent binding of the label was inhibited by an excess of unlabeled psoralen indicating that covalent psoralen binding was saturable. Fractionation studies revealed that the binding sites were present in membrane and cytoplasmic fractions of the cells and were sensitive to protease but not nuclease treatment. Using SDS-polyacrylamide gel electrophoresis, the labeled psoralen binding sites were found to have a molecular mass of 22,000 daltons. The identification of a specific cellular protein which binds psoralen supports our model that the biological effects of these compounds are receptor mediated. Supported by NIEHS ES 03647.

STUDIES ON THE MECHANISM OF PSORALEN INDUCED PHOTOTOXICITY USING HUMAN EPIDERMAL A431 CELLS. F. Meremelstein, M A Gallo, and J D Laskin, UMDNJ-Robert W. Johnson Medical School, Piscataway, NJ.

The psoralenes are furocoumarins that, when combined with ultraviolet light (UVA), cause skin phototoxicity. We have previously shown that psoralens and UVA light are potent inhibitors of epidermal growth factor (EGF) binding in several different cell types. This inhibition appears to be modulated by a high affinity psoralen receptor. The human epidermal cell line, A431, expresses over $10^6$ EGF receptors/cell. The EGF receptor in these cells has been characterized as a 160-170 kd transmembrane protein with an intrinsic tyrosine kinase (TK) cytoplasmic domain. Binding of EGF to A431 cells stimulates TK activity. We found that the psoralen analog 4,5'O-trimethylpsoralen in combination with UVA inhibits EGF binding to A431 cells by 20-25%. Using an antiphosphotyrosine monoclonal antibody, we purified a population of tyrosine phosphorylated EGF receptors from control and psoralen/UVA treated cells. Treated cells were found to have decreased levels of EGF stimulated tyrosine phosphorylated EGF receptors. Our data provide evidence that psoralen modulate EGF receptor binding and function. Supported by NIEHS ES 03647.

ALLYLAMINE (AAM)-INDUCED ALTERATIONS IN THE PHOSPHOINOSITIDE/INOSITOL PHOSPHATE PROFILE OF CULTURED AORTIC SMOOTH MUSCLE CELLS. L R Cox, S K Murphy, and K Ramas, Philadelphia College of Pharmacy & Science, Phila, PA and *Texas Tech University Health Sciences Center, Lubbock, TX.

Previous studies in our laboratory have shown that smooth muscle cells (SMC) cultured from AAM-treated rats exhibit morphologic features and synthetic capabilities characteristic of a proliferative phenotype. As an increased turn-over of membrane phosphoinositides has been implicated in cell proliferation, the present studies were undertaken to examine the phosphoinositide/inositol phosphate profile of SMC cultured from control and AAM-treated animals. Adult male Sprague-Dawley rats were dosed daily with AAM-HCl (70 mg/kg) or tap water for 21 days. The levels of phosphatidylinositol 4-phosphate, phosphatidylinositol 4,5-bisphosphate and phosphatic acid in SMC from AAM-treated rats were lower than those of control SMC by 31, 35 and 22%, respectively (n=10). Inositol phosphate levels were similar in cells cultured from control and treated animals (n=7-9). Our results show that the cellular toxicity of AAM is associated with changes in the phosphoinositide/inositol phosphate profile. AAM-induced alterations are affected by manipulation of cyclic AMP levels. The phenotypic expression of vascular SMC upon AAM exposure may be modulated by changes in phosphatidylinositol turnover.
Is Liver Endonuclease Activity Stimulated by Elevated Cytosolic Ca++ Induced by Halogenated Hydrocarbons? II. In Vitro Studies. R M Long, D R Schoenberg, and L Moore, Dept. of Pharmacology, USUHS, Bethesda, MD.

We have previously reported (Pharmacologist 29:122, 1987) that rising cytосolic Ca++ concentrations do not activate endonuclease in liver exposed in vitro to carbon tetrachloride (CCl4) and 1,2-dichloroethylene (DCE). We have now examined endonuclease activation following in vitro exposure of liver cells to halogenated hydrocarbons. CCI4 (1.5 mM) and DCE (6 mM) were added to primary cultures of rat hepatocytes. DNA was prepared and separated on agarose gels. No generalized DNA fragmentation was observed until very late times (2 hr.) when plasma membrane integrity was disrupted as measured by enzyme release. Endonuclease activity was further examined by specifically monitoring hypersensitive sites in serum albumin gene. This gene is very actively transcribed in liver and thus should be extremely sensitive to nucleolytic attack. DNA was digested with restriction enzymes Eco RI or Hind III, electrophoresed on agarose gels, and blotted onto nitrocellulose. Almond sequences were detected by hybridization to a 3'P labelled 1400 bp genomic clone of rat albumin. No cleavage at hypersensitive sites was observed at early times (15 to 45 min.) when Ca++ homeostasis was first disrupted. Thus as had been found in vivo, there is no evidence to suggest that activation of endonucleases by Ca++ is responsible for mediating the hepatotoxicity accompanying halocarbon exposure. (Supported by ES03437 and GM30270 from NIH.)

FORMATION OF CARBON DIOXIDE FREE RADICAL BY LIVER MITOCHONDRIA FROM KREBS CYCLE INTERMEDIATES WHEN HYDRAZINE IS PRESENT. P K Wong, J L Foyer, C M DuBoise, and R A Floyd Oklahoma Medical Research Foundation, Oklahoma City, OK.

Isolated rat liver mitochondria incubated with malate-glutamate in the presence of hydrazine and spin-traps yielded free radical spin adducts. Studies with mitochondrial inhibitors and the use of succinate as substrate showed that free radical formation was dependent upon mitochondrial electron transport in the NADH dehydrogenase region. The spin adducts showed that the free radicals trapped were carbon centered. The coupling constants suggested the trapped radical was CO2. This was proven to be the case by synthesizing the CO2 trapped radical in a chemical system and isolating the authentic compound and the one produced by mitochondria using EPLC and conducting EPR measurements on the isolated fractions. Studies with mitochondria substrates demonstrated pyruvate produced much more of the CO2 radical, than malate/glutamate. Studies with 1-13C-pyruvate demonstrated that the hydrazine dependent CO2 production arose from the carboxyl carbon of pyruvate.

FORMATION OF FREE RADICAL PRODUCTS FROM HYDRAZINE BY RED BLOOD CELLS AND OXY-HEMOGLOBIN. J L Foyer, C M DuBoise, and R A Floyd, Oklahoma Medical Research Foundation, Oklahoma City, OK.

Human red blood cells treated with hydrazine (3-phenylazaline hydrazine) produced free radicals which could be spin-trapped, and observed using electron paramagnetic resonance (EPR) techniques. The free radical trapping agent DMPO (3,3-dimethyl-1-pyrroline-N-oxide) trapped a nitrogen-centered free radical whereas the free radical trapping agent PBN (α-phenyl-tert-N-butylnitronitrone) trapped a carbon-centered radical as well as the hydrogen free radical. Free radical formation was dependent upon the presence of oxygen, indicating the involvement of oxy-hemoglobin. One of the spin-trapped free radicals which could be identified was the 3-phenylazaline radical. This radical could be formed chemically by the reaction of potassium ferricyanide with hydrazine and trapped with PBN. Bovine oxy-hemoglobin plus hydrazine yielded an EPR spectrum similar to that for red blood cells indicating that the free radicals were similar. Carbon monoxide inhibited free radical formation in both the red blood cell and bovine hemoglobin systems. This work was supported in part by NIH Grant No. 2-R01-ES03067-04.

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Isolated rat liver mitochondria incubated with malate-glutamate in the presence of hydrazine and spin-traps yielded free radical spin adducts. Studies with mitochondrial inhibitors and the use of succinate as substrate showed that free radical formation was dependent upon mitochondrial electron transport in the NADH dehydrogenase region. The spin adducts showed that the free radicals trapped were carbon centered. The coupling constants suggested the trapped radical was CO2. This was proven to be the case by synthesizing the CO2 trapped radical in a chemical system and isolating the authentic compound and the one produced by mitochondria using EPLC and conducting EPR measurements on the isolated fractions. Studies with mitochondria substrates demonstrated pyruvate produced much more of the CO2 radical, than malate/glutamate. Studies with 1-13C-pyruvate demonstrated that the hydrazine dependent CO2 production arose from the carboxyl carbon of pyruvate.

6-METHYL-1,3,8-TRICHLORODIBENZOFURAN (MCDF) AND RELATED ANALOGS AS 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ANTAGONISTS: STRUCTURE-ACTIVITY RELATIONSHIPS. B Astroff and S Safe, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

1,3,6,8-Substituted dibenzofurans, including MCDF, antagonize a broad spectrum of 2,3,7,8-TCDD-mediated biologic and toxic responses in mice and rats; however, MCFP is the only compound which specifically inhibits the induction of rat hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and the associated cytochrome P-450 isozymes. The requirement of the 1,3,6-substituted Cl and the 6-CH3 substituents for this inhibitory activity has been investigated using a series of analogs in which a single substituent (Cl or CH3) has been replaced with a H. The most active antagonist of AHH induction was MCDF; however, replacement of the 1- or 8-Cl group with H did not eliminate the antagonist activity. In contrast, replacement of the 3-Cl or 6-CH3 group with H or the 6-CH3 group with Cl gave congeners with no inhibitory activity. These results correlated in part with the Ah receptor binding activities of these homologs and suggest that the antagonists interact stereospecifically with the receptor. (Supported by the National Institutes of Health.)

Wistar Rats (4 groups of 70 males and 70 females) were given formaldehyde in their drinking water at doses of 0, 5, 25 or 125 mg/kg b.w./day for a period of 2 years. Significant effects were only seen in the high-dose group and comprised markedly decreased liquid intake, lower body weights and food intake, decreases in urinary PH, plasma protein and cholesterol level, and increased potassium concentration in blood plasma. Pathological changes in the stomach (raised limiting ridge, ulcers and epithelial hyperplasia in the forestomach, and gastritis and glandular hyperplasia in the glandular stomach) and kidneys (necrosis of the papilla) were also observed. Some of these changes might partially or entirely be due to the strongly reduced liquid intake.

No compound-related tumors were found. The no adverse effect level was judged to be 25 mg/kg b.w./day. Chronic oral administration of formaldehyde to rats at the NTD caused severe damage to the gastric mucosa but did not result in gastric tumours or tumours at other sites.

EARLY CELL PROLIFERATIVE AND CYTOTOXIC EFFECTS OF ORAL PHENACETIN ON RAT NASAL MUCOSA. N S Bogdanoff, T J Mazaika, and W J Pasano. E I du Pont de Nemours & Co, Inc, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

Phenacetin binds covalently to rat nasal olfactory mucosa (OLF) and causes nasal tumors in rats when administered in the diet for 18 months. The purpose of this study was to examine changes in cell proliferation during 1 or 2 weeks of phenacetin dosing. Rats were gavaged with phenacetin at 100, 625, or 1250 mg/kg for 7 (group A) or 14 (group B) days and were implanted with osmotic minipumps containing [3H]-thymidine during days 1-7 (group A) or 8-14 (group B). Noses were then processed for histosautoradiography. No lesions were seen at 100 mg/kg in groups A or B, but there was a slight increase in cell proliferation in OLF. In both groups, 625 or 1250 mg/kg caused extensive necrosis of Bowman's glands, a loss of PAS positive material in the subepithelium of OLF, and a dose and time related increase in cell proliferation in OLF.

In Group B at 1250 mg/kg, the labeling index was increased approximately 10 fold. In the neuronal cell layer, this was accompanied by disorganization and the appearance of clusters of PAS positive cells. Phenacetin had no effect on cell proliferation in respiratory mucosa. These results demonstrate that phenacetin causes early changes in cell proliferation in the nasal olfactory mucosa consistent with covalent binding and carcinoma formation.

NASAL TUMOURS AND DAMAGE TO THE OLFATORY EPITHELIUM IN FORMALDEHYDE-EXPOSED RATS WITH A SEVERELY INJURED NASAL MUCOSA. V J Peron, R A Woutersen, A van Garderen-Hoetmer, J B Bruijinjtes and A Zwart. TNO-CIVO Toxicology and Nutrition Institute, Zeist, The Netherlands.

To study the significance of damage to the nasal mucosa of rats for the induction of nasal tumours by formaldehyde (FA), a study was conducted in male rats with an injured nose (bilateral intranasal electro-coagulation) or with an intact nose exposed to 0, 0.1, 1.0 or 10 ppm FA for 6 h/day, 5 days/week during 28 months or during 3 months followed by a non-exposure period of 25 months. The damaged nose was more susceptible to 10 ppm FA than the undamaged nose as appeared from increases in incidence of rhinitis, hyper- and metaplasia of the respiratory epithelium, and degeneration and hyper- and metaplasia of the olfactory epithelium in rats with a damaged nose. In addition, exposure to 1.0 ppm FA for 28 months resulted in a much higher incidence of nasal squamous cell carcinomas in rats with a damaged nose (17/60) than in rats with an intact nose (1/29). No significant differences in tumour incidence between rats with a damaged and undamaged nose were found at the 0.1 or 1.0 ppm level or after exposure to 10 ppm for only 3 months. In conclusion: severe damage to the nasal mucosa may be an important factor for the induction of nasal tumours by FA.

TISSUE HYDROXYLATION OF METHYL-N-AMYL-NITROSAMINE (MMAN) IN NEONATAL TO ADULT RATS AND HAMSTERS. S S Mirvish, C Ji, and S Rosinsky. Eppley Inst Res Cancer, Omaha, NE.

In studies to help explain carcinogen organotropy, fresh adult rat tissues converted the esophageal carcinogen MMAN into 2- to 5-hydroxy-MMAN and some 3- and 4-hydroxy-MMAN. In assays, 20-250 mg tissue slices were incubated (3 h, 37°C) with 23 μM MMAN/5 μl Eagle's medium. CHLCl₂ extracts were analyzed by g.l.c.-thermalf energy analysis. We measured sum of hydroxy- and oxo-MMANs, expressed as % yield/100 mg tissue, that were produced by MRC-Wistar rat and Syrian hamster tissues from animals 1 day prenatal to age 6-8 weeks. In rats, metabolism peaked at 9.7% in 6-day esophagus, 1.4% in 3-day forestomach, and 14.9% in 9-day liver (adult levels 3.3, 0.02, 4.2%, respectively). In hamsters, yield peaked at 10.0% in neonatal esophagus, 4.4% in 3-day forestomach, 5.1% in 6-day trachea, and 3.7% in 1-day-prenatal lung (adult levels 0.5, 0.3, 1.9, 1.4%). Metabolite ratios varied with conditions. Incubation for 3 h with varied [MMAN] showed apparent Kₚ of 150 μM for esophagus and 300 μM for liver of adult rats. Differences between adult and 6-day esophagus, and between adult and 9-day liver of rats disappeared when incubation was with 300 μM (esophagus) or 100 μM (liver) MMAN. Hence metabolism in young tissues was increased only with 23 μM MMAN. Support: NIH grants R01-CA-35628 and CA-36727.
RESIDENCE TIME AND TUMOR-INITIATING ACTIVITY OF BENZO(A)PYRENE AND A COMPLEX MIXTURE. 
D D Mahlum. Battelle, Pacific Northwest Laboratory, Richland, WA

It is of both basic and pragmatic interest to know how long a carcinogen needs to be in contact with the skin in order to be effective in initiating tumors. We studied the effect of residence time on initiating activity of BaP and a complex organic mixture on mouse skin. BaP (25 μg) or a coal-derived heavy distillate (HD; 25 mg) was applied to the skin of female C57I mice. At times varying from 10 minutes to 24 hours, the skin was washed with soap and water. Control mice were initiated but not washed. After two weeks all animals were promoted twice weekly with 5 μg of TPA for 24 weeks. Tumor incidence and tumors/mouse were monitored biweekly. BaP controls had a 93% incidence with a mean of 5.3 tumors/mouse; HD controls had an 83% incidence with a mean tumor yield of 2.1 per mouse. Washing BaP mice 10 min after administration reduced the incidence and the tumor yield; washing after 30 or 60 minutes reduced the tumor yield, but not the incidence. Washing HD mice up to 4 hr after application reduced both the incidence and the tumor yield significantly. These data suggest that initiation occurs relatively quickly when BaP is used in μg amounts. However, washing is effective for longer periods when a mixture of carcinogens is applied in mg amounts. U.S. Dept. of Energy Contract DE-AC06-76RL0 1830.

EFFECT OF HEATING OR THE PRESENCE OF VEGETABLES AND FRUIT IN HUMAN DIETS ON THE SPONTANEOUS TUMOR RATES IN RATS. C M Alink, H A Kuiper, R B Beens, and J H Roosjen. Department of Toxicology, Agricultural University, Wageningen, Institute for Quality Control of Agricultural Products (RIKILT), Wageningen, "TNO-CIVO Toxicology and Nutrition Institute, Zeist, The Netherlands. Sponsor: V J Renn.

To study the influence of diet factors like total composition, thermal processing and vegetables and fruit on tumor rate a chronic experiment was designed. Five diet groups were tested in 100 Wistar rats each. Diet A was a semisynthetic animal diet, diet B an animal diet to which vegetables and fruit were added, diet C a human diet with raw products, diet D a human diet with fried and baked products and diet E a complete human meal consisting of heated products, vegetables and fruit prepared according to mean consumption figures in the Netherlands. The animal diets consisted of 25 E % (Energetic) protein, 14.5 E % fat, 60.5 E % carbohydrate and 3.5 % (w/w) fibre. For the human diets these figures were 13, 40, 47 and 5 % respectively. Animals were treated for 142 wk and fed ad libitum. In male rats but not in female rats human diets gave a higher tumor incidence than animal diets (p < 0.01). Frying and baking, and vegetables and fruit induced minor differences in tumor rate, although these differences were not statistically significant (p > 0.1) further research is needed to get more conclusive results.

FAT, VITAMIN A DEFICIENCY AND CALORIC INTAKE COLON CARCINOGENESIS. PM Newberne, D Bueche, and TF Schroeder. Mallory Institute of Pathology. Boston, MA

Epidemiological studies have correlated high fat intake with increased risk of colon cancer. Experimental studies have indicated that high fat intake of unsaturated fat after carcinogen exposure can significantly increase colon tumor incidence suggesting a promotional effect. In this laboratory no enhancing effect of high fat diets on colon carcinogenesis was demonstrated, after using both direct and activated carcinogens and different types of fat at different stages of the carcinogenic process. However, in rats on a high fat diet (24% corn oil) and made vitamin A deficient, tumor incidence was significantly increased from 52% to 75%, p < 0.01. High levels of vitamin A were not protective. This suggests a more complex interaction; in some studies vitamin A levels may not have been controlled. In a separate set of experiments, the effect of postnatal alteration of caloric intake on colon cancer was examined by reducing litter size at birth from 8 to 4. This allowed greater access to milk (calories). Rats at weaning ate ad libitum and then were caged with DMM. Increased access to food (calories) only from birth to weaning significantly increased colon tumor incidence (85% vs 48%), even when rats were pair fed to control rats. This indicates the complexity of dietary factors on colon carcinogenesis.


Lipid lowering agents in the rat produce liver enlargement due to proliferate of peroxisomes and smooth endoplasmic reticulum; long term administration of these compounds to rodents produces liver cell tumors. Clofibrate is a hypolipidaemic agent which has been used in man for more than twenty years; it has been shown to proliferate peroxisomes and produce liver cell tumours in the rat; in the non-human primate, the marmoset Callithrix jacchus the effect of administration of clofibrate at levels of 0.7, 1.7, 2.18 and 265 mg/kg for 343 weeks was studied. There was no evidence of toxicity from daily observation, body weight records, veterinary examination, ophthalmology or pathological examination. No carcinogenic effect was seen in the liver or any other organ.
Dissolved organic compounds (DOC), such as humic acid (HA), can affect the speciation of trace metals in natural waters. The physico-chemical form of metals may, in turn, affect their bioavailability and toxicity. Although adequate information is available concerning the influence on HA on the speciation of several trace metals (e.g., Cd, Cu), there is a paucity of information available on chromium. Dialysis and diffusion cell studies were used to determine the influence of HA (0, 0.5, 5 and 50 mg/L) on the bioavailability of selected chromium species (Cr III, Cr VI and chrome lignosulfonate). Daphnia pulex was used as an animal model to resolve HA's influence on toxicity. The dialysis studies revealed that increasing concentrations of HA decreased the bioavailability of Cr III while having no significant effect on Cr VI and chrome lignosulfonate. These results were reflected by a significant decrease in toxicity of Cr III in the presence of the 5 and 50 mg/L HA concentrations and the absence of any effect of HA on the toxicity of Cr VI. However, the 50 mg/L concentration of HA did significantly decrease the toxicity of chrome lignosulfonate. These results indicate that the influence of HA is dependent on HA concentration as well as metal speciation.

Sequestration of environmental cadmium in Gill and liver cytotoxic proteins of the freshwater teleost, Lepomis macrochirus. C F Watson, K N Baer, and W H Benson, College of Pharmacy and Health Sciences, Northeast Louisiana University, Monroe, LA.

Industrial processes, agricultural practices, and fossil fuel combustions provide a multiplicity of point and diffuse sources of cadmium contamination. Differential sensitivity to environmental cadmium has been observed in freshwater teleost species. There is, however, a paucity of information concerning the biochemical basis for such differential susceptibilities in aquatic species. It has been suggested that metallothionein (MT) and other metal-binding proteins function in the metabolic detoxification and storage of cadmium. It is further postulated that "spill-over" from the MT-like proteins to low-molecular weight (LMW) proteins may correlate with the onset of pathological damage. Recent investigations in our laboratory have indicated that Cd exposure of bluegill sunfish (Lepomis macrochirus) to 1, 10, and 100 μg Cd/L produced a characteristic profile of cadmium distribution in the cytosolic fraction of the gill and the liver. Of the three classes of cadmium-binding proteins identified, the MT-like proteins consistently sequestered the highest percentage of metal. Cadmium-, copper-, and zinc-cytosolic protein interactions reported may further delineate the cellular aspects of cadmium toxicity in freshwater teleosts.

The disposition of I4C-dicofol in the ring dove: the question of DDE and eggshell thinning. B A Narloch, S E Schwarzbach and J R Shull, Departments of Environmental Toxicology and of Avian Science, University of California, Davis, CA.

The disposition of I4C-dicofol in the ring dove was determined following a single oral dose of pure compound. Ring doves maintained on commercial feed and a 16-hour light/day reproductive cycle were exposed to a single dose of 50.0 mg (4.5 uCi)/kg b.w. I4C-dicofol by gastric intubation. Fecal samples were collected at 24-hour intervals and analyzed for parent compound, degradation products and metabolites. Eggs laid post-exposure were collected and shell thickness was measured. Groups of birds were sacrificed at 24, 48 and 72 hours, necropsied and organs and tissues were analyzed for radioactivity. By 72 hours post-exposure, 35.7% of the dose was eliminated in feces. The organs/tissues with the highest amounts of radioactivity present after 72 hours were liver (0.8% of dose), gastrointestinal tract (4.6%), fat pad (5.2%), and carcass (55.4%). The oviduct retained 0.2% of the dose after 72 hours, with the highest amounts of radioactivity present in the shell gland and the developing follicle. Metabolic profiles were obtained for liver, fat, oviduct, eggs and feces, with emphasis on the presence or absence of DDE.

Effect of reference hepatotoxins upon hepatic integrity and P-450 enzymes in the winter flounder (Pseudopleuronectes americanus). K M Kleinevo, B F Droy, D R Buhler, and D E Williams, Louisiana State University, Baton Rouge, LA, West Virginia University, Morgantown, WV, Oregon State University, Corvallis, OR. Sponsor: J J Lech.

Induction of hepatic P-450 in fish exposed to environmental xenobiotics has received attention as a biological monitoring tool for the assessment of aquatic environments impacted by pollutants. Often hepatotoxins are present in conjunction with inducers in polluted waters and therefore reference information is required on this possible interaction. The inducer beta-naphthoflavone (BNF) (100 mg/kg IP) and reference hepatotoxins [carbon tetrachloride (CCL₄), allyl formate (AF) (758 mg/kg via gavage)] were administered alone and in combination as single administrations to 150-450 g flounder. The BNF, CCL₄ and AF were administered respectively 2, 1 and 2 days prior to sacrifice. Polyclonal antibodies against BNF inducible trout LM and constitutive LM areozymes were utilized for immunological staining of western blots. CCL₄ exposure resulted in western blots in LM, collagen and nearly complete loss of LM when compared to controls. BNF resulted in an induction of LM and appeared to be partially protective against CCL₄ mediated reductions in LM and LM. No significant differences were noted for AF or BNF. Serum transaminase levels and histology reflected the isozyme data. These data suggest that at the dosage utilized, inducer-hepatotoxin interactions may occur for select agents in the flounder. Support to KMW, by NIEHS Center for Membrane Toxicity Studies and, Markey Foundation - Mount Desert Island Biological Laboratory; Medical College of Wisconsin BSRG.
One of the non-chemical methods of protecting stored-product commodities is disinfestation by irradiation. An understanding of the environmental factors like temperature and humidity with reference to insect mortality due to irradiation is essential for effective pest control. The mechanism of radiation damage to adult insects is poorly understood. As such, studies were undertaken by exposing grain weevil to different dose rates (0.05, 0.15 and 0.30 kGy) of gamma radiation, maintaining at different temperatures, humidity and analyzing the epicuticular hydrocarbons by gas chromatography-mass spectrometry (GC/MS).

The results indicate that gamma radiation induced greater water loss leading to desiccation and early death of weevils. Low humidity environment (17% R.H) accelerates lethal effects. GC/MS analysis showed significant variations in the n-Alkanes (C23 to C35) of epicuticular hydrocarbon mixture between the irradiated and control weevils. Quantitative changes among the treated groups were observed (Department of Energy Grant No. DE-FG03.86CH10288.A001).

The lipophilic waxy surfaces of pine needles from several urban, non-urban and industrialized areas contained complex mixtures of PCDD and PCDF congeners and the highest concentrations of these toxins were detected in extracts from industrialized areas. Gas chromatographic-mass spectrometric (GC-MS) analysis showed that PCDD/PCDF levels in dry pine needles were as high as 650 pg/g (parts per trillion). Utilizing the induction of aryl hydrocarbon hydroxylase (AHH) in rat hepatoma H-4-II-E cells as a bioassay, the "2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxic equivalents" of the pine needle extracts were determined and showed an excellent correlation with the GC-MS data. The results clearly illustrate the utility of the bioassay for assessing toxic equivalents and also suggest that plant surfaces may be useful monitoring systems for lipophilic atmospheric pollutant (Supported by the Environmental Protection Agency.)
We describe our procedures for assessing risks from chemical contaminants in fish and issuing health advisories (consumption guidelines) for public health protection. Requests for evaluations come from both within and without the Department as a result of accidental contamination situations, routine monitoring programs, and mandated CDHS studies. Review considerations include chemical concentrations in fish, consumption patterns, toxicological data, safety factors, acceptable daily intake, sources of exposure, and sensitive subpopulations. The chemicals involved have included methylmercury, selenium, PCBs, and DDT. Methylmercury affects the nervous system and the fetus. Selenium can cause gastrointestinal disturbances, loss of hair and nails, nervous system effects, skin lesions, and, based on animal data, may cause adverse reproductive and developmental effects. Certain PCBs and DDT are animal carcinogens. Maternal ingestion of PCB contaminated fish is reported to be associated with low birth weight and small head circumferences in newborn infants. Health advisories published in the USA and elsewhere recommend that pregnant women and children avoid eating fish. Fishing Regulations have been issued for persons consuming fish taken from 13 sites in California. News releases and posting may be made with local health departments or the Department of Fish and Game.

High concentrations of the carcinogen aflatoxin B1 (AFB1) are commonly found in respirable, airborne grain dusts. Since the pulmonary epithelium metabolically activates AFB1, the characteristics of AFB1 exposure via the respiratory tract is of interest in the determination of occupational risk. The pharmacokinetic disposition of intratracheally-administered (i.t.) AFB1 either in microcrystalline form (MC) or adsorbed onto grain dust particles (GD) was studied. Blood and tissues were sampled for three weeks at selected intervals following the administration of a single dose of MC or GD [6] 300 μg/kg in male Sprague-Dawley rats. The blood concentration data from both groups approximated a two-compartment open model with first-order absorption. The time-to-peak for the blood concentration of label was significantly greater in the animals given GD than those receiving MC AFB1 (12 hr vs 2 hr), although the first-order elimination rate constants for both groups were nearly identical (0.00928 and 0.00921 hr⁻¹, respectively). Tissue concentrations of label closely followed those in the blood. (Supported in part by PHS grant ES 05391.)

The kinetic constants of chemical metabolism are needed to develop physiologically-based pharmacokinetic (PB-PK) models which predict the time course distribution of volatile chemicals in a mammalian system. Gas uptake proved useful in determining kinetic constants for a variety of volatile chemicals, but low vapor pressure compounds could not be examined and an alternative gas phase method was developed. Rats were first exposed by constant concentration inhalation for six hours and then placed in a 2.5L chamber with a forced air flow (120ml/min). The chamber effluent was serially analyzed for test chemical. The resulting elimination behavior is extremely sensitive to metabolism and kinetic constants can be estimated by simulation with a PB-PK model containing equations describing the experimental conditions. Optimized constants were obtained for 1,1,2-trichloroethane by allowing the model to vary only the kinetic constants until a best least squares fit was achieved between predicted and experimental results (VmAx = 51 μmol/hr/kg; Km = 3.0 μM). This technique has also been applied to several other chlorinated ethanes with still lower vapor pressures (the two tetrachloroethanes isomers, and penta- and hexachloroethane).

The water-soluble metabolites of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) have been studied. IQ, MeIQ and related compounds, a new class of chemical carcinogens, are formed during thermal processing of protein rich food. Due to widespread human exposure, studies on the mode of action are important. Metabolism was studied in rats, isolated rat hepatocytes and rat liver microsomes. PCB pretreated hepatocytes (10⁻⁶ M) metabolized IQ and MeIQ to three main groups of metabolites. After 3 h of incubation with 100 μM or 1-C labelled substrate, 616 pmol IQ/mg prot. and 835 pmol MeIQ/mg prot. was found covalently bound, more than 96% of IQ/MeIQ had been metabolized into water soluble products, while only 2-4% could still be extracted into ethyl acetate. For both compounds 3 major pult conjugates were found in the aqueous phase: one glucuronide, one sulfamate and most likely one sulfate. The sulfates were acid labile and the parent substances were recovered after hydrolysis. The glucuronide (B-glucuronidase treatment) and sulfate (acid hydrolysis) conjugates yielded different kinds of ethylacetate extractable hydrolys products, both more polar than the parent compounds. The main water soluble conjugates were also found in urine and bile of IQ or MeIQ exposed rats. The glucuronides were also formed from IQ and MeIQ in vitro with microsomes in the presence of NADPH and UDPGA. Among the nonpolar compounds (ethylacetate extractable) the N-acetylderivatives were found as well as several other metabolites. Some of these seem to correspond to the hydrolysis products of the conjugates. Further work on the chemical identification of the metabolites are in progress.

Cyclopiazonic acid (CPA), an indole tetramer acid, is produced by several Aspergillus and Penicillium species and co-occurs with aflatoxin B1 in feeds and foods. The toxicity of CPA may be due to its chelating activity; the compound is found unchanged in muscle tissue. Two 28 wk old male broiler chickens were anesthetized and a cannula was placed in the hepatic portal vein. Cyclopiazonic acid, 167 μg/ml (2.5 mg/kg BW), was infused at a rate of 333 ul/min for 90 min. Blood was taken at time 0 and every 15 min for 2 hr. Serum was extracted and CPA quantified by HPLC. After the first 15 min of infusion, an average of 96.1% of the CPA had been distributed to organs, tissues, bile, and urine. During the 90 min infusion period, the average concentration of CPA in serum was 589 ng/ml; more than 75% of the residual CPA was removed from plasma 30 min after termination of infusion. The rapid accumulation of CPA in tissues, particularly muscle, may play a role in the mechanism of toxicity.

CHANGES IN MORPHINE PHARMACOKINETICS AFTER ETHANOL INDUCTION OF UDP-GLUCURONYLTRANSFERASES. S Narayan, D J Kunz, and G S Yost. Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

Chronic ethanol treatment (10% in drinking water for 2 weeks) has been shown to increase hepatic microsomal morphine glucuronidation rates. To correlate in vitro work with in vivo disposition of morphine, male New Zealand white rabbits were dosed with 15 mg/kg ip morphine sulfate, treated for 2 weeks with ethanol, and dosed again with 15 mg/kg ip morphine sulfate. Arterial blood samples were collected from the rabbit ears from 5 min to 6 h after dosing. HPLC analyses of plasma samples demonstrated a reduction in the area under the plasma concentration curve (AUC) for morphine and a concomitant increase in the AUC of morphine 3-glucuronide after ethanol treatment. These results demonstrate that ethanol induction of hepatic UDP-glucuronosyltransferases may be responsible for changes in xenobiotic disposition, which may result in altered pharmacokinetics. Supported by USPHS Grant AA06555. GSY is a USPHS Research Career Development Awardee (HL02119)

SPECIES-SPECIFIC OPTIC NEUROPATHY BY METHYLTIAOACETATE IN RABBITS. D W Rosenberg, G M Ciszon, Z A Wong. Chevron Environmental Health Center, Inc., Richmond, CA.

Methylthioacetate (MTA) has been found to produce optic neuropathy in rabbits but not in rats or monkeys after acute exposure. This effect may be due to species differences in the metabolism of MTA. An in vitro metabolism study was conducted to determine the relative esterolytic activity of liver, kidney and plasma on MTA in the rat and rabbit. In both species, liver microsomes had higher levels of MTA thioesterase activity than in any other tissue tested. At an MTA concentration of 15 nM, thioesterase activity in rabbit liver was 3 to 4 times greater than in the rat. Maximum thioesterase activity was observed with MTA concentrations between 5 and 10 nM in the rat, whereas in the rabbit, activity still increased at the highest concentration of MTA examined (25 nM). The Km for rat liver microsomes was 2.25 nM, with a corresponding Vmax of 74 umoles product/min/mg protein. In contrast, the rabbit had a Km of 13.4 μM and a Vmax of 343.6 umoles product/min/mg protein. The higher MTA thioesterase activity in rabbit tissues, especially in the liver, may be a significant factor in the toxicity of MTA in the optic nerve of this species.

SUBSTRATE SELECTIVITY OF PURIFIED ETHANOL-INDUCED UDP-GLUCURONYLTRANSFERASE FROM RABBIT HEPATIC MICROSONES. R M Hutabarat and G S Yost. Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

We have previously shown that chronic ethanol consumption results in induction of microsomal UDP-glucuronosyltransferase (UDP-GT) activities. We have isolated and purified an ethanol-induced UDP-GT isozyme to homogeneity as determined by electrophoresis. The ethanol-induced isozyme trypsic digest HPLC profile was compared to the trypic digest HPLC profile from a similar isozyme from untreated animals and these were found to be different. The ethanol-induced enzyme form does not appear to have high substrate specificity. The highest specific activity for this isozyme was found with the GT1 substrate naphthol (5/4.92 nM/min/mg protein with a Km of 42.6 μM). The specific activity for the GT2 substrate morphine was 10.26 nM/min/mg protein with a Km of 108.73 μM. The steroid substrates, estrone, and testosterone had specific activities of 3.22 nM/min/mg protein and 2.46 nM/min/mg protein, respectively. The increase in Vmax/Km (naphthol) for the ethanol-induced isozyme compared to the control isozyme activity was two-fold. The large increase in catalytic efficiency of the ethanol-induced isozyme for naphthol, coupled with only small increases in turnover numbers for other substrates, demonstrates a partial selectivity of this enzyme form for GT1-type substrates. Supported by USPHS Grant AA06555. GSY is a USPHS Research Career Development Awardee (HL02119)

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Glutathione S-transferases (GST) are involved in the detoxification of many xenobiotics. A genetic tri-modal distribution of GST activity in human mononuclear leukocytes has been found toward trans-stilbene oxide (TSO). It has been suggested that leukocyte GST activity toward TSO may be used as a marker for genetic susceptibility to cancer. The aim of this study was to determine if leukocyte GST activity expressed toward TSO is characteristic of activity toward environmentally relevant carcinogenic substrates. HPLC assays were developed to measure GST activity in leukocytes towards aflatoxin-8,9-oxide (AFBO), benzo(a)pyrene-4,5-oxide (BaP), and p-nitrosodimethylnitrosamine (NDS). Activity toward CDNB was also assayed. These assays were conducted on leukocyte preparations from 31 apparently normal individuals, with the exception of the aflatoxin assay, in which no measurable GST activity was found toward AFBO in a subset of these samples. Comparisons of GST activity toward TSO yielded statistically significant correlations between GST-TSO activity and GST-BaP activity (r=-0.74); and GST-TSO activity and GST-CDNB activity (r=0.48). GST-PNS0 activity was not significantly correlated with GST-TSO. No apparent modality in GST activity toward these substrates was observed. These results suggest that it may be misleading to make inferences about GST activity towards environmentally relevant compounds from the use of surrogate substrates. (Supported by The Dana Foundation).

ANALYSIS AND SCREENING OF XENOBIOTIC MERCAPTURIC ACID CONJUGATES USING NEGATIVE IONIZATION AND TANDEM MASS SPECTROMETRY. C K Winter, A D Jones*, and H J Segall, Department of Veterinary Pharmacology and Toxicology and *Facility for Advanced Instrumentation, University of California, Davis, CA.

The mass spectra and fragmentation pathways of the mercapturic acid conjugates of 1,3-dichloropropene, styrene oxide, trans-4-hydroxy-2-hexenal, trans-4-hydroxy-2-nonenal, naphthalene oxide, and benzyl chloride were investigated using chemical ionization and fast atom bombardment mass spectrometry in conjunction with linked scan and mass-analyzed ion kinetic energy spectrometric techniques. Fragmentation patterns of the pseudomolecular ions of these mercapturic acids were simple and consistent, with the dominant mode of decomposition of [M+H]⁺ involving scission of C-S bonds with loss of a neutral fragment of mass 129 u. Screening for mercapturic acids in urine samples was performed by introducing samples via desorption chemical ionization probe, and neutral loss scanning was performed to obtain selective detection of mercapturates. Limits of detection were determined to be at low nanogram levels.

CHICK EMBRYO RETINA CELL CULTURE: TERATOGEN SCREEN AND MECHANISTIC PROBE. G P Daston, J E Tonker, D Baines and J I Pointer, Miami Valley Laboratories, Frocter & Gamble, Cincinnati, OH.

We have determined that a suspension culture of aggregated neural retina cells derived from incubation day 6 chick embryos is a useful screen for teratogens, and may provide information on the mechanisms of action of teratogens. In culture, the embryonic cells undergo a number of fundamental processes of development, including cell-cell interactions, pattern formation, histogenesis, growth and division, and selective expression and suppression of genes to produce a differentiated phenotype. We have established objective, measurable endpoints for these developmental events. Perturbation of any endpoint by a chemical is indicative of potential for developmental toxicity. To date, we have tested 17 known teratogens and non-teratogens, and the screen has correctly classified all but one (a false negative). We have successfully used an endogenous metabolizing system (rat hepatic S-9) to activate an indirect teratogen, cyclophosphamide. Developmental toxicants alter different sets of endpoints depending on teratogenic mechanism. For example, inhibitors of gene expression alter endpoints of differentiation; enzyme inhibitors or mitoclasts interfere with growth; inhibitors of cell-cell interaction disturb pattern formation. Thus, this assay is a predictive teratogen screen and may be useful in identifying putative mechanisms of action.

THE HYDRA ASSAY AS A PRESCREEN FOR TERATOGENIC MYCOTOXINS. E E Smith, E A Maull, M A Taylor, B A Clement and J D Phillips, Veterinary Public Health, Texas A&M University, College Station, TX.

More than 200 mycotoxins have been discovered and structurally characterized. A number of these chemicals have been reported to be teratogenic, but for most, the developmental hazard is unknown. This study was designed to evaluate the ability of the hydra assay to accurately detect and predictively rank teratogenic mycotoxins and/or homologs, according to their developmental hazard index (A/D ratio). Hydra attenuata were maintained in culture. Mycotoxins, (i.e., T-2 toxin, diacetylstilbene, aflatoxin B₁ and G₁, ochratoxin A, and a racemic mixture of ochratoxin alpha) were diluted in an appropriate vehicle and tested according to established procedures to determine the minimal affective concentrations of each for the adult hydra (A) and the developing hydra "embryo" (D). The calculated A/D ratios ranged from 1.0 (for aflatoxin B₁, suggesting maternal toxicity at concentrations shown to affect the embryo) to 5.5 (for T-2 toxin, suggesting more of an effect on the embryo and less maternal toxicity). These findings are in general agreement with previous studies in vivo, supporting the conclusion that the hydra assay may be useful as a prescreen for other important mycotoxins (Supported by AH 6830, USDA Project 84CRSR-2-2434 and USAID CRSP 02-50305-2).
We have characterized the in vitro developmental toxicity of aflatoxin B1 via preimplantation mouse embryo culture to compare in vitro the embryotoxicity of these compounds with prenatal toxic effects observed in vivo. Whole mouse embryos, explanted 48 hours after conception, were cultured in serum-supplemented Eagle's basal media for 96 hours with each test compound and ultrastructurally evaluated for toxic indices. Over a 10 fold dose range, AFBl alone demonstrated minimal embryotoxicity; after culture supplementation with rodent hepatic S-9 fractions isolated from animals preadministered Aroclor 1254, AFBl embryolethality as well as embryotoxicity was observed. Ultrastructural effects of AFBl in culture included decreased embryo hatching, attachment, and inner cell mass. These findings parallel reports in vivo of the prenatal toxic effects associated with AFBl and strongly support application of this test system for testing environmental agents suspect as prenatal and abortivitc toxicants.

2-Methoxyethanol (ME) is oxidized to MAA which is the proximate toxin. ME is ineffective in whole embryo culture (WEC), while MAA is embryotoxic. We have shown that the teratogenic effects of ME in vivo are attenuated by simple physiological compounds, among them acetate (A) and formate (F), which provide carbon for purine and pyrimidine bases in DNA. Label from 1,2-14C-ME was present in all macromolecular fractions of embryos and dams, and dams exhaled CO2, suggesting that MAA was further metabolized and that this might be relevant to embryotoxicity. The present WEC experiments were designed to distinguish maternal from embryon effects of MAA. Embryos on gestation day 11 (40-44 somites; maximally sensitive to MAA in vivo) were maintained in WEC for 6 hr in serum free medium. The WECs were exposed to MAA, A, F or MAA-A and MAA-F for 5 hr before supplementation. Incorporation into DNA was measured during the last hour. MAA reduced (5 mM) the incorporation into DNA by 50%. Neither A (5 mM) nor F (1 mM) affected DNA synthesis. However, when added to WEC together with 5 mM MAA, both A and F completely abolished MAA's effects. These findings suggest that A and F block the entry of MAA into reactions that inhibit DNA synthesis in embryos.

The placental function of the visceral yolk sac (YS) of the early post-implantation rat conceptus is well established as a target for certain teratogens. Especially sensitive to insults are the processes of pinocytic uptake and degradation of protein by YS. Interference with either of these processes is considered to produce a similar nutritional deficit and consequent embryonic malformation: this latter point however has not previously been demonstrated. Using 48 hr rat whole embryo cultures, we have compared the dysmorphicogenic action of 2 agents that are teratogenic through an effect on YS: leupeptin (proteolysis inhibitor) and anti-YS antisera (pinocytosis inhibitor).

At the lowest dysmorphicogenic doses (1 µg/ml leupeptin; 10 µg/ml anti-YS) the pattern of abnormalities was similar: retardation or absence of optic vesicles and some growth retardation but no apparent YS pathology. At higher doses (2 µg/ml leupeptin; 15 µg/ml anti-YS) gross brain deformities and severe growth retardation were evident but still YS appeared normal. These data demonstrate that, as anticipated, disturbance of YS placental function in either of 2 ways produces similar embryonic dysmorphism and, furthermore, occurs in the absence of gross effects on YS appearance.

The ethylene glycol ether 2-methoxyethanol (ME) is selectively embryotoxic and teratogenic in several species. Results from other studies led to the conclusion that methoxyacetic acid (MAA), the oxidation product of ME, is the proximate teratogen. However, our data revealed that incorporation of label derived from 1,2-14C-ME into macromolecular fractions of the embryo suggested that MAA enters into further biochemical reactions. Sarcosine (S) is an intermediate in the biosynthesis of glycine and a structural analogue of MAA; MAA inhibits S oxidation to glycine. The entire backbone of glycine is incorporated into the purine ring. In the present experiments mice received concurrent oral doses of 3.3 mmol ME/kg and 43 mmol 5/kg on gestation day 11. Animals dosed with ME alone exhibited digit malformations (any digit) in 47% of the fetuses or 83% of litters. S significantly reduced the digit malformation incidence (p < 0.05) to 8% of the fetuses or 42% of litters. This outcome suggests that MAA might inhibit purine biosynthesis by depleting glycine pools in the embryo. Another mechanism leading to disruption of purine and pyrimidine supply, supported by earlier results, indicates that a product of further MAA metabolism might interfere with one-carbon moiety availability.
As part of ongoing studies of the teratogenicity of diphenyl ethers, four compounds that can be unambiguously ranked with respect to embryotoxicity in vivo were also evaluated in an in vitro assay for cytotoxicity. The 4 compounds and the dose required to cause statistically significant perinatal mortality in CD-1 mice were [in ascending order of effective dose]: 2,4,5-trichlorophenyl 4-nitrophenyl ether (TCN), 4 mg/kg/day; 2,4-dichlorophenyl 4'-nitrophenyl ether [nitrofen], 50 mg/kg/day; 2,4,6-trichlorophenyl 4'-nitrophenyl ether (CNP), 250 mg/kg/day; phenyl 4-bromophenyl ether (4BR), > 1000 mg/kg/day. The same order pertains for teratogenic potency, but decreased Harderian gland size is seen at lower doses than is perinatal mortality.

In short-term culture, 100 μg toxicant per ml culture medium, the toxicity of these compounds to human lymphocytes was: 4BR > CNP > nitrofen > TCN, both in the absence and in the presence of rat microsomes. Cytotoxicity of 4BR decreased, and that of the remaining diphenyl ethers increased, with addition of microsomes to the system. At cell mortalities in excess of 50%, discrimination between compounds was unsatisfactory because total cell counts were too low.

PHARMACOKINETIC MODELS FOR FETAL EXPOSURE TO DEVELOPMENTAL TOXICANTS. A H Marcus, P Feder, D Hobson. Battelle Columbus Division. Research Triangle Park, NC and Columbus, OH.

Time-dependent compartmental models with physiological parameters are used to study the effects of pregnancy and lactation on the delivery of toxic substances and their metabolites to the fetus and neonate. Examples are shown for lead, hexachlorobenzene, and ethanol. The model shows lead accumulation in growing fetal organs. The model also illustrates the effects of differing acetaldehyde metabolism of ethanol in mother and fetus, and may be useful in studying effects of time-varying exposures e.g. "binge drinking". Parameters used in modeling rat data are adjusted statistically to provide good-fitting models.


Highly sensitive probe-substrate analyses revealed that three separate tissues of day 11 rat conceptuses contained xenobiotic-oxidizing P450 isozymes. The embryo proper contained constitutive P450(s) that catalyzed readily measurable O-depentylatation and O-debenzylation but no measurable O-demethylation and barely detectable O-deethylatation of phenoxazone ethers. Higher activities for O-depentylatation and O-debenzylation were measured in the yolk sac which also contained constitutive P450(s) for readily detectable O-deethylatation. O-deethylatation could not be detected in the yolk sac. Only O-debenzylation was detected in the amniotic cavity. Treatment with 3-methylcholanthrene (MC) significantly increased O-deethylatation in yolk sac and embryo but not placental conceptus. Demethylatation was not detectable in the same preparations. Phenobarbital, pregnenolone 16 β-carbonitrile or isoasafrole produced no effect on any reaction. CO markedly inhibited all reactions and inhibition was reversed by O2. Decarbamylatation and debenzylation were inhibited by anti-P450A1 IgG but not MC induction but were not affected by the same IgG fraction in untreated conceptuses. Depentylatation was not inhibited by anti-P450A1 or anti-P450OH1B1/2 under any conditions utilized. Decarbamylatation was strongly inhibited by 1.0 μM 7,8-benzoflavone in MC-treated but not untreated conceptuses. Metyrapone (1.0 μM) failed to inhibit depentylatation reactions. The results indicated four (or more) functional P450 isozymes in the conceptus during organogogenesis: a constitutive depentylatation(s) in yolk sac and embryo, a constitutive deethylatation(s) in yolk sac, an MC-inducible deethylatation(s) in embryo and yolk sac and constitutive debenzylation(s) in all three tissues. No O-demethylation was detectable in any tissues, even after exposure to inducers. Supported by NIH grants ES-04041 and ES-04342.
Treatment of neonatal male rats with phenobarbital (PB)-like inducers results in irreversible effects on reproductive function and hepatic monoxygenase activities. The present study was carried out to determine whether maternal exposure to 6-CB influenced T levels in male perinatal rats, thereby modulating CNS “ imprinting” of these activities. 6-CB (100 mg/kg) or corn oil (CO) was administered ip to d14 pregnant rats. On d19 of gestation (d19g) and d4 and 15 postpartum, plasma was collected from individual male offspring. Hepatic microsomes were prepared from individual litters and assessed for ethoxyucoumarin-O-deethylase activity (ECOD), benzhxenamine-N-demethylation (BND) and P-450 content. No differences were seen on d19g correlating to the minimal placental transfer of 6-CB. Activities were elevated in nursing offspring of 6-CB treated mothers (d4-ECOD:19-fold; BND:25-fold; P-450:4-fold; d15-ECOD:5-fold; BND:6-fold; P-450:2.5-fold). T levels (pg/ml) as determined by RIAs were not different following 6-CB exposure (d19g:CO=1038±222; 6-CB=1073±225; d4:CO=752±159; 6-CB=852±172; d15: CO=556±86; 6-CB=474±114). This suggests that the neonatal endocrine system can compensate for elevated hepatic PB-like monoxygenase activities.

Glycol ethers (GEs) are known male reproductive toxins in a number of species, including man. Groups of male CD-1 mice received p.o. either distilled water or EGME at one of two dose levels (100 or 500 mg/kg per day) for one or two weeks. The males were euthanized, and the testes, epididymides, seminal vesicles, liver, kidneys, and spleen were removed and weighed. The testes were homogenized, pelleted, and resuspended in a Triton X-100 solution. After 12 hours of incubation at 4°C, supernatants were filtered, and aliquots analyzed for LDH or LDH activity. For LDH assays, sodium pyruvate substrate and NADH in buffer were added; LDH activity was measured as a change in optical density at 340 nm. For SDH, fructose substrate was added, and the measurements made at 366 nm.

Testes weights decreased with increasing EGME doses after both one and two weeks of exposure. No differences were observed in other organ weights between any of the groups. Decreases in LDH and SDH activity were observed in groups exposed to 500 mg/kg EGME for one and two weeks.

These results confirm previous observations that EGME exhibits toxic effects on the male mouse reproductive system at the testis level.

The effect of neonatal exposure to Aroclor 1254 on adult rat hepatic microsomal testosterone hydroxylases was determined in 60-, 90- and 120-day-old rats. Most alterations in testosterone metabolism were observed in 90-day-old male and 60-day-old female rats. Males at 90 days exhibited decreased basal 7α-hydroxylation and increased basal 16α-, 2α-, 25α- and 15β-hydroxylations and androstenedione formation. 60-Day-old females treated neonatally with Aroclor 1254 exhibited decreased basal 16α-, 2α-, 6α-, 15β- and 16α-hydroxylase activities and androstenedione formation. Testosterone hydroxylase inducibilities by Aroclor 1254 were also modulated by neonatal exposure to this commercial mixture. In many cases, the lower dose of Aroclor 1254 (100 umol/kg) was more effective than the higher dose (500 umol/kg) in imprinting hepatic testosterone hydroxylases and further research on the neonatal effects of lower "environmental" doses of C08s is required. (Supported by the Texas Agricultural Experiment Station.)

Reproductive toxicity studies are routinely conducted using laboratory animals, or more rarely using non-human primates, which are thought to be better models for humans. Testicular xenobiotic metabolism was compared in humans, monkeys, rats, and mice to determine the validity of using these animals as models for humans in reproductive toxicity. Human tissue samples were obtained from adult male organ donors. Rhesus monkey tissues were obtained at necropsy from adult males. Rodents were sexually mature male Fisher 344 rats and CD-1 mice. The activities of epoxide hydratase (EH) in microsomes (m) and cytochrome c and cytosolic glutathione S-transferase (cGST) were measured using a radiometric technique. Testicular cEH activity in humans was comparable to mice, as opposed to 3.5-fold greater than rats, and 15-fold greater than monkeys. Testicular cEH activity in humans was comparable to rats but 2-fold greater than mice. Testicular cGST activity in humans was comparable to mice, however it was 60-fold lower than rats and 35-fold lower than mice. These results indicate that none of the animals studied here are an accurate model for testicular xenobiotic metabolism in man.
LDH-C4: A SPECIFIC MARKER OF ACUTE-PHASE TESTICULAR DAMAGE. S C J Reader, C Shingles and M D Stonard. ICI plc, Central Toxicology Laboratory, Macclesfield, Cheshire, U.K. Sponsor: E A Lock

1,3 dinitrobenzene (DNB) and ethylene glycol monomethyl ether (EGME) markedly affect the testis Sertoli and germ-cells respectively. We have monitored biochemical responses to such damage including the testis specific isozyme LDH-C4. Rats (6/group) were dosed F.O. with DNB (0-30mg/kg) or EGME (0-500mg/kg), after 48h plasma/tissue samples were analysed. Further studies used a single oral dose of DNB (25mg/kg) or EGME (500mg/kg) for 16, 24, 48, 96h and 14 days. LDH isozymes were profiled using electrophoresis, C4 was assayed using a kinetic NAAD-linked reaction with a ketocaprate as substrate. Testicular C4 activity was reduced after DNB (25-30mg/kg) from control 24±14 IU/mg protein to test 19±36 IU/mg protein; P<0.02, with a concomitant increase in plasma C4 activity at 10mg/kg DNB from control 3±2 IU/L to test 31±1 IU/L; P<0.05. C4 was increased in plasma and decreased in testis after 6h (P<0.02) remaining so for up to 14 days (P<0.05). Similar results were found with EGME up to 96h. Testicular lesions were seen at 16h with 20mg/kg and 300mg/kg for DNB and EGME respectively. In conclusion, these results suggest disruption of the blood-testis barrier allowing C4 to be measured in blood as an early diagnostic marker of acute-phase damage before apparent pathological lesions.

CATIONIC MODULATION OF ADENYLATE CYCLASE (AC) ACTIVITY IN A HETEROGENEOUS POPULATION OF MALE GERMLINE CELLS (NGC). L Beebe, K Pendino, R O Warwick Jr., and D A Barsotti. Philadelphia College of Pharmacy & Science, Phila., PA and A.T.S.D.R., Atlanta, GA.

Germ cell specific AC is located in the cytosol of haploid germ cells, where it demonstrates an obligatory requirement for manganese, and insensitivity to hormones or estrogens. Investigations in our laboratory have demonstrated AC activity in a membrane enriched fraction isolated from a heterogenous population of NGC. These studies have focused on the cationic requirements for activation of this membrane associated enzyme, and its responsiveness to activators of the Gs protein. NGC membranes are incubated in the presence of manganese (10 μM), magnesium and manganese (5 μM each), or manganese (10 μM) alone, in an assay buffer containing HEPES (50 μM), pH 7.5, EDTA (1 μM) and IMX (1 μM). The conversion of DPEP to 32P-AMP is quantitated utilizing successive chromatography over Dowex and alumina columns. Our results indicate that membrane associated AC is stimulated by both manganese and magnesium, although the magnitude of this stimulation is greater in the presence of manganese. Responsiveness to GTP (10-100 μM) is demonstrated only in the presence of magnesium, and is abolished upon the addition of magnesium to the incubation buffer. These data suggest that the membrane bound AC in MGC has a divalent cation requirement which may be fulfilled by either magnesium or manganese. Furthermore, the expression of G protein activation is magnesium dependent and attenuated by the presence of manganese ions.


The Cellsoft computer-assisted sperm motion analysis system (CRYO Resources) has been used for the evaluation of motion characteristics of sperm. Using previously published sample setting in our analyses, we identified the potential for error in evaluating dose effects in rats exposed to ECH, a compound previously shown to reduce fertility in experimental animals. We then examined the effect of changes in Cellsoft system settings on motion endpoints. In controls, sperm tracking was significantly shortened at a maximum velocity setting of 300 μm/sec, resulting in artificially lower mean velocities. Mean linear velocities appeared to level off when maximum velocity was set to 600 μm/sec. Using a paired t-test, Cellsoft values for the percentage of motile sperm were not significantly different from manually determined values when the minimum sampling motion parameter setting was 10 frames rather than 1. ECH dose-response relationships were then evaluated at these settings and compared to those determined at the previously published settings. Use of the new settings reduced system biases and expanded the range of measurements to allow a greater sensitivity in measuring toxic dose effects. (Abstract does not necessarily reflect EPA policy).
SILICONE IMPLANTS IN THE RAT VAS DEFERENTES. D P Waller, A Martin, M Szarley, A R Nikurs, N A Nuzzo, and L J D Zaneveld, University of Illinois at Chicago, and Rush Presbyterian-St. Lukes Medical Center, Chicago, IL.

A silicone plug for the vas deferens (SHUG) is being developed as a new reversible male contraceptive device. Previous studies have demonstrated its ability to block the flow of spermatozoa when placed in the monkey vas deferens. Our laboratory evaluated the effect of chronic exposure of the rat vas deferens to the presence of the silicone material used to make the SHUG. Four hundred rats were divided into two equal groups. One group had sham operations and the other group had silicone tubes implanted in the vas deferens and secured by a suture. Animals were euthanized, and the vas deferens removed, fixed, and histologically evaluated after six, twelve, or 22 months of implantation. Changes in the vas deferens attributed to the presence of the silicone were minimal-to-mild epithelial hyperplasia in the proximal, surgical, and distal segments. The incidence and severity were directly related to the length of exposure to the silicone. An increased incidence of spermatoceles, aspermatic granulomas, and chronic inflammatory changes within the inner walls of the vas deferens were also observed in all implanted groups. These vas deferens changes represent normal tissue responses to the presence of a foreign body. Supported by the program for Applied Research on Fertility Regulation (Agency for International Development, PARFR 339).


CGS 15863, a thromboxane synthetase inhibitor, was evaluated for effects on fertility and reproductive performance in rats. The compound was administered orally via gavage to groups of male and female rats in two dosages, 75 or 225 mg/kg. Females were dosed for 14 days prior to mating and throughout gestation and lactation. Males were dosed for 63 days prior to mating and received a total of either 98 or 105 daily doses. Following the dosing period, selected males from the control and 225 mg/kg groups were placed on recovery (untreated) for 70 days and then mated with a separate group of untreated females. Compound-related effects were not evident at daily doses of 25 or 75 mg/kg. In contrast, rats from the 225 mg/kg group exhibited compound-related reproductive toxicity, which included: a) reduced fertility, decreased numbers of implants and viable neontates, and no litters at the end of the recovery/retaining period; b) reduced testes weights; c) gross testicular atrophy; and d) microscopic evidence of testicular vacular degeneration, progressing to tubular atrophy. These effects were increased in incidence and severity in recovery animals, indicating non-reversibility. The etiology of these degenerative testicular changes in response to CGS 15863 has not been established.
2-Methoxyethanol (2-ME) was applied to the backs of Sprague-Dawley male rats, at dose levels of 0, 625, 1250, and 2500 mg/kg on occluded sites, and 0, 1250, 2500, 5000 mg/kg on nonoccluded sites for 7 consecutive days. Changes in testicular histology were evaluated on weeks 4, 7, 10, and 15 of the study. In an attempt to examine the time needed to induce testicular toxicity, the testes of animals exposed to 1250 mg/kg (occluded) and 2500 mg/kg (nonoccluded) were examined on days 2 and 5 and weeks 1 and 2 of the study (Day 1 = 1st day of dosing). After fixation in 10% formalin, the testes were embedded in paraffin and stained in glycol methacrylate (GMA). The paraffin sections were stained with hematoxylin and eosin (H&E) and the GMA sections stained with H&E or Periodic Acid Schiff. Toxicity to spermatoocytes was apparent in Occluded and Nonoccluded groups after one day of dosing. Recovery (presence of spermatocytes in a majority of the seminiferous tubules on week 15) from 2-ME toxicity was apparent in 50% (2/4) of the Occluded group animals exposed to 2500 mg/kg and in 100% of the Nonoccluded group animals exposed to 5000 mg/kg. Histological evaluation of the testes of the mid- and low-dose Occluded groups is underway in an attempt to establish the dosage for which recovery is apparent.

Dominant lethality studies indicated that the exposure of male rats to ACR produced significant pre-implantation loss at Weeks 1-4 after treatment (spermatogonial and meiotic stages of spermatogenesis). The present study was designed to determine if ACR-induced fertilization failure might contribute to a reduced rate of implantation. Adult male Long Evans rats received 0, 15, or 45 mg/kg ACR (p.o.) for 5 days and were then mated to proestrous females on Weeks 1-4 after ACR. Ova were flushed from the oviducts 12 hours after mating. The presence of a sperm head and tail or two pronuclei in the oocyte provided evidence of fertilization. Females mated to males at Week 1 after exposure showed 84%, 41%, and 29% fertility for the 0, 15, and 45 mg/kg ACR, respectively. At Week 3, fertilization rates were 65%, 75%, and 12% for 0, 15, and 45 mg/kg ACR, respectively. These rates indicate significant reductions in fertilization at both ACR exposures at Week 1 and at the 45 mg/kg dose of ACR at Week 3. During Weeks 2 and 4, fertilization was not affected. These data suggest that fertilization failure may, in part, account for the subsequent reduction in implantation associated with ACR exposure. (This abstract does not reflect U.S. EPA policy or opinion).

Diaceptoxyscrinenol (DAS, angudine) administered ip at 7% of the LD50 causes testicular degeneration in CD-1 mice and Lewis rats. The objective of this study was to characterize the testicular damage in a strain of rats in which testicular structure and function have been more fully characterized. Mature (12 wk) male F344 rats were given DAS in 10% aqueous DM50 by ip injection at 50% (0.8 mg/kg) or 75% (1.2 mg/kg) of the LD50 and studied 7, 45 or 90 days later. DAS caused dose-dependent decreases in testicular and epididymal weights, sperm production and epididymal sperm reserves. These decreases were significant at all times studied after exposure to 1.3 mg/kg but only at 45 and 90 days after exposure to 0.8 mg/kg. Morphologic changes were mainly dose- and time-dependent decreases in diameter of seminiferous tubules and increases in the percent of hypocellular tubules. These results are consistent with damage to proliferating cells early in the maturation sequence and to mature spermatids. (Supported by contract DAMD 17-82-C-2235 from the U.S. Army Medical Research and Development Command.)


Previous studies have shown that methylxanthines (caffeine and theobromine) exert testicular and thymic toxicity in rats when fed at high dietary levels (0.6-0.8%). This study determined the effects of niacin and/or zinc supplementation on theobromine (TBR)-induced thymic and testicular atrophy in weanling and adult male Sprague-Dawley rats fed control, 0.6% TBR, 0.6% TBR + 0.12% zinc, 0.6% TBR + 0.2% niacin, or 0.6% TBR + 0.12% zinc + 0.2% niacin in a purified diet. After 28 days of TBR exposure, body weights were reduced in all TBR treatment groups although effects were more severe in the weanlings. Relative thymus and testes weights in weanlings treated with TBR were 30% and 70% of controls, respectively. Adult animals exhibited no testicular atrophy from TBR, but relative thymus weights were 40% of control. Analytical results following TBR exposure indicated: 1) reduction in sperm motility, 2) decreased plasma/testes testosterone, 3) increased testes malate dehydrogenase, and 4) decreased testes sorbitol dehydrogenase. Plasma/testes alkaline phosphatase and zinc levels were similar among all animals. Supplementation with niacin and/or zinc had no significant positive effects on TBR-induced organ or body weight changes.
We have previously developed a model to investigate the toxicity of promotional agents on a heterogeneous population of MGC. Exposure regimens in vitro have necessitated the quantitation of cell viability as determined by trypan blue exclusion dye and lactate dehydrogenase (LDH) leakage from these cells. ATP production has also been quantitated utilizing a purified luciferin/luciferase enzyme assay. MGC have been exposed to the promotional agents tetradecanoylphorbol-13-acetate (TPA) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) over time (1.7 mM, 30–130 min) and concentration (1nM–1μM, 60 min) ranges. DMSO (0.5% v/v) and PBSG were incubated as vehicle and non-vehicle controls, respectively. Viability was statistically reduced at 150 and 180 minutes for the DMSo treatment. Neither TPA nor TCDD decreased viability at any time interval or concentration investigated, as compared to vehicle controls. ATP production was statistically decreased by all treatments at 60 minutes, with recovery by 90 and 120 minutes. Incubations exceeding 120 minutes reduced ATP levels as compared to the 30 minute value. Neither TPA nor TCDD inhibited ATP production at any time interval or concentration investigated. These data suggest that TPA and TCDD are not directly cytotoxic under our exposure conditions. Additionally, incubations should not exceed 120 minutes utilizing this model.

The pesticide Linuron (LRN) has been reported to cause testicular damage in rats. Consequently, the present study was designed to explore the effects of LRN on the brain-testicular reproductive axis (H-P-T) axis after short term exposure. Adult male rats were gavaged with LRN (50, 100, or 200 mg/kg) in corn oil for 4 days. Controls received corn oil only. Rats were killed 1, 3 or 7 days post-dosing and serum was collected for analyses of pituitary, thyroid and gonadal hormones. Pituitary homogenate and hypothalamic were assayed for tissue hormone concentrations. Testicular and epididymal (epi) measures were recorded and testosterone and androgen binding protein (ABP) were assayed in individual testicular compartments. Data analysis showed a reduction in caput epi weight and interstitial fluid volume on day 1 at the highest doses. A dose-dependent reduction in the thyroid hormone, T4, occurred on day 1. No other hormonal alterations were seen. The data indicate that LRN under these conditions has minimal effects on the H-P-T axis. Additional research is needed on the influence of more prolonged exposure to LRN.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
IN VIVO PERCUTANEOUS ABSORPTION OF 4 PESTICIDES, AS AFFECTED BY ANATOMIC REGIONS OF THE RAT. L L Tromp, C Brownie, and F E Guthrie. Toxicology Program, N.C. State University, Raleigh, NC.

A comparative study was carried out in 7 week old Sprague-Dawley rats (150 ± 5 gms) with 14C-labeled pesticides. The comparative variable was anatomic site (back, stomach, feet, ear and tail) with dose (33 mg/g sq cm), application site (+ 4.5 cm²), solvent (50 µl acetone), and methodology being constant. Quantitative measurement was based on excreta (urine and feces) and caecum recovery. The penetration of radioactive malathion, carbofuran, lindane, and fentervate was determined in the rat after separate applications. After 48 hr the percent of malathion absorbed was greater than absorption of other pesticides for all anatomic sites except the ear where lindane was the highest penetrant. There was no consistent pattern of penetration among the 4 insecticides studied although malathion absorption was greatest after 48 hr. Absorption at 48 hr was generally greatest by the feet and lowest by the tail.


Certain mineral oils and hydrocarbons require repeated topical applications to cause irritation. A structure activity relationship of pure n-alkanes in a mouse ear edema model was undertaken to investigate the mechanism of cumulative irritancy. Alkanes were applied twice daily over a 96 hr period. Dodecane was non-irritating. Tridecane (C13) elicited a response only at 96 hr. Tetradecane (C14) was the strongest irritant with significant increases at 48 hr. Hexadecane, octadecane and eicosane showed progressively decreasing activity. Permeability of the ears to cortisol was monitored in vitro during C13 and C14 induced irritation. Significant increases in permeability were observed 24 hr before edema formation. Larger increases were observed with C14 than C13. Loss of barrier function resulted in increased cutaneous availability of the alkanes. Increased permeability prior to edema formation indicates that induction of barrier dysfunction may be a factor in the mechanism of alkane induced irritation. Appreciation of such factors is important in new method development such as in vitro alternatives.

DERMAL ABSORPTION KINETICS OF VAPORIZED VOLATILE ORGANIC CHEMICALS. J L Proctor, J R Lusseh, K Kutzman, Northrop Services, Inc, RAN, and D S Mattie, ARMRL/TH, WPAFB, OH.

The objective of this study was to determine the dermal absorption kinetics of volatile organic chemicals detected in contaminated water. Male Fischer 344 rats were dermally exposed to neat, saturated, 2/3 saturated, and 1/3 saturated aqueous solutions of trichloroethylene (TCE), perchloroethylene (PCE), and toluene for 24 h. Blood samples (100 µl) were withdrawn prior to exposure and after exposure for 0.5, 1, 2, 4, 8, 12, and 24 h. Samples were analyzed by headspace gas chromatography. The volumes and concentrations of the chemical solutions remaining in the exposure cells were determined after exposure for 24 h. Dermal exposure to neat PCE produced higher peak blood levels than exposure to neat TCE or neat toluene. Blood levels of each chemical reached a peak value rapidly and remained elevated throughout the 24 h exposure. Exposure to saturated aqueous solutions resulted in rapid absorption. Blood levels did not remain elevated but decreased back to control levels by 24 h. Exposure to 1/3 and 2/3 saturated solutions of TCE, PCE, and toluene resulted in very low, but potentially significant levels of these chemicals in the blood. These data will be used in the development of a refined model of dermal absorption. (Supported by ARMRL/TH contract No. F33615-85-I-0332).


Rat skin was exposed in vivo to 6 organic solvents to correlate ultrastructural changes with the location of an electron dense tracer, lanthanum (La), which is normally excluded by the permeability barrier in the stratum corneum. Male F-344 rats were exposed for 24 h to sterile saline, trichloroethylene, perchloroethylene, toluene, xylene, hexane or benzene using dermal cells developed in this laboratory (surface area of 2.54 mm²). The cells were filled with 2 mL of saline or neat chemical. After exposure, the cells were rinsed with saline and filled with 1% La in saline for 1 h. After exposure to La, rats were sacrificed, cells removed and the exposed area of skin prepared for light and electron microscopy. Rat skin exposed to saline for 24 h was normal. La was found only on the surface of the epidermis indicating that the permeability barrier was intact. In rat skin exposed to an organic solvent for 24 h, La was found throughout the epidermis and upper dermis indicating that these six solvents altered the barrier properties of the stratum corneum.

Chronic dermal treatment of C3H/HeN mice with a mildly hydrotreated light petroleum middle distillate (MD) resulted in pronounced skin irritation. In an atypical response to many animals developing malignant tumors late in the study (mean onset: 79 wk). Results from subsequent subchronic studies indicated that Sencar BR mice are more resistant to MD-induced skin irritation than C3H mice. In order to better understand the effects of MD on skin, in vivo percutaneous absorption studies with the MD were performed in C3H and Sencar mice. Absorption of the MD was measured over 96 hr following a single topical dose containing [3H]-n-dodecane and [14C]-2-methyl naphthalene as surrogates representing the aliphatic and aromatic components, respectively, of the MD. The results indicate that there is no significant difference in the permeability of C3H and Sencar mice skin to the MD. This similarity suggests that the different MD-induced skin irritations observed in C3H and Sencar mice are a result of biological and/or immunological variations between the two strains.


Age dependence in percutaneous absorption of 14C-nicotine was assessed in 33 and 82 day old Fischer 344 rats. Nicotine was applied in acetone to the previously clipped back skin and the radioactivity in the treated skin, tissues, urine and feces was determined at 24, 48, and 72 hours. In vitro dermal absorption was also measured in the same aged rats by static and flow through methods. In vivo dermal penetration at 48 hrs. was 81% in young and 79% in adult rats. Adults excreted 67% in urine and 5% in feces in 48 hours while excretion in the young was 73% urinary and 5.5% fecal. Age dependence in tissue content was found in kidney, blood, and carcass. Age dependence in tissue concentration was seen also in kidney, blood, and carcass. Dermal absorption was slightly higher in young rats while retention was greater in the adult. Skin absorption by the in vitro methods was somewhat less than the in vivo absorption and was greater in young than adults. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

DERMAL AND TRANSDERMAL TOXICITY OF THE CALCIUM IONOPHORE, A23187. R W Wannemacher, Jr., D L Bunner, and R E Dintzian. U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

A23187 is an antibiotic which has properties as a Ca++ ionophore. Its dermal toxicity and transdermal lethality was assessed in a guinea pig model. For the dermal toxicity studies, a 2-μl sample of varying concentrations of A23187 in DM50 or ethyl acetate was applied to a shaven area of the skin. At 1, 2, 3, 7, and 14 days, the resultant lesions were scored for erythema, edema, or necrosis. Six hours after dermal exposure to A23187, we observed a severe erythema and edema, with a minimal effective dose of 20 ng. A severe necrosis developed by day 3, and the lesion was still detectable by day 14. Various concentrations of A23187 in DM50 (0.1 ml) were applied to the shaven area of skin to assess transdermal lethality. We used a protective barrier to prevent oral ingestion. We did not observe transdermal lethality with doses of A23187 up to 13 mg/kg in DM50. In mice, the minimum lethal dose by the i.p. route was approximately 7 mg/kg, while doses up to 25 mg/kg s.c. were not lethal. Thus, we conclude A23187 is a severe dermal irritant but has a low transdermal toxicity.
DERMAL ABSORPTION OF POLYCHLORINATED DIBENZOFURANS (PCDFs) AND TCDD. Y B Banks-Case, D W Brewster, and L S Birnbaum. NSHS, Research Triangle Park, NC.

PCDFs and polychlorinated dibenzodioxins are toxic environmental contaminants which accumulate in human tissues. In order to examine the potential for systemic exposure following dermal exposure, the absorption, distribution and elimination of 1,2,3,7,8- (1-PCDF) and 2,3,4,7,8-pentachlorodibenzofuran (4-PCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDD), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were evaluated in male F344 rats. The compounds were applied to the back at doses of 0.1, 0.5, and 1.0 μmol/kg in 60 μl acetone and covered with a perforated cap; rats were held in individual metabolism cages for 3 days. The percentage of dose absorbed decreased between 0.1 and 1.0 μmol/kg. Absorption of 1-PCDF, 4-PCDF and TCDD was similar: 22% of the dose at 0.1, 11% at 0.5, 10% at 1.0 μmol/kg. Absorption of TCDD at 0.1 μmol/kg was 44% which was significantly greater than that of the other compounds. Absorption at the higher doses resembled the other compounds. Major tissue deposits for all 4 compounds were liver, fat, skin and muscle. Very little of the absorbed dose of 1-PCDF, 4-PCDF and TCDD was eliminated, however at the low dose of TCDD, 56% of what was absorbed was excreted as metabolite(s) in 3 days. Results indicate that all the compounds are poorly absorbed after dermal exposure and that metabolism is required for elimination.


In vitro percutaneous absorption studies have been undertaken to investigate the development of appropriate surrogate compounds to represent the major classes of compounds found in refinery streams. The dermal penetration of a series of three through five fused-ring FNA has been determined using a modified in vitro procedure using anthracene as a model for 3-fused-ring aromatics and benzo[a]pyrene (BaP) as a model for 4- and 5-fused-ring aromatics. The extent of absorption of nearly all the compounds in both classes fell within a factor of two of the chosen standards. In order to evaluate the "operational window" of anthracene and BaP as surrogates for other FNA, a mathematical model was developed based on structure-activity relationships and multiple linear regression. Molecular descriptors (independent variables) were generated for each of the compounds whose dermal penetration (dependent variable) had been experimentally determined. A model was developed which correlated well with the experimental absorption data. The descriptors that best described the variance in the FNA study set seem to support the suggestion that percutaneous absorption can be assessed in terms of molecular shape and size and other common physicochemical properties.

IN VIVO AND IN VITRO SKIN ABSORPTION OF PCBs. R C Wester, H I Maibach, D A W Bucks, J McMaster, M Mobayen, R Sarason and A Moore. Department of Dermatology, University of California at San Francisco, and the California Primate Research Center at Davis, CA.

PCBs are a major contaminant of the environment. Knowledge of their entry through the skin into the body and subsequent disposition is necessary for estimating human health hazard. [14C]-42% PCBs and [14C]-54% PCBs were separately administered intravenously and applied to rhesus monkeys. Following i.v. administration, 30 day excretion was 39.4±5.9% and 16.1±8.3% of the total feces (total 55.5±5.1%) for 42% PCBs and 70.2±2.1% for 54% PCBs. Skin absorption of 42% PCBs was 20.4±8.3% and 18.0±3.8% in trichlorobenzene. Absorption of 54% PCBs was 20.8±8.3% in mineral oil and 14.6±3.6% in trichlorobenzene. Mineral oil and trichlorobenzene are common PCB vehicles in transformers. Therefore, in vivo skin absorption of PCBs is considered high and disposition from the body is considered low.

In vitro skin absorption in human cadaver skin was not predictive of the in vivo data in the rhesus due to lack of PCB partition from skin to water reservoir fluid, even with addition of 6% oleic 20 globulizer.

DESIGN OF CHEMICALS TO TEST PERCUTANEOUS ABSORPTION (PA) PARAMETERS. D H Gould, US Environmental Protection Agency, Washington, DC.

It is known that the rate of PA (flux) is related to the octanol-water coefficient (logP) of test chemicals; and also to the molecular weight (MW). Thus, for the first few members of a homologous series, the logP and MW increase with each additional -CH2- and so does the flux. However, the increasing size (MW) operates to decrease the PA after a maximum is reached. In a series of phenols and nitrophenols, the maximum flux is reached at logP of 2-2.5. Clearly at some maximum MW and/or logP, the flux will become negligible. To determine the maximum MW which can show PA, a series of polyesters was designed with a monomer core, with a calculated logP increment of 0.15 and a MW increment of 144, malonylpropylnyl. This was capped as nitrate and benzyl esters giving a monomer of MW 357 and ClogP 252. This series leads to the pentamer of MW 933 and ClogP 1.92. Thus, the log P is held relatively constant while the MW almost triples. This should allow determination of MW maximum for PA independent of change of logP.
The in vitro dermal penetration of $^{14}$C-labeled parathion, fenvalerate, carbofuran, and lindane through fresh full-thickness human newborn foreskin was determined at 1, 6, 24, and 48 hours. The pesticides were applied to a constant dosing area (0.031cm$^2$), and a fixed dose (1.13 µg) for each of the compounds studied. Ninety percent, or greater, of the labeled pesticides were recovered in all cases. Carbofuran showed the greatest mean penetration of 82% followed by parathion and lindane with mean penetrations of 79% and 66%, respectively. Fenvalerate exhibited a mean penetration of 9% which is significantly lower than that of the other three compounds. No difference was noted in the penetration of pesticides through human skin from blacks and whites.

The potential for toxicity to result from dermal absorption of benzethonium chloride (BTC) exists because it is widely used in cosmetics, disinfectants, herbicides, and topical antimicrobials. The objectives of this study were to determine the amount and rate of percutaneous absorption, tissue distribution, and elimination of BTC after a single IV administration (0.15 mg/kg) and a single (0.15 or 1.5 mg/kg) or 10-day repeated (1.5 mg/kg) dermal application. Serial blood samples collected for 24 hrs following IV administration of $^{14}$C-BTC were used to estimate bioavailability and determine pharmacokinetic parameters of BTC. Blood samples collected following the single or repeated dermal application of $^{14}$C-BTC were generally undetectable (low dose) or slightly above (high dose) detection limits (5.6 ng/mL). Low levels of $^{14}$C-BTC equivalents were measured in selected tissues from animals sacrificed at 6, 24, 48, 96 or 168 hrs after dosing. Urine and fecal samples were collected at 24 hr intervals after dosing. The urine contained detectable levels (>2.29 ng/mL) and the feces low levels of $^{14}$C-BTC equivalents. These results suggest that BTC penetrates the skin after dermal application. (Supported by Contract No. N01-ES-7-5177 from NTP).

Evaluation of first-pass xenobiotic metabolism in in vitro percutaneous absorption studies requires the use of a receptor fluid which maintains skin viability. The fluid should allow extraction of parent compound and metabolites. Organ culture media (minimal essential media (MEM) ± 10% fetal bovine serum (FBS)), physiological buffers and balanced salt solutions (BSS) were evaluated for these properties. Skin viability was determined through aerobic C14 glucose utilization, maintenance of estradiol and testosterone metabolism for 24 hr and histological appearance of skin. Results show HEPES-buffered Hank’s BSS with 0.1% glucose was equivalent to MEM in maintaining metabolic activity for 24 hr in the diffusion cell at a flow rate of 1.5 ml/hr. Phosphate buffered saline was unable to maintain skin viability as exhibited by declining glucose utilization and steroid metabolism. The exclusion of FBS and other organic compounds from perfusion fluids simplified extraction of parent compound and metabolites and precluded masking of metabolism due to protein binding of steroids. Recovery of the metabolite, estrone, was enhanced 5 fold by exclusion of FBS. Thus a HEPES-buffered BSS is adequate for metabolic maintenance of dermated skin (200 µm) in low-through cell absorption/metabolism experiments.
SENGAR mice are especially susceptible to epidermal tumorigenesis. The basis for this is unknown, but perhaps greater metabolic activation of initiators (Phase I metabolism) and/or decreased detoxication of reactive intermediates (Phase II metabolism) in skin are involved. Thus, metabolic capacity of SENGAR mouse skin was compared with another mouse strain and two rat strains. Benzo(a)pyrene (BP) was applied to skin in flow-through diffusion cells and was measured with metabolites in receptor fluid. Skin of SENGAR mice metabolized substantially more BP to ethyl acetate- and water-soluble metabolites than skin of BALB C mice, Osborne Mendel rats or Fuzzy rats; e.g., in SENGAR mice 73% of BP penetrating the skin was present as ethyl acetate-extractable metabolites while in Fuzzy rats the value was only 18%. In enzyme kinetic studies, SENGAR mouse skin cytosol had substantially more glutathione transferase activity than Fuzzy rat skin cytosol. Apparent Vmax for the conjugation of dinitrochlorobenzene in SENGAR mice was nearly 2.5X that in Fuzzy rats. These results together are evidence that skin of SENGAR mice has substantially more metabolizing capacity than another mouse strain and species; and, that both Phase I and Phase II metabolism is involved.


In exploratory studies using a photohemolysis model, substantial species differences were noted in susceptibility of red blood cells to phototoxics and to UVA light alone. We examined the effects of UVA light (1300 w/cm²) on catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPH) in dog and mouse washed red blood cells. UVA induced hemolysis was more rapid in the mouse than in dog. Control levels of CAT and GPH were approximately 10 and 3 times greater in the mouse than in dog. SOD levels were higher in the dog (2x), however, individual variation was high. Mouse CAT and GPH activity declined rapidly. Enzyme degradation was more pronounced in UVA irradiated samples. Dog CAT was stable for 5 hours but activity was significantly decreased after 3 hours of UVA irradiation. SOD levels were unaffected by UVA irradiation in both mouse and dog. These results suggest that species differences in susceptibility to hemolysis by phototoxics may be due to differences in oxygen scavenging enzyme activities and their inactivation by UVA light. Therefore, in vitro phototoxicity screens from differing species should be interpreted with caution.

Dermal Toxicity of a High Boiling BP 250-450°C Coal Liquefaction Product in the Rat. I Chu1, D C Villetenuve2, M Cote2, V Secours3, R Otten4, and V E Vaill1.1, 2Environmental Health Directorate, Ottawa, Ontario, 3Department of Pharmacology, University of Montreal, Montreal, Quebec and 4Biopath Analysts Ltd, Guelph, Ontario.

Coal liquefaction products (CLP) have been considered as an alternate source of energy to replace conventional crude oil. The present study was designed to investigate the dermal toxicity of a heavy fraction of CLP (bp 250-450°C) in the rat. Groups of 10 male and 10 female Sprague-Dawley rats (180-200 g) were painted dermally with the CLP at dose levels of 0, 100, 200, 400 or 800 mg/kg b.w./day for 6 weeks. Growth suppression was observed in all CLP treated groups of males and in the 2 highest dose groups of females. Increased liver and kidney weights were observed in the diesel fuel (400 mg/kg) as well as CLP treated groups of females. Decreased red cell count, hemoglobin and packed cell volume and mild bone marrow hyperplasia occurred in some CLP and diesel fuel groups of both sexes. Mild histological changes were observed in the thyroid, liver, bone marrow and skin of rats of both sexes treated with CLP and diesel fuel. Based on the data presented, CLP produced systemic toxicity at a dose level of 100 mg/kg b.w., and both CLP and diesel fuel possess toxic effects of a similar nature and magnitude.

The toxicity of DGHE from repeated cutaneous exposure was assessed. New Zealand White rabbits (3/sex/group) were exposed to DGHE by included cutaneous exposure at doses of 0, 100, 300, or 1000 mg/kg body weight/day for a total of 9 applications (6 hours/day) over 11 days. Local clinical signs of toxicity from DGHE included edema, erythema, fissure formation (100-1000 mg/kg), encrustation, and ecchymoses (100 mg/kg). Microscopic changes in the skin included acanthosis, hyperkeratosis (100-1000 mg/kg), suppurative epidermitis (300-1000 mg/kg), hemorrhage (females, 300-1000 mg/kg) and dermal fibrosis (300-1000 mg/kg). Transient depressions in body weight and food consumption (1000 mg/kg) possibly related to the stress caused by the skin lesions, were observed during the first week of the study. No treatment-related effects on hematology, clinical chemistry measurements, or organ weights were observed. Based on this study, DGHE is considered to be a mild skin irritant at 100 mg/kg/day and a severe skin irritant at 1000 mg/kg/day. However, short-term cutaneous exposure to DGHE did not cause systemic toxicity in this species.
The degree and course of irritation from dermally applied acrylic acid (AA) at 0%, 1%, and 4% in acetone were assessed in 3 strains of mice. For each level, groups of 30 male ICR, 30 male C3H and 30 female B6C3Fl mice were treated 3 times weekly for 13 weeks, with skin irritation graded daily. Five mice per group were necropsied after weeks 1, 3, 4 and 8; the remaining mice after week 13. Four percent (4%) AA in acetone caused significant skin irritation in ICR, C3H and B6C3Fl mice. Desquamation, fissingure, and eschar were observed with 4% AA after 1 or 2 weeks (depending on strain), and continued through week 13, peaking between weeks 3 and 5. Little or no gross irritation occurred in any strain dosed with 0% (acetone only) or 1% AA. Microscopic findings of proliferative, degenerative, and inflammatory changes in the epidermis and dermis were seen in all strains dosed 4% AA, beginning at week 1 and continuing through week 13 with little change in severity. A low incidence of minimal proliferative changes were noted at 1% AA, but not in control animals. Skin irritation and histological changes caused by AA did not differ significantly among three different strains of mice.

TEA and DEA are amine alcohols frequently included in cosmetic preparations as emulsifiers and thickeners. Twelve doses of TEA (unlabeled) or DEA (in ethanol) were administered to the interscapular region of F344 rats or B6C3Fl mice during a 16-day period. Dosages ranged from about 0.1 to 2.0 g/kg body weight in rats and 0.2 to 3.0 g/kg in mice. Administration of TEA did not cause any deaths. Necrotizing inflammation of the epidermis and dermal fibrosis were observed at the site of application in rats of both sexes (but not mice) at doses of 0.6 g/kg and above. DEA caused mortality in the highest dose groups of rats and mice. Chronic active inflammation, ulceration, and acanthosis of the epidermis were observed at the site of application in rats (0.5 g/kg and higher) and in mice (at 1.25 mg/kg and higher). Acute acanthosis was also present in mice in the lower dose groups. Renal tubular necrosis and necrosis of the seminiferous tubules were observed in high dose rats. These data indicate that rats are more susceptible to ethanolamines than mice, and that systemic toxicity can result from dermal DEA administration. (Supported by Contract No. N01-ES-45068 and N01-ES-65148 from NTP.)

Thirteen week studies were conducted by applying 1,2-dihydro-2,2,4-trimethylquinoline (monomer) in acetone to rats (0, 20, 50, 100, and 200 mg/kg) or mice (0, 2.5, 5, 10, 20, and 50 mg/kg) by skin-paint (1x/da., 5 da/wk). All animals survived to the end of the study. Male rats treated with 200 mg/kg had reduced body weights (7%) and increased liver/body weight (BW) ratios. Thymus to BW ratio in male rats was increased at the 20 mg/kg and greater doses. Female rats had dose-related increased liver/BW and kidney/BW ratios. Histopathology revealed treatment related lesions of the skin in rats at all doses. Hepatocellular vacuolization was observed in 200 mg/kg treated male rats. For mice, there were no significant decreases in survival, body weights, or organ weights due to treatment. Body weights of female mice were increased in the 2.5 mg/kg and greater dose levels. Treatment related skin lesions at the dose site were observed microscopically in all dose groups of mice. Based on these results, long term toxicology and carcinogenesis studies in F344 rats and B6C3Fl mice and study designs to determine the initiation and promotion potential of TMQ are proposed in male F344 rats and female SENCAR mice.

Animal skin irritation testing is normally conducted following governmental regulatory agency procedures. Often more than one test must be conducted in order to meet the various requirements. Test in methods were compared with identical human tests to evaluate potential alternatives to the current regulatory procedures. Variables evaluated in the study were 19 mm Hill Top chambers vs. standard gauze patches (DOT procedure), intact vs. perforated chambers, and time of exposure (1 and 4 hours). Variability in the irritation responses produced in both species was attributed to subject susceptibility, and not procedural factors. Most rabbit responses were conservative estimates of human test responses. The use of Hill Top chambers or equivalent offers the potential to 1) reduce the number of animals used for skin irritation screening (smaller group size and up to eight test substances/concentrations per animal), 2) eliminate the need for conducting multiple tests to satisfy governmental requirements and 3) reduce animal stress by reducing exposure times. It appears that these benefits could be achieved without compromising the value of the irritancy patch test for making human dermal safety judgments.
SUPPLEMENTING the diet with Vitamin A acetate (VAA) enhances the ability of laboratory mice to develop delayed contact hypersensitivity. We evaluated the effect of VAA on irritant inflammation in unsensitized mice. One ear of groups of five to six female Balb/c mice, maintained on normal diet or diet supplemented with 0.477g/kg VAA, were dosed with 25 ul of the irritants in a 1:1 acetone:corn oil mixture. Six concentrations of 1 chlo-ro-2.4 dinitrobenzene (DNCB), 1 chlo-ro-2.4.6 trinitrobenzene (TNB), and 4-ethoxyethylene-2 phenyloxazoline (oxazolone) were tested. Inflammation was quantified as change in ear thickness measured with a micrometer at 1, 2, 4, 6 and 24 hours after application of the irritants. Ear thickness changes in animals maintained on VAA diet were significantly greater than controls dosed with DNCB or TNB. Early thickness changes produced by oxazolone were not different but 24 hour responses were significantly greater in animals fed VAA. Histology of responses and expression of immune response antigens in ear tissue of animals fed each diet were compared to investigate the mechanism of differences in irritant responses to DNCB, TNB and oxazolone.

MODIFICATION OF THE NON-IMMUNOLOGIC CONTACT URTICARIA PREDICTIVE ASSAY IN GUINEA PIGS
H I Maibach and E. Patrick, Department of Dermatology, University of California, San Francisco, CA.

The predictive assay for non-immunologic contact urticaria (NICU) in the guinea pig used change in ear thickness to evaluate the inflammatory response. A significant increase in ear thickness was evidence for a positive response. We performed assays of 27 consumer products and ingredients, not previously shown to produce urticaria. Evaluation by change in ear thickness measured with a micrometer and visual grading for the presence of discrete lesions and vasodilation were compared. Ears of ten 300-400 gm Hartley strain guinea pigs were dosed with 0.1 ml of the test material; the opposite ear was dosed with a suitable control. Thickness measurements and visual examinations were performed 15, 30, 45, and 60 minutes and 24 hours after application of the test materials. No significant change in ear thickness was produced by any of the test materials. Seven of the 27 materials produced visible lesions which were reproducible on the same animals when retested at 48-96 hours. Human testing or use tests have confirmed that three of the seven materials, visually positive in the NICU, produce urticaria in man. This comparison demonstrates that incorporating visual evaluation by a trained observer will increase the sensitivity of the NICU assay.

CORRELATION OF AN IN VITRO KERATINOCYTE MODEL WITH THE RABBIT PRIMARY DERMAL IRRITATION MODEL

Studies were conducted to determine the feasibility of using an in vitro system as a predictor of dermal irritation potential. Commercially obtained normal human epidermal keratinocytes were grown in the presence of various concentrations of ten chemicals. These chemicals were selected for their known effect in vivo, ranging from non-irritating to severely irritating on intact rabbit skin. In vitro toxicity was determined by the inhibition of DNA or protein synthesis as measured by either the incorporation of 3H-thymidine or 3H-leucine, respectively. Parallel studies with the same chemicals were conducted using the standard in vivo rabbit primary dermal irritation test.

The test compounds were ranked in terms of severity. The preliminary results indicated a good correlation between the in vitro and in vivo tests. We are encouraged by the possibility that this in vitro system offers an alternative to the animal assay for identifying potential dermal irritants.

CONTACT SENSITIZATION FOLLOWING APPLICATION OF A PYRIDOSTIGMINE BROMIDE TRANSDERMAL DRUG DELIVERY SYSTEM
G. L. Harris, J. L. Allen, and H. I. Maibach. Pathology/Toxicology Department, Riker Labs/3M Co. St. Paul, MN and University of California, San Francisco, CA.

Pyridostigmine bromide (PB), is a useful pre-treatment for organophosphate poisoning. PB transdermal formulations were developed since this route provides more consistent blood levels than oral administration. The formulations contained 30 or 50% PB in a gel matrix and some also contained a surfactant (eg, 0.2% sodium laurel sulfate) as a penetration enhancer. Guinea pig sensitization studies (Split Adjuvant Method) were conducted as part of this formulation's safety evaluation. Eleven groups of 10 animals were induced and challenged with combinations of PB and excipients such that the sensitizers could be identified. Four of nine animals induced with 50% PB alone had positive responses to a PB challenge dose. Most animals induced with PB-surfactant had positive responses to PB and PB-surfactant formulations. Surfactants appeared to potentiate the response since the incidence of sensitization was greater. PB is structurally similar to quaternary ammonium compounds which are known sensitizers. Thus, PB may represent an example of contact sensitization from a cationic tertiary ammonium compound. This work supported by the U.S. Army, but the findings and conclusions are not necessarily the official position or policy of the U.S. Army.
512  G. W. Trimmer and J. J. Freeman. Exxon Biomedical Sciences, Inc., East Millstone, NJ

The skin sensitizing potential of five chemicals was assessed in guinea pigs using both the Maximization Test (MT, Magnusson and Kligman, J. Invest. Dermatol. 52:266, 1969) and the Closed Patch Test (CPT, Duelli et al. Arch. Dermatol. 97:17, 1963). The test materials included two unknowns ("A", magnesium alkylphenol sulfide and "B", 3,4,5-trichloropyridazine), two sensitizers ("C", formalin and "D", mercuribenzoalbumin) and a primary irritant ("E", sodium lauryl sulfate). Female Hartley albino guinea pigs (15/group, 310-470 g) were used for both procedures. Dermal responses were graded 24 and 48 hr after challenge (and after rechallenge) according to the method of Draize. The average rate of positive responses (Test values—concurrence irritation control values) in the MT and CPT, respectively, were: "A": 0%, 9%; "B": 93%, 51%; "C": 71%, 78%; "D": 97%, 25%; "E": 10%, 0%. Stronger responses were observed for "A" (24%) and "D" (53%) after re-challenge in the CPT. The evidence of sensitization was observed for "E" in either assay or for "A" in the MT; "A" appeared to have sensitizing potential in the CPT (but has tested negative in a human patch test) and "E", "C" and "D" exhibited sensitizing potential in both assays. In addition, the sensitizing potentials of "B" and "D" were more readily detected with the MT.

513  E. I. Fort and R. V. Kotz. Abbott Laboratories, Abbott Park, IL

Euthymic hairless, Crl: IAF(Wa)HR, and Hartley albino guinea pigs were compared to determine the suitability of hairless guinea pigs as a model for investigation of phototoxicity. 8-Methoxypsoralen (8-MOP) given intraperitoneally was used to induce phototoxicity. Hartley guinea pigs were shaved and treated with a depilatory 24 hrs. prior to treatment. Groups composed of 2 animals per sex each of hairless or martley guinea pigs were given a single treatment: no treatment, UV (ultraviolet) irradiation only, 50 mg/kg 8-MOP plus UV, or 10 mg/kg 8-MOP plus UV. UV irradiation was a 30 min. exposure to a GE L4 Sunlamp positioned 15 inches above the animals beginning 1 hr. after injection of 8-MOP. The skin of the back and ears was evaluated for phototoxicity (erythema) at 1, 2, 3 and 5 days after irradiation. Hairless and Hartley guinea pigs proved to be equally sensitive to phototoxicity under the conditions used. Hairless guinea pigs offer the advantages of 1) eliminating the need for hair removal prior to irradiation or evaluation of the erythema response, thus avoiding the potential for interference with the erythema response and 2) when an erythematous response was observed on the back, a definite line of demarcation separating the exposed and unexposed skin facilitated grading the response.

514  A. C. Johnson & Son, Racine, WI

The FHSRA test for eye irritation, using the Draize technique, has been used for many years as the industrial standard for assessing potential irritancy of test materials. The assay uses a dose of 100 μl. The scoring results in a number of time dependent eye scores which creates complex data relationships. The present study integrates the time dependent data by using a simple time-weighted average over 7 days by applying Simpson's rule. This method produces a single value which improves comparisons and enables rankings between different products and different studies on similar products. It is our experience that a reduced dose of 30 μl can improve discrimination between the potential eye irritancy of new formulas. Thus, 30 μl of each of 15 marketed shampoos was instilled into rabbit eyes without washing and standard Draize readings were performed. The shampoos were ranked according to this new integration method. Irritation comparisons were made by observing the shape of time dependent plots and time-weighted average scores ranging from 0.4, non-irritant, to 35.0, a severe irritant. In all cases, the time-weighted averages provided simplified, single-figure comparisons of eye irritation. This new method enables a graphic presentation of complex eye irritation scores that can be easily understood by non-specialists.


Concern for the use of animals in safety testing must be balanced with the need to ensure the safety of new products and compliance with regulatory requirements. To meet these needs an alternative to the in vivo Draize eye irritation test was investigated which uses the choroidalantiotic membrane (CAM) of a fertile chicken egg. Various methods have been published using this membrane but have shown limited correlation with in vivo. One only report addressed the application of their method to predicting regulatory labelling requirements for eye irritation (Kong, B. N. et al. In Vitro Toxicology - Approaches to Validation. Vo. 5, A. N. Goldberg (Ed.) M. A. Liebert Inc., NY (1987)). However, a shortcoming of the CAM assay was the occurrence of false-positive reactions. Investigating the time course of the CAM response to irritants suggested that the initial event of hemorrhage could be used as an endpoint. The time of onset and the incidence of hemorrhaging were related to the ocular irritancy of the test material. Determining the incidence of CAM hemorrhaging at 30 min. after treatment resulted in a better separation of irritants from non-irritants. This modified CAM assay also offers the advantages of a shorter assay completion time, a more easily defined endpoint and the use of a less developed.
REDUCTION IN THE NUMBER OF RABBITS USED TO ASSIGN EYE IRRITATION CLASSIFICATIONS WITH CORNEAL PACHYMETRY. R L Morgan, S S Sorenson and I R Castles. Stauffer Chemical Co. Toxicology Department, Richmond, CA.

Reduction of the number of rabbits used for an eye irritation test is important for animal welfare considerations and cost effectiveness. Corneal pachymetry has been shown to be an accurate method for assigning EPA and EEC eye irritation classifications. Due to the precision inherent to pachymetry it was reasonable to assume that a smaller number of rabbits per test could be used to reliably assign eye irritation classifications. To test this hypothesis, a variety of materials were tested for eye irritation. Each material was tested in a group of seven to eight rabbits and in a group of three rabbits. Both left (treated) and right (control) eyes were evaluated for irritation and corneal thickness each day for three days. EPA and EEC classifications were assigned using the corneal thickness ratios (left corneal thickness/right corneal thickness). Approximately 85% of the irritation classifications obtained with 3 rabbits were in agreement with or 1 grade higher than those obtained with a minimum of 7 rabbits. The mean coefficient of variations for the ratios were 19% and 10% for the tests using three rabbits and 7 to 8 rabbits, respectively. From these results corneal pachymetry has been shown to accurately assess eye irritation with approximately a 50% reduction in the number of rabbits required.

MULTISPECIES COMPARISON OF CORNEAL LESIONS PRODUCED DURING A 2-WEEK VAPOR EXPOSURE TO PROPYLENE GLYCOL MONOETHYL ETHER (PGE). D R Kennon, D F Dodd, R Ballantyne, and F E Losco. Rody Run Research Center/Union Carbide Corp., Export, PA.

A previous 2-wk study in male and female F-344 rats indicated the cornea to be the primary target organ from exposure to PGE vapor. The present study evaluated the potential ocular toxicity in male Sprague-Dawley rats (SDR), F-344 rats (FR), Hartley guinea pigs (HGP), and New Zealand white rabbits (NZW). Exposures were 6 hr/d, for 9 days during a 2-wk period to mean concentrations of 0, 105, 486, or 1824 ppm PGE. At 1824 ppm ocular irritation, ataxia, and narcosis occurred in all animals except HGP; 3 NZW died; FR had opacities, necrosis, and vascularization of the cornea, with stromal mineralization, splitting, and fibrosis; SDR had corneal opacities and keratitis; NZW had opacities (transient), conjunctivitis, keratitis, and corneal degeneration; HGP had no lesions. Similar ocular lesions were observed in FR, SDR, and NZW at the lower PGE concentrations, but at a lesser incidence. Only lesions (mineralization, vascularization, stromal splitting and fibrosis) found in the FR were considered to be nonreversible. The results of this study indicate that the FR is the most sensitive species with regard to eye lesions produced by exposure to PGE.

EFFECTS OF BETA-ADRENERGIC AGONISTS ON THE RETINA OF RATS AND HAMSTERS. G R Lankas and H L Allen. Merck Sharp & Dohme Research Laboratories, West Point, PA. Sponsor: R T Robertson

Clameterol and L-644,469 are potent, non-selective beta-adrenergic agonists with significant growth-promoting activity. Both compounds produced a focal retinopathy in hamsters, but not in pigmented or albino rats after 4 weeks of treatment at dosage levels > 0.5 mg/kg/day. These lesions were characterized as a focal loss of rods and thinning of the retinal outer nuclear layer. Subsequent studies showed that these lesions could be inhibited by coadministration of propranolol, a beta-adrenergic blocking agent, implicating beta-receptors in the induction of the retinopathy. To determine if the hamster was unique in the accumulation of drug in the retina compared to rats, 14C-L-644,469 was given IP to both rat strains and hamsters. Autoradiographic images made 3 hours after dosing showed that despite marked accumulation of drug in the pigmented ocular tissue of both rats and hamsters, retinopathy was found only in hamsters. No drug accumulation or lesions were found in albino rats. These studies indicate that the retinopathy induced in hamsters by beta-agonists involves more than just accumulation of drug in the retinal pigmented layer.


We have recently reported that a portion of butyl 2-chloroethyl sulfide (BS), a potent vesicant and a bifunctional analog of bis-2-chloroethyl sulfide (vultur mustard) which comes in contact with skin remains free to disperse through various tissues and organs, including the retina and lens of the eye. Because of the potential for occupational exposure and our concern to establish an effective antidote, we studied metabolic and histologic changes in the eyes of rats systemically exposed to BS. Young rats were injected (ac) with 20 ul of neat BS. At various times (24 and 48 hrs) after injection, rats were killed, eyes were excised, dissected, and prepared for histologic and biochemical evaluation. Treated rats had multiple foci of dark, angulated, and vesiculated cells in the ganglion cell layer of the retina. Photoreceptors had an increased number of autophagosomes and disarrayed outer segments. Endothelial cells of the optic nerve and choroidal vessels were elevated from the retina and aggregates of activated platelets were present in the lumens. There was an increase in lipid peroxidation during the first hr which returned to values for untreated rats at 4 hr after injection. The results of this and our previous study show that systemic administration and perhaps cutaneous penetration of BS, produce metabolic and ultrastructural changes in the eye.

Keratoconjunctivitis Sicca (KCS) is an inflammatory eye condition affecting the cornea and conjunctiva, caused by the deficiency in the aqueous fraction of tears. A number of sulphophenamides including salicylazosulphapyridine (SA) have been associated with KCS in the dog. KCS has been noted in a 1-year dog toxicity study with the non-sulphonamide 5-aminoisalicylic acid (5-ASA), with females being more affected than males (Incidence: Males 12%, Females 71%).

There was a close correlation between KCS and reduced lacrimation. Primary atrophic changes were observed in the lacrimal and nictitating glands and parotid salivary gland of treated dogs. These changes were associated with lymphoid cell infiltration suggestive of a cell-mediated immune reaction. Lesions were also present in the cornea, eyelids and nictitating membranes. In contrast to the effects noted in dogs, there have been no reports of KCS in man following SA administration (the prodrug of 5-ASA) during four decades of clinical use. Therefore, it is highly probable that the dog is not a predictive model for man for this effect.

DEFEROXAMINE ON ANOXIA-INDUCED INJURY IN RAT RENAL SLICES. W Hewitt and A Silver. Smith Kline & French Labs, King of Prussia, PA.

Deferoxamine (DEF) has been shown to reduce the renal dysfunction in rats produced by ischemia/reperfusion. It is unclear if DEF acts directly on renal tubular cells or on extrarenal cells (e.g., neutrophils). The objective of this study was to determine if DEF prevented proximal tubular dysfunction produced by an in vitro anoxic insult. Longitudinal renal slices prepared from male CD rat kidneys were incubated at 37°C. One-half of the slices were subjected to a 45-min anoxic (100% N₂) period followed by 8 hr of reoxygenation (100% O₂); control slices were incubated continuously under 100% O₂. Proximal tubular function was estimated hourly during reoxygenation by slice organic ion (PAH, TEA) accumulation; viability was assessed by LDH leakage. Malondialdehyde (MDA) generation was used as an index of lipid peroxidation. PAH accumulation by slices subjected to anoxia was reduced 40% and 60% after 1 and 8 hr of reoxygenation, respectively; TEA uptake was reduced approximately 30% after 8 hr reoxygenation. LDH leakage and MDA generation increased in a time-related fashion and were greater in slices exposed to 100% N₂. DEF (1 or 200 µM) did not ameliorate the anoxia-induced alterations in slice organic ion accumulation, LDH leakage or MDA generation. Thus, DEF may not exert a direct protective effect on renal cells damaged by anoxia/reoxygenation.


The mechanism by which subchronic feeding of DPT causes renal cysts and the observed changes in renal enzyme activities is unknown. Because DPT is metabolized in vivo, we examined the ability of DPT and its para-hydroxylated metabolites to alter various enzyme activities in vitro. Using enriched preparations of mouse liver microsomes and cytosol, the microsomal and cytosolic forms of EH were assayed using the specific substrates styrene oxide and trans-stilbene oxide, respectively. cEH was inhibited by the 4-hydroxy and 4,5 dihydroxy metabolites, but not the 3-hydroxy or parent compound. In contrast, only the 4-hydroxy form inhibited mEH. 5-hydroxy DPT actually stimulated mEH activity. When the known renal cystogens nordihydroguaiaretic acid and diphenylamine were tested, only inhibition of cEH was observed. The hydroxylated metabolites of DPT also inhibit catalase activity in a hydrogen peroxide-facilitated manner. Thus, metabolites of DPT share with two known cystogens the capability of directly altering the activity of cEH in a manner consistent with pro-oxidative mechanisms of cellular stress. (Supported by PHS grants AM33003 and CA34455.)

RENAL CYSTEINE CONJUGATE θ-LYASE (θ-LYASE)-MEDIATED TOXICITY STUDIED WITH PRIMARY CULTURES OF HUMAN PROXIMAL TUBULAR CELLS (HPTC). J C Chen, J L Stevens, and T W Jones. Univ. of MD School of Medicine, Balto., MD and W. Alton Jones Cell Science Center, Lake Placid, NY.

θ-Lyase-mediated, S-cysteine conjugate-induced nephrotoxicity has been described in a variety of in vitro and in vivo animal models. In this study, we have extended these observations to include human by investigating the toxicity of S(1,2-dichlorovinyl)-glutathione (DCVG) and S(1,2-dichlorovinyl)-L-cysteine (DCVC) to HPTC. Primary HPTC were treated overnight with either DCVG or DCVC at concentrations ranging from 100 µM to 1.0 mM. In each case, a dose-dependent toxicity was seen with both DCVG and DCVC. Despite a wide degree of variation in sensitivity observed between cases, DCVC was consistently found to be more toxic than DCVG. However, inclusion of glycylglycine (10 mM) or γ-glutamyltranspeptidase (0.5 U/ml) increased the toxicity of DCVG to that of an equimolar dose of DCVC, indicating that metabolism to the cysteine conjugate is an important rate-limiting step. Aminoxyacetic acid (250 µM), an inhibitor of pyridoxal phosphate-dependent enzymes such as θ-lyase, provided complete protection, suggesting a critical role for θ-lyase in the HPTC toxicity of DCVG and DCVC. (Supported by ACS BC-570.)
A decline in the viability and functional capabilities of isolated RPT limits their utility for many in vitro studies. Tubules were isolated from adult male F-344 rats by a one-step collagenase perfusion method and were incubated in Waymouth's 752/1 + 2% BSA + 5 mM lactate in gas-tight flasks with an atmosphere of 95%, 19%, or 10% O2:5% CO2:balance N2. Tubule viability was determined by measuring lactate dehydrogenase (LDH-R). LDH-R at 4 hr increased progressively with higher O2 tensions. Butylated hydroxytoluene (0.1 mM) in the medium of RPT incubated with 95% O2 decreased LDH-R significantly from 26.6 ± 2.6% to 11.2 ± 1.3%. Deferoxamine (DEF; 0.1 mM) decreased 4-hr LDH-R (27.0 ± 3.2% to 22.4 ± 1.5%) and ascorbic acid (1.0 mM) was ineffective. Modification of the perfusion buffer significantly improved tubule quality. LDH-R at 4 hr was 10.6 ± 1.7% vs 21.0 ± 4.7% for the previous best isolation method. The basal rate of O2 consumption decreased about 10% in 4 hr compared with the change in viability. Nystatin stimulation of O2 uptake was maintained for 2 hr and decreased 15-20% between 2 and 4 hr. (Supported by NIEHS contract ES-65145.)

Some cysteine conjugates are potent nephrotoxins. Metabolism by a renal β-lyase is thought to mediate the toxicity of some of these conjugates. There is evidence to suggest that mitochondria are the ultimate site of toxicity. We have studied the inhibition of α-ketoglutarate/malate (site I) and succinate (site II) stimulated respiration in rat kidney mitochondria by pentachlorobutadienyl- (PCBDC), dichlorovinyl- (DCVC), tetrafluoroethyl- (TFEC), chlorotrifluoroethyl- (CTFE), and hexafluoropropyl-L-cysteine (HFPC). The IC50 (concentration which gives 50% inhibition) values for site I respiration are 65 ± 11 μM, 76 ± 26 μM, 191 ± 80 μM, 265 ± 67 μM and 979 ± 203 μM for PCBDC, DCVC, TFEC, CTFE and HFPC respectively. For site II the IC50 values are 45 ± 8 μM, 236 ± 40 μM, 216 ± 81 μM, 236 ± 72 μM and 779 ± 142 μM. (Aminoxy)acetic acid, a β-lyase inhibitor, blocks the toxicity. Only PCBDC was shown to uncouple oxidative phosphorylation. DCVC shows a significant difference in inhibition of site I vs. II stimulation. The other conjugates inhibit both sites to about the same extent. The data may suggest more than one mechanism for the mitochondrial toxicity of cysteine conjugates. Metabolism by β-lyase will be discussed with regard to inhibition of mitochondrial respiration.

The effect of the fungal metabolite, OTA, on the transport of p-[1H]aminohippurate (PAH), a prototypic organic anion was examined in canine renal brush border (BBMV) and basolateral membrane vesicles (BLMV). OTA was an effective inhibitor of PAH transport in both membranes as was probenecid, a competitive blocker. The IC50 values for PAH inhibition by OTA in BBMV and BLMV were 20 μM and 32 μM, respectively. The effect was specific since the transport of the organic cation N-methyl-nicotinamide was not affected. The phenomenon of counterflow was studied to evaluate PAH translocation. OTA produced trans-stimulation of PAH transport in both BBMV and BLMV. The observation that OTA produced trans stimulation of PAH transport is conclusive evidence that it is translocated across both of these membranes. The data suggest that the reason OTA accumulates in the kidney is because it is a substrate for the renal organic anion transport system. (Supported by NIH 502835.)

Benazepril is a structurally novel nonsulfhydryl inhibitor of ACE. Toxicology studies demonstrated that Benazepril is well tolerated in mice, rats, and dogs at doses well in excess of those needed for effective ACE inhibition. Acute toxicity was low in all three species. Clinical signs appeared in the rat at 3000 mg/kg and in the dog at 250 mg/kg. In toxicity studies of 2-3 weeks duration, doses as high as 100 and 90 mg/kg were considered to be nontoxic in the rat and dog, respectively. Thirteen week rat and dog toxicity studies revealed no drug-related toxicity at high multiples (up to 75x) of the intended human dose. Chronic administration for 6-12 months to rats and dogs produced no unexpected findings or adverse target organ changes. Minimal and mostly reversible compound-related effects were observed in rats and/or dogs after repeat exposure, and included: reductions in body weight, erythrocytic parameters, and heart weight; increases in serum BUN; and microscopic evidence of hyperplasia and/or hypertrophy of renal juxtaglomerular cells, and hypertrophy of afferent arteriolar and interlobular arterial walls. The majority of these findings were pharmacologic in nature and have been previously reported for other ACE inhibitors.


The nephrotoxicity of D- and L-arginine was investigated. Each enantiomer was infused at 6 g/kg over 75 min into the caudal vein of eight male rats. Kidney function was evaluated by measuring several constituents of serum and urine. Structural changes were assessed by light and electron microscopy. Both D- and L-arginine caused changes in kidney function. Urinary protein and glucose were increased and urinary pH was decreased. Glomerular filtration rate was reduced as evidenced by reduced creatinine clearance, and increases in serum urea and creatinine. In addition, half of the rats treated with D-arginine but none treated with L-arginine had marked increases in urinary malate dehydrogenase activity, probably indicative of injury to epithelial cells of the proximal tubules. D-arginine caused obvious swelling and vacuolation of the epithelial cells of the proximal tubules, but L-arginine caused less obvious changes. In conclusion, D-arginine was moderately nephrotoxic, based on tests of kidney function and structure, while L-arginine caused less serious changes.

THE TOXICITY OF 2-METHYL-1,4-NAPHTHOQUINONE (M) AND TWO THIOETHER CONJUGATES. P C Brown and J W Jones. Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

The cytotoxicity of M has been associated with depletion of cellular thiols due to direct covalent interaction and oxidation as a result of redox cycling. Excretable thioether conjugates represent important quinone detoxication products. However, since the quinone nucleus remains intact, these conjugates may pose the threat of oxidative damage to tissues involved in their elimination. In the present study, the toxicity of M and its glutathione (GSH) and N-acetyl-L-cysteine (MNAC) conjugates was studied using subcellular fractions and freshly isolated renal epithelial cells (IREC). Unlike M, GSH and MNAC did not react with soluble or protein thiols in a cell-free system indicating a lack of alkylating potential. However, all three compounds were capable of redox cycling in the presence of renal microsomes (MMNAC-MG) or mitochondria (MMNAC-MG). All three compounds were cytotoxic to IREC (MMNAC-MG). In all cases, cell death was associated with a depletion of soluble and protein thiols. M and MNAC resulted in a similar degree of cell death at 2 hr, but M caused more rapid initial cell killing and was associated with a larger reduction of thiols. These results indicate that the thioether conjugates of M are toxic to IREC by a mechanism involving oxidative stress.

STUDIES OF METHYLCYCLOHEXANE INDUCED NEPHROTOXICITY AND METABOLISM IN MALE FISCHER 344 RATS. M J Parmell, G W Henningssen, K O Yu, M P Servie, and G M McDonald. Harry G. Armstrong Aerospace Medical Research Laboratory, Toxic Hazards Division, Wright-Patterson AFB, OH.

Methylcyclohexane (MCH), a common solvent and a component of many fuels, was examined for its ability to produce a type of nephropathy characteristic of branched chain and cyclic hydrocarbons. Additionally, in a continuing effort to understand what effects specific hydrocarbon metabolism may play in the pathogenesis of this nephrotoxic syndrome, MCH urinary metabolites were isolated and identified.

Fischer 344 male rats were dosed with MCH over a 14 day period. Histopathologic examination of the rat kidneys revealed only a minimal amount of renal damage. Forty-eight hour rat urine metabolites of MCH identified were cyclohexylmethanol, t-3-methylcyclohexanol, t-4-methylcyclohexanol, 2'-hydroxy-4'-methylcyclohexanol, 2'-hydroxy-4'-methylcyclohexanol and 2'-hydroxy-4'-methylcyclohexanol.

Availability of Na$_2$S$_2$O$_3$ for mitochondrial rhodanese is the rate-limiting factor for the detoxification of cyanide to thiosulfate (SCN$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$$^-$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The nontoxicity of 2,2,4-trimethylpentane (TMP) in male rats is characterized by an increase in protein droplet formation and renal concentration of α2u. These changes correlate with an increase in cell proliferation noted in male rat kidney after TMP exposure. TMP-OH has been identified as the metabolite of TMP reversibly bound to a protein fraction containing α2u isolated from TMP-treated male rat kidney cytosol. To investigate whether TMP-OH is bound specifically to α2u and not other low molecular weight proteins, α2u was purified from [3H]TMP-treated male rat kidneys using gel filtration and ion exchange chromatography. Purified α2u, identified using SDS-PAGE (18,000 Da) along with Western blot analysis (anti-α2u), co-eluted with TMP-derived radiolabel when injected onto an HPLC anion exchange column. To evaluate whether TMP-OH could potentially bind to other low molecular weight proteins, in vitro binding studies were carried out. [14C]-TMP-OH was incubated with either purified α2u (untreated male rats), β-lactoglobulin (β-lact), β2-microglobulin (β2-mic), α-glyco which are members of the same family of proteins as α2u. There was no detectable binding of [14C]-TMP-OH to unrelated proteins such as β2 or α-glyco. Although TMP-OH binds to proteins in the same family as α2u in vitro, only the protein found to be associated with radiolabel derived from [3H]-TMP-treated male rat kidney cytosol was α2u.

2,2,4-Trimethylpentane (TMP) causes an increase in the renal concentration of α2u, a male rat-specific low molecular weight protein. A reversible association between a metabolite of TMP and α2u has been observed in kidney cytosol of TMP-treated male rats. This study assesses protease hydrolysis of [14C]-α2u, with or without the bound TMP metabolite. Male F-344 rats were treated (po) with TMP (500 mg/kg) or corn oil at 0, 3, 6 and 9 hr, and [14C]-labeled amino acids (ip) at 2.5, 5.0 and 7.5 hr. [14C]-α2u was isolated from kidney cytosol using gel filtration and ion exchange chromatography. Hydrolysis was performed using a mixture of purified proteases (PP), proteinase K (PK) or kidney (untreated male rats) lysosomal enzymes (LE) at their respective optimum pH. [14C]-α2u was incubated at 37°C for 2 hr with or without the PP, PK or LE. The reaction was stopped by addition of perchloric acid, and protein hydrolysis assessed by measuring the radioactivity in the supernatant. [14C]-α2u from both control and TMP-treated rats was poorly hydrolyzed by PP or LE, and moderately hydrolyzed by PK. However, both [14C]-high and low molecular weight hepatic proteins were hydrolyzed by PP, PK and LE. These results show that α2u is resistant to protease hydrolysis. Under these in vitro conditions, α2u hydrolysis was not altered by the binding of the TMP metabolite.

COMPARISON OF ALPHA-2U GLOBULIN ISOLATED FROM THE URINE OF ALBINO AND NON-ALBINO MALE RATS. T E Eurell, M J Parnell*, and G M Henning*., Dept. of Vet. Biosci., Univ. of IL, *AMRL, Wright-Patterson APF, OH, and**NIOSH-DBS, Cincinnati, OH.

Alpha-2u globulin has been associated with the hydrocarbon-induced proximal tubular cell degeneration reported in the male rat kidney. The Fischer 344 strain has been used most often in these studies, and may be particularly susceptible to the toxic effect. Alpha-2u globulin was isolated from the urine of albino (Fischer 344 and Sprague Dawley) and pigmented (Long-Evans and Fawn-Hooded) male rats for protein characterization by isoelectric focusing techniques. An alpha-2u globulin isoelectric variant profile distinguishing albino from non-albino male rats was not apparent, however, strain differences were revealed. Fischer 344 male rats appear to have higher levels of the (pI)=5.4 and 5.5 isoelectric variants than the other strains studied. These findings suggest that if a strain susceptibility to the hydrocarbon-induced nephrotoxic lesion exists, it may be associated with the alpha-2u globulin isoelectric variant profile. (Supported by APOS grant # 86-0313.)

LOCALIZATION OF α2u-GLOBULIN WITHIN RENAL PROTEIN DROPLETS OF MALE RATS EXPOSED TO 2,2,4-TRIMETHYL-PENTANE (TMP). V L Burnett, B G Short, J A Swenberg, Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

A novel immunohistochemical technique was developed to assess the relationship between protein droplet formation and α2u-globulin accumulation induced by light chain hydrocarbons in the male rat kidney. This procedure utilized a mouse monoclonal antibody to localize α2u within the kidney. Seventy hours after oral dosing of male rats with 50 mg/kg TMP, kidneys were perfusion-fixed, cold processed into glycol methacrylate, and cut into 2u sections for staining. Results showed that localization of α2u stained by immunohistochemistry corresponded to the localization of protein droplets seen in serial sections stained with Lee's methylene blue-basic fuchsin. Quantitative morphometry by image analysis showed the area of staining of both renal α2u and protein droplets of the treated animals increased 1.5- to 2-fold over respective controls, which was comparable to the increases seen using biochemical and morphological evaluations. Thus, by using this immunohistochemical technique, a strong correlation between protein droplet formation and α2u accumulation has been observed in control rats and rats treated with TMP.

Young male rats treated with certain hydrocarbons, including gasoline, develop nephropathy characterized by accumulation of HD in cells of the renal proximal tubules (PCT). However, no information is presently available on possible age-dependent susceptibility to HD accumulation. PCT of young (3 mo.), untreated, male Fischer rats had numerous HD in the P1 and P2 segments. By 12 mo. in age, the number of droplets had declined markedly; at 22-26 mo., no HD were visible. Electron microscopy of PCT of aged male rats showed that phagolysosomes, equivalent to HD, were reduced in number. We have also shown that a major constituent of HD is the male rat urinary protein, α,2-globulin, for which the rate of hepatic synthesis declines during aging. Levels of both hepatic and renal α,2-globulin in 25 mo. old male rats were about 1.5% of those in young adult. Unleaded gasoline (0.4 mL/kg/d, 5 d) caused a 3-fold increase in the renal content of α,2-globulin in young males whereas the same treatment did not increase renal α,2-globulin in old rats. We conclude that age is a major determinant in the development of hydrocarbon-induced nephropathy in male rats and only rats which produce large amounts of α,2-globulin are susceptible.


α,2-Globulin (α2G) is a protein thought to be integral to the development of male rat-specific nephrotoxicity caused by unleaded gasoline. A gold-labeled second antibody was used to determine the distribution of αG within cells of the renal proximal convoluted tubules (PCT). αG was localized almost exclusively in PL of saline- or gasoline-treated (2.0 mL/kg/d, 9 d) male rat kidney; no α2G was apparent at sites other than PL, microvilli or endocytotic vacuoles. Further, treatment of male rats with leupeptin, to inhibit cathepsin B, caused accumulation of PL identical to those observed following gasoline administration and rapidly increased the renal content of αG. αG in PL of control rats was distributed uniformly. However, the enlarged, angular PL characteristic of gasoline and leupeptin intoxication contained deposits of αG-locating preferentially over crystalline PL inclusions. In addition, numerous PL, unreactive with anti-αG, were observed in PCT of gasoline- or leupeptin-treated rats. Thus, it is concluded that a defect of lysosomal proteolysis is induced by gasoline and results in sequestration of large amounts of protein, including αG, in PL of PCT.


Several nongenotoxic carcinogens produce increased protein droplets and cell proliferation in the male rat kidney, the target organ. These protein droplets contain α2u-globulin (α2u), a male rat-specific low molecular weight protein that is absorbed by proximal tubule cells. In a two-year exposure, IPH has been shown to cause a low incidence of renal tumors in male, but not female rats or either sex of mice. Our objective was to test whether IPH would induce protein droplet formation and to determine whether IPH or the metabolites, isophorol and dihydrosophorone, were associated with α2u. Male rats treated with 0.5 or 1.0 g/kg IPH exhibited a significant increase in protein droplets. Gel filtration chromatography was used to isolate α2u from other proteins in male rat kidney cytosol 24 hr after treatment with IPH, isophorol or dihydrosophorone. Samples were extracted with ethyl acetate. GC/MS of the organic phase positively identified IPH (parent compound) and dihydrosophorone in the α2u samples from kidney cytosol of the IPH and dihydrosophorone treated animals, respectively. IPH was identified in samples from animals treated with isophorol. This may suggest that isophorol is metabolized to IPH, via the alcohol dehydrogenase system, prior to binding to α2u. Biochemical alterations induced by IPH resembled those of 2,4-dimethyl pentane, and 1,4-dichlorobenzene, and thus suggest the same mechanism of nephrotoxicity and carcinogenicity in male rats.


Rat lung and right heart weights increase following injection of 40 - 100 mg/kg monocrotaline or following continuous ingestion of 20 mg/L monocrotaline in drinking water. We have investigated the relationship between dose and toxicity by giving male rats (60 or 100 g) monocrotaline in drinking water for 1, 2, 4, 6, 10 or 20 days. Organ weights and pulmonary arterial contractility were measured 20 days after initiating treatment. Rats given 20 mg/L monocrotaline for 4 or more days developed the same degree of right ventricular hypertrophy as rats given 40 mg/L monocrotaline. Likewise, rats given 20 or 40 mg/L monocrotaline for 6 or more days developed similar and significant increases in lung weights. Pulmonary arteries were removed to assess norepinephrine-induced contractility in tissue baths. Maximum force of contraction was significantly decreased by monocrotaline treatment, but only if rats were exposed for 4 or more days. There was no difference in D50 between treatment groups. The threshold exposure which produced changes in pulmonary arteries, lung and heart weights was ingestion of 20 mg/L or more of monocrotaline for more than 45 hours (a total dose of less than 15 mg/kg). If damage occurred during the first 48 hr of treatment, it was reversed by day 20. Monocrotaline-induced damage to rat lung and heart appeared to be quantitatively rather than graded. Supported by USPHS HL25285 and NIAMS ES-070-91.
Erythrosin B is an inhibitor of high affinity ouabain binding to guinea pig cardiac membrane preparations and Na⁺(K⁺)ATPase activity. The present studies set out to investigate the actions of EB on spontaneously beating MMR and compare them with those of ouabain. Chick MMR were prepared from 10 day old embryos and superfused with salt solutions containing test compounds whilst being filmed under the microscope. Superfusion with ouabain (0.25μM-5μM) increased contraction amplitude. Ouabain also inhibited ββ⁺ accumulation (IC₅₀ 2.5μM). EB similarly increased contraction amplitude (0.1μM-10μM) and inhibited ββ⁺ accumulation (IC₅₀ approx 4μM). In contrast, EB (1μM) caused unusual contraction patterns in MMR leading to eventual cessation of contraction which were not observed with ouabain toxicity. These results indicate that EB, like ouabain, is a positive inotrope and can inhibit Na⁺(K⁺)ATPase in MMR. However the unusual contraction patterns observed in EB toxicity suggest that Ouabain and EB may have different mechanisms of toxicity.

Sixteen organics in 0.2% glucose-Ringer solution at 10⁻⁷ to 10⁻³ M concentrations were tested for acute toxicity on isolated frog hearts (R. catesbeiana). The heart was perfused with glucose-Ringer solution at 25°C. The force of the heartbeat was measured as full isometric contractions from aorta to apex by longitudinal pull with a force transducer (Grass FT03) and an externally applied 3g load. The contractions were recorded with a Grass 7D polygraph. The maximum tension was compared in each titration for 5 or 10 minutes with controls. Acute toxicity was found at up to 10⁻⁴ M with 1,2,3-trichloropropene, acetonitrile, 2-chlorophenol, 2,4-dimethylphenol, 1,2,3-trichlorobenzene and 1,2-dichloroethane, causing reversible decrease in heartbeat tensions or heart stoppage. Some organics increased the force of contraction. Other benzene isomers had no effect up to 10⁻³ M. The compounds were also tested for interference with isolated sarcoplasmic reticular Ca²⁺ ATPase. No effect was seen up to a 10⁻³ M. The ease of reversibility of effects indicated no interactions with either with the glycolytic or the oxidative ATP generating systems. (Abstract does not necessarily reflect EPA policy).

The effects on the electrocardiogram of administration of high doses of indolator phosphodiesterase type III inhibitors, SKF 94120 [5-(4-acetamidophenyl)pyrazin-2(1H)-one] and related compounds SKF 95654, SKF 94836 and SKF 94418 were assessed in toxicity studies. In the dog, i.v. administration of SKF 94120 (1.5mg/kg), SKF 95654 (2mg/kg) and SKF 94418 (2mg/kg) gave rise to a transient increase in heart rate (HR) (maximum 290 beats/min) and T wave amplitude, also a slight reduction in P-R interval (0.01-0.03secs). SKF 94836 did not cause these changes when given p.o. to dogs (up to 800mg/kg). T waves were observed infrequently following exposure to SKF 94418 (i.v.), SKF 95654 (i.v.) and SKF 94836 (p.o.). Ventricular extrasystoles were observed after administration of SKF 94120 at 120mg/kg and SKF 94418 at 4mg/kg. I.v. infusion of SKF 94120 (15mg/kg) gave rise occasionally to atrioventricular dissociation with acchoochage. Papillary muscle necrosis was observed in dogs given SKF 94120, SKF 95654 and SKF 94418, but not SKF 94836. These changes correlated with the incidence of tachycardia for these compounds and suggested that tachycardia may be a contributory factor in the pathogenesis of this lesion.

PIFA has a high order of acute toxicity. The oral LD₅₀ is 49 mg/kg in rats and <25 mg/kg in dogs. Rapid and extensive gastric absorption is shown by comparison to the rat iv LD₅₀ of 20 mg/kg. The dermal LD₅₀ is between 200 and 2000 mg/kg in rabbits and the inhalation LC₅₀ is 1.75 mg/l in rats. PIFA applied to the eye at 50 mg/kg causes lethality in rabbits. Structure-activity studies show that the diphenyldionium cation is responsible for the toxic effects. These are expressed by an all-or-nothing response and exhibit a steep dose-response curve. Death is associated with respiratory failure and generalized engorgement of blood in the organs. Single iv doses in anesthetized dogs cause hypotension. Repeat oral doses in unanesthetized dogs cause fatty degeneration of cardiac muscle. Subacute oral studies in rats and hairless mice and dermal studies in rabbits and hairless mice confirm the high degree of toxicity. Effects include lethality, gastrointestinal inflammation, myocardial hypertrophy and renal tubule degeneration.
SUBCHRONIC CARDIOVASCULAR EFFECTS OF SOMAN INTOXICATION. R Moutvic, C R Hassler, *R L Hamlin. Battelle Columbus Div., Columbus, OH; *Ohio State University, Columbus, OH. Sponsor: G L Fisher

This study investigated the effects of a single, non-lethal dose of soman on the mechanical function, electrical properties, and nervous control of the canine heart. Chronically instrumented animal models were prepared to examine irritability, nervous control, hemodynamic properties and arrhythmic activity. All animals were observed for one month post-exposure. The electrophysiology data suggest a temporary decrement in response to direct sympathetic stimulation and direct sympathetic stimulation hypotension. Chemical denervation experiments suggested a direct effect of soman upon cardiac activity. Repetitive ventricular response data indicated an increase in irritability. Cardiovascular data indicated a mild decrease in cardiac performance; however, none of these decrements were life threatening and indicated the cardiovascular system was capable of providing sufficient blood supply. Holter arrhythmia analysis indicated animal-specific increases in ectopic events post-Soman exposure. Overall, a prolonged state of autonomic imbalance was characterized primarily by parasympathetic activity. The autonomic imbalance was further suggested by the appearance of potential life-threatening arrhythmic events in Soman-intoxicated animals. (Supported by U.S. Army Contract No. DAMD17-85-C-5038).

EFFECT OF PRENATAL EXPOSURE TO SODIUM SALICYLATE (NaS), ASPIRIN (ASA), OR GENTAMICIN (G) ON BLOOD PRESSURE IN RATS. G L Johnson, F R Alleva and T Balazs. FDA, Washington, DC.

Prenatal exposure to NaS or G has been reported to result in elevated blood pressure (BP) in female rats. To confirm these findings, pregnant Sprague-Dawley rats were given either NaS (125 or 175 mg/kg, p.o., q.d), ASA (175 mg/kg, p.o., q.d) or G (25 or 75 mg/kg, i.p., b.i.d.) on days 9-11, 9-11, and 14-18 of gestation, respectively. BP was measured by the tail-cuff method (indirect) in: 1) lightly anesthetized (pentobarbital, 30 mg/kg, s.c.) 3-month-old male and 5-month-old female pups from the NaS and ASA groups and 1-year-old female pups in the G group; and 2) conscious 3-month-old male pups from the high-dose NaS group and 5-month-old females from the NaS and ASA groups. BP was also measured by cannulation of a carotid artery (direct) in more deeply anesthetized 5-month-old females from the NaS and ASA groups. Heart rate (HR) was determined during all BP measurements by counting pulses. In no instance was either the BP or HR of rats exposed prenatally to NaS, ASA or G significantly different from that of respective controls. Also, there was no drug-related histopathologic change in kidneys from 5-month-old pups exposed prenatally to NaS or ASA. Treatment with G resulted in renal histopathologic changes in dams at 21 days of gestation but not in 21-day-old fetuses or 1-year-old offspring.

CARDIOGENALY IN NEONATAL RATS EXPOSED TO 500 PPM CARBON MONOXIDE. F J Clubb, Jr, D G Panney, and S P Bishop. Dept Path, UTHSCD, Dallas, TX; Dept Physiol, WSU, Detroit, MI; Dept Path, UAB, Birmingham, AL. Sponsor: Z Ruben

Carbon monoxide (CO) hemodynamic workload was induced in growing neonatal rats to study changes in myocyte (MC) structure and number to determine if CO produces volume-overload cardiomyalgia. Newborn rats were exposed to 500 ppm CO for up to 32 days of age (d), at which time the remaining CO exposed rats and ambient air controls continued development in room air until 200d. In the CO group, ventricular weight: body weight ratio was 26% greater than controls at 6d, more than 100% at 15d and 47% greater at 28d. Although absolute MC volumes were not different between the two groups at any time period, the CO group did have greater MC:body weight ratio at 6, 15 and 28d. Binucleated MCs of 15 and 28d CO rats were both longer and had increased length:width ratios than controls. By 200d, MCs from LV+S of CO exposed rats were significantly shorter. CO exposed rats from 200d had more total MCs (36X10⁶ versus 32X10⁶ for controls, p<0.05). Cardiomyalgia induced by 500 ppm CO from birth to 32d was primarily due to MC hypertrophy, with MCs having increased length:width ratios (i.e., changes consistent with a volume-overload model). Regression of cardiomyalgia occurred after removal from CO, with smaller MC length and volume and greater MC number.

REVERSAL OF PROPRANOLOL TOXICITY WITH AMINOPHYLLINE, AMINORONE OR FORSKOLIN. Vick, J, Whitehurst, V, Joseph, X and Balazs, T. Food and Drug Administration Washington, DC.

Hypotension and bradycardia leading to irreversible shock and death can be the result of overdoses of propranolol, a widely used beta blocking agent.

We attempted to reverse the effects of lethal overdoses of propranolol with one of three agents, aminophylline, aminorone or forskolin. Anesthetized beagle dogs were given a ten minute infusion of propranolol at a dose of 1 mg/kg/min. The five control dogs exhibited profound hypotension and severe bradycardia which lead to cardogenic shock and death at 15-30 min. Treatment with each compound was initiated within five minutes following the end of propranolol infusion. Aminophylline (20 mg/kg iv) increased heart rate and blood pressure to near normal levels in 7 treated dogs within 30 to 60 seconds. Both aminorone (2-3 mg/kg, iv) in 5 dogs and forskolin (1-2 mg/kg, iv) in 4 dogs were also effective; however, recovery was slower than with aminophylline. All treated dogs survived with no apparent residual damage. Results of this study show that each of these drugs is capable of reversing the otherwise lethal effects of propranolol overdose.
Chlorinated drinking water decreased HDL cholesterol and increased low density forms of lipoproteins in the serum of two species of non-human primates maintained on an atherogenic diet consisting of a normal high protein monkey chow containing 15% top lard and 1% free cholesterol. Food and water was administered ad libitum, and weekly fasting serum samples were collected for determination of cholesterol, triglycerides, HDLc and lipoprotein electrophoresis. The effect on lipoproteins appeared within four weeks after start of exposure and persisted after cessation of exposure to hypochlorite. Similar effects however could not be elicited by comparable doses of NaOCl when the monkeys received a non-atherogenic, low lipid and high protein diet (Purina Monkey Chow). This observation raises suspicion about the possible role of residual chlorine in drinking water as a co-atherogen which may exacerbate the atherogenic risk of dys-lipoproteinemia in susceptible or diet-stressed human populations. (Abstract does not necessarily reflect EPA policy).

In order to assess the feasibility of route to route extrapolation of pharmacokinetic data for volatile organics, the uptake and elimination of DCE were contrasted in male S-D. rats subjected to inhalation and equivalent oral DCE exposures. Unanesthetized rats of 325-375 g with an indwelling arterial cannula inhaled 100 or 300 ppm DCE for 2 hr through a 1-way valve. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently and analyzed for DCE by GC. Based on cumulative uptake at the end of the 100 and 300 ppm inhalation exposures, an equivalent oral dose of 10 or 30 mg/kg, respectively, was given in an aqueous emulsion as a single oral bolus to rats and serial blood samples taken from an arterial cannula. Peak blood levels of DCE were achieved within 4 min of the oral bolus, but levels declined rapidly thereafter. Rats inhaling DCE attained near steady-state blood levels during the 2-hr exposure that were 3 to 4 times lower in magnitude than peak levels achieved after an equivalent oral bolus dose. Nevertheless, AUC values were higher for each inhalation group than for the corresponding ingestion group. (Supported by U.S. EPA CR 812267 and U.S. Air Force AFOSR 870248)

A variety of materials have been developed as replacements for asbestos but the potential health impact of these materials is largely unknown. The purpose of this study was to evaluate and compare the cytotoxicity of crocidolite, amosite, and chrysotile asbestos, with two asbestos fiber substitutes, calcium sodium metaphosphate 70% (Monsanto) and Fiberfrax (Sohio), as well as semi-metallic substitutes and drum brake lining residues generated using a dynamometer under simulated driving conditions in a macrophage-like cell line, J774a.1. Following 24-hour incubations of cells with the test materials, changes in cell morphology, number and viability were assessed. A viability index was calculated to reflect changes in both cell number and viability and used to establish the relative cytotoxicity of the various materials under study. At equivalent concentrations (µg/mL), the cytotoxicity of the test materials was amosite, crocidolite, chrysotile > calcium sodium metaphosphate fibers >> semi-metallic drum brake residue >> semi-metallic disk brake residue, Fiberfrax fibers > semi-metallic disk and drum brake lining fillings.
It is well recognized that nitrogen dioxide (NO₂)-induced lung damage results in the proliferation of type II pneumocytes, presumably in response to type I cell injury and death. The objective of the present study was to assess the level of expression of type II cell hyperplasia in the lungs of rats following brief exposures to relatively high concentrations of NO₂. Groups of F344 rats were exposed to NO₂ in concentrations ranging from 25-250 ppm for durations of 15 or 30 min. Sham-air-exposed rats served as controls. Animals from the various exposure groups were sacrificed 24 and 48 hrs after exposure and their lungs were subjected to quantitative histopathologic analysis for evidence of type II cell hyperplasia. NO₂ type II cell hyperplasia was observed at the two sacrifice times following the 15 min, 25 ppm NO₂ exposure whereas all other 15 min exposures to 50-200 ppm NO₂ resulted in levels of type II hyperplasia that correlated with NO₂ exposure concentration. Following the 250 ppm NO₂, 15 min exposure, type II cell hyperplasia was not apparent at 24 hrs but did appear as of 48 hr post-exposure. A similar delay in the appearance of type II cell hyperplasia was also observed following the 30 min exposures to the higher NO₂ concentrations. Our results suggest that brief exposures to high concentrations of NO₂ may injure or otherwise compromise the resident population of type II pneumocytes and thereby limit their ability to proliferate.

Nitric oxide (NO) is the major oxide of nitrogen that can be formed during high-temperature combustion processes and, accordingly, represents a potentially hazardous air contaminant in settings where such processes occur. Although the biological toxicity of NO is thought to be less than that of nitrogen dioxide (NO₂), little information as to the toxicity of NO in the respiratory tract, or the relative toxicities of inhaled NO and NO₂, is currently available. The objectives of this study were to: 1) assess the toxicologic effects of NO in the lung, and 2) obtain information on the relative toxicities of NO and NO₂ when inhaled at relatively high concentrations for short durations. Groups of adult Fischer-344 rats were exposed to 500-1500 ppm NO or to 25-100 ppm NO₂ for 5-30 min durations and subsequently sacrificed 24 hrs later for lung gravimetric and quantitative histopathologic analyses. Groups of rats exposed to filtered air served as controls. The NO exposures did not bring about detectable histologic changes in the lung or cause significant alterations in lung wet or dry weights, whereas lung injury was detected following exposure to 25 ppm NO₂ for 30 min. Our over-all findings suggest that the lung toxicity of acutely inhaled NO is at least 30-40 times less than that of NO₂. It remains possible that NO per se is totally innocuous in the lung.

An information gap continues to exist regarding the induction and expression of pulmonary injury as a function of nitrogen dioxide (NO₂) exposure concentration and exposure time when NO₂ is acutely breathed. The objective of this study, accordingly, was to examine the relative importance of exposure concentration of NO₂ versus exposure duration in producing pulmonary injury. Groups of adult Fischer-344 rats were exposed to 25-250 ppm NO₂ for durations ranging from 2-30 min and subsequently sacrificed 24 hrs later for lung gravimetric and quantitative histopathologic analyses. These exposure conditions were expressed and analyzed in terms of exposure equivalents, i.e., NO₂ ppm-min. Control groups consisted of animals exposed to filtered air only. In all instances where significant increases in lung wet and dry weights were found, the most pronounced increases in these gravimetric parameters were produced by the shorter duration exposures involving the higher or highest concentrations of NO₂ administered at each of the exposure equivalents studied. The predominant importance of exposure concentration versus time was also demonstrated histologically, with the most conspicuous cell hyperplasia being most pronounced with the highest NO₂ concentration studied in a given set of exposure equivalent exposures. The results of this study indicate exposure concentration plays a more important role in inducing lung alterations than exposure time when NO₂ is inhaled for brief durations.

To investigate the development of cross-tolerance to O₃ after preexposure to CdCl₂ aerosol, rats were preexposed to CdCl₂ (1 hr., 1.4 mg/m³) and one day later bronchoalveolar lavage fluid (BALF) was analysed for protein and albumin accumulation. The results were compared with an O₃ preexposed group (4 hrs, 3 mg/m³, 3 days recovery) and with a non-preexposed group. Exposure to O₃ after preexposure to CdCl₂ or O₃ resulted in lower levels of protein and albumin in BALF compared to non-preexposed lungs (resp. 47% and 29% for protein, 40% and 24% for albumin). These biochemical data and light microscopic examination of lung cross sections indicate that the lungs suffered less damage when the exposure to O₃ was preceded by an exposure to either CdCl₂ or O₃. The results suggest that the development of (cross-)tolerance to pneumotoxic compounds is related to a non-specific response of the lung tissue. Proliferation of alveolar type II cells has been observed after exposure to O₃ and CdCl₂ in this and in many other studies. Various authors (1) have suggested that it may be a part of the tolerance mechanism.

The effects of inhaled BeO were studied in Beagle dogs to evaluate the suitability of a dog model for human chronic beryllium disease. Groups of dogs exposed to single inhalation exposures to respirable radiolabeled aerosols of 7BeO calcined at either 500 or 1000°C resulted in lung burdens of 18 μg Be/kg and 6 μg Be/kg. Serial sacrifices through one year after exposure were conducted to permit quantitation of tissue Be content and histological examination. BeO calcined at 500°C was cleared more rapidly from lung, and Be was translocated more rapidly to blood, liver, and skeleton, compared with BeO prepared at 1000°C. Pulmonary histological abnormalities demonstrated marked individual variation between dogs and were most severe at 64 days after exposure. Animals scheduled for sacrifice at 2 years after exposure underwent pulmonary lavage for cytological evaluation at intervals up to 18 months after exposure. Pulmonary lymphocyte stimulation responses peaked at 7 months after exposure then declined. This study, still in progress, indicates that the Beagle dog is a useful model for examining the induction of chronic beryllium disease. (Research sponsored by the U.S. DOE Office of Health and Environmental Research under Contract No. DE-AC04-76EV01013.)

SEX AND SPECIES DIFFERENCES IN THE INHALATION TOXICITY OF THIPHENE. R Irwin,* M Heitmancik, M Ryan, D Craig, and A Peters. Battelle Columbus Division, Columbus, OH and *NIEHS, Research Triangle Park, NC.

Thiophene (CAS No. 110-02-1) is widely used as an industrial solvent and pharmaceuticals synthesis intermediate. A two-week inhalation study (12 six-hour exposures) was conducted in F344 rats and B6C3F1 mice at concentrations of 0, 500, 1000, 2000, 4000 and 8000 ppm. Thiophene was vaporized by nebulization into a common plenum distribution system, and chamber concentrations were measured using a Miran-600 Infrared analyzer. The first exposure to thiophene produced 100% mortality in male mice at all exposure concentrations and in female mice at the two highest levels. Clinical signs of toxicity included hypotension, prostration, and dyspnea. A single exposure to thiophene also produced complete mortality in the high dose male and female rats. Mortality also occurred in male rats at the 2000 and 4000 ppm dose levels. Rats of both sexes at the 4000 ppm dose level showed an abnormal gait immediately following exposure, possibly indicative of cerebellar dysfunction. Thiophene exposure produced reduced weight gain, gross liver abnormalities, and an increased liver-to-brain weight ratio in survivors of both species. Long-term studies suggest that mice are more sensitive than rats to similar thiophene concentrations by the inhalation route, and that males of both species are more sensitive than females. (Supported by Contract No. N01-ES-65163 from NTP.)

EVALUATION OF CARBON DIOXIDE RESPONSE CURVES IN GUINEA PIGS. M Schaper, K Detwiler and Y Alarie, University of Pittsburgh, Pittsburgh, PA.

It is well-recognized that humans increase their ventilation during exposure to a mixture containing CO2. Furthermore, the level of ventilatory response to CO2 is proportional to the concentration of CO2 in inspired air. This is the premise of the test in which the CO2 response curve is generated. From this curve, the slope and intercept are then obtained. We have simulated this test in unanesthetized, unrestrained guinea pigs. Animals were placed in whole-body plethysmographs, to which sensitive pressure transducers were attached. These animals were first exposed to room air and then mixtures containing increasing levels of CO2 (1.5, 2.8, 4.4, 7.0, 10.0 or 15.0% in CO2 in 20% O2, balance N2). Tidal volume (VT) and respiratory frequency (f) were continuously monitored and all data were collected by an IBH-AT PC. The level of change in VT and f was assessed at each concentration of CO2 and plots of CO2 concentration vs. response level for VT and f were obtained. For each plot, the slope and intercept were determined. Our results indicate low variation in the responses to CO2 in normal guinea pigs. This approach may be useful in assessing lung function or changes in central chemoreceptor sensitivity to CO2 in animals acutely or chronically exposed to toxicants. Supported by NIEHS grant ROI-ES02747.
SIMULTANEOUS MEASUREMENTS OF WHOLE BODY PLETHYSMOGRAPHIC PRESSURE AND TRANSPIRATORY PRESSURE DURING AIR BREATHING AND CO₂ CHALLENGE

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When using a whole body plethysmograph, the minima and maxima of the pressure changes (P) due to respiration are taken to indicate the beginning and end of each breath and the amplitude is taken to represent tidal volume (VT). We reported that this is correct in normal guinea pigs but not during intense bronchoconstriction. To verify this, guinea pigs were anesthetized and a catheter tip pressure transducer was secured into the thoracic cavity to measure transpulmonary pressure (TPP). Two days later P and TPP were measured during air breathing, CO₂ challenge, and during challenge with histamine (H) or carbamylcholine (C). During air breathing or CO₂ challenge P and TPP were in phase for the beginning and end of each breath. With H or C, the P minima were shifted in time, occurring much before the end of expiration as indicated by TPP. The minima shifted in time and also were much lower due to gas compression. Thus, if the amplitude from minima to maxima is taken to represent VT, overestimation of up to 100% can occur. However, this can be used to indicate bronchoconstriction during challenge with a constrictor without measuring TPP. Supported under NIHES 1R01-ES02747.

DETERMINING THE LC50 FOR RATS EXPOSED BY INHALATION TO DIMETHYLPHOSPHORCHLORIDOThIOATE

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This inhalation study with Dimethylphosphorochloridothioate (DMPCt) was conducted to determine the 1-hour LC50 using whole body exposures. DMPCt is an intermediate in the production of pesticides. Well's adaptation of the moving average method (Drug and Chemical Toxicology, 6(6); pp. 595-603, 1983) was used which allowed the calculation of an LC50 using small group sizes and concentrations producing 0 or 100% mortality. Five male Sprague-Dawley rats per dose group were exposed to mean concentrations of DMPCt of 0.43, 0.90, 1.06 and 3.00 mg/l. Mortality was 100% for both sexes at 3.00 mg/l and 40% for males at 1.06 mg/l. Transient body weight loss was observed in all groups exposed to DMPCt except at 3.00 mg/l where 100% mortality occurred by day 2. Clinical and gross pathologic signs of toxicity suggested that pulmonary insufficiency and/or systemic toxicity may have contributed to mortality. This method calculated LC50 values for both sexes to be less than 2 mg/l. It was an acceptable one for calculating an inhalation LC50 with as few as 5 rats per group.

COMPUTERIZATION OF PULMONARY FUNCTION STUDIES IN LABORATORY ANIMALS

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A series of computer programs were developed to permit real time display and storage of pulmonary function data. Changes in pressure due to the respiration of animals can be detected by pressure transducers or microphones and displayed on a recorder. These respiratory signals are digitized, allowing the calculation of the following parameters: respiratory rate, amplitude of the respiratory wave (i.e., tidal volume), time of inspiration, and time of expiration. The data are stored on disk of an IBM personal computer AT®. Respiratory frequency and mean amplitude of the signal every 15 seconds can be seen on the video display and a graph of these parameters can be generated on a printer while the experiment is in progress. A second computer program splits the data file into individual animal files to allow for processing of the data. The third computer program smooths the data over pressure signals due to animal movement. The final computer program in the series calculates mean values and standard deviations. This software can be used in many different pulmonary function applications including meumitization and pulmonary hypersensitivity testing. Supported in part by NIHES 1 R01-ES02747.

INFLUENCE OF THE LUNG TOXIN PARATQUAT ON THE FREQUENCY AND FORM OF SPONTANEOUSLY GENERATED AUGMENTED BREATHS IN UNANESTHETIZED RATS

D J Murphy and D C Lintott

Changes in the frequency and form of spontaneously generated augmented breaths following the administration of a single ip dose of parquat were measured using unanesthetized rats. The ventilatory parameters were measured in unanesthetized rats using a head-exposed plethysmograph chamber. Parquat treatment caused dose-dependent decreases in volume and increases in the inspiratory time of augmented breaths. Significant changes were first noted at doses of 16 and 20 mg/kg for volume and expiratory time, respectively. The frequency of augmented breaths was least affected by parquat, with a slight decrease noted only at the highest dose of 24 mg/kg. Changes in tidal volume and respiratory rate (tachypnea) were first detected at 16 mg/kg and were highly correlated with the decreases in the volume of augmented breaths (r = ± 0.7). The changes in frequency and expiratory time of augmented breaths, however, were poorly correlated with the changes in respiratory rate and tidal volume (r < ± 0.5). Thus, changes in the volume of augmented breaths are as sensitive as the changes in respiratory rate and tidal volume in assessing the effects of parquat on the lung, and all of these changes appear to be influenced by a common factor(s).
Changes in the inspiratory volume of augmented breaths (IVAB) caused by pulmonary damage resulting from a single dose of SKRF 104524 (7.5 mg/kg i.v.). ConA (4 mg/kg i.v.) or Paraquat (8 mg/kg i.v.) were determined at various times after dosing in anesthetized Sprague-Dawley rats using a head-exposed plethysmograph. Maximum decreases in IVAB were noted 2-5 hours post dose for SKRF 104524, 2 days post dose with Con-A, and 3 days post dose with Paraquat. Changes in the esophageal pressure (EP), IVAB, peak inspiratory flow (PIF), and peak expiratory flow (PEF) were subsequently measured in anesthetized animals using a whole body plethysmograph at the time of maximum effect. Augmented breaths were initiated by manually occluding the trachea. Decreases in the IVAB of treated animals were not associated with decreases in EP or PIF, thus the neuro-muscular mechanism responsible for governing the augmented breaths was apparently unaffected, and the reduced IVAB in the treated animals appears to be due to a restrictive lung disorder. A slight decrease in the PEF of Con-A treated rats was apparent suggesting a possible obstructive disorder. This obstruction did not influence EP or PIF and was apparently not associated with the treatment-related decrease in IVAB.

Effect of particle size on inhalation toxicity of fenthion. H Tsuda, M Yoshida, N Murao, M Iwasaki, and Y Shirasu. Institute of Environmental Toxicology, Tokyo, Japan.

In order to examine the effect of particle size, rats were exposed for 6 h to fenthion mists of two different particle size distributions. The study was conducted using the size-selective nose-only inhalation chamber developed in our laboratory. Mass median aerodynamic diameter (μg) for the larger and smaller mist were 6 μm (1.5) and 1 μm (2.1), respectively. The LC50 for the larger mist (0.9 mg/1) was 3 times as low as that for the smaller mist (2.8 mg/1). The signs of toxicity of rats exposed to the larger mist developed more slowly and persistent compared with the smaller mist. During the exposure to fenthion (0.6 mg/1), the larger mist caused similar inhibition of erythrocyte acetylcholinesterase activity (AChE) as the smaller mist, but produced much more persistent inhibition. This slower recovery was also noted in the brain AChE of rats exposed to the larger mist. The area under the plasma concentration-time curve during the 6 h exposure and subsequent 18 h was 1.3 times higher for the smaller mist than that for the larger mist. These data suggest that the persistent inhibition of AChE may be a reason for the greater and persistent toxicity of the larger mist of fenthion, but the persistent AChE inhibition was not related to the absorbed amount of fenthion.
ASSOCIATION OF OLFACTORY FUNCTION AFTER INHALATION EXPOSURE OF RATS TO METHYL BROMIDE. M. Hurtt, D. A. Thomas, K. T. Morgan, and P. K. Working. CIIT, Research Triangle Park, NC.

Olfactory function was evaluated in methyl bromide (MeBr)-exposed adult male F-344 rats using the buried food pellet test. Animals (n=6 per group) were trained to locate and retrieve a food pellet buried under 2-4" of bedding in a 2x4-ft. open field. The average retrieval time (RT) ± SEM for 4 replicates in untreated rats was 32.2 ± 4.4 sec. Animals were retested 24 hr after a single 6 hr inhalation exposure to either 90 or 200 ppm MeBr. The animals' ability to locate the buried pellet was unaffected by exposure to 90 ppm MeBr (RT = 31.0 ± 2.9 sec), consistent with the absence of morphologic damage to the olfactory epithelium seen after this exposure. The exposure to 200 ppm MeBr rendered the animals temporarily incapable of locating the hidden pellet, consistent with the severe destruction of the olfactory epithelium observed immediately after such an exposure. Four to 6 days after treatment, the rats recovered sufficient olfactory function to find pellets (RT at day 5 = 39.8 ± 11.9 sec), even though histological examination of the epithelium revealed that morphological damage was still extensive at that time. These data indicate that functional recovery precedes complete morphological recovery and, thus, may not be a good indicator of the morphological state of the olfactory epithelium.

A COMPARATIVE STUDY OF TEST METHODS USED TO DETERMINE THE TOXIC POTENCY OF PVC SMOKE. W. G. Switzer, and H. L. Kaplan, Southwest Research Institute, San Antonio, TX, and M. M. Hirschen, BFGoodrich, Avon Lake, OH.

Toxic potency of smokes produced by combustion of two standard (SI, SJ) and two experimental (EX B, EX C) polyvinyl chloride (PVC) wire coating materials were evaluated by the National Bureau of Standards (NBS), Radiant Heater and University of Pittsburgh (UPitt) test methods. Analysis of CO and HCl in the smoke were correlated with lethality data in order to assess the role of these toxicants for each method. In the NBS method, EX B and C produced atmospheres containing 78% less HCl than equivalent quantities of SI, resulting in significantly higher LC50 values (p<0.05) for the experimental materials. The Radiant Heater method markedly decreased HCl content of smoke from all materials, possibly due to HCl decay at high humidity. Thus, lethality was mostly caused by CO, but the method still differentiated between materials. With the UPitt method, HCl content of smoke from both EX B and C was reduced in comparison to the standard materials, but differences between LC50 values were statistically significant only for EX C compared to SI and SJ. The lack of differentiation in LC50 values between EX B and the other three materials was determined to be due to an extreme sensitivity of mice to even low concentrations of HCl. This work indicates that toxic potencies determined by the UPitt method may not accurately reflect the relative toxic hazard of smoke from some materials to humans. (Sponsored by the BFGoodrich Geon Vinyl Division)

LIMITATIONS OF THE UPITT METHOD FOR THE SCREENING OF MATERIALS FOR THE TOXIC POTENCY OF SMOKE. H. L. Kaplan and W. G. Switzer. 1) Southwest Research Institute, San Antonio, TX; 2) BFGoodrich, Avon Lake, OH.

New York State's requirement for submission of LC50 values of materials, using the University of Pittsburgh (UPitt) combustion toxicity screening method, may lead to the selection of materials based exclusively on these values. In a comprehensive evaluation of the UPitt method, LC50 values were obtained for four PVC and one nylon materials. The relative toxic hazards to humans of the corresponding smoke atmospheres were estimated from measurements of the concentrations of the major toxicants in the smoke and compared with UPitt LC50 values. UPitt LC50 values underestimated the relative toxic hazard of smoke from nylon and overestimated that of smoke from two PVC materials. Determinations of LC50 values for CO, HCN, HCl and CO + HCl for mice proved the excessive sensitivity of this animal to HCl. Deficiencies in the UPitt method identified by this study include: the rapid evolution of gases resulting in a brief exposure of animals to very high levels, non-linearity between sample weight and gas evolution with some materials and, particularly, the extreme sensitivity of mice to HCl. The results show that a ranking based on LC50 values obtained by the UPitt method may lead to wrong identifications of those materials that produce smoke most toxicologically hazardous to humans. (Sponsored by the BFGoodrich Geon Vinyl Division)
EFFECT OF CHLORPROMAZINE ON PARAQUAT AND NADPH-DEPENDENT LIPID PEROXIDATION IN LUNG MICROSOMES. P Ogunbiyi and HP Miera. VA-MD Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Chlorpromazine (CPZ) has been shown to ameliorate or exacerbate paraquat (PQ) toxicity in experimental animals. The involvement of lipid peroxidation in PQ toxicity is controversial and the effect of CPZ on the process is inconclusive. In this study, the role of PQ and the effect of CPZ on lipid peroxide formation by lung microsomes were investigated using thiobarbituric acid reactive substance (TBARS) assay. NADPH-stimulated lipid peroxidation with or without exogenous Fe^{3+}. PQ (10^{-7} to 10^{-5} M) inhibited microsomal TBARS production under aerobic condition irrespective of the presence of Fe^{3+}. In the absence of PQ, CPZ (10^{-7} to 10^{-2} M) significantly inhibited microsomal lipid peroxidation. In PQ-treated microsomes, CPZ at 10^{-7} to 10^{-5} M, significantly inhibited, while at 2.5x10^{-4} to 10^{-2} it enhanced microsomal lipid peroxidation. Addition of Fe^{3+} (5x10^{-5} to 5x10^{-3} M) potentiated these responses. These results indicate that PQ-induced lung injury in guinea pig does not involve lipid peroxidation. The reported beneficial effect of CPZ in PQ toxicity probably involves mechanisms other than inhibition of peroxidation of lung lipids. (Support: NIH HL 36366, HL 35656).

PERTURBATION OF LUNG SUBCELLULAR CALCIUM TRANSPORT BY PARAQUAT. J W Coleman and A K Agarwal. Toxicology Research and Training Center, John Jay College of CUNY, New York, NY. Sponsor: H M Mehendale

Pervious studies from this laboratory indicated a perturbation of lung mitochondrial and microsomal calcium transport by paraquat. In the present study, male Sprague-Dawley rats were given paraquat ip at doses from 10 to 30 mg/kg. The animals were sacrificed at 72 hr following paraquat. Lungs were removed and mitochondria or microsomes were isolated by differential centrifugation. In vitro 45Ca uptake and ATPase were measured. There was a severe inhibition of mitochondrial and microsomal calcium uptake but no dose response relationship was observed. The rate of calcium uptake in lung mitochondria and microsomes was almost negligible at higher doses of paraquat. A dose dependent decrease was observed in ATPase. The results suggest that perturbation in subcellular calcium transport might be associated with lung damage.

SENSORY IRRITATION STRUCTURE ACTIVITY RELATIONSHIPS OF SOME BENZYLCHLORIDE CONGENERS. B R Dudek, M V Roloff, R D Short, M A Council. Monsanto Company, St. Louis, MO.

Inhalation sensory irritation causes stimulation of trigeminal nerve endings in the nasal mucosa. One of the reflex reactions in mice resulting from such stimulation is a decrease in respiratory frequency. The concentration of chemical that causes a 50% reduction in respiratory rate is referred to as its RD50. The RD50 values (ppm) for benzylchloride and some of its congeners were determined in mice by whole body plethysmography to be: benzylchloride (27), benzylobromide (5.2), benzylidone (4.1), ortho- (5.7), meta- (27), and para- (14) chlorobenzylchloride, and a,a-di-chlorotoluene (20). The structure activity relationships can be rationalized in terms of the reaction of the irritant chemical with a receptor protein in a lipid layer. A good correlation (.92) between the RD50's and the bond strength of the benzyl-halogen bond was found for the benzylhalides. For the ortho-, meta-, and para-substituted congeners, the RD50's appear to be related to the electron withdrawing ability of the ring halogen during a nucleophilic substitution reaction at the alpha carbon by a nucleophilic group such as a sulphydryl group associated with the receptor protein.

INDUCTION OF OXIDATIVE-STRESS IN UPPER RESPIRATORY TRACT (URT) TISSUES. D G Cavanagh and J B Morris. Toxicology Program, School of Pharmacy, University of Connecticut, Storrs, CT.

Oxidant gases deposit efficiently in the URT, but it is not known if this site is sensitive to these gases, or to oxidants in general. To provide preliminary data on the sensitivity of URT tissues to oxidative stress, the URT of the anesthetized F344 rat was exposed to the known oxidant tert-butyl hydroperoxide, via continuous lavage. This system allowed for precise control of dosage, and also for assessment of lactate dehydrogenase (LDH) leakage over the course of the exposure. The URT was lavaged at a flow rate of 2 ml/min for 60 min with 0.9% NaCl-1% dextran containing 0.0, 0.2, 1, or 5 mM TBHP. Immediately post-exposure URT tissues were isolated for biochemical analysis. At the highest level TBHP was cytotoxic as evidenced by increased lavage LDH content (p<0.01). Enzyme leakage became apparent 20-30 min after initiation of exposure. Tissue nonprotein sulhydryla were decreased in a dose-dependent manner by TBHP (p<0.01). TBHP also produced dose-dependent elevations in tissue malondialdehyde levels, but the changes were not statistically significant. These results indicate URT tissues are sensitive to TBHP-induced oxidative stress. (Supported by NIH grants ES 03676 and ES07163, and a Sandoz Institute Fellowship in Toxicology to DGC.)
Isolated, enriched fractions of alveolar type II cells, non-ciliated bronchiolar epithelial (Clara) cells, endothelial cells, and pulmonary macrophages were examined to determine relative xenobiotic metabolism parameters for rat lung cell types. Male F-344 rats were given β-naphthoflavone (50 mg/kg) ip 48 hours prior to sacrifice. Perfused lungs underwent protease-digest yielding a heterogeneous cell suspension (102 x 10⁶/rat) containing 33.8% type II cells and 3.6% Clara cells. Enriched fractions of type II cells (73.8%) and Clara cells (21.6%) were prepared by centrifugal elutriation and discontinuous gradient centrifugation. The final Clara cell fraction (97% viable) possessed higher levels (per 10⁶ cells) than the type II cell and endothelial cell fractions, respectively, for cytochrome P-450 (P-450) (33% and 14%), glutathione S-transferase (GST) (33% and 51%), ethoxyresoruvin-0-deethylation (EROD) (51% and 104%), and GST-S-transferase (69% and 277%). Macrophages contained non-detectable P-450, EROD, and GST-S-transferase, but possessed 50% greater cellular GST content than the Clara cell fraction. The biochemical characterization of these metabolically active isolated, enriched pneumocyte fractions will help elucidate cell specificity of pulmonary toxicants in vitro.

The objective of these studies was to determine the accumulation and fate of the disulfide, cystamine, by rat lung slices. Cystamine was accumulated by two uptake systems that obeyed saturation kinetics with apparent Ka values of 12 and 503 nM, and maximal rates of 330 and 5900 nmol/g wet weight/hr respectively. The high affinity system was competitively inhibited by the diamine, putrescine, and the herbicide, paraquat, which are themselves accumulated. Thus, this pulmonary uptake process appears to be identical for all three compounds. The low affinity process was not inhibited by putrescine and results from the diffusion of cystamine into the cell and its subsequent metabolism. Cystamine was metabolised predominantly to the sulfonic acid, taurine, with 10-20% of the intracellular cystamine covalently binding to protein. Conversion to taurine was unaffected by amine oxidase inhibitors, but was decreased after GST depletion, suggesting that pulmonary metabolism is GST-dependent, and is not mediated by diamine oxidase. We have concluded that cystamine is taken up by the lung, whereupon it may protect against oxidative stress, since both cystamine and taurine have been implicated as antioxidants.

ITII has been proposed as a possible route of exposure in assays to detect pulmonary carcinogens. For a preliminary toxicity assessment, suspensions of ARH (4%, w/v), which is rich in poly-cyclic aromatic hydrocarbons, and MC (2.5%, w/v) were prepared in L (5% w/v in phosphate buffered saline) by sonication. Once/wk for 5 wks male F-344 rats (285-310g, 5/group) were anesthetized with enflurane and administered 0.2 ml of ARH, MC or L using a small animal laryngoscope. ARH- and L-treated animals were sacrificed 1, 4 and 8 wks later. The MC group was sacrificed at 6 wks. Transient rales was frequently observed post-dosing. Mortality was spread across treatment groups and appeared to be related to the static anesthetic procedure. (Mortality was altogether eliminated in a subsequent study using a dynamic anesthetic exposure system). Histopathological evaluations of the lungs and tracheas revealed no adverse effects in L treated rats. A single focus of squamous metaplasia was noted in the lungs of a MC-treated rat. ARH elicited accumulations of foamy macrophages in the alveoli, multifocal interstitial pneumonitis and terminal bronchiolitis. Similar inflammatory changes have been reported to occur following inhalation studies with mineral oils and might be expected to occur in chronic ITII studies.
Studies were conducted on a number of ethoxylated alcohols (EAL), nonylphenols (NP) and poly-ethylene glycols (PEG) to investigate their potential to cause lung injury by aspiration. Male Sprague-Dawley rats received single endotracheal doses of test chemicals to determine minimal lethal doses (MLD). The doses were administered in constant volume (0.5 ml/rat) by diluting the substances with saline. The MLD ranged from 20 ul to 640 ul/kg for the surfactants (EAL and NP), while MLNs for PEGs were greater than 1 g/kg. In a subsequent study, groups of 10 rats of each sex received a single sub-lethal dose of each material. Two animals from each group were sacrificed at 1, 2 and 3 days post-dose, and the remaining 4 at 14 days post-dose. For EALs and NPs, the most commonly occurring gross observation on lungs was color change associated with inflammatory infiltrates, edema, and atelectasis at dosages of 10 to 160 ul/kg. Microscopic findings included fibrinopurulent pneumonia early in the course of the reaction, fibrosis later, and subsequently atelectasis accompanied by bronchoalveolar mucus accumulation. Pulmonary injury was seen with PEGs only at much higher dosages (1 g/kg). Significant aspiration hazards exist for ethoxylated surfactants but not for PEGs.

The long-term pulmonary effects induced by OOS-TMP, an impurity in organophosphorus insecticides, was examined by morphological and biochemical means. Weanling, female WAG/Rij rats received either corn oil or 40 mg OOS-TMP dissolved in corn oil/kg body weight by gavage and were studied at the following time intervals: 10 d, 30 d, 90 d, 6 mos and 1 yr. No animal died spontaneously. The 14 d LD<sub>50</sub> in pilot studies was 80 mg/kg body weight. OOS-TMP treatment resulted in the following morphological changes: hypertrophy of type II alveolar epithelial cells which contained abundant and enlarged osmiophilic lamellar bodies, and increased interstitial fibrosis accompanied by basement membrane alterations. There was also a significant increase in pulmonary hydroxyproline content in OOS-TMP treated animals as compared to controls at all times examined. Collagen deposition was associated with the interalveolar septa rather than being oriented around airways. The results of this study indicate that a single, sublethal dose of OOS-TMP induces long-term pulmonary structural alterations in rats.
587 PULMONARY RESPONSE TO AMIODARONE (AD) IN RATS.
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Humans treated chronically with the antiarrhythmic drug AD may develop pulmonary damage. To characterize the pulmonary response to AD, male Fischer 344 rats were treated with AD, 150 mg/kg p.o., for varying periods of time. Throughout 13 wks of treatment, AD and its principal metabolite, desethylAD, accumulated in lung tissue to approximately equal levels. Bis-desethylAD was also present, but was minimal by comparison. Two other unidentified polar metabolites were also detected. By 3 wks of treatment, total pulmonary phospholipid was increased 2-3 fold and was not further increased through 13 wks of AD. The molar ratios of AD to phospholipid and desethylAD to phospholipid were both approximately 0.05. All classes of phospholipids measured were elevated. The activity of pulmonary Na+K+ATPase was inhibited in a time-dependent manner by AD treatment with about 35% of the activity remaining after 9 wks of AD. Alkaline phosphatase levels in the lungs were not changed by AD treatment. Additionally, lysosomal phosphatase activities were not markedly affected by AD treatment. The results indicate that selective biochemical changes occur in rat lung following AD treatment. (Supported by a grant from the American Heart Assoc.)

590 THE ROLE OF METABOLISM IN CARBON TETRACHLORIDE-MEDIATED IMMUNOSUPPRESSION.
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The role of metabolic bioactivation for carbon tetrachloride-mediated suppression of humoral responses was investigated in B6C3F1 mice. Comparisons of immune responses were performed between in vivo systems and in vitro systems with and without metabolic generating capability. Treatment of mice i.p. with 500, 1000 or 1500 mg/kg of carbon tetrachloride (CCL4) for 7 consecutive days followed by in vivo sensitization with SRBC resulted in a dose-dependent suppression of the T-dependent antibody response (36, 48 and 53%, respectively). The in vitro T-independent antibody response to DNP-Ficol was suppressed only at 1500 mg/kg (16%). Antibody responses by splenocytes from mice treated with 1500 mg/kg and sensitized in vivo were suppressed by 65% to SRBC and 25% to DNP-Ficol. The polyclonal response to LPS was unaffected by 1500 mg/kg CCL4. CCL4 added directly to culture at a 3.0 μM concentration resulted in decreased antibody responses to SRBC, DNP-Ficol and LPS, which were directly attributable to cytotoxicity. Direct addition of CCL4 to naive splenocytes co-incubated with a S9 metabolizing system did not enhance the suppression of humoral responses. Direct addition of CCL4 to naive splenocytes co-cultured with metabolically active hepatocytes also did not enhance the suppression of humoral responses. Studies utilizing the cytochrome P-450 inhibitor methoxsalen are in progress. (Supported by NIH ES03564 and NRSA ES05415).

588 PULMONARY CHANGES FOLLOWING INTRATRACHEAL INSTILLATION OF GALLIUM ARSENIDE AND ARSENIC AND GALLIUM OXIDE IN HAMSTER LUNGS.
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Male Syrian Golden hamsters and Fischer-344 rats (n=9) were dosed to assess the effects of gallium arsenide (GaAs) and arsenic (III) and gallium trioxide (Ga(III)) on several lung antioxidants. The specific activity of the enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX) plus sulfhydryl and ascorbate levels were measured following intratracheal instillation of GaAs (100 and 10 mg/kg), As(III) (5 mg/kg) and Ga(III) (50 mg/kg) at 1-56 hours postexposure. In both species the lung/body weight ratio and total lung protein increased compared to the control values for all test groups. GaAs (after 1 day) and As(III) (up to 3 hours) treated animals showed lower CAT activity. SOD-PX activity was lower than control levels at tissue levels at day 1 with the GaAs and As(III) only. SOD activity was below control levels for As(III) and GaAs after 3 hours and up to 2 days. Sulfhydryl and ascorbate levels were different from control levels at the earlier time points for all test compounds. Lung response to GaAs exposure involves changes in enzymes and biochemicals important in oxidant metabolism.

590 HPLC SEPARATION OF BENZO(a)PYRENE [B(a)P] METABOLITES GENERATED BY SPLENIC MICROSONOMES OF UNTREATED MICE.
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Immunosuppression produced by B(a)P is thought to be mediated by its reactive metabolites rather than the parent compound, as demonstrated for its carcinogenic effects. Thus, metabolism of B(a)P by splenocytes to reactive intermediates would be a requisite of B(a)P-induced suppression of the splenic immune response. Although murine micromesosomes were demonstrated to be capable of metabolizing B(a)P to water soluble compounds (Cancer Res. 47:2317, 1987), the types of metabolites generated are not known. To identify the B(a)P metabolites produced by splenmic microsomes, 3H-B(a)P was incubated with micromosomal preparations and the metabolites were separated by reverse-phase HPLC. The metabolites were then compared by elution time to standard metabolites. The 9,10- and 7,8-dihydrodiol B(a)P were the primary diol metabolites formed, while only low levels of the 4,5-diol were detected. The addition of the epoxide hydrolase inhibitor, trichloropropylamine oxide (0.1 mM), was found to abrogate the formation of the diol metabolites. Splenic microsomes were also found to generate the generation of the 9, 7-, and 3-hydroxy and 3,6- and 6,12-diones metabolites. The cytochrome P-450 inhibitor, alpha-naphthoflavone (0.1 and 1.0 μM), inhibited the generation of all B(a)P metabolites. These results suggest that the spleen is capable of generating reactive B(a)P metabolites that may alter the immune response. (Supported by NIH grant ES03434).
ROLE OF ADRENAL CORTICOSTEROID (CS) IN SUPPRESSION OF CYTOTOXIC T LYMPHOCYTE (CTL) RESPONSE FOLLOWING EXPOSURE TO 3,4,5,3',4',5'-HEXACHLORO-BIPHENYL (HxCB). N I Kerkvliet, R B Smith and L B Stepan. College of Veterinary Medicine, Oregon State University, Corvallis, OR.

The CTL response of C57Bl/6 mice to allograft is sensitive to suppression by HxCB, a toxic Aα-receptor binding PCB isomer. However, suppression of the CTL response occurs only at doses of HxCB that also produce thymic atrophy. Since thymic atrophy can result from elevated levels of CS and PCB exposure has been shown to elevate serum CS levels, the possibility that suppression of the CTL response was an indirect effect of HxCB on CS production was examined. Serum CS levels were elevated 3-fold (p < 0.01) in mice treated orally with a single dose of 10 mg/kg HxCB. Allograft challenge alone did not influence serum CS levels nor alter the elevation of CS induced by HxCB. Plasma ACTH levels were not altered by HxCB suggesting an effect at the level of the adrenal gland rather than the pituitary. Adrenalectomized (Adx) mice treated with 10 mg/kg HxCB exhibited a high incidence of mortality when challenged with allogeneic tumor cells. Survivors showed suppressed CTL responses. Since Adx did not prevent the suppression of CTL activity by HxCB, a cause-effect relationship between HxCB-induced elevation of CS and suppressed CTL is doubtful. Studies with 2,3,4,5,6,7,8- and 2,3,4,5,6,7,8,9-tetrachlorodibenzo-p-dioxin (TCDD) exposed mice, R A Tomar and N I Kerkvliet. College of Veterinary Medicine, Oregon State University, Corvallis, OR.

Antibody production to T-dependent antigens is highly sensitive to suppression by polychlorinated dibenzo-p-dioxins. Previous studies from this laboratory have shown that the cellular mechanisms of suppression is related to effects on T cells. The present study provides evidence for a defect in T helper (Th) cells in TCDD-exposed mice. Because spleen cells from non-immune TCDD-exposed mice did not show suppressed antibody response in the adoptive host, we used a hapten-carrier (TNF-SRBC) system with cell separation/reconstitution techniques to determine the effects of TCDD on carrier specific Th cells. Lethally irradiated syngenic recipients, reconstituted with virgin B cells (non-immune) and T cells primed to sheep red blood cells, were immunized with TNF-SRBC. Mice reconstituted with carrier primed T cells from TCDD-exposed mice produced fewer anti TNF-PFC than compared to controls. In vitro assay of Th cells produced similar results. The findings suggest reduced Th cell activity in mice exposed to TCDD. The inability to show suppression upon transfer of unprimed spleen cells suggests that resting T cells are not sensitive to TCDD. Supported by NIH Grant ES00042.

ALTERATION IN PGE2 PRODUCTION FOLLOWING IN VIVO EXPOSURE TO DIMETHYLASITOSAMINE (DMN). M J Myers, J F Lockwood, and L B Schook. Laboratory of Molecular Immunology, Dept. of Animal Sciences, University of Illinois, Urbana, IL.

Previous results from this laboratory have shown DMN depressed T cell responses through changes in macrophage (MPH) functions. As PGE2 is an important MPH derived mediator affecting both MPH and T cell functions, it was of interest to ascertain if DMN affected PGE2 production. Peritoneal exudate MPH elicited with either thioglycollate (TC) or Con A (CA) were cultured in vitro with medium, LPS or IFN-γ. Both TC and CA MPH obtained from DMN exposed animals showed a 3-fold increase in PGE2 levels following either LPS or IFN-γ as compared to vehicle control responses. The production of PGE2 induced by LPS and IFN-γ in both vehicle and DMN treated animals were completely inhibited by the addition of indomethacin. In contrast, bone marrow derived MPH cultured with either medium, LPS or IFN-γ for 24 h prior to examination showed no differences in PGE2 production between vehicle and DMN treated animals at either 2, 5, or 9 d of culture. These results suggest the DMN induced decrease in MPH dependent T cell responses may be due to increased PGE2 production by MPH. (Supported by NIH grant ES-04348).

INHIBITION OF ANTI-HAPTEN ANTI BODY RESPONSE IN ADAPTIVE HOST RECONSTITUTED WITH T CELLS FROM 2,3,7,8-TE TrAC HLORODIBENZO-P-DIOXIN (TCDD) EXPOSED MICE. R A Tomar and N I Kerkvliet. College of Veterinary Medicine, Oregon State University, Corvallis, OR.

DIMETHYLASITOSAMINE (DMN)-INDUCED CHANGES IN TNF-α EXPRESSION AS DETECTED BY NORTHERN BLOT ANALYSIS. J F Lockwood, M J Myers & L B Schook. University of Illinois, Urbana, IL.

Previous results from this laboratory have shown exposure to DMN in vivo resulted in increased tumoral activity of thioglycolate elicited macrophage (MPH) following activation in vitro and cultured bone marrow derived macrophage (BMM). An TNF-α (TNF) is the predominant MPH effector cytokine molecule, it was necessary to determine if DMN exposure was altering the regulation of this cytokine. Three Tc-MPH and BMM were examined for TNF RNA by Northern Blot analysis. Examination of TNF-MPH from vehicle and DMN exposed animals revealed similar TNF levels. However, following LPS stimulation in vitro, Tc-MPH from DMN animals demonstrated enhanced transcrational activity of TNF. Additionally, the kinetics of TNF expression by BMM also was affected by DMN exposure in vivo. BMM from control animals demonstrated highest accumulation of mRNA at day 7 following LPS treatment whereas BMM from DMN exposed animals demonstrated greatest mRNA accumulation at day 5 of differentiation. These results suggest that the previously observed increase in MPH tumoral activity are due in part to enhanced levels of TNF mRNA. (Supported by NIH grant ES-04348).

Rats were exposed to an average human equivalent of 1 pack of cigarette/day (10 puffs of fresh cigarette smoke) for a period up to 41 weeks. Several parameters of their immunological response were compared to age and sex matched sham control and/or normal control groups. Studies show that cigarette smoke preferentially inhibits the antibody response of lung-associated lymph nodes (LAL) compared to anatomically distant lymph nodes or the spleen. Prolonged exposure to cigarette smoke may induce similar changes in other lymphoid tissues. The smoke-induced inhibition of the immune response does not appear to be nonspecific because the response of LAL cells from smoke-exposed animals to T cell mitogens (Con A and PHA) remains comparable to that of the control animals. Moreover, the ratio of T cells to B cells does not appear to be altered in the smoke-exposed animals. Preliminary results indicate that cigarette smoke under these conditions may alter B cell function in LAL.

These studies were supported in part by grants from NIH-DA04208 and CTR-1957.

PERIPHERAL BLOOD (PB) T CELL PHENOTYPES IN HUMANS AFTER TREATMENT WITH A T CELL MONOCLONAL ANTIBODY (OKT3). G M Shopp, A M Harford, L M Ashmore, J E Seppelt, L J Gibel, and W A Sterling, Jr. Lovelace Medical Foundation, and University of New Mexico School of Medicine, Albuquerque, NM. Sponsor: J M Benson.

Kidney transplant patients undergoing rejection were treated with 5 mg OKT3 IV daily for 14 days. Flow cytometric techniques were used to assess the T cell phenotypes of the patients during treatment. The cell populations analyzed included: pan-T cells (T4+), T helper/inducer cells (T3+ T4+), and T suppressor/effecter cells (T3+ T8+). Also analyzed were T4+ cells and T8+ cells that did not have detectable amounts of the T marker on their cell surface: T3+ T4+ cells and T3+ T8+ cells, respectively. The results showed the following: [1] After OKT3 treatment there was a decrease in the number of T3+ cells in the PB (12% of pre-treatment levels). [2] T3+ T4+ cells and T3+ T8+ cells decreased to about 90% of pre-treatment levels. Cells that were labeled T3+ T4+ and T3+ T8+ were seen to have values of 30% and 40% of total lymphocytes, respectively. (Normal values for these two cell populations are 1% or less.) T3+ T4+ cells and T3+ T8+ cells began to return to the PB within 24 hours after the time of treatment. [3] OKT3 treatment resulted in a decreased fluorescent intensity of the T3+ cells, indicating a decrease in the amount of T3 marker on the surface of these cells. These data support the hypothesis that one mechanism of action of OKT3 treatment is the down regulation of the T cell receptor/T3 complex.

DELTAPA-9-TETRAHYDROCANNABINOL INHIBITS Macrophage Functional Competence. G A Cabral and E M Mishkin. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA. Sponsor: G G Bradley

The objective of this study was to determine the effect of Delta-9-tetrahydrocannabinol (Delta-9-THC), the major psychoactive component of marijuana, on macrophage functional competence. Its effects on tumoricidal activity against retrovirus-producing neuroblastoma cell line, on Ia antigen expression, and on the synthesis of proteins associated with macrophage activation was determined. Propionibacterium acnes (P. acnes) peritoneal macrophages of (B6C3F1)F1 mice treated with Delta-9-THC (15mg/kg to 100mg/kg) exhibited decreased tumoricidal activity, and secreted less soluble tumoricidal factors, when compared to P. acnes-macrophage vehicle controls. In addition, protein profiles associated with P. acnes macrophage activation reverted to those of unactivated macrophages. In vitro exposure of P388D1 macrophages to drug (10^{-7}M to 10^{-3}M) resulted in inhibition of differential protein synthesis induced by lymphokines and by bacterial lipopolysaccharide. Delta-9-THC was shown, also, to suppress the expression of Ia antigens on P388D1 cells. These results suggest that Delta-9-THC inhibits the activation of macrophages. The drug alters "full activation" as exemplified by decreased tumoricidal activity and suppresses the expression of proteins induced by stimuli which drive macrophages to full activation.
Phosgene (COCl₂) is a highly toxic gas used in the synthesis of isocyanates. Its target organ of toxicity is the lung. The effects of phosgene on the in vivo generation and kinetics of the influenza virus-specific cytotoxic T lymphocyte (CTL) response were investigated. Male Fischer-344 rats were infected with influenza/Port Chalmers/1/73 (H3N2) virus via intranasal administration 24 hr following acute exposure to 1.0 ppm phosgene or clean filtered air for 4 hr. Single cell suspensions of lung cells were prepared by collagenase digestion of finely minced lung tissue. The lung cell suspensions were assayed for influenza-virus specific CTL activity using an 18 hr ⁵¹Cr release assay. The pulmonary CTL response was detected 5 days post-infection and reached a peak response 10 days post-infection. Acute phosgene inhalation resulted in the enhancement of the CTL response on day 7 post-infection and was followed by a suppression in activity by day 10 post-infection. The CTL response in phosgene exposed animals did not differ from those of air exposed infected animals at days 15 and 20 post-infection. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

The guinea pig has acute and chronic responses to inhalation of cotton dust (c.d.) resembling those reported for cotton workers. Effects of sub-chronic exposure to a non-irritating concentration of c.d. (1.6 mg/m³) were examined by exposing 30 guinea pigs 6 hrs/day, 5 days/wk for 12 wks. Respirable particles were generated from bulk c.d. The sham-exposed group contained 20 animals. Daily measurements included: weight, respiratory rate and breathing volume pre- and post-exposure in air and in 10% CO₂. Blood was collected monthly for blood chemistry and antibody measurement. After 12 wks groups were pre-treated with atropine (0.015g), anesthetized with Nembutal and the lungs perfused with glutaraldehyde. Heart, liver, and kidneys were removed for histology. The rate of weight gain of the exposed group was decreased compared to controls. Five exposed animals had produced antibodies. No differences were found in respiratory rate or volume. These results indicate chronic changes due to exposure to a low c.d. concentration and may have implications for establishing a safe workplace exposure level which is currently based on acute changes in pulmonary function. Supported under USDA Cooperative Agreement 58-434K-0050.
The effects of subchronic exposure to an acutely non-irritating concentration of cotton dust (c.d.) were examined using histopathologic, electron microscopic, and morphometric techniques. Examination of the lungs, trachea, major bronchi, and smaller bronchioles, nasal cavity, and peribronchial lymph nodes from guinea pigs exposed by inhalation to c.d. at 1.8 mg/m³ for 12 weeks failed to reveal significant morphologic changes when compared with controls. However, when tissues from the deep lungs were examined by transmission electron microscopy, differences in the lungs of c.d.-exposed guinea pigs when compared to controls were: 1) increased numbers of alveolar macrophages, many with phagocytosed particles; 2) increased numbers of type II cells, particularly around the end alveoli; 3) recruitment of leukocytes; 4) focal thickening of the alveolar wall caused by deposition of collagen fibers and infiltration of leukocytes. Morphometric analysis revealed slight thickening of the bronchiolar smooth muscle layer. Numbers of alveolar macrophages were increased 40% and type II cells increased 10% over the controls. Supported by USDA Coop. Agric. # 49NA-5-0056.

Full-scale assessment of the smoke toxicant revealing the combustion of Teflon® FEP in a wood fire was studied. Fires were started in a 40 m³ burn room; the smoke was extracted from the end of a 13 m corridor and conducted to the animal exposure chambers. Groups of 10 Crl:CD® rats were exposed nose-only for 30 minutes to the smoke formed from burning 110 kg of wood with 20-30 kg of Teflon® FEP cable. Smoke samples were monitored for hydrolyzable fluoride (FH), CO, CO₂, and fluorocarbon gases. Blood was obtained from 4 rats immediately after exposure for carboxyhemoglobin (COHb) analysis. Tetrafluoroethylene, hexafluoropropylene, perfluorocyclobutane or perfluorosubstituted hydrocarbons were not present in lethal concentrations. Time-integrated CO concentrations ranged from 70,000 to 110,000 ppm; O₃ concentrations ranged from 54-66%; some animal deaths were attributed to CO exposure. FH concentrations ranged from 7000 to 35,000 ppm·min and were better correlated with animal deaths. The data suggested that the toxic components present in the smoke include CO and fluorochemical(s) with a toxicity similar to acrylonitrile fluoride; the presence of unusually toxic substances was not experimentally confirmed.

Thermal decomposition products of certain perfluorinated polymers are toxic to experimental animals in small-scale combustion toxicity tests. Our studies were designed to investigate the time course of pulmonary effects in rats after a 30 min exposure to Teflon (FEP-100) comp. products. FEP was combusted in a quartz beaker at 500°C (0.03 mg/l). Five groups of rats were exposed under varying conditions. Groups A & B were exposed to FEP smoke during 1-3 and 15-30 min intervals, respectively. Group C was exposed to FEP for the entire 30 min period, while group D was exposed to a filtered (particle-free) atmosphere of FEP for 30 min. Group E was sham-exposed to room air. Lung wet wts. were recorded and cells and fluids recovered by lavage were assessed for toxicity. Our results showed that lung wts., inflammation, hemorrhage, alk. phosphatase, β-gluc, LDH, protein, and angiotensin convert. enzyme levels were significantly elevated in groups B & C compared to group D (filtered) and group E (sham). Group A rats were intermediate for all parameters. The data suggest that the lung injury observed in groups B & C occurs during the later phase of exposure (i.e., 15-30 min segment of exposure), and that animals exposed to a filtered atmosphere were protected. Secondary decomposition products following pyrolysis of FEP may be responsible for the observed lung toxicity.

The acute and subchronic inhalation toxicity of DMFA vapor was evaluated. The 4-hr LC₅₀ value (95% confidence limits) for Wistar rats was 1641 (862-3125) ppm with signs of nasal and ocular irritation, respiratory distress, and decreased body weight. F-344 rats exposed to 98, 288, or 566 ppm DMFA for 9 days (6 hr/d during an 11-day period) had signs of respiratory and ocular irritation (except at 98 ppm) and body weight loss, with 100% (586 ppm) and 16% (288 ppm) mortality. Microscopic lesions (only 98 and 288 ppm groups evaluated) occurred in ocular and nasal tissues. Subsequently, F-344 rats exposed to 0, 8, 24, or 76 ppm DMFA for 6 hr/d, 5 d/wk for 13-wk, with a portion of each group B,), showed transient ocular opacity (24 and 76 ppm), decreased body weight gain (76 ppm), lesions of the respiratory and olfactory epithelium within the anterior nasal cavity (76 ppm), and lesions of the eye (76 ppm females). Recovery rats had nasal tissue lesions which were decreased in incidence and severity. DMFA is an ocular and upper respiratory tract irritant and toxicant in rats at vapor concentrations of 76 ppm, while 24 ppm or less produced no biologically significant toxicity.
3-FMP is a semi-volatile by-product from the synthesis of a process intermediate. Single or multiple exposure of rats to 3-FMP vapour resulted in lesions in the nasal olfactory epithelium and liver. Pathogenesis and progression of both lesions were followed in a series of experiments ranging in duration from a single 15 min exposure to 13 weeks exposure for 6 hours/day. The olfactory lesion, a focal necrosis/desquamation of the epithelium, was evident within 24 hours of a single 6hr exposure to 3-FMP at 1ppm or higher. The liver lesion, typically a hydropic degeneration of hepatocytes, was seen one day after a similar exposure to 50ppm. Recovery of both lesions was evident even with continued exposure.

Additional studies investigated the toxicity of 3-FMP in the mouse, rabbit and monkey. There was considerable interspecies variation in the severity of the lesions seen, particularly with respect to the nasal lesion. The cynomolgus monkey was the least affected with no nasal lesion evident following exposure to 50ppm 3-FMP for 10 days. Effects on bodyweight gain, food consumption, clinical signs and blood biochemistry occurred in one or more species but are considered to be secondary to those in the target organs.

Vinylidene fluoride (VF₂) is a flammable gas, nominated for carcinogenicity testing because of its large production volume, possible occupational exposure, and chemical relationship to known carcinogens. The high exposure level of 50,000 ppm for rodent inhalation studies was very close to the lower flammable limit (LFL) for VF₂ of 55,000 ppm. Therefore, the inhalation exposure room was modified to comply with the National Electrical Code for Class I, Division I, Group D atmospheres. All metal parts of the inhalation system were grounded to prevent static discharge; the chambers were modified to include explosion relief panels, and all possible ignition sources were removed from VF₂ areas. A specially designed safety control system that closely monitored VF₂ pressures and flows and chamber air flows was incorporated in the inhalation dosing system. The VF₂ delivery system automatically activated audible and visible alarms, and shut off the VF₂ supply if pressure or flow increased, or air flow decreased, increasing VF₂ concentration toward the LFL. Monitoring pressures, flows and VF₂ concentrations was done remotely. The delivery system was operated safely without incident and all concentration parameters were well within acceptable limits.

1Now at Battelle Columbus, Columbus, OH.

To determine the influence of tissue hypoxia on the acute toxicity of Halon 1211, groups of ten Fischer 344 rats were subjected for 4 hours to one of the following exposure conditions: 1) 100% Halon 1211; 2) 15% Halon and 12% O₂; 3) 12% O₂ w/o Halon. Unexposed rats were used as colony controls. Asemotransferase (AST), alkaline phoshatase (ALP), blood urea nitrogen (BUN) and serum creatinine (Crt) were measured on all rats. AST and ALP levels in group 2 (15% Halon and 12% O₂) were increased significantly when compared to control groups. The BUN and Crt levels remained unchanged in treatment and control groups. Histopathological examination showed moderate to marked centrilobular vascular degeneration and necrosis in the liver of all group 2 rats (15% Halon 1211 + 12% O₂). This lesion was not observed in rats in the other three groups. In another experiment, groups of six Fischer 344 rats were exposed for 4 hours to the same exposure conditions as described above and immediately after the exposure the livers were examined for the formation of thiobarbituric acid (TBA) reactive product. TBA reactive product formation in group 1 or 2 was not significantly different from controls. Our data suggest that hypoxia enhances the toxicity of Halon 1211 but the mechanism does not involve lipid peroxidation.

The generation and control of vinylidene fluoride, a flammable gas, for inhalation toxicology. D K Craig, and W J EASTIN, Jr., Litton Bionetics, Inc., Rockville, MD and NIEHS, Research Triangle Park, NC.

The influence of hypoxia on the acute toxicity of Halon 1211 fire retardant. S Ugwu, J Thillat2 and R R Smith. The College of Pharmacy, University of New Mexico, Albuquerque, NM. *Veterinary Diagnostic Services, New Mexico Dept. of Agric. Albuquerque, NM.

Interaction of amphiphilic drugs with phospholipid vesicles. U M Joshi, K S Prasad Rao, B Coudert, T M Dyer and M Mehendale. Department of Pharmacology Toxicology, University of Mississippi Medical Center, Jackson, MS.

Binding characteristics of several amphiphilic drugs to L-a-dipalmityl phosphatidylcholine (DPPC) was studied using fluorescence probes, 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-ami- lino-5- naphtalelenesulfonate (ANS) for hydrophobic and hydrophilic interactions, respectively. Drug binding to DPPC was quantitated using Scatchard equations. The drugs used, showed varied binding capacities to DPPC with respect to the probe. The order of binding capacity using DPH was promazine(PZ)<chloramphenicol(CRP)<amiodarone(AM)<tridiprime(TIP)<chlorpromazine(CP)<imipramine(IM)<propranolol (PP). Two binding affinities were calculated for some of the drugs. The order of the drug binding capacity using ANS was chlorphenter- mine(CP)<CRP<TIP<PMZ<PP. IM and CP2 showed intense fluorescence with ANS in the absence of DPPC. CP did not bind at the hydrophobic site of DPPC and AM did not bind to the hydrophilic site of DPPC. CRQ, did not bind to DPPC. These results suggest that induction of pulmonary phospholipidosis could be either binding of the drug to phospholipids or by inhibiting enzymatic break-down of phospholipids, or both, depending on the molecular charge of the drug. (Supported by HL-20692.)
The relationship between the development of coal workers' pneumoconiosis (CWP) and the geologic variables in coal has been a subject of debate and study. The differences in the prevalence of CWP between geographic regions and mines can only be partly explained by the rank of coal, mineral composition, or its cytotoxicity potential. Increased levels of organic-free radicals were shown to be present in the freshly fragmented coal (Artmoro and Resnik, 1980; Delal et al., 1986). These studies have postulated a potential role for organic-free radicals in the pathogenesis of CWP. This present study was initiated to test this hypothesis with the aid of cytotoxicity bioassay to determine whether organic-free radicals generated are associated with an increased potential for cellular injury. To accomplish this task, we ground coal and measured the concentrations of organic-free radicals using electron spin resonance. Allquots of same coal dust used in bioassays showed that freshly ground coal with greater concentrations of organic-free radicals induced increased cytotoxic effect. The organic-free radicals generated by grinding decayed at a slow rate over time with a corresponding loss in toxicity. The half-life of reactivity patterns of free radicals detected by ESR correlated with cytotoxicity of coal in bioassays.

Guinea pigs were exposed to $^{14}$CHNCO for 1 to 6 hrs at 0.5 to 15 ppm. A carotid artery cannula was implanted two days prior to exposure. Each animal was exposed in a glass whole body plethysmograph. Blood samples taken during exposure revealed immediate and rapid uptake of $^{14}$C. This uptake continued following exposure, followed by gradual clearance over a period of 3 days. The uptake was linear during exposure and, when expressed in cpm/min/ppm exposure concentration was 10 times lower at 15 ppm than at 0.5 ppm. We conclude that nasal secretions which increase with exposure concentrations of this irritant are responsible for this phenomenon. Animals fitted with a tracheal cannula and exposed while under anesthesia showed a much reduced arterial blood $^{14}$C uptake, too low to be due to the reduced minute ventilation due to anesthesia. The reduced uptake is due primarily to bypassing the upper respiratory tract which we conclude to be the major site from which $^{14}$C was absorbed in normal animals exposed to $^{14}$CHNCO. The results show that it is impossible to extrapolate from the concentration range used to lower or higher exposure concentrations. Supported under NIEHS 1801-ES02747.

MIC is a highly reactive gas with documented physiological effects. Analysis of the distribution, cellular damage and fate of MIC will add to our understanding of its mechanism of injury and physiological response. To examine the effects of MIC at the subcellular level, guinea pigs were exposed, through inhalation, to a range of sublethal concentrations of radioactive MIC from 0.5 to 15 ppm. The relative organ distribution of radioactivity, which was concentration independent, showed high levels in trachea, lung and blood, and lower but significant levels in brain, liver, kidney and spleen. In all cases, clearance of radioactivity was rapid. Histological examination of trachea and lung sections of exposed animals showed minimal epithelial damage except at 15 ppm. Inflammatory cell migration was also studied. Autoradiography of these sections showed that the label penetrated to the substrahe beneath the respiratory epithelium and rapidly cleared in vivo. Biochemical analysis of respiratory tissues revealed that 90% of trachea and 70% of lung associated radioactivity was saline extractable. This indicated a significant noncovalent interaction of MIC or covalent reaction with a soluble respiratory factor. This work was supported by grant OH-02214 to NIEHS.

APPLICATION OF SHORT-TERM LUNG BIOASSAYS TO RISK ASSESSMENT FOR METALS B D Beck 6 J D Brain 6, Gradient Corporation, Cambridge, MA 6 and Harvard Schi. Pub. Hlth., Boston, MA 6 Short-term pulmonary bioassays of metals were used to evaluate dose-response relationships and to assess complex mixtures. Published data were obtained on in vitro macrophage tests and on mouse infectivity models for the following metals: As, Pb, Mn, Ni, Zn, V, Cd, Fe and Cu. Dose extrapolation from in vitro studies to maximum concentrations in human lungs was performed assuming the volume of 1 gm of tissue was 1 ml and metals were evenly distributed in the lungs. Comparison of estimated in vivo concentrations to in vitro cytotoxic concentrations showed in vivo concentrations to be much less than those required to reduce in vitro macrophage viability by 50%. Extrapolation from infectivity models based on deposited dose showed that Cd, Cu and Zn (assuming metals were present as salts) were present in human lungs at levels at those required to increase mortality during infection by 20% in mice. Mixtures were evaluated by comparing effects of mixtures with those of individual metals. For oil fly ash, V could account for in vitro toxicity and Zn and Ni for infectivity. For Al smelter dust, Cu and As were greater than necessary to enhance infectivity. In contrast, levels of metals in steel mill furnace particulates and in coal combustion fly ash could not account for toxicity, suggesting a role for other constituents.
FACTORS INFLUENCING THE ESTIMATION OF HAZARD FROM AN ACCIDENTAL ARSINE RELEASE. C. V. Alexeoff, California Department of Health Services, Berkeley, CA

Arsine is an extremely hazardous substance that requires evaluation for the potential consequences of an accidental release. Reported rat LC50s indicate that arsine is of similar toxic potency as methyl isocyanate. Although over 470 human cases of arsine poisoning have been reported in the literature, quantitative dose-response data are lacking. Thus, available data reported for laboratory animals were evaluated to calculate concentrations that could produce fatal or severe toxic effects (hemolysis) in humans. Reported data for mice indicate that the toxic response to arsine varies as a function of concentration to the 0.5 power. Calculations based on the administered concentrations indicate that the mouse (10-min LC50 = 85 ppm, 10-min LOEL = 12 ppm) is more sensitive than the rabbit (10-min LC50 = 250 ppm, 10-min LOEL = 16 ppm). However, calculations based on the estimated quantity of arsine absorbed per RBC indicate that the rabbit may be 5 to 10 times more sensitive to the effects of arsine than the mouse. Based on evaluation of pharmacokinetic parameters, children would receive approximately twice the arsine dose per RBC compared to adults breathing the same concentration.


A number of cytotoxicants (C) are carcinogenic in rodent bioassays. Their carcinogenicity may be due to continued regenerative hyperplasia (RH) following repeated toxicity. Evaluation of this hypothesis requires, in part, quantitation of necrosis and RH after C exposure. We have developed a simulation model describing C pharmacokinetics and a biochemical mechanism leading to cell death. In this model a metabolite of C attacks a target macromolecule (T) and cells die when T falls below a threshold concentration. Soluble enzymes are released by viable, leaky while membrane-bound enzymes are only released when cells die. Computer simulations of hepatotoxicant inhalation and resultant cytotoxicity are presented. One aspect of model validation is the quantitation of hepatic enzyme activity in situ and of its clearance from blood. SGPT, a soluble hepatic enzyme, had in situ activity of 19,450 ± 3,700 SF units/g liver in male Osborne-Mendel rats and was cleared from the blood by a biphasic process with half-lives of 3.4 and 35 hr, respectively. Full validation of the model may enable activities of membrane-bound hepatic enzymes in blood to be used as quantitative indices of hepatocyte death.


Predicting the shape of carcinogen D-R curves at low doses is a long-standing problem which can be addressed by computer simulation. We have developed a simulation model for a cytotoxicant (C), including its pharmacokinetic behavior, biochemical mechanism of toxicity, and a linkage between toxicity and tumor formation. Target tissue cells have basal birth and death rates. Mutations occur during replication and cause transition of normal cells to intermediate and then malignant genotypes (0, 1, and 2 mutations, respectively). For cytotoxicity, a metabolite of C wreaks a target macromolecule (T) and cells die when T falls below a threshold concentration. Individual cell thresholds are normally distributed. Cell death is followed by regenerative hyperplasia which increases the transition rates. Six hr/day, 5 day/wk for 2 yr inhalation exposures were simulated. The threshold for cytotoxicity was varied and D-R curves obtained from 1 ppb through 100 ppm. As expected, shapes of carcinogen D-R curves were a function of cytotoxic threshold. This study illustrates the use of computer simulation to integrate information on carcinogen exposure, pharmacokinetics, mechanism of action, and tumor formation.


The disposition of TCDD in the mouse is primarily determined by high affinity hepatic binding to a cytosolic receptor and a microsomal binding domain. Distribution studies provided estimates of the binding constant for the latter, but not for the former. We developed and validated a physiological pharmacokinetic model for the mouse which included the 2 hepatic binding sites. We then modified the model to include enzyme induction, which was assumed to be related to the fractional occupancy of the cytosolic receptor. This model was scaled up for the rat to evaluate literature data for enzyme induction by TCDD. The cytosolic receptor binding affinity in vivo was estimated by simulation to about 0.03 nM. This rat model also accurately predicted the tissue distribution following repeated dosing as described by Rose et al. (Toxicol. Appl. Pharmacol. 36 (1976) 209). In both instances, the behavior was extremely sensitive to binding affinities, but much less sensitive to binding capacities in the dose range studied. This physiological model for TCDD which accounts for hepatic binding and enzyme induction is useful for cancer risk assessments when it is coupled with biologically-based models for tumor promoters.
A BIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR DERMAL ABSORPTION. C B Frederick and I M Chang-Mateu. Rohn and Haas Co., Spring House, PA.

A biologically-based computer model has been developed to examine chemical absorption into skin. The model includes the metabolic characteristics of the stratum corneum, viable epidermis, dermis, and either the circulating blood in the capillaries in vivo or the bathing solution of an in vitro cell in vitro. The partitioning of a compound between skin compartments, the rapid initial absorption of compounds through hair follicles, and the evaporation of volatile compounds are included. This model is developed in a simulation language that is simple to use and easy to modify so that biochemical pathways specific to a compound may be included. The model has been applied to several sets of data illustrating various metabolic and reactivity profiles. This approach may be useful for risk assessment and the simulation of exposure scenarios involving dermal exposure to xenobiotics.


Andersen et al. (Tox. Appl. Pharm. 87, 188, 1987) developed a PB-PK model for MEC risk assessment. To confirm the human metabolic rate constants used in this model, cytosolic and microsomal enzymes were prepared from lung and liver of F344 rat, B6C3F1 mouse, hamsters, and humans. Radiochemical assays (36Cl-MEC) of glutathione S-transferase (GST) and mixed function oxidase (MFO) gave kinetic parameters for these enzymes. Michaelis constants (liver enzymes) were 1-2 mM (MFO) and >50 mM (GST). MFO activities (nmol/min/mg at 5 mM MEC) for mouse, rat, hamster, and human were 11, 4, 14, and 5 nmol/min/mg (liver) and 5, 0.2, 1, and 0.1 (lung). GST activities (at 40 mM MEC) were 26, 7, 1, and 2 nmol/min/mg (liver) and 7, 1, 0.2 and 0.4 (lung) respectively. These data provide support for the human constants used in the PB-PK model of Andersen et al. and suggest that the lung and liver enzymes observed in mice exposed to MEC vapor may result from the high exposure concentrations (which saturate MFO) and the high levels of GST enzymes found in mice but not hamsters or humans.

INSIGHT INTO THE INTERSPECIES DIFFERENCES IN BENZENE TOXICITY PROVIDED BY A PHYSIOLOGICAL MODEL. M A Medinsky, P J Sabourin, R F Henderson, G Lucier*, L S Birnbaum*, Lovelace ITI, Albuquerque, NM and *NIEHS, RTP, NC.

Studies on the chronic toxicity of benzene indicated that B6CF, mice are a more sensitive species than are F344 rats. A physiological model was developed to describe the uptake and metabolism of benzene in rats and mice and to determine if differences in toxic effects are consistent with differences in pathways for metabolism or by differences in total metabolism. Compartments incorporated into the model included lung, blood, liver, fat, a group of poorly perfused tissues such as skin and muscle, and a group of richly perfused tissues such as kidney, bone marrow, and heart. Simulations of 6-hr inhalation exposure to benzene indicated that up to 1000 ppm, mice metabolized 2-3 times more benzene than rats per kg body wt. Simulations of oral exposure to benzene up to 150 mg/kg body weight resulted in similar amounts of benzene metabolized in both species per kg body wt. However, patterns of metabolites formed were different. Rats formed primarily the detoxification metabolite, phenyl sulfate. Mice formed more hydroquinone glucuronide and muconic acid which are associated with formation of putative toxic metabolites. (Research supported by the NIEHS through Interagency Agreement ES20092 with U.S. DOE Contract No. DE-AC04-76EV01013.)

PHYSIOLOGICAL PHARMACOKINETIC MODEL FOR HEXACHLOROBENZENE (HCB) IN THE SPRAGUE-DAWLEY RAT AND RHESUS MONKEY. A C E Wilson, K K Rozman, J D Wilson, and R A Freeman. Monsanto Company, St. Louis, MO and University of Kansas Medical Center, Kansas City, KS.

Numerous studies have been performed on the absorption, distribution and elimination of HCB in the rat. In addition, studies have also been conducted in the rhesus monkey. However, no unifying model appears to have been developed which would permit examination of HCB pharmacokinetics in different species, including man. In this paper we describe the development of a physiologically-based pharmacokinetic model (PB-PK) for HCB in the rat. The PB-PK model simulations for HCB in the rat were shown to be in excellent agreement with the published data. The use of PB-PK in species extrapolation was demonstrated by scaling the rat model to the rhesus monkey. Model simulations for the pharmacokinetics of HCB in the rhesus monkey were found to be in good agreement with the experimentally determined data.
PHYSIOLOGICALLY-BASED COMPUTER SIMULATION OF CHLOROPENTAFLOLOROBENZENE (CPF8) PHARMACOKINETICS AND ITS QUANTITATION IN EXPIRED BREATH: A NON-INVASIVE TOOL FOR EVALUATING EXPOSURE HISTORY. A. Vinegar, D W Winsett, R B Conolly, and R E Andersen. Northrop Services, Inc., Dayton OH and ANNHI/NIH, Wright Patterson AFS, OH.

A physiologically-based computer simulation (PB-PK) model has been developed to describe the relationship between CPF8 concentrations in expired breath and body tissues. In this model ventilation and cardiac output were initially both set at 14 L/min/kg x body weight$^{3/4}$ (Eq. 1). For model validation rats were exposed to CPF8 vapor (300, 600, 1200 ppm for 1 hr) and expired CPF8 tracked for 1 hr post-exposure. Ventilation was measured with a combination restrainer plethysmograph. It showed significant minute-to-minute variation and was higher than predicted by Eq. 1. PB-PK model simulation of expired CPF8 levels improved dramatically when the real ventilation data were substituted for Eq. 1 and when cardiac output was set at one half the ventilation rate. This study shows that PB-PK modelling, used in conjunction with quantitation of toxicant in expired breath, can function as a non-invasive, retrospective exposimeter for CPF8 exposures. The technique should be generally applicable to other volatile materials and for those with a low blood-air partition coefficient it could also be utilized as a probe of cardiac output.


Cobalt is a toxic and carcinogenic metal emitted by the combustion of fossil fuels and by industrial processes. Sprague-Dawley rats were exposed to various CoCl$_2$ aerosol concentrations for two hours to estimate human lung and body burdens. Co aerosols were deposited in the NPR and lung with efficiencies of 2.3 and 5.4%. Kinetics of NPR Co removal were first-order with estimated clearance rate of 0.16 hr$^{-1}$. Consistent with a two-compartment model, lung Co removal decreased over time. Co uptake into compartment 1 was 425 ng/hr at 656 mg/cm$^2$ exposure concentration. Elimination from compartment 2 was 0.075 hr$^{-1}$. Intercompartmental transitions were 0.022 hr$^{-1}$ ($k_{12}$) and 0.018 hr$^{-1}$ ($k_{23}$) derived from characteristic relaxation times of 10.3 hr and 59.9 hr, respectively. The relaxation time for the NPR was 6.37 hr. Since these clearance rates are faster than those for insoluble particles removed by mucociliary clearance, the two compartments probably represent the lung epithelium and capillary endothelium, respectively. These data and simulations have been incorporated into a model of Co body deposition which predicts organ and body burdens following several exposure scenarios. (Supported by NIH grants ES07031, GA14236 and RR01693 and by the ILSI Risk Institute.)

PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PB-PK) MODEL OF INHALED METHANOL: A SPECIES COMPARISON. V L Horton and D E Rickert. CUIIT, Research Triangle Park, NC, and Curriculum in Toxicology, Univ. N. Carolina, Chapel Hill, NC.

There are minimal data on the disposition of methanol (MeOH) after inhalation. MeOH PB-PK models were developed for P-344 rats, rhesus monkeys, and humans using ACSL software and compared to in vivo results. In rats, a double pathway for MeOH metabolism, using catalase Km and Vmax for one enzyme and high affinity, low capacity values for the second and MeOH, simulated blood concentrations for 6h exposures to 200, 1200, or 2000 ppm MeOH. Simulation of 100 mg MeOH/kg intravenously required only the catalase values, suggesting the second pathway was induced during inhalation exposure. In monkeys, modeling of blood MeOH levels after 6h exposures to 200, 1200, 2000 ppm required catalase parameters; alcohol dehydrogenase values employed as a second enzyme did not greatly influence the simulation, suggesting an interspecies metabolic similarity at low doses. The human urine excretion model used monkey metabolism parameters, an extraction coefficient of 0.007, and a urine production rate of 0.4ml/hr/kg to simulate published data acquired for 8h exposures to 78, 158, or 231 ppm. These studies suggest that there is a high probability of accurately predicting MeOH blood and urine concentrations under varied exposure conditions in various species using PB-PK models.


The insulin-secreting cell lines RINm5F and HIT-T15 have been used to investigate mechanisms of insulin synthesis and secretion. Cyproheptadine (CPH) is a pancreatotoxic chemical that reversibly inhibits insulin secretion and depletes pancreatic insulin in the rat. Studies were undertaken to determine if the RIN and HIT cell lines respond to CPH in a similar manner to rat pancreatic $\beta$-cells.

CPH produced similar alterations in both cell lines. After a 48 hr. culture period in the presence of 0, 1.0, or 10.0 $\mu$M CPH, cellular immunoreactive insulin (IRI) stores and media IRI levels decreased in a concentration-dependent manner. At 10 $\mu$M CPH, RIN and HIT cell insulin declined by 77 and 87%, respectively. Cellular IRI returned to control levels 48 hours after removal of CPH. In experiments designed to assess a direct inhibitory effect on stimulated IRI secretion, 1-10 $\mu$M CPH was found to inhibit glucose-stimulated release from HIT cells, and K$^+$, alanine and glycerol-dehydrostimulated release from RIN cells.

The results show that these cell lines exhibit sensitivity to the IRI-depleting and anti-secretory effects of CPH, and will serve as useful models in which to conduct mechanistic studies into the diabetogenic actions of this compound. (Supported by the Juvenile Diabetes Foundation, Grant #185206.)
COMPARISON OF CHROMIUM-INDUCED DNA LESIONS IN CULTURED HUMAN AND MOUSE CELL LINES. H S Park and C M Witmer, Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ

Chromium-induced DNA lesions were compared in L1210 mouse leukemia cells and A549 human lung carcinoma cells. The latter have properties of Type II alveolar epithelial cells. An alkaline elution assay was used to measure DNA damage after in vitro treatment with chromium compounds. Treatment of L1210 cells with 5uM sodium chromate (hexavalent) for 2 hrs caused the formation of DNA-protein crosslinks at a frequency equivalent to 145 rad exposure. No single strand breaks were found. Results with A549 cells treated with 5 uM hexavalent potassium dichromate for 3 hours showed single strand breaks and a suggestion of DNA-protein crosslinks. Doses of chromate of 5, 10 and 20 uM resulted in a dose-related response of single strand breaks. In contrast, neither single strand breaks nor DNA-protein crosslinks were present in the A549 cells exposed for 3 hours to 20 uM trivalent chromium, as CrCl3. These results support the indications that hexavalent chromium crosses the cell membrane while trivalent chromium is excluded from the cells. They also demonstrate differences in the DNA lesions in different types of cells following treatment with hexavalent chromium, which may be related to the long-term toxic and carcinogenic effects of chromium.


Allylamine (AA) is a specific cardiac toxin in many species causing aortic, valvular and myocardial lesions. Myocardial necrosis can occur 24 hours after a single dose. Toxicokinetic studies with [14C]AA (150 mg/kg) have shown 50-45% of AA in coronary and myocardial tissue 5 minutes after gavage dosing (Toxicology 35:167, 1985). AA has been shown to bind to mitochondria from aorta and heart, suggesting that the subcellular site of injury may be at or near the mitochondrion. The present investigation evaluated the effect of AA on mitochondria isolated from rat (RHM) and beef heart (BHM). Concentrations necessary for 50% inhibition of control succinoxidase State III activity (IC50) were 3.0 mM in RHM and 4.7 mM in BHM. The inhibition could not be reversed with dinitrophenol or carbonyl cyanide m-chlorophenylhydrazone. In frozen-thawed BHM and beef heart sub-mitochondrial particles (BHSMP), reversal of AA inhibition could be demonstrated with tetramethylenethylene diamine. Treatment of BHSMP with AA resulted in the accumulation of reduced cytochrome. These data suggest that AA is an inhibitor of heart mitochondrial electron transport at or near Complex 2. This type of biochemical lesion could be responsible for the observed cardiotoxic sequelae reported from AA exposure.

BIOCHEMICAL BASIS OF ALLYLAMINE (AA)-INDUCED VASCULAR CYTOTOXICITY. K Ramos, S L Grossman*, and L R Cox*, Texas Tech University Health Sciences Center, Lubbock, TX and *Philadelphia College of Pharmacy & Science, Phila, PA

Benzylamine oxidase catalyzes the oxidative deamination of AAM to form acrolein (ACR) and hydroxide peroxide (H2O2). Although this metabolic conversion appears to mediate AAM-induced vascular toxicity, the role of ACR and/or H2O2 in the production of cytotoxicity is not yet clear. The present studies were conducted to assess the contribution of H2O2 to AAM-induced cytotoxicity in primary cultures of rat aortic endothelial (VEC) and smooth muscle cells (SMC). Experiments were also conducted to determine if AAM toxicity is dependent upon the presence of serum in the culture medium. Lactate dehydrogenase (LDH) release was used as an index of cytotoxicity. Confluent cultures were exposed to 200 uM AAM for 4-24 hr. The effects of catalase (CAT) (2500 U/ml), a H2O2 scavenger, were evaluated 4 hr after AAM exposure. AAM alone caused a significant increase in LDH release in cultures of both cell types. In the presence of CAT, the release of LDH was reduced by 35.4% and 26.7% in cultures of VEC and SMC, respectively (n=5). The occurrence of cytotoxicity was not serum-dependent since maximal enzyme leakage was observed after exposure to AAM for 24 hr in the absence of serum. These results suggest the concept that both ACR and H2O2 mediate the vascular toxicity of AAM.

COCAINE TOXICITY IN PRIMARY CARDIAC MUSCLE AND NON-MUSCLE CELL CULTURES. AA Welder, IMA Smith, *K Ramos, and P Acosta. University of Texas College of Pharmacy, Austin, TX University of New Mexico College of Pharmacy, Albuquerque, NM and *Texas Tech Health Sciences Center, Department of Pharmacology, Lubbock, TX

A growing number of reports have related cocaine use with the onset of myocardial infarction in young, otherwise healthy individuals. Traditionally, the cardiac effects of cocaine have been attributed to sympathomimetic stimulation. However, some pathological data suggest that cocaine may be directly cardiotoxic. The purpose of this study was to determine the direct cardiotoxic effects of cocaine in an in vitro preparation devoid of sympathetic innervation. Primary cultures of rat cardiac muscle and non-muscle cells were prepared from hearts excised from 3-5 day old Sprague-Dawley rats. Cultures were exposed to various cocaine concentrations (1 x 10^-7 to 1 x 10^-3 M) for 1 to 24 hrs. Beating activity, morphological status, lactate dehydrogenase leakage (LDH), and 45Ca+ uptake were evaluated following cocaine exposure. A decrease in the beating activity of cultured cardiac muscle cells was observed after exposure to the highest cocaine concentrations tested. Upake of 45Ca+ was depressed by high concentrations of cocaine. This effect was consistent with the depression of beating activity induced by cocaine. Morphological alterations were evident after exposure to 1 x 10^-3 M cocaine. Vacuoles appeared 1 hr after cocaine exposure. These vacuoles were replaced by dark granules within 24 hrs. LDH release was significantly elevated by 1 x 10^-3 M cocaine at 24 hrs. The pattern of cocaine-induced morphological alterations and enzyme leakage was similar in non-muscle cells. These data suggest that cocaine induces direct cardiotoxic effects which may be associated with tonic disturbances.
631 ISOLATION AND CHARACTERIZATION OF FOUR SUBGROUPS OF MAMBA (DEENDROASPIOS) CARDIOTOXINS USING PRIMARY CULTURES OF RAT MYOCARDIAL CELLS. P M Mbuga*, A A Welder, D Acosta. University of Texas, College of Pharmacy, Austin, Texas and University of Nairobi, Nairobi, Kenya.

African mamba (Dendroaspis) snakes produce neurotoxic venoms like other snakes in the Elapidae family (cobras, kraits). Cobras produce venoms containing cardiotoxins as the main toxic components, whereas mamba venoms have been suggested to lack cardiotoxins based on data obtained from isolated tissue preparations. Preliminary results obtained in our laboratory show that all four African mambas are potentially cardiotoxic when screened in primary cultures of rat heart cells. The purpose of our further work was twofold: first, to isolate and purify cardiotoxins from venoms in three mambas found in Kenya, Dendroaspis jensesoni (Dj), D. polyphemus (Dp) and D. angusticeps (Da); secondly, to determine the relative effects of the different range of mamba cardiotoxins in primary cultures of rat myocardial cells. Mamba cardiotoxins were isolated and purified from Dj, Dp, and Da venoms by a combination of gel filtration and cation exchange chromatography. Primary cultures of spontaneously contracting myocardial cells, isolated from neonatal rat hearts, were used to evaluate the cardiotoxic effects of the basic non-enzymatic polypeptides purified from the three mamba venoms. The cardiotoxic actions of the polypeptides were assessed on the basis of inhibition of spontaneous beating activity, leakage of lactate dehydrogenase, changes in morphology, cell membrane lysis and loss of viability. Four arbitrary subgroups of mamba polypeptides are suggested: cardiotoxins, Da1-Da3, Dj1-Dj2, Dp1-Dp3; cardiotoxin-like polypeptides, Da4-Da12, Da14-Da18, Dj3-Dj8, Dp16-Dp18; less active membrane lytic polypeptides, Da13, Da15-Da17, Dj9-Dj13, Dp15, Dp19; and membrane lytic polypeptides, Da18, Dj14, Dp20. It is suggested that the toxic effects of mamba cardiotoxins may directly contribute to the pronounced cardiovascular distress in victims of mamba bites, particularly green mamba (D. angusticeps) and Jameson's mamba.

632 GROWTH OF HUMAN LUNG FIBROBLASTS FOLLOWING EXPOSURE TO SODIUM SULFIDE. LJ Hayden, SN Faust and SH Roth, Division of Toxicology, University of Calgary, Calgary, Alberta, Canada. Sponsor: FG Biddle

The growth of fibroblasts derived from fetal human lung (W-38) was monitored following acute exposure of the cells to 250 μM sodium sulfide (Na₂S). Trypsinized confluent cells were plated at 10 cell/well in 1.5 cm multwell dishes and allowed to attach for 24 hours. The medium was then changed to F12 with and without 250 μM Na₂S (pH 7.4), placed in a sealed environmental chamber and gassed with 5% CO₂-95% O₂. The cell cultures were then maintained at 37°C. Medium from the monolayer cultures was changed after 24 hrs and then every three days thereafter. Accumulation of protein in each well was monitored using a quantitative Naphthol yellow S stain; DNA was assayed using hydroxethylidone fluorescence incorporated into viable cells. After 24 hr exposure protein content of cultures incubated with Na₂S was suppressed by 12%. Cellular DNA accumulation in exposed cells was 30% of control after 24 hrs and then recovered to a level of 70% of control at 120 hrs. These studies demonstrate that a single exposure to Na₂S for 24 hrs inhibits the growth of human lung fibroblasts. Supported by Alberta Occupational Health; Heritage Fund.

633 SUPEROXIDE ANION (O₂⁻) PRODUCTION INDUCED BY CHRYSTOLITE ASBESTOS IN THE GUINEA PIG ALVEOLAR MACROPHAGE (AM). P L Roney, and A Holian. Univ. of Texas Health Science Center, Houston, TX. Sponsor: E J Fairchild II

O₂⁻-production by AM has been suggested to play a role in asbestos-related diseases. The purpose of this study was to investigate whether chrysotile induced O₂⁻-production by AM via the specific pathway involving phospholipase C (PLC) activation. 10, 25, or 50 μg/ml chrysotile was added to 10⁶ AM/ml suspended in a cuvette containing 100 μM cytochrome c to measure O₂⁻-production at 550 nm. When chrysotile was added, there was an initial rapid drop in absorbance, followed by an increase within minutes. Examination of the AM revealed that the AM had formed aggregates around the fibrils, accounting for the drop in absorbance. Superoxide dismutase eliminated the increase in absorbance establishing O₂⁻-production. Asbestos treatment stimulated efflux of 45Ca from preloaded AM and increased 32P/Pi labeling of phosphatidylinositol, indicating that PLC was activated. 1 hour pretreatment of AM with 1 μg/ml pertussis toxin blocked chrysotile stimulated O₂⁻-production, but had no effect on phorbol dibutyrate stimulated O₂⁻-production, suggesting that chrysotile stimulation is mediated through a coupling protein. We propose that chrysotile stimulates AM through a PT inhibitable coupling protein activating PLC leading to O₂⁻-production.


Many hypolipidemic drugs, industrial plasticizers and pesticides cause peroxisome proliferation in rat liver, and induce hepatic neoplasms in rats and mice. The peroxisome proliferators (PPs) show little or no evidence of direct interaction with DNA. In the present study effects of the PPs clofibrate (Clo), diethylhexyl phthalate (DEHP) and 2,4-dichlorophenoxyacetic acid (2,4-D) have been studied on different marker enzymes of Wistar rat embryo (WRE) cells, and Syrian hamster embryo (SHE) cells.

Clo and DEHP induced morphological transformation of SHE cells and enhanced the catalase activity (peroxisome) of both SHE and WRE cells. The catalase activity increased more in the WRE cells than in the SHE cells. 2,4-D seemed not to induce transformation or increase the catalase activity of SHE cells. The PPs did not enhance the activity of glucose-6-phosphatase (endoplasmic reticulum) or acid phosphatase (lyso-somes). Electron microscopical studies demonstrated the presence of a very small number of peroxisomes in the SHE cells. Supported by the Norwegian Cancer Society.
ALTERNED IN VITRO GROWTH CHARACTERISTICS OF CANINE TRACHEAL EPITHELIAL CELLS FOLLOWING EXPOSURE TO N-METHYL-N'-NITRO-N'-NITROSO-GUANIDINE (MNNG). AF Hubbs, FF Hahn, and DG Thomassen. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM and Colorado State University, Fort Collins, CO. Sponsor: J M Benson

To investigate the stepwise development of neoplasia in a higher mammalian species, canine tracheal epithelial cells have been cultured in serum-free medium and exposed to the direct-acting carcinogen, MNNG. Up to 6% of the normal canine tracheal cells plated in serum-free medium formed colonies of 30 or more cells. Following exposure to MNNG, cultures were switched to serum-containing medium. In this medium, most cells ceased proliferation and underwent squamous differentiation. A fraction of carcinogen-exposed cultures contained colonies of proliferative cells which approached confluence 3 weeks after exposure. Cells isolated from these colonies were capable of repeated passage in vitro, showed a decreased tendency to differentiate in culture, and were shown by electron microscopy to be composed of epithelial cells. These results indicate that altered growth patterns can be identified in vitro in canine epithelial cells following exposure to MNNG. (Research supported by the U.S. Department of Energy's Office of Health and Environmental Research, under Contract No. DE-AC04-76EV010013.)


p-Dichlorobenzene (pDCB) was found to be carcinogenic in mouse liver and male rat kidney in two-year gavage studies conducted by the NTP. pDCB (97.7% pure) was administered in corn oil via gavage to B6C3F1 mice 16 and 48 hr prior to sacrifice and to Fischer-344 rats 16 and 96 hr prior to sacrifice. UDS and SPS were quantitated by autoradiography in mouse hepatocytes and rat kidney cells. All dose levels of pDCB and the negative control yielded >0.75% DNA/nucleus (NG). Positive control groups yielded >15 NG (liver) and >4 NG (kidney). All control groups yielded <0.52 percent of cells in S-phase. In treatment groups, mice with 300, 600, or 1000 mg/kg pDCB yielded 0.46, 1.90, and 1.55% NG (males) and 2.61, 1.18, and 4.45% NG (females) in hepatocytes. The same doses yielded 0.87, 0.67, and 1.01% NG (males) and 0.48, 0.43, and 0.32% NG (females) in rat kidney. These results indicate that pDCB is not genotoxic in the mouse liver or rat kidney following single administration of doses comparable to the daily doses given in the NTP bioassay. Increases in SPS may indicate a possible mechanism of tumor induction.

Sponsored by the Chlorobenzene Program Panel, Chemical Manufacturers Association, Washington, DC 20037

CHRONIC TOXICITY AND ONCOCENICITY STUDY OF 2,6-DIETHYLAMINOLE. TG Pullin, RW Naismith, JF Hardisty, EB Whorton Jr, GL Ter Haar. Ethyl Corporation, Baton Rouge, LA.

Diethylaniline (DEA) is used in the production of chloroacetanilides which are important herbicides used for corn, soybeans, and other crops. It is a mammalian metabolite for the chloroacetanilide herbicide, alachlor. This study was conducted to investigate the potential chronic toxicity/oncogenicity of DEA. The test material was administered in the diet ad libitum at 0.02, 0.16 or 0.32% to groups of 80 Sprague-Dawley rats of each sex for 104 weeks. The vehicle control group, consisting of 100 rats per sex, received diet alone. DEA had no effect on the survival of the rats. There were no consistently significant alterations in food consumption, hematologic or blood chemistry values or organ weight data. Body weights, male and female rats were consistently reduced over each 6 month interval. Histopathological examination of tissues showed no increased incidence of neoplasms. An increase in the incidence and relative degree of severity of eosinophilic foci in the liver was present in the male rats receiving 0.16% and 0.32% DEA. Eosinophilic foci in livers of males receiving 0.02% DEA were similar to controls. No treatment related changes were observed in the female rats. This study demonstrated no evidence of a chronic toxicity or oncogenic effect of DEA in rats.
639 COMPARISON OF DOSE BY MASS AND NUMBER OF MINERAL FIBERS IN THE INDUCTION OF MESOTHELIOMA IN RATS. D L Coffin, 1 L D Palekar, 2 P M Cook, 3 and A G Stead, 1 IU S Environmental Protection Agency, RTP, NC, 2Northrop Services, Inc., RTP, NC and 3IU S Environmental Protection Agency, Duluth, MN.

Human studies suggest that mineral fibers differ in their potency to induce mesotheliomas. Erionite, crocidolite, chrysotile and asbestiform fibers were injected into the pleural cavities of male Fischer 344 rats. The rats were necropsied upon their death. The survival time and the incidence of tumors were determined for each group and compared. The administered dose was calculated according to both mass and number of minerals of various categories of length and widths. The results indicated: 1) there was an inverse correlation between the survival time and tumor incidence; 2) the average survival time of erionite treated animals was lower than that of asbestos treated animals; 3) the average tumor incidence was higher in the erionite treated animals than in those treated with asbestos. The tumorigenic potencies per mass dose ranked in the decreasing order for erionite, chrysotile, crocidolite and asbestiform fibers. When the dose was computed according to the number of fibers, the ranking was erionite, crocidolite, chrysotile and asbestiform. This latter ranking correlates with that noted in human exposure for erionite and asbestos.

640 A 90-DAY STUDY OF PETROLEUM MIDDLE DISTILLATE-INDUCED DERMAL IRRITATION. J J Freeman, R D Phillips, R H McKee, R T Flumlik and R A Scala, Exxon Biomedical Sciences, East Millstone, NJ.

Some petroleum middle distillates (PMDs) elicit skin tumors in mouse skin painting studies. This tumorigenic activity is not always explained by polycyclic aromatic hydrocarbon (PAH) content; many PMDs contain low concentrations of PAHs. However, the dermal irritation elicited by PMDs may play a role in tumor formation. This study was conducted to study the patterns of dermal irritation elicited by PMDs of varying aromatic content: a steam cracked gas oil (SCCO), a mineral seal oil (MSO) and a Jet Fuel (JF), (aromatic content: 95%, 11%, 21%). Male C3H mice (25/group) were treated topically (37.5 ul 2x/week for 15 weeks) with 10%, 50% or 100% undiluted concentrations of each PMD. The vehicle was a 90/10 mineral oil/gasoline. Dermal changes were evaluated by gross observations and light microscopy. The vehicle and the 10% PMDs were essentially nonirritating. The 50% PMDs elicited slight to moderate proliferative and inflammatory changes. 100% SCCO caused evidence of necrosis on Days 1-7, but not later in the study. 100% MSO did not cause necrosis but with time induced inflammatory and marked proliferative changes. The effects of 100% JF were similar to 100% MSO but less severe. Thus the SCCO elicited a different pattern of dermal effects than MSO or JF. The possible relationships of these dermal changes to epidermal carcinogenesis are under study.

641 COMPARATIVE EFFECTS OF TWO MOUSE SKIN TUMOR INITIATORS IN THREE MOUSE STRAINS. W C Eastin Jr., M R Heitmannick, G E Wilkinson, and A C Peters. NIEHS, Research Triangle Park, NC, Battelle Columbus Division, Columbus, OH.

Two-stage mouse skin initiation/promotion studies are routinely used to identify chemicals with initiating and/or promoting properties. For these tests, it is important to select a mouse strain to minimize total study time and to maximize the probability of identifying a promoter. In the current studies, the sensitivities of both sexes of Sencar, Swiss (CD-1) and B6C3F1 mice strains to different combinations of known initiators (DMBA and MNN) and promoters (TPA and benzoyl peroxide) were compared using the two-stage mouse skin protocol. One week after initiation with DMBA (0.25, 2.5, 25, or 50 μg) or MNN (100, 500 or 1000 μg), promotion was begun with TPA (5 or 1 μg) or benzoyl peroxide (20 mg) and continued for up to 52 weeks. Gross observations of tumor appearance and number were recorded weekly. Results indicate that males respond similar to females of the same strain, that all three strains respond to a low dose of initiator given repeatedly, that there is an initiator dose response, and that there is a difference between strains in mean time to first tumor appearance. These data suggest that the sensitivity of the three strains in the two-stage mouse skin protocol is: Sencar > Swiss (CD-1) > B6C3F1.

642 DERMAL INITIATION/PROMOTION STUDY OF o-BENZYL-p-CHLOROPHENOL (BCP) IN SWISS CD-1 MICE. M Heitmannick, M Ryan, A C Peters, W C Eastin, and L J Birnbaum. Battelle Columbus Division, Columbus, OH and NIEHS, Research Triangle Park, NC.

o-Benzy1-p-chlorophenol (CAS No. 120-32-1) is a biocide which is used as a phenolic disinfectant with a high potential for human exposure. The chemical was tested for activity as an initiator (with TPA promotion), a promoter (after DMBA initiation), and as a complete carcinogen using a mouse skin two-stage protocol. Positive control groups that received DMBA only or DMBA/TPA and negative control groups were included for comparison with test groups. All doses were applied to the back in acetone in a volume of 100 microliters. Initiator doses were administered once during the first week and promoter doses were applied three times weekly for 51 weeks thereafter. BCP was administered once at a dose of 10 mg (for initiation) or three times weekly at doses of 0.1, 1, or 3 mg. The location and dimensions of tumors were recorded weekly. The survival rate of mice was depressed in the positive control groups that developed tumors. BCP showed little activity as an initiator. A dose-related increase occurred when BCP was administered as a promoter, and the time required for the first appearance of a tumor was inversely related to dose. The incidence of tumors produced by BCP was similar in male and female mice. Thus, BCP appears to be a promoter of mouse skin tumorigenesis. (Supported by Contract No. N01-ES-45042 from NTP).
643 THE DERMAL CARCINOGENIC POTENTIAL OF LUBRICANT
BASE OILS AND CUTTING FLUIDS. R H Maexes, R A
Scala, and C Chauzy. Exxon Biomedical Sciences,
Inc., East Millstone, NJ and Centre Henri
Beaurepaire, Rouen, France.

Epidermal cancer in humans has been associated
with industrial exposure to products derived from
unrefined petroleum distillates. Because of this
association, many of these products are now
normally formulated from highly refined (e.g.,
solvent-extracted) lubricant base oils which are
normally inactive in dermal carcinogenesis
bioassays. The current studies assessed the
carcinogenic potential of solvent-extracted
lubricant base oils and several products
including fresh and used cutting fluids which
were prepared from these oils. All materials
were tested for tumorigenic potential in mouse
skin, and selected samples were analyzed for
polycyclic aromatic hydrocarbon content. None of
the solvent-extracted base oils induced skin
tumors, and, similarly, the cutting fluids
prepared from these oils were not carcinogenic.
Additionally, there was no evidence that
industrial usage influenced either the
carcinogenic potential or the PAH levels in these
fluids.

644 EFFECT OF DURATION OF DERMAL EXPOSURE TO BENZO-
a-PYRENE ON THE CARCINOGENIC RESPONSE IN MICE.
Mobil Oil Corporation, Princeton, NJ.

The risk of skin cancer from lifetime exposure
to Benzo-a-pyrene (Bap) is well established in mice.
The present study was conducted to
assess the risk of tumors from shorter periods
of exposure. Twice weekly applications of
0.05% Bap in acetone were performed in groups
of 50 male C3H mice for 6, 13, 26, or 41 weeks.
Tumor incidences after 80 weeks from the ini-
tiation of exposure were 6, 74, 100, and 94%,
respectively. In addition, twice weekly
applications of 0.01% Bap in acetone were per-
formed in groups of 50 male C3H mice for 26,
52, or 80 weeks. Tumor incidences after 80 weeks
from the initiation of exposure were 84, 96, and
96%, respectively. For all groups, tumors have
continued to appear long after treatment
stopped. The incidence was not proportional
to the total dose applied, e.g., 0.01% for 26
weeks = 260 ug total, 84% tumors; 0.05% for 6
weeks = 300 ug. 63% tumors; 0.01% for 52 weeks =
520 ug, 96% tumors; 0.05% for 13 weeks =
650 ug, 74% tumors. Thus the duration of
exposure is a more important factor than the
exposure level in the risk of tumor development
from dermal exposure to Bap.

645 CHRONIC TOXICITY AND CARCINOGENESIS STUDIES OF
SULFAMETHAZINE IN FISCHER 344 RATS. N A
Littlefield, D W Gaylor, R R Allen, and W G.
Sheldon. National Center for Toxicological
Research, Jefferson, AR. Sponsor: C L Wolff

A lifespan dosing study using Sulfamethazine in
the diet of Fischer 344 rats at dose levels of
10, 40, 600, 1200, and 2400 ppm in the diet for
up to 24 months was conducted to determine its
toxicity and carcinogenicity. Sacrifices were
conducted at 18, 24, and 24 months. A total of
810 males and 810 females were allocated to the
study. Food consumption was equal among
the dose groups and the control. A dose effect was
noted at all dose levels for body weight gain.
An inverse dose effect was noted for mortality,i.e., the high dose groups survived longer
than the controls. Mortality for the controls,
10 and 40 ppm dose groups was about 38 to 40% at
24 months, while the mortality in the 600, 1200,
and 2400 ppm groups was only about 20 to 26%.
Low-level statistically significant neoplastic
responses consisting of follicular cell adeno-
carcinoma (male and female) and adenomas (male
only) of the thyroid gland were noted only in
the 24 month sacrifice group. While over-all
trends were noted, the effect was positive only in
the high dose groups for adenocarcinomas in
males and the 1200 ppm group in females. Non-
neoplastic dose-related responses were noted as
thyroid gland hyperplasia and focal cellular
changes, atrophy of the retina and of the
pancreas, and dilatation of the uterus lumen.

646 CHRONIC ORAL TOXICITY AND CARCINOGENICITY STUDY
OF VINYL ACETATE ADMINISTERED IN DRINKING WATER
DC Shaw, and AJ Zubaidy Hazleton UK, Harrogate,
England; JJ Clarke, RW Rickard, TR Tyler, MB
Vinegar and F Carpanini

This study was designed to evaluate the chronic
toxicity and carcinogenic potential of vinyl
acetate (VA). The uninhibited commercial VA
monomer was administered in drinking water,
prepared fresh daily, at nominal concentrations
of 0 (control), 200, 1000 or 5000 ppm (v/v).
Sixty male and 60 female Sprague Dawley rats at
each dose level were used for the 2 year study
following in utero exposure. An additional 30
animals of each sex and group were similarly
treated and used for interim clinical pathology
and post-mortem investigations at 52 and 78
weeks. Chronic oral exposure to VA caused a
statistically significant reduction of water and
food intake and reduced body weight gain at the
highest dose level tested. There were no
effects of treatment with VA on mortality,
 morbidity, clinical signs, haematology, clinical
chemistry, urinary constituents or pathology.
The only treatment-related organ effect was an
increase in relative kidney weight in the
high-dose males which was not accompanied by
toxicologically significant pathological
changes. There was no evidence of
treatment-related systemic toxicity or of an
oncogenic response.
Three isomeric amino-nitrophenols, 4- amino-2-nitrophenol (4A2NP), 2-amino-4-nitrophenol (2A4NP), and 2-amino-5-nitrophenol (2A5NP) have been evaluated for genotoxic activity in bacterial and mammalian systems and for carcinogenic potential in two year studies in rats and mice. Each compound was mutagenic in S. typhimurium and in the mouse lymphoma forward mutation assay. During two year studies 4A2NP was administered in feed, 2A4NP and 2A5NP by gavage in corn oil. 4A2NP induced transitional cell carcinomas of the urinary bladder in male rats; 2A4NP caused tubular cell hyperplasia and adenomas in the kidneys of male rats; 2A5NP caused an increase in actin cell adenomas of the pancreas in male rats and marginal increase in the incidence of preputial/corporal gland neoplasms but had no effect on the kidney or bladder. The differing patterns of carcinogenic responses noted for the three isomers indicate a dependence on the relative position of the -NH2 and -NO2 on the phenol ring. 2A4NP and 2A5NP, which differ only in the position of the -NO2 group, were administered to rats at the same dose levels and by the same route of administration, but produced different carcinogenic responses. None of the isomers produced a carcinogenic response in mice at the dose levels employed for these studies.

Carcinogenic activity associated with 1,3-dichloroacetone in the mouse skin assay. M Robinson, and B.A Merrick. Toxicology and Microbiology Division, HEEL, USEPA, Cincinnati, OH.

1,3-dichloroacetone (DCA) has been found in drinking water and is produced when humic acid solutions are chlorinated. DCA was a potent, direct-acting mutagen in the Ames assay when tested in this laboratory. Recent work has shown that DCA initiates tumors in the SENCAR mouse skin assay but a dose response was not seen due to cytotoxicity at higher dose levels. To determine if a tumor initiating dose response effect could be observed with DCA, it was applied topically to SENCAR mice (40/group) at doses of 37.5, 75, 150, and 300 mg/kg. Doses were administered once in 0.2 ml ethanol. After two weeks, 1.0 µm TPA in 0.2 ml acetone was applied 3X weekly for 20 weeks. After 40 weeks, the % of animals with tumors for 37.5, 75 and 150 mg/kg groups were 50%, 60% and 73% respectively, compared to controls with 10% tumors. Only 13% of mice treated with DCA at 300 mg/kg developed tumors. These data demonstrate that DCA does initiate skin tumors in the mouse in a dose-response manner when applied at non-cytotoxic levels. (Abstract does not necessarily reflect EPA policy).

Butenedial - a predicted metabolite of 1,3-butadiene. M C Bird, D Lewis, C Witz, D V Parks. Exxon Biomedical Sciences, Inc., E. Millstone, NJ. Surrey University, Guildford, Surrey, UK. 3UMDN-Jobert Wood Johnson Medical School, Placatay, NJ.

Benzenes was recently reported to be metabolized by mouse liver microsomes to the open-ring six carbon diene dialdehyde trans,trans-muconaldehyde. The latter compound was also reported to decrease bone marrow cellularity, hematocrit, and increase spleen weight when administered to mice. Similar hematotoxic effects reported to occur in mice exposed to 1,3-butadiene (BD) led us to hypothesize that BD may be metabolized in the mouse to the homologous 4 carbon dialdehyde, butenedial. To explore this idea, computer graphics and electronic structure calculations using the MINDO method were carried out. The results show that the structures of the two dialdehydes as well as the parent compounds have similar shapes and electronic parameters, suggesting similar lipophilicities and toxicities. Hence, it is predicted that, like benzene, BD may be metabolized to a reactive alpha,beta-unsaturated dialdehyde responsible, in part, for the observed hematotoxic effect of BD. Confirmatory analytical studies of BD hepatic metabolism are planned.


Monuron is a phenyl substituted urea herbicide, widely used as a broad-spectrum herbicide for control of grasses and weeds along ditches and rights-of-way. Monuron was not mutagenic in S. typhimurium, E. coli, B. subtilis, and yeast tests. Monuron induced chromosomal aberrations and sister chromatid exchanges in CHO cells and unscheduled DNA synthesis in human lung fibroblasts. Toxicology and carcinogenesis studies of monuron were conducted because of potential for human exposure. Groups of 50 F344/N rats of either sex, 7-8 weeks old, were fed diet containing monuron at 0, 750, or 1,500 ppm and groups of 50 B6C3F1 mice of either sex, 7-9 weeks old, at 0, 5,000, or 10,000 ppm for 103 weeks. Throughout the study, body weight gains of dosed rats and mice of either sex were lower than those of their respective controls. Survival of dosed male and female rats and mice was compatible to that of controls. Associated with monuron administration there were increased incidences of renal tubular cell adenomas and adenocarcinomas (controls, 0/50; low dose, 3/50; high dose, 15/50) and hepatic neoplastic nodules and carcinomas (1/50, 6/49, 9/50) in male rats and cytomegal of renal tubular epithelial cells in male and female rats. No increased incidences of neoplastic and non-neoplastic lesions were observed in male and female mice. Thus, monuron was carcinogenic for male F344/N rats but was not carcinogenic for female F344/N rats and B6C3F1 mice of either sex.

Microsomal hepatic hydroxylation of the colon carcinogen AZO to MAM and colonic metabolism of MAM by cytoytic alcohol dehydrogenase (ADM) was assayed in young (2-4 mo old aged 22-26 kg) male Fischer 344 rats. AZO hydroxylase (AZO-H) was quantified by reversed-phase HPLC, and metabolism of MAM was measured by reduction of NAD+ at 340 nm. Activity of AZO-H declined by 33% in aged rats on a per mg protein basis, and by 15% on a per nmol cytochrome P-450 basis. The latter decline in activity is in agreement with the 21% decrease in hepatic P-450 content found in aged rats. In young rats, phenobarbital (PB), 80 mg/kg x 5 days, caused a 2.7-fold induction of P-450 and a 1.8-fold induction of AZO-H. Thus, activity on a per nmol P-450 basis in PB-induced rats was decreased 42% relative to control. This evidence, coupled with the decline in AZO-H seen in aged rats and data that their ycholanthrene, 30 mg/kg x 5 days, showed no inductive effect, implies indirectly that the decline in AZO metabolism is due to a decreased content of a specific isozyme(s) of P-450 responsible for metabolism of AZO. Colonic metabolism of MAM by ADM, in contrast to AZO-H, showed no age-related decline in activity. These results suggest a greater role for extrahepatic metabolism of AZO in aged rats.


Modifying effects of five phenolic antioxidants on N-methyl-N'-nitro-N'-nitrosoguanidine (MNNG)-induced stomach carcinogenesis were investigated in F344 male rats. Groups of 20 rats were given an intragastric dose of 150 mg/kg by MNNG. Starting one week later they received diet containing 0.8% catechol (CC), 1.2% 2-t-butyl-4-methylphenol (TBMP), 1.5% p-t-butylphenol (PTBP), 1.5% methylhydroquinone, 1.5% 4-methoxyphenol (4MP) or basal diet alone for 51 weeks. Histological examination revealed that CC, TBMP and PTBP strongly enhanced the development of squamous cell carcinoma of the stomach, whereas AMP inhibited induction of carcinoma in situ. In the pyloric region of the glandular stomach, CC strongly enhanced the development of adenomatous hyperplasia and adenocarcinoma; in addition, CC, TBMP and PTBP strongly induced adenocarcinoma in 100% and 20% of rats, respectively. These results clearly show that CC, TBMP and PTBP are strong promoters for stomach carcinogenesis, and that CC, which is known to be widely present in our environment as a natural or industrial product, is a new glandular stomach carcinogen with strong promoting activity for the stomach.


Cardiotoxicity is a major cause of morbidity and mortality following high dose cancer chemotherapy. The antineoplastic drugs, bis-chloroethyl nitrosourea (BCNU) and melphan, are alkylating agents known to reduce intracellular glutathione (GSH) in some cell types. A reasonable hypothesis is that the decreased GSH increases the cardiac cell's sensitivity to cytotoxic effects of the drugs or decreases its ability to detoxify peroxides or free radicals formed during drug metabolism. To establish a relationship between GSH and cardiotoxicity, we report here human GSH levels from cardiac biopsies. Cardiac biopsies from cancer patients were analyzed for GSH content before and immediately after high dose cyclophosphamide, cisplatin and either BCNU or melphan. Cardiac GSH levels in biopsies of less than 1 mg/kg were determined by the HPLC method of Keller and Menzel (Anal. Biochem. 131, 418, 1985). A 60-80% reduction in cardiac GSH following chemotherapy was observed in four of five patients examined. Although initial GSH levels varied 100-fold between individuals, a similar degree of reduction occurred. Initial GSH content and percent decrease after chemotherapy appear correlated with cardiotoxicity. (Supported by NIH Grants CA14236 and BR01693 and NCI Grant 5-PO1-GA32672.)


The effects of various chemicals on BHA-induced rat forestomach hyperplasia was evaluated in 2 experiments. In the first, groups of male F344 rats were given diet containing 1% BHA together with 50 ppm indomethacin (IM), 1.5 ppm dexamethasone (DEX), 0.15% 6-aminocaproic acid, 0.15% FOY 305, 0.25% diethylmaleate (DEM) or 0.015% retinyl acetate for 52 wks. Histological examination revealed that the development of forestomach epithelial hyperplasia was completely inhibited by DEM, and significantly inhibited by IM or DEX. In experiment 2, groups of F344 male rats were given diet containing 1% BHA together with 0.7% BH, 1% sodium ascorbate (SA), 1% propyl gallate (PG), 2% alpha-tocopherol or 0.2% ethoxyquin for 52 wks. SA enhanced the induction of hyperplasia in the prefundic region, whereas PG enhanced it in the mid-region. These results indicate that glutathione may be involved in the induction of forestomach hyperplasia by BHA, and that some antioxidants (e.g. SA, PG) enhance the induction of BHA-induced hyperplasia at different sites of the forestomach epithelium.
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**EFFECT OF AGING ON PROSTATE CARCINOGENESIS INDUCED BY 3,2'-DIMETHYL-4-AMINOBIPHENYL (DMAB) IN F344 RATS.** A Nakamura, T Shirai, S Fukushima and N Ito. 1st Department of Pathology, Nagoya City University Medical School, Nagoya, Japan.

DMAB, a multipotential carcinogen, induces prostate carcinoma in F344 rats. The effect of aging on DMAB prostate carcinogenesis was examined in male F344 rats. Rats 5, 35 and 65 weeks of age were given DMAB s.c. at a dose of 200 or 150 mg/kg once a week for 4 weeks. Initially, the experiment was started with 200mg DMAB. Since this dose was too toxic for 65-week-old rats, additional groups of 65-week-old and 5-week-old rats were treated with 150mg DMAB for comparison. All animals in the 5- and 35-week-old groups were killed 60 weeks after the beginning of the experiment, but rats in the 65-week-old group were killed after 46 weeks. Carcinomas of the prostate in each group were found in incidences of 8 to 19%. The incidences of atypical hyperplasia in the prostate were 33% to 75%. An age-related decline in DMAB induction of small intestine tumors. The incidences of tumors of the preputial and mammary gland were the highest in the 35-week-old group. There were no clear differences in the frequencies of tumors of the large intestine, Zymbal gland, subcutis and urinary bladder between groups of prostatic glands (NPG) in experimental conditions, injection of DMAB in rats at different ages had no effect on prostate carcinogenesis.

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**CELL KINETICS OF PEPSINOGEN DECREASED PYLORIC GLAND CELLS, A PUTATIVE PRENEOPLASTIC LESION, IN RATS TREATED WITH MNNG.** M Mutai, M Tatekatsu, K Imada, and N Ito. 1st Dept. Pathol., Nagoya City Univ. Med. Sch., Nagoya, Japan.

Pepsinogen 1 (Pgl) decreased pyloric gland cells (PDPC) represent the earliest histopathological-ly detectable preneoplastic change during MNNG induced rat gastric carcinogenesis. In this study, we evaluated the cell kinetics of the PDPC compared with glandular (NPG). Forty male WKY rats, aged 7 weeks, were divided into two groups. One was given 100 mg/kg MNNG in the drinking water for 10 days and sacrificed at the end of 12 days. The other was given tap water as a control. Bromodeoxyuridine (BrdU) was applied to both groups as a single injection or by continuous labeling using osmotic mini-pumps for 10, 7, or 4 days before sacrifice. The stomachs were fixed and routinely processed. The sections were double-stained immunohistochemically for BrdU incorporation and Pgl. The labeling indices after a single injection were 0.12% in NPG and 1.7% in PDPC. More than 90% of PDPC cells incorporated BrdU by 7 days of labeling, but NPG cells incorporated only 7.8% by 10 days. These results suggest that NPG cells have little cell proliferating activity and are replaced by newly formed cells in about 14 days. In contrast, PDPC cells have increased proliferating activity and are replaced in 7-10 days. PDPC show an independent proliferation pattern as an early preneoplastic event.

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**MARKED ENHANCING POTENTIAL OF PRIOR N-METHYL-N-NITROSODESAME (MN) TREATMENT ON RAT THYMIC NEOGENESIS IN VARIOUS ORGANS INDUCED BY 5 DIFFERENT CARCINOGENS.** S Uwagawa, K Imada, T Tsuda, T Masui and N Ito. 1st Dept. Pathol., Nagoya City Univ. Med. Sch., Nagoya, Japan.

The enhancing effects of 6 different carcinogens on two stage carcinogenesis initiated with MN on rats were investigated. Male, 6-week-old F344 rats were divided into 7 groups of 25 rats each. Rats were injected with MN (20mg/kg, 1,1) twice a week for 4 weeks, then treated with DMAB (3',2'-dimethyl-4-aminobiphenyl, 50mg/kg, s.c. 1x/week), DBN (N-nitroso-di-n-butylamine), DBN (N-nitroso-di-n-butylamine), DBN (N-nitroso-di-n-butylamine). All animals in the 5- and 35-week-old groups were killed 60 weeks after the beginning of the experiment, but rats in the 65-week-old group were killed after 46 weeks. Carcinomas of the prostate in each group were found in incidences of 8 to 19%. The incidences of atypical hyperplasia in the prostate were 33% to 75%. An age-related decline in DMAB induction of small intestine tumors. The incidences of tumors of the preputial and mammary gland were the highest in the 35-week-old group. There were no clear differences in the frequencies of tumors of the large intestine, Zymbal gland, subcutis and urinary bladder between groups of prostatic glands (NPG) in experimental conditions, injection of DMAB in rats at different ages had no effect on prostate carcinogenesis.

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**VINYLACETATE: INHALATION TOXICITY AND CARCINOGENICITY STUDY IN RATS AND MICE.** PE Owen and CA Thompson Hazleton UK. Harrogate, England; JClarity, JW Rickard, TR Tyler and MB Vinegar.

A two year inhalation study was conducted in Sprague-Dawley rats and CD-1 with vinyl acetate (VA). Groups of 90 male and 90 female rats and mice inhaled VA at 0, 50, 200 or 600 ppm for 6 hrs/day, 5 days/week. 10 animals/sex/species were designated for interim clinical and pathological investigations at weeks 53 and 83. Similar groups were removed from treatment at week 70 and examined 16 weeks later.

No effect on rat survival was noted. A few early deaths in mice were possibly associated with inhalation of 600 ppm of VA. Body weight gain was lower in both species at higher concentrations. Treatment-related pathology in both species was limited to the respiratory tract (hyperplasia atrophy) at 200 and 600 ppm. Generally these changes were evident at 53 weeks and showed little progression. Following recovery, hyperplasia reversed but not atrophy. 11 nasal tumours and 1 larynx squamous carcinoma were observed in rats at 600 ppm. A nasal papilloma occurred in 1 rat at 200 ppm. One squamous carcinoma and one squamous nodule were noted in lungs of mice at 600 ppm. There was no evidence that exposure to VA caused adverse systemic effects and 50 ppm was a clear no observable effect level.
INFLUENCE OF VIRAL INFECTIONS ON TUMOR INCIDENCES, BODY WEIGHT AND SURVIVAL OF FISCHER 344 RATS. Q N Ho, J Edmondson, and J K Haseeman. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Sialodacryoadenitis virus (SDAV), Sendai virus (SV), and pneumonia virus of mice (PVM) are the common viral infections used in rats. Influence of these viral infections on the incidence of some common tumors (>20%) such as leukemia, pituitary tumors and adrenal pheochromocytomas in the male rat and pituitary tumors, mammary tumors and leukemia in the female rats along with the body weights and survival in 29 diet control groups at 5 different laboratories with and without the viral infections were evaluated. The PVM and SV but not the SDAV infections significantly (P<0.05) decreased the body weights. The rats with PVM infection had significantly lower incidence of leukemia and this effect can be explained by laboratory to laboratory variability. Male rats with SDAV infection had significantly higher (P<0.01) incidence of pituitary tumors and this difference can be explained on the basis of time-related trends and laboratory to laboratory variability. All other tumor incidences evaluated in this study and the survival were not affected by the viral infections.

PERINATAL CARCINOGENESIS INDUCED BY INHALED VINYL CHLORIDE. M J Radke, J Warkany, K Stemmer, E Bingham. University of Cincinnati College of Medicine, Institute for Developmental Research, Children's Hospital, Cincinnati, OH. Sponsor: D Marshawsky

In order to investigate the effects of vinyl chloride (VC) on perinatal carcinogenesis, three groups of pregnant Sprague-Dawley rats were exposed by inhalation to 600 ppm VC 4 hrs/day, from the 9th to 21st day of gestation. One VC group received 5% ethanol (EtOH) in water, and one group, with neonates, was additionally exposed to VC through weaning of pups. Offspring, including those of EtOH and filtered air control groups, were observed for life. The development of angiosarcoma (liver, lung, muscle) in VC-treated groups indicates the transplacental potential of VC to initiate cancer in utero. Ingestion of 5% EtOH by VC-treated dams did not enhance the incidence of treatment-related malignancies. Post-natal exposure of pups to 600 ppm VC increased the incidence of liver tumors, 10 of 72 offspring with angiosarcoma and 48 of 72 with carcinoma. In comparison, exposure to VC in utero alone induced liver angiosarcoma in 1 of 71 rats and hepatocellular carcinoma in 11 of 71. Treatment-related malignant tumors included angiosarcoma in the liver, lung and muscle, and carcinoma in the liver, bile duct and mammary tissue. Supported by USEPA 88-03-2402

TRANSPLENTAL (TP) TUMORIGENESIS BY N-NITROSO-ETHYLUREA (NEU), N-NITROSODIETHYLAMINE (NDEA), AND N-NITROSODIMETHYLAMINE (NDMA) IN MICE. E M Anderson, J M Rice, and A Hagihara, National Cancer Institute, Frederick, MD

Childhood cancers including brain tumors are postulated to be associated with exposure to nitrosamine-containing materials. Mice develop capacity for metabolic activation of nitrosamines by gestation day (g.d.) 16, and those of the C3H strain show neurogenic tumors after tp NEU. To test this strain as a model for tp formation of neurogenic tumors by nitrosamines pregnant C3H/HeNCR mice were treated ip on g.d. 16 or 19 with NDEA, NDMA, or NEU (0.5, 0.1, 0.4 mmole/kg, resp.). In the offspring, NEU caused multiple alveologenic lung tumors, increased hepatocellular tumors, and schwannomas in 12% (g.d. 16) or 35% (g.d. 19). Unusual cranial tumors were also noted, including 4 glioblastomas. NDMA was more effective after g.d. 19 than 16, causing a significant increase in both liver and lung tumors compared with controls. NDMA, being tested for the first time as a tp carcinogen in mice, resulted in an increased incidence of liver tumors in both sexes after g.d. 19 exposure, but had no effect on lung tumors. Five hepatocellular sarcomas and a schwannoma—like neoplasm also occurred. These results confirm that nitrosamines can be effective tp carcinogens in rodents once fetal activating enzymes have developed, but do not have a pronounced neurotropic effect analogous to that of the nitrosoureas.
663 RELATIONSHIP OF LYSOSOMAL PROTEIN OVERLOAD IN THE KIDNEY AND TUBULAR TUMORIGENESIS.
C L Alden, R Parker, M F Ezra, E VonBargen, G M Ridker. The Procter & Gamble Company, Cincinnati, OH.

Chemicals such as d-limonene and unleaded gasoline cause renal tubular tumors in male rat kidneys associated with alpha-globulin lysosomal overload. This chemically-mediated alpha-globulin lysosomal overload induces increased apoposis with consequent increased replication in proximal convoluted tubular epithelium.

We have developed evidence that alpha-globulin overload results in lysosomal instability. This lysosomal instability may lead to leakage of lysosomal enzymes as the cytotoxic mechanism causing increased apoptosis. Furthermore, lysosomal enzymes have been demonstrated to be genotoxic by Zajac-Rayne, suggesting an initiating indirect genetic mechanism in the tubular tumor development associated with long-term unleaded gasoline and d-limonene treatment. This initiating influence may act in concert with the replicative promotional influence consistently leading to tubular tumor formation in this alpha-globulin nephropathy syndrome. These chemicals are now recognized as inducers of this unique syndrome.

665 DICHLOROACETATE (DCA) INDUCED DNA STRAND BREAKS APPEAR BEFORE PEROXISOME PROLIFERATION
M A Nelson, R J Bull, and D L Springer. Pharmacology/Toxicology Program, College of Pharmacy, Washington State University, Pullman, WA. Battelle-Pacific Northwest Laboratory, Richland, WA.

DCA increases the incidence of hepatic tumors in B6C3F1 mice. Previous work has shown that DCA produces single-strand breaks (SSB) in hepatic DNA in vivo. DCA also induces hepatic peroxisomes; a response associated with hepatocarcinogenesis. The present study examined whether SSB arose from peroxisome proliferation (PP). Male B6C3F1 mice were exposed to a single oral dose of 3.8 mmol/kg DCA. At various time points, between 1 and 24 hours after administration of DCA, the livers were removed and SSB measured using the alkaline unwinding assay of Morris and Shertzer (1985). Peroxosomal β-oxidation of palmitoyl-CoA was used to determine PP (Lazarow, 1981). DCA significantly increased the SSB at 1 hour, 2 hours, and 4 hours and returned to control levels at 8 hours. No increase in peroxisomal β-oxidation was observed at any of the time points. This data indicates that the SSB induced by DCA do not secondary to PP. (Supported by NRCUS and DHE Contract DE-AM06-76-RLO2225, Battelle-PNL, and USAF grant # AFOSR 86-0284)

664 RELATIONSHIP OF OXIDATIVE DAMAGE TO CARCINOGENICITY WITH THE PEROXISOME PROLIFERATORS DEHP (2-ETHYLDIETHYLPHthalate) AND WW-14,643 (WW).

DEHP (1.2%) and WW (0.1%) in the diet produce similar degrees of peroxisomal proliferation but WW produces a much higher incidence of hepatocellular cancer in rats than DEHP (Toxicologist 7:166, 1986). To investigate further this difference in carcinogenicity, H2O2 detoxification enzymes and oxidative damage were measured in livers of male F-344 rats fed 1.2% DEHP or 0.1% WW for up to one year. Both DEHP and WW increased catalase activity (25%) and decreased glutathione peroxidase activity (50%) from 8-365 days. Reduced glutathione concentrations were not affected by 151 days of DEHP or WW. Autofluorescent lipofuscin, quantitated morphometrically in sections of liver, was increased 3-fold after 39-365 days of DEHP feeding. In contrast, lipofuscin was increased 4-fold after 18 days and was 30-fold above controls after 365 days of WW. Conjugated dienes were increased (49%) only in rats receiving WW for 151 and 365 days. Thus, changes in lipofuscin and conjugated dienes but not enzyme activities correlated with the relative carcinogenicity of WW and DEHP. The data indicate that oxidative damage is a key component in peroxisome proliferator-induced carcinogenesis.

666 INHIBITION OF MOUSE HEPATOCYTE INTERCELLULAR COMMUNICATION BY ACTIVATED OXYGEN. P A Nigrovic, R J Ruch, and J E Klirig. Dept. of Pathology, Medical College of Ohio, Toledo, OH.

Intercellular communication through gap junctions (IC) is important in several cellular processes including growth control, development, electrical synapsing, and homeostasis. The purpose of this study was to determine if activated oxygen could inhibit IC between primary cultured male B6C3F1 mouse hepatocytes. IC was assayed in 24 h-old hepatocyte cultures by microinjection of fluorescent Lucifer Yellow CH dye and detection of dye spread to adjacent hepatocytes (dye-coupling). Treatment of hepatocytes with sublethal concentrations of paraquat (PQ, 0.5-5 mM); hydrogen peroxide (0.5-2 mM); glucose oxidase (0.1 U/ml), or xanthine oxidase (0.2 U/ml plus 1 mM xanthine) at durations of 2-8 h resulted in concentration-dependent decreases in dye-coupling. Addition of the antioxidants DPPD (25 U/ml) or vitamin E (100 U/ml) to PQ-treated cultures decreased the inhibitory effect of PQ on dye-coupling. Pretreatment of the PQ-treated cultures with aminoguanidine (20 mM) or diethylmaleate (25 U/ml) potentiated inhibition of hepatocyte dye-coupling by PQ. Thus, exposure of hepatocytes to sublethal concentrations of activated oxygen results in the loss of IC. This effect may be an important mechanism in the in vivo toxicity of prooxidant compounds.

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KINETICS OF THE INHIBITION OF MOUSE HEPATOCYTE INTERCELLULAR COMMUNICATION BY THE LIVER TUMOR PROMOTER PHENO Barbital. R J Rucb and J E Klauning. Medical College of Ohio, Toledo, OH.

The inhibition of intercellular communication through gap junctions (IC) by tumor promoters may permit initiated cells to escape growth regulation by adjacent cells and proliferate. In this study, the kinetics of the inhibition of male B6C3F1 mouse hepatocyte IC by phenobarbital (PB) were studied. Hepatocyte IC was evaluated by microinjection of fluorescent Lucifer Yellow CH dye into one hepatocyte and visualization of dye spread into adjacent hepatocytes (dye-coupling). Dye-coupling was detected in untreated hepatocytes after 0.5 h in culture, reached a maximum level in 24 and 48 h old cultures (95.2%), and then decreased over the next 72 h. PB (20-500 Mg/ml) decreased dye-coupling in a dose-related manner when added to freshly plated cultures over 0.5-12 h of treatment but was not seen after 24 treatment. Dye-coupling in hepatocytes pre-treated with PB for 24 h was not inhibited by a secondary application of PB. PB also decreased dye-coupling within 30 min when added to established (24 h-old) hepatocyte cultures. This effect was maximal after 2 h treatment, was dose-dependent, and was reversible within 15 min after removal of PB. Thus, PB inhibited mouse hepatocyte dye-coupling rapidly and reversibly and the cells became refractory to the inhibitory effect after prolonged PB exposure.

MECHANISM OF INHIBITION OF INTERCELLULAR COMMUNICATION BY THE PROMOTERS DOT, PHENO Barbital, AND LINDANE IN MALE B6C3F1 MOUSE HEPATOCYTES. M E Schutz, R J Ruch, and J E Klauning. Dept. of Pathology, Medical College of Ohio, Toledo, OH.

Intercellular communication (IC) through gap junctions may regulate cellular proliferation. The liver tumor promoters, DOT, phenobarbital (PB), and lindane inhibit IC between mouse hepatocytes in vitro. In the present study, we have examined the inhibitory effects of these promoters on the formation of IC in freshly plated mouse hepatocytes and on established IC in 24 h old hepatocyte cultures. Isolated individual male B6C3F1 mouse hepatocytes were plated onto glass dishes. At the same time, sublethal doses of DOT (1-10 ug/ml), PB (20-500 ug/ml), or lindane (5-15 ug/ml) were added to the cultures. IC was then evaluated by microinjection of fluorescent dye (Lucifer Yellow CH) (dye-coupling) 2, 4, and 6 h later. In addition, 24 h old cultures were treated with the promoters and evaluated for dye-coupling 2, 4, and 6 h later. In freshly plated cells, DOT, PB, and lindane inhibited dye-coupling after 2, 4, and 6 h treatment, suggesting that these promoters can interfere with gap junction formation. In contrast, only PB inhibited dye-coupling in 24 h old cultures, suggesting that PB was also capable of inhibiting IC in preformed gap junctions. These data suggest different mechanisms of action of the three promoters on the inhibition of mouse hepatocyte IC.

PHENO Barbital PROMOTION IN INFANT B6C3F1 MICE: INFLUENCE OF GENDER AND INITIATOR. J E Klauning, C M Weghorst, M A Pereira, E Lin, and T R Weghorst. Dept. of Pathology, Medical College of Ohio, Toledo, OH and HRL, USEPA, Cincinnati, OH.

Phenobarbital (PB) has been shown to promote hepatic tumorigenesis when chronically administered to male B6C3F1 mice after initiation with diethylnitrosamine (DENa) at 30 d of age. However, if male B6C3F1 mice are initiated with DENa at 15 d of age followed by long term PB treatment, an inhibition of tumor formation occurs. This study evaluated the role of the initiating compound and gender on the ability of PB to promote hepatocellular lesions in the initiated infant B6C3F1 mouse. At 15 d of age, male B6C3F1 mice received either a single ip initiating dose of DENa (5 ug/gbw), dimethyl-nitrosamine (DNNA; 5 ug/gbw) or saline. Female mice received either DENa or saline. All mice were killed at 28 weeks of age. Males treated with DENa and PB displayed a significant decrease in hepatic tumors compared to males receiving DENa only. Females exposed to DENa + PB exhibited an enhancement of liver tumors compared to DENa only treated females. PB treatment in males initiated with DENNA also showed hepatic tumor promotion. Foci and adenomas in PB-treated mice were predominantly eosinophilic in phenotype, while non-PB promoted lesions were basophilic in appearance.

MODIFICATION OF AFLATOXIN B1 BIOTRANSFORMATION IN VITRO AND DNA BINDING IN VIVO BY DIETARY BROCCOLI IN RATS. H S Ramsdell and D L Eaton. Department of Environmental Health, University of Washington, Seattle, WA.

Aflatoxin B1 (AFB1) is a potent hepatotoxin and carcinogen and a contaminant of the human food supply. Cruciferous vegetables and some of their chemical constituents have been shown to reduce the incidence of tumors in animals given carcinogens. In this study, rats were fed a purified diet, purified diet containing 25% freeze-dried broccoli; or rodent chow for 3 weeks. The animals were then treated with 3H-AFB1 (0.25 mg/kg) and hepatic AFB1-DNA binding was determined. Rates of hepatic microsomal AFB1 oxidation and cytosolic AFB1-epoxide GSH conjugation were measured by HPLC analysis. Microsomal dealkylation of resorufin ethers, epoxide hydrolysis of p-nitro styrene oxide and benzo[a]pyrene-4,5-oxide (BaPO), and cytosolic GSH transferase activity toward 1-chloro-2,4-dinitrobenzene (CDNB), 3,4-dichloronitrobenzene (DCNB) and BaPO were measured. Cytosolic conjugation of AFB1-epoxide with GSH was increased 2.8-fold in rats fed broccoli relative to the purified diet group, whereas GSH conjugation of CDNB, DCNB and BaPO was increased by 63%, 31% and 27%, respectively. The rate of microsomal epoxidation of AFB1 was not affected by the dietary treatments. Binding of AFB1 to DNA in vivo in the broccoli group was decreased to 43%, whereas DNA binding in chow-fed rats was not significantly reduced. These data indicate that broccoli contains substances that result in a reduction in the binding of AFB1 to DNA, apparently through increases in GSH transferase(s) with greater activity toward AFB1-epoxide than the surrogate substrates. (Supported by NIH Grant T32 ES-07032.)
IN VIVO DNA BINDING DOSE-RESPONSE STUDIES WITH 671
AFB1 AND THE ANTI-CARCINOCEN INDOLE-3-CARBINOL
(I3C). R Dashwood, D Arbogast, A Pong, J Hendricks and G Bailey. Oregon State University, Corvallis, OR. Sponsor: D Sellenevich

Several recent reports describe inhibitor-mediated reductions in the covalent binding of various carcinogens to DNA in vivo. Most show inhibitory effects after testing at one inhibitor and one carcinogen dose-level only. Thus, the detailed relationships between carcinogen dose, inhibitor dose, and in vivo inhibitory potency have not been clearly delineated in any species. Therefore, inhibitory potencies were assessed in rainbow trout (Salmo gairdneri) given a range of carcinogen (AFB1) and inhibitor (I3C) doses by concurrent dietary exposure. In vivo binding of AFB1 to liver DNA was used as an end-point; linear increases occurred with increasing AFB1 dose and with time of I3C/AFB1 co-treatment, at each I3C dose-level. Successive increases in I3C dose gave dose-related decreases in DNA binding, yielding a series of curves of decreasing slope, with 95% inhibition at the top dose of 4000ppm I3C. Linear inhibitory responses observed at low I3C doses indicate the possibility of a threshold for any significant threshold for I3C protection against AFB1-DNA binding. Thus, even low levels of I3C may offer some protection against chemically-induced neoplasia. (Studies supported in part by grants CA34732, ES03850, ES00210).

672 EXAMINATION OF EVIDENCE FOR THE INTRACELLULAR FORMATION OF AN ADDUCT, N'-HYDROXYMETHYLDIADEOXY
ADENOSINE (hm6da), BY FORMALDEHYDE. M. Casanova and H d'A Hock. CIIIF, Res. Triangle Park, NC

Formaldehyde (FA)-induced DNA-protein cross-links (DPX) are unstable to enzymatic hydrolysis of the DNA and dissociate, releasing free HCHO. The released FA may react with any DNA nucleosides (DNAs) in the hydrolyzate to form hydroxymethyl adducts (hmNAs). To test whether this mechanism can explain the detection of hm6da in hydrolyzates of DNA from mammalian cells incubated with FA (Beland et al., 1984), a mixture of the four major dmNs was incubated with [14C]FA (1 μM) in the Tris buffer, pH 8. Tris. All of the dmNs formed hmNAs in this buffer, which were detectable by HPLC. The labeled compounds were identified from the elution profile and spectra of products obtained by reacting the dmNs with FA (1 and 82 μM). No hmNAs were formed when [14C]FA was incubated with the dmNs in the 14C amine buffer, Tris, due to preferential reaction of FA with the amine group of the buffer. The yield of [14C]hm6da obtained in big-Tris buffer, adjusted for differences in reaction concentrations, was almost identical to the yield of [14H]hm6da reportedly found in DNA hydrolyzates (2.7 vs 2.3 hm6da per 10^10 da). These results strongly suggest that the hm6da was not an intracellular product, but was formed in the hydrolyzate by release of [14H]FA from DPX and reaction of the released [14H]FA with da.

673 FORMATION OF CROSS-LINKED ADDUCTS ON REACTION OF AMINO ACIDS WITH FORMALDEHYDE AND DEOXYRIBONUCLEOSIDES
OR DNA. T R Fennell, F H Deal, and J A Swenberg. Chemical Industry Institute of Toxicology, Research Triangle Park, NC

Formaldehyde (HCHO) is a nasal carcinogen which is known to form DNA-protein cross-links both in vitro and in vivo, and little is known about their chemical nature. Previous studies have identified unstable adducts formed by reaction of HCHO with deoxyguanosine (dG) and lysine. We have investigated the cross-linking reaction of HCHO with nucleosides and other potentially reactive amino acids (gly, thr, and N-acetyl derivatives of arg, asp, cys, glu, his, phe, ser, trp and tyr). After incubation of HCHO (100 mM) with nucleosides (10 mM) and amino acids (10 mM) at 37°C and pH 7.4 for 12 h, products were analysed by reverse phase HPLC. Glycine reacted with dG and HCHO to form two unstable products. On reaction of N-acetyllysine with HCHO and dG (but not dA, dC, or dT), a cross-linked adduct was found which was stable in aqueous solution. It was characterized by proton NMR spectroscopy as N'-methylene-(N-acetyllysine-5-yl)deoxyguanosine. Reaction of N-acetyllysine with HCHO and dmNAs under similar conditions in vitro resulted in the formation of the adduct, resolved on HPLC of a nucleoside digest. The formation of a similar cross-linked adduct produced on reaction of DNA with cysteine from proteins in nuclei is under investigation.

674 DETECTION OF N'3-ETHENOQUANINE AND 7-(2-OXOETHYL)QUANINE IN DNA FROM RATS CHRONICALLY EXPOSED TO ACRYLONITRIL.E. S A M Koch, V E Walker and J A Swenberg. CIIIF, Research Triangle Park, NC

Acrylonitrile (AN) has been shown to be carcinogetic in rats. DNA from target and non-target organs of rats chronically exposed to AN was analysed for the presence of two postulated promutagenic adducts, N'3-ethenoquanine (ethenoG) and 7-(2-oxyethyl)guanine (7-OEG). Tissues were assayed at termination of a 2-year study in which rats had been exposed to 500 ppm of AN in drinking water. DNA was extracted from brain, stomach and Zymbal's gland (target tissues), as well as liver and spleen (non-target tissues). The DNA was either acid-depurinated to release ethenoG or reduced with NaBH4 and subjected to neutral thermal lysis to release 7-OEG. The adducts were measured using HPLC with fluorescence detection. Adduct concentrations were highest in the brain (1.6 mmol ethenoG and 1.2 mmol 7-OEG/mmol guanine) followed by Zymbal's gland. Only 7-OEG was detected in stomach DNA. Much lower concentrations of both adducts were found in liver and neither adduct was detected in spleen. Both adducts were formed in calf thymus DNA incubated (37°C, 18 h) with 2-cyanoethylene oxide (ANO), a metabolite of AN. These data suggest that ANO is a proximate carcinogetic metabolite and that one or both adducts are relevant to AN carcinogenesis. (Supported in part by NIH grant NS 20023)
FORMATION AND PERSISTENCE OF DNA ADDUCTS IN RAT HEPATIC TISSUE FOLLOWING PRETREATMENT WITH 3,3'-DICHLOROBENZIDINE. S Nessel and M N Iba. Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

3,3'-Dichlorobenzidine (DCB) is hepatocarcinogenic in several species, including mice, rats, and dogs. In order to assess the potential of DCB to form DNA adducts in vivo, rats were administered 7.8 umol 14C-DCB (specific activity = 0.32 mCi/mmol) ip and sacrificed 3 or 14 days following treatment. Hepatic DNA was isolated by precipitation and solvent extraction and subjected to enzymatic hydrolysis. Modified nucleosides in the hydrolysate were separated from unbound nucleosides by Sephadex LH-20 chromatography. Chromatographic, radiometric, and spectrophotometric analysis indicated that several persistent 14C-DCB adducts are formed in hepatic DNA following administration of this compound. The binding (molecules of DCB : nucleosides) was 1:19000 and 1:70000 at 3 and 14 days, respectively. Two of the adducts formed in vivo co-chromatographed with deoxyguanosine (dG) adducts formed in NADPH-supplemented rat hepatic microsomal incubations containing 14C-DCB and dG. These results suggest that DCB is bioactivated to reactive intermediates capable of forming persistent adducts with DNA, two of which may be deoxyguanosine-derives. (Supported by US EPA R-812459)

PREPARATION OF DNA ADDUCTS FOR CHEMICAL CHARACTERIZATION STUDIES, USING ISOLATED RAT HEPATOCYTES. D A Dankovic, D L Springer, D B Mann, B L Thomas, and R M Bean. Pacific Northwest Laboratory, Dept. of Biology and Chemistry, Richland, WA 99352.

Preparation of carcinogen adducts to DNA in quantities sufficient for chemical characterization (1e, µg) is frequently difficult. Bio-synthetic methods are often employed, using rat liver microsomes plus calf-thymus DNA (CT-DNA). However, it has previously been shown that the HPLC profile of benzo[a]pyrene (BAP) adducts obtained in this way differs substantially from that observed in vivo. In contrast, the adduct profile obtained from isolated rat hepatocytes is very similar to that seen in vivo. It has also been previously shown that BAP adducts are formed with CT-DNA added to the hepatocyte incubation medium. We now report that the HPLC profiles of BAP-DNA adducts prepared using isolated rat hepatocytes plus exogenous CT-DNA are essentially identical to the profiles of mouse skin adducts; in both cases the predominant peak is the BAP-anti-diol-epoxide adduct of deoxyguanosine. An experiment using 60 µl of cell suspension and 180 µM BAP, for 3 hours with 54 µg of CT-DNA, yielded 1.58 µg of BAP added to 28 µg of DNA. The binding level was 217 pmol BAP bound/mg DNA. For comparison, a typical result using mouse skin in vivo is approx. 20 pmol BAP bound per mg DNA, yielding 2 - 5 µg of adduct/mouse. Supported by U.S. Department of Energy Contract DE-AC06-76RLO 1830.

CHARACTERIZATION OF NON-CLASSICAL BAP ADDUCTS TO DNA. D L Springer, B L Thomas, D A Dankovic, D B Mann, E K Chess and R M Bean. Battelle, Pacific Northwest Laboratory, Richland, WA.

Tumor Initiation by BAP and other PAH correlates with covalent binding of the diol-epoxide to the exocyclic nitrogen group of deoxyguanosine. Classically, these adducts have been studied by isolation of DNA from mouse skin about one-day after treatment with radiolabeled BAP, enzymatic hydrolysis to nucleosides and separation of adducted from normal nucleosides by LH-20 chromatography. Using this procedure, about 50% of the radioactivity associated with the DNA elutes as classical adducts, which are derived from reactions with BAP-diol-epoxides. The other 50% is more polar and elutes from the LH-20 column near the solvent front (non-classical fraction). When treated with acid, the polar material releases radiolabeled compounds having reverse phase HPLC retention times coincident with BAP-quinones. Similar treatment of the classical fraction produces BAP-tetrols characteristic of adducts formed from BAP-diol-epoxides. Hydrolysis of intact adducted DNA yields both quinones and tetrrols. The presence of quinone precursors in the non-classical fraction suggests a radical cation mechanism for their formation and represents a previously uncharactized class of DNA adducts. Supported by U.S. Department of Energy Contract DE-AC06-76RLO 1830.

IN VIVO INDUCTION OF DNA-PROTEIN CROSSLINKS IN RAT TRACHEAL IMPLANTS EXPOSED TO FORMALDEHYDE (HCHO) AND BENZO(A)PYRENE (BAP). G N Cosma, A S Wilhite, and A C Marchok, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN. Sponsor: M Costa.

HCHO and BAP are ubiquitous atmospheric pollutants with known or suspect carcinogenicity in animals and humans. To complement our study of their initiation of carcinogenesis in rat tracheal implants, the single and combined effects of HCHO and BAP on DNA damage were measured by alkaline elution of DNA coupled with a fluorometric assay in order to detect DNA-protein crosslinks (DPC) in the tracheal epithelium. A maximal response of 65% total DNA retention was obtained from single or multiple exposure (2X/wk) to a 0.2% HCHO solution, while 43% DNA retention resulted from a 0.01% HCHO exposure. The effect was reversed by protease treatment of DNA prior to elution which revealed the protein nature of the crosslink. Removal of HCHO-induced DPC was complete 3d after both single and multiple exposures. Tracheas exposed to BAP exhibited no DPC, regardless of exposure time (24-48h) or concentration (20-40 µg). Pre-exposure to 20 µg BAP followed by 0.05% HCHO diminished HCHO-induced DPC, but the combined effect was lost at higher HCHO concentrations. (USPHS Grant CA-43857 and ONR U.S. DOE under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.)

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Strain differences in susceptibility to liver carcinogens were investigated using a medium-term (B6C3F1) liver carcinoma bioassay system developed in this laboratory using male F344 rats. Six-week-old male rats of 7 different strains (65 rats each) were given a single i.p. injection (200mg/kg) of diethylnitrosamine (DEN) for initiation, and starting 2 weeks later, they were treated with 0.02% 2-acetylaminofluorene (2-AAF) or 0.05% phenobarbital (PB) mixed in basal diet for 6 weeks and then sacrificed. All rats were subjected to two-thirds partial hepatectomy (PH) at week 3. Quantitative values (No., or area of foci/cm²) of immunohistochemically demonstrated glutathione S-transferase P type (GST-P) positive liver cell foci were compared to those of a control group given DEN (+PH) alone. Strains used were F344, Wistar, Lewis, L-ascorbic acid synthesizing-defect rats (ODS), Nagase aluminemic rats (NAR), spontaneous hypertensive rats (SHR), and diabetic rats (WBN). Results demonstrated distinct differences in susceptibility to 2-AAF or PB among the 7 strains of rats. Lewis and F344 rats were most susceptible to 2-AAF and PB hepatocarcinogenesis. Thus, the use of F344 rats for a bioassay model was validated.

CARCINOGENIC RISK ASSESSMENT OF VINYL CHLORIDE. J P Christopher, F Cavender, and J H Brantner. California Department of Health Services, Toxic Substances Control Division, Sacramento, CA, and Dynmac Corporation, Rockville, MD.

The carcinogenicity of vinyl chloride (VC) was assessed to provide health-based criteria to risk managers at hazardous waste sites. Human epidemiological studies on VC did not include adequate measures of exposure, so 14 data sets from animal studies were used: Peron et al. (1981), Til et al. (1983), Drew et al. (1983), Melton et al. (1975, 1981, 1984), and Bi et al. (1985). Metabolized dose of VC was estimated using saturation kinetics (Watanabe et al., 1976; Gehring et al., 1978). Following the method of Allen et al. (1987) with "no averaging," 95% lower confidence bounds on dose at a risk of 10⁻⁶ were estimated for each positive site using GLOBAL82. The lowest lower bound was selected for each data set; from these the median lower bound was selected for each species: mice - 3.31 x 10⁻⁶ mg/kg/day (Drew et al., 1983); hamsters - 0.02 x 10⁻⁵ mg/kg/day (Ibid.); rats - 1.04 x 10⁻⁵ mg/kg/day (Til et al., 1983). Selecting hamsters as the median species, a "bias-correcting conversion factor of 3.4 (Allen et al., 1987) was applied to extrapolate to humans on a body weight basis. The 95% lower bound on the dose associated with an incremental lifetime risk of 10⁻⁶ for human cancer induced by VC was estimated to be 1.4 x 10⁻⁵ mg/kg/day.

A COMPARISON OF GUIDELINES IN THE CARCINOGENIC RISK ASSESSMENT OF CHLORODANE. F Cavender, N Page, and B Cook. Dynmac Corporation, Rockville, MD.

Carcinogenic risk is often calculated using bioassay data, even for known human carcinogens, primarily because exposure data are usually inadequate in epidemiological studies. Methods of using bioassay data and the parameters involved in calculating the human equivalent dose were compared. The carcinogenicity of chlorodane was assessed using guidelines developed by the International Agency for Research on Cancer (IARC), the U.S. EPA's Carcinogen Assessment Group (CAG), the National Toxicology Program (NTP), and the California Department of Health Services (DHS). Since the carcinogenicity of chlorodane in humans has not been established, eight sets of rat and/or mouse bioassay data were considered using the CAG guidelines: Ambrosi (1953), Becker and Sell (1979), Ingel (1952), IROC (1973), NCI (1977), and RIASBT (1983). Four mouse and one rat study were used to estimate the carcinogenic potency of chlorodane at a risk of 10⁻⁶ using the linearized multistage model (GLOBAL82). Estimates ranged from 0.25 to 4.74 per mg/kg/day. Major differences in applying the various guidelines are in the set of acceptable studies and the length of study/lifetime ratio. For example, NTP and DHS utilize only those studies with a statistically significant incidence of a malignant tumor while IARC and CAG also utilize studies with a significant increase in combined benign and malignant tumors.

CARCINOGENIC RISK ASSESSMENT OF VINYL CHLORIDE. J P Christopher, F Cavender, and J H Brantner. California Department of Health Services, Toxic Substances Control Division, Sacramento, CA, and Dynmac Corporation, Rockville, MD.

The carcinogenicity of vinyl chloride (VC) was assessed to provide health-based criteria to risk managers at hazardous waste sites. Human epidemiological studies on VC did not include adequate measures of exposure, so 14 data sets from animal studies were used: Peron et al. (1981), Til et al. (1983), Drew et al. (1983), Melton et al. (1975, 1981, 1984), and Bi et al. (1985). Metabolized dose of VC was estimated using saturation kinetics (Watanabe et al., 1976; Gehring et al., 1978). Following the method of Allen et al. (1987) with "no averaging," 95% lower confidence bounds on dose at a risk of 10⁻⁶ were estimated for each positive site using GLOBAL82. The lowest lower bound was selected for each data set; from these the median lower bound was selected for each species: mice - 3.31 x 10⁻⁶ mg/kg/day (Drew et al., 1983); hamsters - 0.02 x 10⁻⁵ mg/kg/day (Ibid.); rats - 1.04 x 10⁻⁵ mg/kg/day (Til et al., 1983). Selecting hamsters as the median species, a "bias-correcting conversion factor of 3.4 (Allen et al., 1987) was applied to extrapolate to humans on a body weight basis. The 95% lower bound on the dose associated with an incremental lifetime risk of 10⁻⁶ for human cancer induced by VC was estimated to be 1.4 x 10⁻⁵ mg/kg/day.

Dietary Factors in Esophageal Carcinogenesis. TF Schrager, D Bueche, M Conner, PM Nowberne. Mallory Institute of Toxicology. Boston, MA.

The incidence of esophageal cancer varies quite dramatically worldwide. In many areas of the world, including the Middle East, this cancer is associated with malnutrition. We previously demonstrated that zinc deficiency significantly enhances esophageal tumor incidence in rats exposed to the esophageal carcinogen, methylbenzyl-nitrosamine (MBN). A 20% riboflavin deficiency combined with MBN increases esophageal tumor number but does not change tumor incidence. A 10% riboflavin deficiency plus MBN significantly increases tumor incidence. Two rats in the 10% riboflavin group without MBN also developed esophageal carcinomas, an event not seen in control diet rats untreated with MBN. Although morphological changes in both dietary groups are similar after MBN dosing—including hyperplasia—other changes preceding dosing are different, suggesting different mechanisms. Zinc deficient animals display early and progressive esophageal hyperplasia, characterized by increased incorporation of 3H-thymidine, whereas in the riboflavin deficient group the esophageal epithelium is normal until dosing. DPHMO, an inhibitor of ornithine decarboxylase, inhibits cell proliferation in many tissues. In this model DPHMO inhibited the enhancement of tumor incidence by zinc deficiency and also inhibited the increased of carcinogen-DNA adducts in that diet group.

Gellan gum (a microbial polysaccharide) was administered orally to Swiss albino mice to determine carcinogenic potential. The gum was given in the diet at dose levels of 1, 2, and 3% for 98 weeks for males and 96 weeks for females. These concentrations represented average daily intakes of approximately 1.6, 3.2, or 4.9 g/kg/day for males and approximately 2.2, 4.2, and 6.2 g/kg/day for females. No treatment-related effects were noted in the clinical examinations, body weights, food consumption, ophthalmoscopic examinations, and gross pathology. There was no increase in the incidences of neoplastic lesions. Non-neoplastic findings were considered to represent spontaneous background changes commonly occurring in mice. In summary, treatment at levels up to 3% of gellan gum produced no overt signs of toxicity and no effect on the spontaneous tumor profile of mice of this age and strain.


Workers (N=229) from an Egyptian pesticide formulation plant were screened for signs of organophosphorus induced delayed neuropathy (OPIDN). Tests included lymphocyte neurotoxic esterase (LTEE), serum cholinesterase (CHE), tactile sensitivity (OPTACON), block design (BD) and Santa Ana Dexterity (SAD) test. Workers from fertilizer (N=181) and textile (N=166) plants were also tested as controls. A one-year follow-up evaluation of workers was also conducted. LTEE activity levels of pesticide formulators were significantly lower than levels of workers from other plants. Tactile thresholds averaged for the two assessments varied significantly among plants (highest in pesticide plant) and for levels of education (inverse relationship) after controlling for age and LTEE activity. There was no significant interaction of education and plant. Tactile threshold increased linearly with age as expected. BD and SAD scores did not vary with LTEE or among plants. The finding of reduced tactile sensitivity in pesticide formulators is consistent with OPIDN, although LTEE levels were inhibited more than 50% relative to controls in only 11% of the pesticide workers. Results suggest that tactile sensitivity testing may provide a useful method for early detection of peripheral neuropathy in workers exposed to pesticides.

DIETARY IRON ENHANCES THE TUMOUR RATE IN DIMETHYLHYDRAZINE-INDUCED COLON CARCINOGENESIS IN MICE. C.P. Siegers, D. Buhmann, and M. Younges. Institute of Toxicology, Medical University of Luebeck, 21534, FRG.

Treatment of male mice with 20 mg/kg dimethylhydrazine (DMH) s.c. for ten weeks caused a mean tumour rate of 3.5 in 13 tumour-bearing out of 19 animals after 20 weeks. Feeding an iron-enriched diet (3.5% Fe-fumarate for 10 weeks) enhanced the number of tumour-bearing mice to 18/19 and the mean tumour rate to 13.9. Deferoxamine (0.1% in the drinking water) was not able to reduce the DMH-induced tumorigenesis in mice under normal diet. All tumours detected were localized exclusively in the distal colon and rectum. The dietary iron load caused a 6.5 fold increase in the mucosal Fe⁺ concentration in the proximal as well as in the distal colon. Enzymes involved in the bioactivation of DMH to the putative ultimate carcinogen as microsomal DMH-demethylase or cytosolic alcohol dehydrogenase (ADH) were neither altered by iron nor deferoxamine treatment in the colonic mucosa. DMH-demethylase activity was not different in the proximal and distal segments of the colon, whereas ADH-activity was 3.3 fold higher in the distal colon and rectum as compared to the proximal segment. This might explain the DMH-induced tumorigenesis in the distal segment only. It is suggested that iron ions might evoke cocarcinogenic activity by a stimulation of cell proliferation.


LTEE, a mipafox-sensitive phenyl valerate hydrolyase found in neuronal and other tissues, is the proposed molecular site for initiation of organophosphorus-induced delayed neuropathy. In an attempt to characterize LTEE, we have solubilized active enzyme from hen brain microsomal membranes with the zwitterionic detergent CHAPS (0.3%, 30 min., 0°C) and chromatographed the soluble fraction (100,000 x g, 60 min) on Sepharse 4B gel exclusion medium. Active LTEE eluted as a protein of Mr=900KDa relative to standard proteins. Microsomal membranes were also preincubated with 100 μM paraaxon for 6 hr (0°C) followed by radiolabeling with [³²P]ATP for 16 hr (0°C) and then solubilized with CHAPS. Chromatography of the radiolabeled soluble fraction on Sepharse 4B followed by assay of column fractions for LTEE activity and radiolabel (by SDS-polyacrylamide gel electrophoresis) revealed coelution of LTEE activity with a DPP-labeled protein of Mr=160KDa. This suggests that LTEE exists as a holoenzyme containing an active site subunit of Mr=160KDa.

Coincidentally, a paraaxon-sensitive phenyl valerate hydrolyase which was solubilized using CHAPS eluted as a protein of Mr=158KDa. The possibility that the paraaxon-sensitive enzyme is the catalytic subunit of LTEE with a prosthetic group imparting differential organophosphate sensitivity needs further study. (*supported by NRC Research Associateship.)

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Rhesus monkey (Macaca mulatta) blood was evaluated for the ability of the monopridinium aldoxime 2-PAM to reactivate cholinesterase (CHE) activity after the inhibition of the enzyme with paraoxon. The Ellman enzymatic procedure (Ellman, et al., Biochem. Pharm. 7, 88-91, 1961) was utilized for monitoring the CHE levels. Each animal served as its own control. Baseline CHE activity in whole blood, RBC, and plasma were established prior to incubation with paraoxon. Samples were incubated at 37°C with varying concentrations of paraoxon for 30 min to achieve CHE activities ranging 10-80% of the baseline value. 2-PAM was then added to a duplicate sample which was incubated at 37°C to allow for reactivation of the enzyme, and then CHE activity was determined. The ratios of paraoxon-inhibited/baseline CHE activity vs. reactivated/baseline CHE activity were compared to control samples. A significant difference was observed with 2-PAM reactivated-paraoxon treated samples when compared to those with no reactivation. The addition of the reactivation step in the CHE procedure may improve the use of this assay in determining anti-CHE exposure in cases in which baseline values are not available or in cases in which CHE inhibition is used for diagnostic interpretation. (Supported in part by the USDA).

The activity of serum paraoxonase, an enzyme which hydrolyzes the organophosphorus paraoxon (PO) is polymorphically distributed in many populations. It has been suggested that in humans exposed to organophosphorus insecticides, the severity and duration of symptoms may correlate with serum paraoxonase activity. To determine the possible role of paraoxonase in the sensitivity to PO, we investigated its toxicity in the rat and the rabbit, two species which have a 7-fold difference in serum paraoxonase activity (measured by a newly developed method at pH 8.0 which eliminates interference by albumin). We determined that the minimum ip doses of PO causing signs of cholinergic intoxication were 0.5 and 2.0 mg/kg in the rat and rabbit, respectively. These doses gave equal inhibition of cholinesterase activity. Thus a 7-fold greater serum paraoxonase activity appeared to provide a 4-fold protection against PO toxicity. Intravenous administration of 150 units of partially purified rabbit paraoxonase to rats raised the rat serum paraoxonase levels by 8-fold and 6-fold at 30 min. and 4 hrs. after administration, respectively, but declined to almost control values at 24 hrs. Rats intravenously injected with PO 30 min. after paraoxonase administration were protected from its toxicity. (This research was supported in part by grants from the Charles A. Dana Foundation and the NEHS ES-03424).

The toxicity of the organophosphorus pesticide, ethion, was evaluated in 90-day and one-year studies to assess potential health hazards associated with repeated dietary exposure. Ethion technical (93.4%) was administered to beagle dogs (4/sex/group) at dietary levels of 0, 0.5, 2.5, 7.5, 25.0, or 300 ppm in the 90-day study, and at levels of 0, 0.5, 1.0, 2.0, 20, and 100 ppm in the chronic study. The 300 ppm dose exceeded a maximum tolerated dose (MTD) in that dogs exhibited marked reductions in body weight gain and severe clinical signs of toxicity (niosis, tremors, salivation), and one female was sacrificed moribund on study day 90. At 300 ppm, plasma, erythrocyte, and brain cholinesterase activities were biologically and statistically inhibited. No treatment-related histopathologic changes were observed. In the chronic study, body weight, food consumption, hematology and clinical chemistry appear unaffected through 9 months of treatment, and there are no clinical signs of toxicity. Erythrocyte (100 ppb) and plasma (20, 100 ppm) cholinesterase activities are biologically and statistically depressed in both sexes; the 100 ppm dose thus represents an appropriate estimation of an MTD. Based on cholinesterase inhibition, the No Observed Effect Level appears to be 2 ppm.

This study was designed to assess the effects of ethion technical, an organophosphate pesticide, on the reproductive performance and fertility over three generations (2 litters/generation) in CD rats. Groups of rats (30 females and 15 males/group/generation) were fed diets containing 0, 2.0, 4.0 or 25.0 ppm ethion technical for 14 weeks (PO) or 17 weeks (F1, F2) prior to mating, and throughout gestation, parturition and lactation periods. Parental animals were mated for a second litter 2 weeks after the weaning of the first litter. There were no effects on reproductive functions of either sex in any generation and no treatment-related anemotoms or macroscopic observations. There was a statistically significant inhibition of serum cholinesterase of 43%, 45% and 53% among the high dose females at the F1 pre-mating, F1-final sacrifice and F2-final sacrifice intervals, respectively. Erythrocyte and brain cholinesterase levels were not significantly inhibited at these intervals. The no observable effect level (NOEL) for toxicity in this study was 4.0 ppm. The NOEL for reproductive effects was 25.0 ppm.
Glutathione-S transferases (GST) are a family of dimeric enzymes responsible for the detoxification of a variety of toxic chemicals, including the widely used insecticide methyl parathion (MP). A mechanism of detoxification of MP proceeds via demethylation and requires glutathione as a cofactor. This study sought to determine the relative ability of GST to demethylate MP in the presence of other methyl and ethyl organophosphate analogs. A reverse-phase ion-pairing high performance liquid chromatography method was developed to separate all the metabolites of MP. The limit of quantitation of demethyl parathion (DMP) was 100 pmol. Incubations of rat hepatic cytosol with 0.50 mM MP resulted in a GST-mediated rate of demethylation of 559 pmol/mg/min. Demethylation of MP displayed saturation kinetics with an apparent Vmax of 1.13 nmol/mg/min and Km of 0.56 mM. Methyl paraoxon demonstrated a similar activity towards GST, but did not exhibit saturation up to an incubation concentration of 4 mM. A wide variety of methyl organophosphates inhibited the GST-mediated formation of DMP, indicating that many methyl organo- phosphates may serve as substrates for GST. Ethyl organophosphates also inhibited the demethylation of MP by GST, however, deethylation was not detected, suggesting an affinity for the active site in GST without serving as a substrate. (Supported by the Dana Foundation).


The dimethyl-substituted organothiophosphate insecticide methyl parathion is thought to undergo glutathione-mediated detoxification in mammals. In the present study, depletion of hepatic glutathione in the mouse by pretreatment with diethyl maleate (DEM) potentiated the acute toxicity of methyl parathion, whereas depletion of hepatic glutathione by pretreatment with bithionol sulfoximine (BSS) did not. Furthermore, incubation of 50 μM methyl parathion with mouse hepatic microsomes for 5 min in the presence of 1 mM DEM led to significantly greater (p < 0.05) production of methyl paraoxon, compared to incubations in the absence of DEM (2.1 ± 0.3 nmol methyl paraoxon/100 mg liver with DEM versus 0.6 ± 0.1 nmol methyl paraoxon/100 mg liver without DEM). These results suggest that normal levels of hepatic glutathione are not required for detoxification of methyl parathion. Moreover, the potentiation of the acute toxicity of methyl parathion following DEM pretreatment could result from enhanced production of methyl paraoxon and not from depletion of hepatic glutathione. Therefore, these data raise doubts about the participation of glutathione in the detoxification of methyl parathion in vivo in the mouse. (Supported by Grant ES04335 from NIEHS).

ACUTE ORAL TOXICITY STUDY IN CYROMULUS MONKEYS WITH ALDICARB RESIDUE IN BANANAS AND WATERMELON. J. A. Tutter, F. E. Reno, R. H. Cox, R. L. Baron, and J. M. Charles. Hazleton Laboratories America, Inc., Vienna, VA, Rhone-Poulenc Ag Company, Research Triangle Park, NC.

The study was performed to determine the acute toxicity and effects on acetylcholinesterase (ChE) activities in cynomolgus monkeys after intake of bananas or watermelon treated with an exaggerated rate of TEMIK 10G to assure delivery of fruit containing high aldicarb residue levels. Twelve monkeys (3/sex/group) were fed test fruits in amounts providing a residue intake of 0.005 mg/kg of body weight, a value equal to the ADI established by the WHO. Equal numbers of controls (0.000 mg of residue/kg) received a similar rate of fruit (20g/kg). Periodic evaluations of clinical signs and ChE activities were made through 24 hours post-feeding. There were no clinical signs of toxicity or inhibition of RBC ChE activity. Plasma ChE activity was inhibited through 2 hours (watermelon) or 4 hours (bananas), with peak inhibition (32-37%) occurring at 1- and 2-hours, respectively. These data fit on the same dose response curve as that which was obtained from human volunteers given an acute aqueous solution of aldicarb corresponding to doses of 0.025, 0.050 and 0.10 mg/kg of body weight.


The more than 1200 illnesses attributed to aldicarb-contaminated watermelons in the summer of 1985 in California and Oregon sparked this project to determine whether acetylcholinesterase (AChE) inhibitions and honeybee mortalities would provide rapid, sensitive assays for carbamate contamination of melons. Aqueous extracts of melons were concentrated with a rotary evaporator; extracts and concentrates were spiked with aldicarb, aldicarb sulfoxide and aldicarb sulfone. Inhibition of electric eel AChE was measured with an automated colorimetric assay and an optical plate reader. Honeybees were fed extracts in syrup and the LD50s for the carbamates determined. Aldicarb sulfone was the most potent inhibitor. Detection limits were within the range expected for contaminated melons. The lower limit for AChE was 30 ppb, competitive with gas chromatography, and that for honeybees was approximately 1 ppm. Supported by contracts from California Department of Health Services.
CHOLINESTERASE INHIBITION IN MICE AFTER 1-, 12- OR 24-MONTH DIETARY ADMINISTRATION OF IMIDAM. A C Katz, D W Frank, J C Turner and G L Exner. Environmental Health Center, Stauffer Chemical Company, Farmington, CT.

This study assessed cholinesterase (ChE) inhibition after short- and long-term IMIDAM exposure. Dietary IMIDAM (N-[mercaptomethyl] phthalimide S-[6,0-dimethylphosphorodithioate]), an insecticide, was administered to male B6C3F1 mice for 1, 12 or 24 months. The calculated doses producing 50% inhibition of plasma and brain ChE activity after 1 month of dietary administration were 15.2 and >51.2 mg/kg/day. Inhibition of plasma ChE after 12 or 24 months of treatment (12 mg/kg/day) was 53.8% and 52.2%, respectively. Inhibition of brain ChE after 12 or 24 months (12 mg/kg/day) was 47.3% and 32.1%, respectively. No significant cholinesterase inhibition resulted at doses lower than 3 mg/kg/day. Few obvious clinical signs of ChE inhibition occurred during the study. In conclusion, the study demonstrated that plasma ChE inhibition after 12 and 24 months of IMIDAM treatment was comparable to the level expected after 1 month. In contrast, brain ChE inhibition at 1 month was not predictive for inhibition after 12 and/or 24 months of administration.

RABBIT BLOOD PRESSURE, TEMPERATURE, BODY WEIGHT AND ERYTHROCYTE AND PLASMA CHOLINESTERASE ACTIVITY DURING SEVEN-DAY SOMAN ADMINISTRATION. C-Y Hu, C-Y Hung and C P Robinson, College of Pharmacy, University of Oklahoma, Health Sciences Center, Oklahoma City, OK.

Soman was given daily to correlate cholinesterase (ChE) inhibition with blood pressure and other changes. Male New Zealand White rabbits (2-3 kg) were given 5 μg/kg of soman (pinacolyl methylphosphonofluoridate) subcutaneously each day for 7 days. Observations were made immediately before and one hour after soman injections. Blood pressure was measured in the central ear artery by a non-invasive method. By the end of the seven-day period, erythrocyte ChE activity was reduced by 93% and plasma ChE activity by 66%. Soman also reduced body temperature significantly on 3 of the 7 days (p<0.05). It did not alter body weight (p>0.05). Blood pressure was reduced (p<0.05) on the first day of the study. Thereafter, it was not different from sham-treated controls. Altered responses to soman doses following the first day seem to indicate induced changes in the vascular neuro-effector system. In central mechanisms controlling blood pressure, or both. Supported by DOD Contract DAMD 17-85-C-5114.

PROPHYLACTIC AND THERAPEUTIC EFFICACY OF MEMANTINE AND ATROPINE AGAINST CARBOFURAN ACUTE TOXICITY IN RAT. R C Gupta and W L Kadel, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY.

Male Sprague-Dawley rats administered with a sublethal acute dose of Carbofuran (CBF, 1.5 mg/Kg, sc) developed cholineric signs of carbamate toxicity within 5-7 min. The peak toxicity signs including severe tremors and generalized muscle fasciculations were evident within 15 min, and persisted for 2-3 hrs. A dose of 2.5 mg CBF/Kg was minimal lethal dose. The time-course of AChE inactivation in discrete brain regions and hemi-diaphragm muscle showed a positive correlation with induced intoxication and recovery. A single dose of memantine RC1, (MEM, 18 mg/Kg, sc), and atropine sulfate (ATS, 16 mg/kg, sc), 60 and 15 min, respectively before CBF completely prevented the expected gross toxic signs and significantly (P<0.01) reduced CBF-induced inhibition of AChE activity. When given therapeutically, this combined treatment completely reversed the clinical evidence of CBF intoxication within 15 min and also markedly reduced AChE inactivation. MEM or ATS when given alone was less effective. However, MEM plus ATS combination was most effective. The results of this study suggested that, in addition to cholinolytic effects of ATS, MEM may prevent and antagonize CBF toxicity by enhancing its degradation and/or by AChE reactivation.


Fenvalerate is a composite of 4 stereo-isomers; SS-isomer exhibits the highest insecticidal activity. Subchronic toxicity of fenvalerate and SS-isomer were comparatively evaluated. Male and female rats were administered fenvalerate or isomer in the diet for 13 week. Rats were fed diets of fenvalerate containing the equivalent of 0, 27.5, 110, 220, 440 ppm active isomer; SS-isomer was fed at 0, 42, 126, 252, 420 ppm. Comparative mortalities were 20/24 and 7/60 at the top dose for fenvalerate and isomer respectively. Neurological signs of toxicity were observed with both compounds, effects occurring at lower doses for isomer compared to fenvalerate. Transient body weight/food consumption changes were seen. No compound related blood chemistry changes were seen for either compound. Higher relative kidney weights were seen with both compounds; greater effect was observed with fenvalerate. Liver weight changes were also seen with fenvalerate. No microscopic tissue effects were seen with fenvalerate, however, hypertrophy of the parotid salivary gland was seen in 10/60 rats at the top dose with the isomer. Metabolism of fenvalerate and isomer was similar.
A 72 hr LD50 study involving the fenvalerate formulation, Pydrin® 2.4 EC, was performed using male Swiss mice in order to compare the relative toxicities of Pydrin® to that of technical grade fenvalerate in corn oil. Fenvalerate was more toxic when administered as the formulated product, than when administered as technical grade material. The LD50 value for Pydrin® was calculated to be 72 mg/kg and 62 mg/kg following PO and IP administration. Administration of 128 mg/kg and 89 mg/kg of technical grade fenvalerate, which corresponded to the amount of fenvalerate contained in the LD50 of Pydrin® for the PO and IP routes respectively, resulted in no deaths. These data suggest a substantial effect of the Pydrin® formulation vehicle on the toxicity of technical fenvalerate. The formulation vehicle, did not result in substantial lethality as compared to no-treatment controls. An alternative interpretation of these data is that the fenvalerate was less bioavailable when administered in corn oil. The comparative bioavailability of the technical grade material and Pydrin® in corn oil, is currently being investigated. (Work supported in part by the Research Institute of Pharmaceutical Sciences).

THE EFFECTS OF A BENZODIAZEPINE RECEPTOR ANTAGONIST AND PICROTOXIN ON FENVALERATE TOXICITY. KM Tolson and Wh Bourn, School of Pharmacy, Northeast Louisiana University, Monroe, LA. Sponsor: FJ Medon.

Pyrethroid insecticides of the Type II class have been linked to the GAABAergic system in mammals. A variety of evidence indicates that fenvalerate (FVN) and other Type II agents interact with the picROTOXIN domain on the GAABA receptor complex. In the present study FVN toxicity was manipulated with a benzodiazepine (BZ) receptor blocker, CCS-8216, and picROTOXIN (PFX).

The time course of FVN toxicity was previously determined for female rats in an electronic activity monitoring system. Onset of the syndrome, as shown by an increase in activity, occurred within one hour following an oral dose of 100 mg/kg FVN. A subconvulsive dose of PFX (2 mg/kg ip) 30 minutes prior to FVN exposure precluded any signs of FVN toxicity until the fourth hour of testing. Pretreatment with CCS-8216, an established BZ receptor antagonist, failed to alter the above measured behavioral aspects of the FVN toxicity syndrome.

Similarly, pretreatment with the subconvulsive dose of picROTOXIN reduced the frequency of electroencephalographic spiking induced by continuous intravenous infusion of FVN. In a parallel experiment, CCS-8216 did not alter EEG spiking.


The toxicity of the organophosphate pesticide ethion, was evaluated in lifespan feeding studies to assess the chronic toxicity and oncogenic potential. Groups of 80/sex/dose C57 mice and Sprague-Dawley rats were administered ethion technical in the diet for 24 months. The dose levels were 0, 0.75, 1.5, and 8.0 ppm for mice and 0, 2, 4, and 40 ppm for rats. Interim sacrifices were conducted at 6, 12, and 18 months (10/sex/group) and a final sacrifice was performed at 24 months (50/sex/group). There were no remarkable differences in body weight, food consumption, clinical observations, hematological or biochemical differences between control and treated animals except for treatment-related depression of plasma cholinesterase levels. In mice, plasma cholinesterase depression of 25-44% was observed in the 8.0 ppm dose group at 6, 12, and 18 months in females and at 12 and 18 months in males. In the rats, cholinesterase depression was 37-57% in the 40 ppm dose group at 12 and 18 months for the males, and at all intervals for the females. There were no treatment-related neoplastic or non-neoplastic histopathological findings. The NO Observable Effect Level (NOEL) is 4 ppm in rats and 1.5 ppm in mice.
CHRONIC TOXICITY AND OCONEGENICITY OF INHALED TECHNICAL GRADE 1,3-DICHLOROPROPENE (DCP) IN RATS AND MICE. WT Stott, KA Johnson, LG Lomax and LL Calhoun, The Dow Chemical Co., Midland, MI 48674

The effects of chronic DCP exposure in rodents were examined via inhalation, an appropriate route of potential human exposure. Male and female Fischer 344 rats and BCG3F1 mice were exposed to 0, 5, 20 or 60 ppm DCP 6 hours/week, 5 days/week for 2 years. Body weights of both sexes of rats and mice exposed to 60 ppm vapors were depressed. A slight degeneration of the olfactory epithelium of the nasal mucosa, occasionally accompanied by submucosal fibrosis, also occurred in both sexes of rats exposed to 60 ppm DCP. In mice, nonneoplastic changes included: hyperplasia of the transitional epithelium of the urinary bladder; slight degeneration of the olfactory epithelium of the nasal mucosa; and minimal hyperplasia and hypertrophy of the nasal respiratory epithelium of both sexes exposed to 60 ppm vapors. Bladder and nasal effects were also observed in mice exposed to 20 ppm DCP. A minimal degree of hyperplasia also occurred in the nonglandular stomach mucosa of high-dose group male mice. The only tumorigenic response observed was an increased incidence of benign lung tumors (bronchioloalveolar adenomas) in male mice exposed to 60 ppm DCP (22/50 vs 9/50 in controls). No treatment-related effects were observed in rats and mice exposed to 20 ppm and 5 ppm DCP, respectively.


Lactofen (L), registered in 1987 for use as a soybean herbicide, was found to induce liver tumors in rodents following long-term dietary administration. Lacking genotoxic activity, an alternate mechanism was sought as a basis for the tumorigenic action of this compound. This project assessed the potential of L to induce liver changes in the reactive rat and in the higher primate comparable to the peroxisomal enhancement seen in the mouse. Rats (M & F) were fed diets containing 0 or 2000 ppm L for a period of 8 weeks and then sac'd. Six male chimpanzees received oral doses of 5 or 75 mg/kg/d of L for 3 months. Serial liver biopsies were taken on Days -12, 92 and 239. Both species were subjected to blood chemistries, liver microscopic and biochemical study for evidence of hepatic effects. Rats exhibited hepatic peroxisome proliferation as evidenced by 2.5 to 3.5 fold increases in palmitoyl CoA oxidase in M and F's respectively, and by microscopic observations. Chimps displayed no changes in these peroxisomal parameters from pre-treatment through 92 days of dosing. These observations indicate that this species is refractory to L induction of peroxisome changes often associated with hepatic tumorigenesis in rodents.
PEROXISOMAL PROLIFERATION IN PRIMARY RAT HEPATOCEYS INDUCED BY LACTOFEN (COBRA HERBICIDE) AND ITS METABOLITES. K Allen, E Tyson*, and P Leber*. #SRI International, Menlo Park, CA and *PPG Industries, Barberton, OH.

Lactofen (L) was found to induce hepatic tumors in mice and rats as well as peroxisomal proliferative changes. This study was undertaken to (1) confirm the peroxisomal findings in an in vitro system, and (2) to examine the potential for de-esterification or nitro-reduced metabolites of L to contribute to the peroxisomal responses seen. L and 4 of its mammalian metabolites were incubated at concentrations of 0.1 to 10 mM in rat hepatocyte cultures. Cells were assayed for CN-insensitive palmitoyl CoA oxidase or subjected to electron microscopy. The hypolipidemic agent clofibrate was employed as positive control for peroxisomal enhancement. The data indicate that L increased peroxisomal oxidase levels 6 times that of control levels whereas clofibrate induced levels 10 fold. All 4 metabolites enhanced the enzyme in rat cells but to a lesser degree than L (3-4 fold above controls). Increased numbers of peroxisomes were observed by EM in L and positive control cultures only. These results demonstrate that L exhibits activity towards elevating palmitoyl CoA oxidase in an isolated cell system, and further suggest that L's activity in vivo cannot be ascribed to specific metabolites. The higher activity exhibited by L versus its metabolites may result from increased transmembrane uptake of the un-ionized parent compound.

PHARMACOKINETICS OF LACTOFEN AND METABOLITES IN THE MOUSE, RAT, RHESUS MONKEY AND CHIMPANZEE. J H Ross and C R Fisher. PPG Industries, Inc., Barberton, OH.

In mouse and rat chronic bioassays of lactofen, a recently registered diphenyl ether herbicide, the liver was found to be a target organ. A pharmacokinetic comparison of the parent compound and/or major metabolites in rodents and primates seemed appropriate in attempting to define factors that would influence the risk assessment extrapolation from rodents to humans. Several experiments were designed to test the kinetics of excretion and/or metabolism of lactofen in mice, rats, rhesus monkeys and chimpanzees at dose levels approximating the dose range used in the rodent chronic feeding studies. Excretion kinetics, clearance of total plasma radiocarbon derived from lactofen, the kinetics of plasma clearance of a major de-esterified metabolite, acifluorfen, and steady state concentrations of several other metabolites of lactofen were compared in rodent species (mouse and/or rat) and primate species (rhesus monkey and/or chimpanzee). The pharmacokinetic parameters that were derived indicate that the primates more rapidly clear metabolites derived from lactofen and achieve lower plasma steady state levels of measured metabolites than rodents. From these comparative pharmacokinetic results, it seems likely that humans would be at less risk from a comparable dosage of lactofen than rodents and, therefore, that the rodent model overestimates risk when extrapolating from rodents to humans.


A technical grade lactofen (1'-[Carboethoxy]ethyl 5-2-chloro-4-[trifluoro-methyl]phenoxy]-2-nitrobenzoate) has been shown to induce liver tumors in mice. To determine a possible mechanism of action, the effect of four dietary concentrations of technical grade lactofen and one concentration of pure lactofen was studied on various liver parameters in groups of male and female CD-1 mice. After a seven week exposure, liver weight to body-weight ratio, liver catalase, liver acyl CoA oxidase, cytoplasmic eosinophilia, nuclear enlargement, hypertrophy and peroxisomal staining were measured. The tumorigenic dose of lactofen significantly increased all the parameters in a fashion similar to a comparison chemical nafenopin, which is a peroxisome proliferator. Doses of lactofen that were reported as non-tumorigenic had little or no effect on these parameters. Equal concentrations of pure and technical grade lactofen resulted in similar changes. These findings suggest that lactofen induces murine liver tumors through a mechanism similar to epigenetic hepatocarcinogens of the peroxisome proliferating type.

MODIFICATION OF HEPATOTOXICITY OF TCPO AND MO BY CHRONIC ETHANOL INGESTION. K Maita, N Nakashima, and Y Shirasu. Inst. of Environmental Toxicology, Tokyo, JAPAN.

The effects of chronic ingestion of alcohol on the toxicity of pesticides were studied. A total of 30 F344 male rats at 5 weeks of age were fed a liquid diet for a week. During the 3 weeks, 12 rats were fed the liquid diet containing alcohol at 5g/100g diet (A1) and the liquid diet (Liq) feeding was continued for 18 rats. Then, each diet group was divided into 3 subgroups, two of which received a single po dosing of either 160 mg/kg B.W. of 2,4,6-trichlorophenyl 4'-nitropheny ether (MO) or 1,500 mg/kg B.W. of tri-ortho cresyl phosphate (TOCP) after one day fasting. The control rats of both diet groups were given saline. A total of 6 subgroups were provided as follows: (1) Liq, (2) A1, (3) Liq-MO, (4) A1-MO, (5) Liq-TOCP, (6) A1-TOCP. The animals fed A1 diet showed ruffled hair, anemia, hyper-nemitsiveness to stimuli, emaciation, and alcoholic smell of the body. When the A1 diet was withdrawn for the fasting, 3 rats died after manifesting withdrawal symptoms such as shivering, jumping, and squeaking. Serum biochemistry disclosed that A1-MO and TCPO in Liq-MO group were 2 and 4 times higher than those in Liq-MO group. In the TOCP treated groups, alcohol pretreatment lowered GOT and GPT at the rates of 1/2 and 1/7 times as compared with those in Liq-TOCP group. These results were consistent with the observations in the histopathology.
THE SUBCHRONIC EFFECTS OF ETHYLENE DIBROMIDE ON CYTOCHROME P-450 LEVELS AND GLUTATHIONE-S-TRANSFERASES IN RAT LIVER AND KIDNEY. J W Hauswirth, Center for Veterinary Medicine, Food and Drug Administration, Beltsville, MD. Sponsor: T M Farber.

This study was undertaken to determine the subchronic effects of ethylene dibromide (EDB) on liver and kidney cytochrome P-450 (P-450) levels and glutathione-S-transferases (GST's). Dosage levels used were 0, 10, 40 and 80 mg/kg, given by gavage to male Sprague-Dawley rats for a period of 90 days. Six animals were killed on days 7, 14, 30, 60 and 90 and liver and kidney tissues were removed for analyses. P-450 levels were induced in the kidney by EDB at all dosage levels at 30 days. However, by 90 days, the percentage induction in kidney decreased. By 90 days at the 80 mg/kg dose, the levels were only 80% of the controls. Hepatic levels of P-450 were unaffected at 7 and 14 days, but by 30 days the levels were decreased by treatment. The reduction reached a maximum at 90 days in the 80 mg/kg group when P-450 levels reached 35% of the control group. GST's were consistently induced in the liver at all dosage levels and in a dose-related manner. In the kidney at 40 and 80 mg/kg, GST's were also induced but not to the same extent and the effect plateaued earlier. At 10 mg/kg in the kidney, there was an initial increase followed by a decrease to or just below control levels. These results indicate a mixed effect of EDB on P-450 levels in liver and kidney whereas, a consistent inductive effect was seen on GST activities.

TOXICITY OF MIXTURES OF HERBICIDES, FOUND IN GROUNDWATER, IN MICE. A K Chaturvedi, L N Dix, W L Liu, I E Berg, and G Padmanabhan, Departments of Civil Engineering, Pharmaceutical Sciences/Toxicology, and Veterinary Science, North Dakota State University, Fargo, ND.

Alachlor(AL), atrazine(AT), and/or picloram(P) have been reported to be present (<1 ppb) in groundwater of certain agricultural areas. Though the toxicity of each of these herbicides (HEs) is individually studied, the toxicity of their mixtures has not been evaluated. Therefore, AL+AT, AT+P, P+AL, and AL+AT+P mixtures (10 ppm of each of HEs) and individual HE in drinking water were given to 1CR male mice (21-24 g) for 30 days. The exposure to HEs or their mixtures did not produce significant change in food intake, water consumption, body weight gain, or liver or kidney to body weight ratio. However, the spleen to body weight ratios in AT and AL+AT groups were higher, respectively, than the control (n=10). Histopathologic examination of the above tissues revealed that the enlarged spleens were moderately congested. The pentobarbital (60 mg/kg, ip)-induced sleep time in AL+P group was lower (24%, p<0.05) than the control. These results suggest that HEs and their mixtures have potential to produce undesirable effects on spleen and to alter liver function. More data, however, from the exposure to HE mixtures for a longer period are crucial in elucidating their toxicity. (Supported by USDGS Grant #14-08-0001-G1441-NDWRIP).

COMPARATIVE TOXICITY OF 4 ALKYI THIOCARBAMATES IN DOGS AND RATS FOLLOWING REPEATED ORAL ADMINISTRATION, M W Sauerhoff, D R Saunders, and G L Sprague, Environmental Health Center, Stauffer Chemical Company, Farmington, CT.

The toxicity of 4, closely related alkyl thio- carbamates was compared in Sprague-Dawley rats (diet) and Beagle dogs (capsule) using repeated, daily administration. The highest dose tolerated, without producing lethality, was the end-point used for comparison. All compounds exhibited relatively low toxicity. They were s-ethyl disopro- pyn thiocarbamate ([I]), s-ethyl di-isobutyl thiocarbamate ([II]), s-propyl dipropyn thiocarbamate ([II]) and s-propyl butylethyl thiocarbamate ([IV]). The order of potency in dogs was [I]>[II]>[IV]; corresponding doses were 60, 100, 125 and 300 mg/kg/day, respectively. The order of potency in rats, ([I]>[II]>[IV]; corresponding doses were 25, 25, 60 and 400 mg/kg/day, respectively) was the same as for dogs except for III. For dosage levels producing lethality, deaths occurred after 1-2 weeks in both species for II and III and for I and IV in rats. In dogs, I and IV produced death within 1 week. For I, III and IV rats were slightly more sensitive than dogs when doses were expressed as mg/kg/day (2-4x). For I, rats and dogs were approximately, equally sensitive. When doses were expressed as mg/m²/day rats were 2-15x more sensitive than dogs. In conclusion, rats were more sensitive than dogs for 3 of the 4 compounds.

COMPARISON OF CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) FOR THE ANALYSIS OF PESTICIDES IN BIOLOGICAL SYSTEMS. E R Campbell, D W Later*, D N Dankovic, R C Zangar* and D L Springer*. *Lee Scientific, Inc., Salt Lake City, UT and Battelle, Pacific Northwest Laboratories, Richland, WA.

Currently, chromatographic methods for the analysis of pesticides and their metabolites in biological systems is tedious, time-consuming, and often lacking in sensitivity. Pesticide compounds were evaluated by capillary SFC and HPLC to determine the feasibility of detecting them in potentially complex cellular matrices. Actual metabolism studies, using radiolabeled pesticides such as aldicarb and carbaryl, were performed with freshly isolated rat hepatocytes. Metabolic products were analyzed by capillary SFC and flame ionization detection and the results compared to HPLC separation and liquid scintillation counting. Results from analysis with these techniques indicated that approximately 75% of the parent, along with its metabolite, was converted to metabolite after 30 min of incubation. It was also shown that aldicarb sulfone and sulfoxide metabolites could be separated by using a selective cyanopropyl stationary phase for SFC and that these separations provided an enhancement in analytical data compared to reversed phase HPLC. It was observed that the data obtained by capillary SFC agree favorably with that of HPLC. Work supported by NCI Contract N44-CP-71086.
DOSE-DEPENDENT PHARMACOKINETICS AND MAXIMUM TOLERATED DOSE OF OXADIXYL IN MICE. Y H Atallah, and C C Yu. Environmental Sciences, Sandoz Crop Protection Corporation, Des Plaines, IL.

This experiment is designed to determine the dose-dependent pharmacokinetics and maximum tolerated dose (MTD) of oxadixyl fungicide in mice (Naval Medical Research Institute strain). Groups of males and females were dosed with 0, 10, 30, 100 and 400 ppm of 14C-oxadixyl equivalent in diet. 14C in the blood was monitored at specific time intervals. Excreta were collected and analyzed. Tissue samples were collected from animals killed at 24 and 96 hr after 14C dosing. Oxadixyl was rapidly absorbed and then rapidly eliminated by mice. The major route of elimination was via urine (95% in 4 days). Fecal elimination accounted for about 5%. The body weight gains in treated male mice was significantly reduced at day 28. The liver weights as percentage of body weight in both males and females were significantly increased. The half-lives of blood radiocarbon in the 100 and 400 ppm treatment groups were about twice or more than that of the 10 and 40 ppm groups in both sexes. The proportion of unchanged oxadixyl was higher in urine of mice at 400 ppm diet level. These results showed that saturation kinetics of oxadixyl in mice had occurred at or about 100 ppm dietary levels. These effects were more definite at the 400 ppm level. Thus, the pharmacokinetic data in mice demonstrate that the MTD for this species is between 100 and 400 ppm in the diet.

THE ROLE OF INTESTINAL MICROFLORA ON DEEPXIDATION OF TRICHOTHECENE MYCOTOXINS. S P Swanson, C Helaas, W B Buck and H D Rood. Dept. of Vet. Biosciences, Univ. of Illinois, Urbana, IL.

Deepoxidation is an important pathway in the animal metabolism of trichothecene mycotoxins. In this study, the role of intestinal microflora on metabolism of trichothecenes was examined. Microflora obtained from feces of six species, or intestinal contents from swine and rats were incubated anaerobically with the trichothecenes deacetoxytigliocarpine (DAT) or T-2 toxin. Metabolites were extracted with reverse phase cartridges and identified by capillary gas chromatography and mass spectrometry. Incubation of DAT with fecal microflora from rat, cow and swine, yielded both deepoxidation and decacylation products. By contrast, fecal microflora isolated from horse, chicken or dog failed to reduce the epoxide group in DAT and yielded only the decylated products monoacetoxytigliocarpine (MAS) and scirpentriol (SCP). Intestinal microflora obtained from rats and swine biotransformed T-2 toxin to a variety of deepoxy and decylated products including: deepoxy HT-2, deepoxy T-2 triol, HT-2 and T-2 triol. Rat intestinal microflora also biotransformed the polar trichothecenes T-2 tetrol and SCP to their deepoxy analogs. These results suggest intestinal microflora could play a significant role in the metabolism and toxicity of trichothecenes.
BILIARY EXCRETION OF 7,12-DIMETHYLBENZ(A)ANTHRACENE (DMBA) METABOLITES IN MALE AND FEMALE SPRAUG-DAYLEY RATS: S. T. Water and D. Warshawsky.
University of Cincinnati College of Medicine, Cincinnati, OH.

Sex differences in the biliary excretion of metabolites of DMBA were examined in Sprague-Dawley (SD) rats using a nonrecirculating perfused liver system. Suspensions of 20 uMoles of DMBA in Krebs-Henseleit buffer were infused into the livers of male (M) and female (F) SD rats (6 rats/group) during a 60 minute period. Bile was collected in 20 or 30 minute intervals during this period and for the next 60 minutes. The rates of appearance of metabolites in the bile were higher in the F than the M in all time intervals. The maximum rates (90-120 minutes) were 178±17 and 73±5 mmole x g⁻¹ x hr⁻¹, respectively. In both groups β-glucuronidase treatment hydrolyzed approximately 40% of the conjugated metabolites while aryl sulfatase treatment had no effect. Extracts of glucuronidase-treated samples were analyzed by HPLC. Metabolites consisted of phenolic and dihydrodiols, hydroxymethyl metabolites ("x") in both groups, but the proportions of individual metabolites differed between the groups. The 12-hydroxymethyl constituted 26% of the total glucuronides in the M, but only 10% in the F. In contrast, "x" made up 19% in the F and 11% in the M. Further characterization of "x" is in progress. Supported by NSF Fellowship and ACS Ohio Section.

National Center for Toxicological Research, Jefferson, AR.

Caloric restriction has been shown to reduce the incidence of both spontaneous and chemically-induced tumors in experimental animals. Modulation of neoplastic disease by caloric restriction may be related to alterations in xenobiotic-metabolizing enzymes. In this study, mechanistic factors of tumor reduction were investigated in moderately restricted animals. Caloric restriction effects on DNA synthesis as well as hepatic enzymes were examined. Hepatic cytochrome P-450 content, aryl hydrocarbon hydroxylation (AH) activity, and thymidine incorporation into DNA were determined in Fischer 344 male rats. Two groups of restricted rats (fed 60% or 70% of ad libitum consumption beginning at 6 weeks of age) were sacrificed after either 2 or 4 weeks of restriction. Compared with ad libitum controls, DNA synthesis was dramatically inhibited (75%) in the 2-week restricted rats, whereas P-450 content and AH activity were not affected. However, after 4 weeks of restriction, DNA synthesis in the 70% group did not differ significantly from the control level, but a 50% reduction of DNA synthesis was noted in the 60% group. Hepatic AH activity of the 60% and 70% restricted groups were increased by 20% and 25%, and P-450 content was also elevated in the 4-week restricted rats.

State University of New York College of Environmental Science and Forestry, Syracuse, NY.

Blood levels and the hepatic behavior, in vivo and in situ, of the insecticide parathion, and its metabolite, paraxon, were examined to determine the hepatic breakthrough threshold. These data were used to evaluate the 10-fold protective effect of DDE pretreatment against parathion toxicity to male Sprague-Dawley rats. Pretreatment increased the oral lethal dose for parathion from about 12 mg/kg to 150 mg/kg. At these doses, venous blood levels of parathion at the onset of toxicity were about 1x10⁻⁶ M in controls and about 1x10⁻⁶ M in pretreated rats. Venous paraxon was about 2x10⁻⁸ M in controls while none was detected in pretreated rats. In situ pulse infusion of 0.5 μM or more parathion during recirculating autologous blood perfusion of control livers yielded an ejection peak with a mean transit time of 4-6 min while smaller doses were totally metabolized. In DDE-pretreated livers an ejection peak was not detected after pulse doses of up to 6.4 μM. Hepatic autoradiography following in vivo doses of 1 and 5 mg/kg or perfusion in situ at 1.7x10⁻⁷ M of ethyl-¹H-parathion clearly showed less extensive translobular migration of parathion and/or paraxon in DDE-pretreated versus control rats. These results suggest that an increase in the hepatic breakthrough threshold dose for parathion and paraxon accounts for the protective effect. (Supported by NIHES grant ES01019)

CHLORDECONE (CD) PREEXPOSURE-ALTERED DISPOSITION (PAD) OF A SUBSEQUENT DOSE EXHIBITS HIGHLIGHTS SATURATION IN MICE. L. R. Curtis and H. M. Carpenter.
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Preexposure of mice with CD (Pharmacologist 28:136) and trout with dieldrin (Environ. Toxicol. Chem. 5:69) produces marked, tissue specific PAD of a subsequent dose of the same agent. This suggests a role of poorly understood, perhaps inducible, processes other than biotransformation in pharmacokinetics of persistent organochlorines. We studied PAD of ip [¹⁴C]CD in DNA and C57BL male mice 3 days after a single ip dose of unlabeled CD. Both strains pretreated with 5 mg CD/kg exhibited lower hepatic concentrations with subsequent 1 and 5 but not 40 mg [¹⁴C]CD/kg doses (satisfaction). Preexposure with 5 and 40 but not 1 mg CD/kg resulted in reduced hepatic concentration after 3 mg [¹⁴C]CD/kg (threshold). Dose/tissue concentration profiles for plasma, fat, kidney, and bile do not indicate simple redistribution explains PAD of [¹⁴C]CD. Pretreatment with 1 mg CD/kg increased kidney and plasma without affecting hepatic [¹⁴C]CD in C57BL but not DBA mice. Hepatic microsomal ethoxyresorufin deethylation was induced 3 days after 40 mg CD/kg in C57BL but not DBA mice indicating CD interaction with the Ah locus. Stimulated hepatic [¹⁴C]CD processing in CD preexposed mice via an inducible, saturable system is consistent with our data. (Supported by AFOSR grant 87-0385).
EFFECTS OF LINDANE ON THE DIURETIC RESPONSE AND HEPATORENAL TOXICITY OF EUROSEMIDE IN RATS. H. Landrau1, G. Strois1 and S. Chakrabarti2. 1Fac. de Pharmacie et 2Dépt. Méd. Trav. et Hyg. Mil. Université de Montréal, Qué. Canada.

To determine whether microsomal metabolic activation of eurosemide (F) is important for its diuretic response and hepatorenal toxicity, we have studied the effect of lindane (L) (a monoxygenase inducer) on such parameters. In one experiment, 4 groups of 5 rats each were treated with either the vehicle, L (20 mg/kg.d ip x 4 d), F (40 mg/kg po) or with or without L. In second experiment, an ip dose of 300 mg/kg of F was given. The urinary (U) volume, U excretion of Na, K, F and its major metabolite (CSA), were determined for 0-6 h and 6-24 h. Rats were killed 24 h after F dosing and the contents of hepatic microsomal cytochrome P-450 (C), proteins and activity of aminopyrine N-demethylase (AND) were measured. Hepatotoxic response was evaluated by SGPT and SDH activities and renal toxicity by BUN and U creatinine and protein. The results showed that L increased the activity of AND and the content of C. An increase (54%) but not significant in the excretion (0-6 h) of CSA in rats treated with L+F(300 mg/kg), while that of unchanged F was not modified. Neither L nor F nor their association produced any significant toxic effect on the liver or kidney. Associations did not produce any significant change in F diuretic response.

BIOAVAILABILITY OF 5-AMINOSALICYLIC ACID (5-ASA) FROM MIXED DIET IN RATS. K. K. Huang, A. K. Mandagere, D. T. Drees and J. P. Lac. Marion Laboratories, Kansas City, MO.

5-aminosalicylic acid (5-ASA) is being developed for use in the treatment of ulcerative colitis and Crohn's disease. As part of a subacute toxicology study with 5-ASA in rats, we evaluated: 1) the plasma pharmacokinetics of 5-ASA and its major acetylated metabolites at various dosages, 2) excretion of unchanged drug and metabolites in the urine to estimate absorption, and 3) the effect of dose and route of administration on 5-ASA bioavailability in rats. Nine groups of rats, 10 per group (5 per sex) were housed individually in cages. 5-ASA was given either by gavage or mixed diet in doses ranging from 20-3000 mg/kg. Blood and urine samples were collected separately for 24 hours. 5-ASA and NAc-5-ASA concentrations were determined by HPLC method. Results indicated that 5-ASA was subject to capacity limited acetylation and can be bioaccumulated when the higher doses (>1000 mg/kg) are repeatedly administered. Data further suggested that when 5-ASA was administered to rats as drug/diet mixtures, an average 30% of total dose was excreted in the urine within 24 hours after dosing, whereas, 56% of the total dose was excreted in the urine after oral gavage administration. Results strongly indicate that the absorption of 5-ASA in rats was decreased when administered in feed in comparison to gavage.

EFFECT OF ORTHOVANADATE ON BILIARY EXCRETORY FUNCTION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS. J. B. Watkins III and M. E. Bauman, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN.

Insulin-dependent diabetes alters the hepatobiliary clearance of several drugs in rats. Sodium orthovanadate promotes glucose uptake in muscle and adipose tissues and has been suggested as a possible oral hypoglycemic agent. This study has determined whether orthovanadate, like insulin, can reverse diabetes-induced changes in the biliary clearance of bile acids and rose bengal. Six groups of male Sprague Dawley rats were used: normal, insulin-treated normal, vanadate-treated normal, diabetic, insulin-treated diabetic and vanadate-treated diabetic. Diabetes was induced by injection of streptozocin (45 mg/kg iv). One week later, insulin (2-4 U/day sc) and vanadate (150-200 umol/kg/day po) treatments were initiated. After four weeks, the clearance and biliary excretion of rose bengal (60 umol/kg iv) and endogenous bile acids were determined for three hours. Bile flow rate, rose bengal excretion and bile acids excretion were unchanged in the three normal groups and the insulin-treated diabetic rats. These parameters were increased in untreated and vanadate-treated rats. Pharmacokinetic analyses indicated that total and biliary clearances of rose bengal were increased in diabetic rats and that orthovanadate did not reverse these changes. These data indicate that the oral hypoglycemic chemical sodium orthovanadate does not reverse diabetes-induced alterations of the hepatobiliary clearance of either rose bengal or bile acids.


1,3-DNB is the only DNB isomer that is a testicular toxicant in vivo and in vitro. To determine if differences in metabolism or distribution are involved in this isomer-specific toxicity, the metabolism of all three isomers was investigated in vitro in F-344 rat testicular S-9 fractions and cultured seminiferous tubules. The tissue distribution of 14C-DNB and metabolites following a single oral dose was also examined. The metabolism of 1,3-DNB was slower than that of the other isomers in both in vitro systems. Only 10% of the added 1,3-DNB was converted to 3-nitroaniline (3-NA) after a 6 hour incubation with S-9, and no metabolites were found following 24 hour tubule incubations. In contrast, 1,2- and 1,4-DNB were extensively metabolized (50 to 90%) in each in vitro system, yielding glutathione conjugates and NA. In vivo testicular metabolites found after 1,3-DNB included 1,3-NA, a glutathione conjugate and 4 unidentified metabolites. 1,2- and 1,4-DNB were present in testis as only glutathione conjugates and parent compound. Five times more 14C from 1,3- than 1,2- or 1,4-DNB was present in the testes 6 hours after the dose. Thus, the testes receive a larger percentage of the 1,3-DNB dose compared to the other isomers, and metabolism of 1,3-DNB in the testes is slow. Either the parent compound or an undetectable metabolite formed in the testis is responsible for the testicular toxicity of 1,3-DNB.

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MBOCA, an industrial arylamine and environmental contaminant, is a suspect human carcinogen by virtue of its carcinogenicity in rodents and dogs, and short-term mutagenicity. Extrapolation of risk to humans is currently difficult due to the paucity of data on metabolic activation in man. Since hemoglobin (Hb) adduct formation may be considered an indication of exposure and metabolic activation, this study was designed to access the binding activity of N-oxidized MBOCA metabolites to Hb in vitro and to determine in vivo Hb adduct formation with MBOCA. Metabolism of MBOCA-Hb adduct formation was determined by electron-capture GLC and confirmed by GC/MS. N-Hydroxy- and mononitroso-MBOCA but not MBOCA itself formed adducts to rat and human Hb in vivo in a dose-related manner. Binding was inhibited by cysteine and GSH but not GSSG or mercaptoethanol. Subcutaneous administration of 0.5 to 500 mg/kg MBOCA to rats and guinea pigs resulted in dose-related formation of MBOCA-Hb adducts. MBOCA-Hb remained elevated in blood for greater than 42 days. These experiments demonstrated that activated MBOCA metabolites covalently bind to Hb and that measurement of MBOCA-Hb adducts in blood may be used to assess dose-related exposure to MBOCA. (Supported by the Mich. Dept. of Public Health.)

CONCURRENT MEASUREMENT OF GLUTATHIONE S-TRANSFERASE AND EPOXIDE HYDROLASE ACTIVITY BY HPLC. F L Stapleton and D L Eaton, Dept. Environmental Health, Univ. Washington, Seattle, WA.

Cytosolic glutathione S-transferase (GST) and microsomal epoxide hydrolase (EH) are important detoxification enzymes for many epoxide xenobiotics. We have developed a rapid, simple and convenient HPLC assay which measures both of these enzyme activities toward benz(a)pyrene-4,5-oxide (BaP-O) in tissue homogenates. Tissue fractions were incubated at 37°C in the presence of 5 mM glutathione. Reactions were initiated by addition of BaP-O and terminated by the addition of ice-cold acetonitrile containing 2-methoxy-naphthalean as an internal standard. Samples were analyzed directly on a 15 cm C18 reverse-phase column at room temperature, with a ternary solvent program which utilized 0.01% ammonium phosphate buffer (pH 3.5), acetonitrile and water. UV absorbance (265 nm) was monitored. Baseline resolution of BaP-O, BaP-O-GSH and BaP-O-diol and the internal standard was accomplished in 10 min. In rat hepatic S-9, production of both BaP-O-GSH and BaP-O-diol was linear with time and protein up to 15 minutes and 500 µg/ml, respectively. Coefficients of variation for replicate analyses were 6.3 and 9.7% for GST and EH activity, respectively. With fluorescence detection (exc 241, em 389 nm), this assay was sensitive enough to measure GST and EH activity in mononuclear leukocytes (MNL) and rat embryonic tissue. GST and EH activity in 109 human MNL samples was 142 ± 74 (mean ± SD; range 21 - 435) pmol/mg/min and 19 ± 9 (mean ± SD; range 3 - 59) pmol/mg/min, respectively. These results demonstrate the simplicity, high sensitivity and applicability of this assay for a broad range of tissues. (Supported by the Dana Foundation.)

COVALENT BINDING OF [14C]-ETHYLENE MERCURATE WITH ALBUMIN. G A S Anwar, B B Kephart, D Suno and J C Gan, Division of Chemical Pathology and Biochemistry, University of Texas Medical Branch, Galveston, TX.

In order to evaluate the possibility that alkylation of plasma proteins by industrial chemicals can be used for biomonitoring of occupationally exposed individuals, rats were given daily oral doses of [14C]-ethylenediamine (EB) [25µg in mineral oil/kg body weight] for 12 consecutive days. Blood plasma of the treated rats was dialyzed and subjected to size exclusion high performance liquid chromatography (SE-HPLC). Only one radioactive protein peak (290,000), with molecular weight of 65 kDa, was observed. Double immunofluorescence test showed that it reacted primarily with antisera to albumin. The binding was found to be 139 picolamol equivalent EB/µg albumin. In vitro experiments of human plasma and reconstituted human albumin with [14C]-EB and phenobarbital treated rat liver microsomes in the presence of NADPH generating system were carried out at 37°C for 2 hrs. The 100,000 g supernatant was dialyzed against water and subjected to SDS-PAGE. The binding was found to be 280 picolamol EB/µg albumin. Reversed phase HPLC has confirmed the radioactivity is associated with albumin only. Trypsin digest of the alkylated albumin showed at least six peptides possessing radioactivity. Further amino acid analysis of the in vitro and in vivo alkylated albumin and their radioactive peptides are in progress to identify the amino acids involved in the binding. (Supported by NIGMS Grant No. CH 02169.)

COMPARISON OF THE MURINE AND HUMAN LIVER CYTOSOLIC EPOXIDE HYDROLASE (CEH). E C Dietze, J Magdalou, R N Wixten, M H Silva, and S B Haanack, Department of Entomology and Environmental Toxicology, University of California, Davis, CA.

CEH has been best characterized in murine liver. Before the murine system is used as a model for human CEH, it should be established that the two enzymes are similar. Both murine and human CEH were purified by affinity chromatography. The purified enzymes were compared by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), antigenic crossreactivity, peptide mapping, and isoelectric focusing (IEF). Antigenic relatedness was assessed by Western blots of murine and human liver CEH using antisera raised against either murine or human liver CEH. Peptide mapping was carried out by separating CEH digests by ion-pair, reverse phase liquid chromatography. The results show that, by SDS-PAGE and Western blotting, murine and human CEH are indistinguishable. Peptide maps of murine and human liver CEH clearly show that the two enzymes are similar but not identical. Murine and human CEH are not identical by IEF but both focus at acidic pI's. Using t-stilbene oxide as a substrate, murine and human CEH have similar kinetic properties. Further characterization of the two enzymes should be undertaken in order to validate the use of murine liver CEH as a model for human liver CEH.

Many endogenous and xenobiotic chemical substances are metabolized to epoxides which can then be enzymatically hydrated to less reactive dihydrodiols via microsomal epoxide hydrolase (mEH). Based on the reported rabbit mEH amino acid sequence, we constructed a 35 base oligonucleotide (35-mer) and used it to isolate a cDNA clone from a rabbit liver library. Nucleotide sequence of the isolated clone was determined, and its deduced protein sequence shows agreement with the rabbit amino acid mEH, although some discrepancies exist. Northern blot analyses using the 35-mer and fragments of the cDNA demonstrate constitutive hepatic expression and induction of mEH messenger RNA (mRNA) following exposure to phenobarbital or polychlorinated biphenyls. Constitutive expression of mEH mRNA is also observed in rabbit testes, kidney, and lung. Activity of mEH shows a positive correlation with the level of mRNA expression. Southern blot analysis of rabbit DNA using a mEH cDNA probe suggests the mEH gene family contains only one member. Supported by NIH Grants GM-32281 and EH-07032.


The present study was designed to identify the forms of glucuronotransferase (GT) involved in the conjugation of MAM and age-related changes in this activity. Activity of GT towards 4-nitrophenol (PNP) and 4-MU was measured in colonic microsomes from young (Y; 2-4 mo), middle-aged (MA; 12-14 mo), and aged (A; 22-24 mo) male Fischer 344 rats, either alone or in the presence of 1.25 and 2.5 uMAM. The K_m for PNP-GT was significantly increased in MA rats, while V_{max} declined significantly in both NA and A rats. In contrast, the K_m for 4-MU-GT showed no significant differences with age while V_{max} rose significantly in both MA and A rats. MAM at both 1.25 and 2.5 uM inhibited 4-MU-GT in Y rats by approximately 30 and 50%, respectively, while in MA and A rats, MAM at both concentrations inhibited 4-MU-GT by approximately 50%. No effect of MAM was observed on PNP-GT; in addition, PNP was without effect on 4-MU-GT. This evidence suggests that MAM is a substrate for GT in the large intestine and that since 4-MU-GT activity increases with age, older animals may more effectively detoxify MAM. These results also show that PNP and 4-MU, although both GT substrates, are conjugated by different forms of colonic GT.

733 EFFECT OF UDP-GLUCURONOSYLTRANSFERASE (GT) INDUCERS ON INTESTINAL UDP-GLUCURONIC ACID (UDP-GA) CONCENTRATION. D. Goon and C. D. Klaassen. Univ. of Kansas Med. Ctr, Kansas City, KS.

This study was conducted to evaluate the effect of various microsomal enzyme inducers upon both intestinal UDP-GA concentration and GT activity. Rats were orally administered dibutylated hydroxyacetophenone (BHA), benz[a]pyrene (BP), 3-methylcholanthrene (3MC), phenobarbital (PB), pregnenolone-16a-carbonitrile (PCN), 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) or trans-stilbene oxide (TSO). Intestinal content of UDP-GA and its precursor, UDP-glucose (UDPG), were quantitated using reverse-phase, ion-pair HPLC and microsomal GT activity toward acetaminophen (AA), harmol (HA) and α-naphthol (NA) assessed in vitro. TCDD and 3MC had no effect upon UDPG or UDP-GA, while PCN and PB minimally increased UDP-GA (16 and 18%, respectively) but not UDPG concentration. TSO tended to increase UDPG and UDP-GA levels (27 and 21%, respectively); while BHA and BP substantially elevated both UDPG (50 and 33%, respectively) and UDP-GA (38 and 58%, respectively). PB and PCN did not enhance intestinal glucuronidation of AA, HA or NA, while TCDD selectively increased GT activity toward NA (30%). In contrast, glucuronidation of AA, HA and NA was markedly increased by BHA (146, 82 and 75%, respectively), BP (123, 80 and 120%, respectively) and TSO (164, 92 and 96%, respectively). 3MC also enhanced glucuronidation of AA, HA and NA (18, 27 and 98%, respectively). In summary, hepatic microsomal enzyme inducers possess the propensity to increase intestinal UDP-GA levels as well as GT activity which further confounds study of capacity-limited glucuronidation. (Supported by USPHS Grants ES-03192 and ES-07079)

734 CIRCADIAN VARIATION IN HEPATIC UDP-GLUCURONIC ACID (UDP-GA) DOES NOT AFFECT GLUCURONIDATION OF XENOBiotics. S. R. Howell and C. D. Klaassen. Univ. Missouri-Kansas City, Kansas City, MO and Univ. Kansas Medical Center, Kansas City, KS.

A diurnal variation in acetaminophen (AA) toxicity has been reported and ascribed to circadian variation of hepatic glutathione levels. However, glucuronidation represents a major competing biotransformation pathway which detoxifies AA and many other xenobiotics. In the present study, hepatic glycogen, UDP-glucuronosyltransferase (UDPG) and UDP-GA were determined every 3 hr for 24 hr in male CF1 mice. Glycogen and UDPG concentrations in liver were found to exhibit a diurnal cycle with maximal levels observed at 5 and 11 AM, respectively. Similarly, UDP-GA exhibited a circadian cycle but with maximal levels at 8 PM. To evaluate the effect of the observed circadian variations on glucuronidation capacity, mice were administered either AA (600 mg/kg, ip) or salicylamide (SAL, 550 mg/kg, ip) at either 8 AM or 5 PM. Blood samples were collected every 15 min for 75 min and urine collected for 24 hr. These samples were analyzed for SAL or AA and their respective metabolites by HPLC. No significant diurnal variation was observed in plasma half-life or apparent rate of glucuronidation for either AA or SAL. In summary, diurnal variations in hepatic glycogen, UDPG and UDP-GA levels were observed. The circadian cycle of UDP-GA was the inverse of that for glycogen and UDPG, with peak levels occurring in the early evening. However, the fluctuations in hepatic content of UDP-GA and its precursors do not appear to have any significant effect upon the glucuronidation of xenobiotics. (Supported by USPHS Grants ES-03192 and ES-07079)

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SIMULTANEOUS ANALYSIS OF MORPHINE AND MORPHINE 3-GLUCURONIDE BY ION PAIR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. D J Kunz, S Narayan, and G S Yost. Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

Chronic ethanol administration has been shown to increase the rate of glucuronidation of various substrates, including morphine, in rabbit hepatic microsomes. A procedure was established to quantitatively determine both morphine and morphine 3-glucuronide from plasma of New Zealand white male rabbits, to evaluate pharmacokinetic changes due to chronic ethanol consumption. One ml of plasma was extracted on a SEP-PAK before analysis by gradient (acetonitrile from 2 to 33%/0.1% trifluoroacetic acid, 10 min) high performance liquid chromatography. Trifluoroacetic acid was used as the ion pair agent. Retention times for morphine 3-glucuronide, morphine, and nalorphine (internal standard) were 5.0, 6.5, and 8.2 minutes, respectively. Extractions were determined to be greater than 90% and linear over the biological range. This method provides a simultaneous determination of both drug (morphine) and major metabolite (glucuronide) for pharmacokinetic determinations. Supported by USPHS Grant A06555. GSY is a USPHS Research Career Development Awardee (HL02119)

MECHANISMS OF PENTACHLOROPHENOL-INDUCED INHIBITION OF CONJUGATIVE ENZYME SYSTEMS. M G Miller and R Singh, Dept of Environ Toxicol, Univ of California, Davis, CA. Sponsor: L Shull

Pentachlorophenol (PCP) is a well known uncoupler of mitochondrial oxidative phosphorylation, and is also thought to selectively inhibit the sulfotransferase enzyme system. The present studies will examine the mechanism by which PCP inhibits the conjugation of 1-Naphthol, using isolated rat hepatocytes and microsomal and cytosolic preparations. In cytosol, Lineweaver-Burk and Dixon plots revealed that PCP was a potent competitive inhibitor of sulfation with a K_i of 0.95 x 10^{-6}M. Similarly, in microsomes PCP was a non competitive inhibitor of glucuronatransferase with a K_i of 8.5 x 10^{-6}M. In contrast, isolated cell studies provided no evidence for a selective inhibition of sulfotransferase. Similar concentrations of PCP (> 5 μm) inhibited glucuronatransferase, cytochrome P450 and sulfotransferase. Further investigation revealed that PCP induced a concentration dependent depletion of ATP the extent of which could be correlated with inhibition of metabolism. Cell viability, as assayed by trypan blue exclusion, was maintained despite ATP depletion to as low as 5% of control levels. Explanation of this was sought by measuring 32P-PCP levels associated with cellular vs. aqueous fractions of the hepatocyte suspension. Cellular radiolabel was approx. X40 that in aqueous media. These data would suggest that the PCP concentration at the soluble sulfotransferase enzyme is much lower than at particulate enzymes. Further studies are underway using additional substrates.

DISTRIBUTION OF ACRYLONITRILE (AGN) IN TISSUES OF CONTROL AND GLUTATHIONE (GSH) DEPLETED B6C3F1 MICE. D E Rickert, A E Roberts and D Plon, CIIT, Research Triangle Park, NC.

AGN has been shown to cause brain, stomach and Zymbal's gland tumors in rats. This study examined the distribution of 14C-AGN in B6C3F1 mice, a species in which the carcinogenicity of AGN has not been determined. The concentration of total radiolabel was highest in all tissues studied 1h after oral administration of [2,3-3H]AGN (4 mg/kg, 4 uCi, 1.75 mcCi/mmole). By 24h, approximately 86% of the total radiolabel had been cleared. Radiolabel was irreversibly associated (not removed by extensive dialysis) with tissue macromolecules of brain, lung, liver stomach, kidney and blood. The concentration of irreversibly associated material was highest in stomach and lowest in brain (40 and 1.0 nmol AGN equivalents/g tissue, respectively). After GSH depletion (L-buthionine-S-R-sulfoximine, ip, 1.5h prior to 14C-AGN), the distribution of radiolabel resembled that observed in control (GSH normal) mice. At 24h, 89% of the total radiolabel present at 1h had been eliminated. The concentration of radiolabel irreversibly associated with stomach and brain macromolecules was equivalent to the concentration in controls. In B6C3F1 mice, the conjugation of GSH with AGN (a route of metabolic clearance in rats) may not be important to the interaction of AGN-derived material with tissue macromolecules.

EFFECT OF ROUTE OF ADMINISTRATION AND GSH DEPLETION ON THE IRREVERSIBLE ASSOCIATION OF ACRYLONITRILE (AGN) WITH TISSUE MACROMOLECULES IN RATS. D Plon, A E Roberts and D E Rickert, CIIT, Research Triangle Park, NC.

The metabolism of AGN has been demonstrated to depend on the route of administration. In order to better understand this phenomenon, the distribution of [2,3-3H]AGN was studied in rats given orally or by inhalation to male F-344 rats. Inhalation exposure yielded more (17 to 65%) AGN-derived radiolabel irreversibly associated with brain, lung, kidney, liver and blood macromolecules than after gavage. Radiolabel was also found to be irreversibly associated with the RNA in brain, stomach and liver. No (1 pmol/mg) radioactivity was detectable in DNA of any tissue. The concentration of AGN-derived material associated with RNA was the same in brain, stomach and liver after either route, suggesting that there is no preferential interaction of the radiolabel with the RNA in the target organs (stomach and brain). Experiments performed using GSH-depleted rats (phorone/buthionine sulfoximine treatment) revealed that the depletion increased the magnitude of 92% in radiolabel irreversibly associated with macromolecules in all tissues after gavage but a decrease (30 to 53%) after inhalation exposure. These data suggest that different pathways of AGN elimination predominate in the organs of entry.
METABOLISM OF THE ACROLEIN-GLUTATHIONE ABDUCT 5-(2-ALDEHYDO-ETHYL)GLUTATHIONE BY SPRAGUE-DAWLEY RATS. D. J. Mitchell and D. R. Peterson. School of Pharmacy, Molecular and Environmental Toxicology Program, University of Colorado, Health Sciences Center, Denver, CO.

Acrolein (AC) is a toxic 3,8-unsaturated aldehyde which reacts spontaneously with glutathione (GSH) to form S-(2-aldehydo-ethyl)GSH (SAEG). Quantitative studies have established that the metabolism of the AC-GSH adduct SAEG by rat liver aldehyde (ALDH) and alcohol (ADH) dehydrogenase. Livers were obtained from male Sprague-Dawley rats for the isolation of mitochondria and cytosol. Kinetic parameters, Vmax and V/K, of SAEG by semi-purified cytosolic (C) and mitochondrial (M) ALDHs and ADH were determined. The Vmax values (nmol NADH produced/min/mg prot.) were respectively 3.9 and 3.0 for high- and low-affinity C-ALDHs, and 9.6 and 4.5 for high- and low-affinity M-ALDHs. The affinity constant V/K (nmol NADH produced/min/mg prot./nmol adduct/mi) for the high- and low-affinity C-ALDHs were 0.014 and 0.00043, respectively, and 0.11 and 0.12 for the high- and low-affinity M-ALDHs. The Vmax and V/K for ADH reduction of SAEG were 50 mmol NADH consumed/min/mg prot. and 0.11 nmol NADH consumed/min/mg prot./nmol adduct/mi, respectively. This study demonstrates the intermediary metabolism of the AC-GSH adduct prior to its excretion as a mercapturic acid. (Grant Support: NIH AM34914, NIAAA AA03527, and NIMH MH16880)

ENHANCEMENT OF THE ACUTE TOXICITY OF 2-ThIOTRAIZONE (TTZ) IN RATS BY GLUTATHIONE DEPLETION. T. M. Tate, and W. F. Filory. Louisiana State Univ. Baton Rouge, LA.

The acute toxicity of 2-thiothiazione in adult male rats has been established in previous studies (oral LD50 of 4.6 mg/kg and intraperitoneal LD50 of 1.4 mg/kg). Male rats become resistant to the toxic effects of TTZ after survival of a single exposure. Female rats are resistant to the toxic effects of TTZ with 40% toxicity being observed at concentrations up to 1000 mg/kg. Immature rats (21-45 days old) are completely resistant to TTZ. The effect of glutathione depletions on the toxicity of TTZ in rats (male, female and immature) was investigated. Glutathione was depleted by diethyl maleate (DEM) treatment. Rats were exposed to DEM (0.5 ml/kg) intraperitoneally (ip), 30 minutes prior to TTZ exposure (1-10 mg/kg) ip. Toxicity in male rats during the 24 hr post-exposure increased from 0-50% when dosed with TTZ (1 mg/kg) ip. after DEM treatment. The resistance in female rats was totally overcome by DEM treatment. All female rats died when dosed with the above concentrations of TTZ after DEM treatment. The resistance in immature rats was also diminished whereby 90% of the immature rats died when dosed with TTZ after DEM treatment. Results from this study show the possibility that glutathione concentration has a definite effect on TTZ toxicity in rats.

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GLUTATHIONE TRANSFERASE-DEPENDENT METABOLISM OF 1,3-BIS(2-CHLOROETHYL)-1-NITROSUREA. C G Evans, P D. Deane-Setzer, and M T. Smith. School of Public Health, University of California, Berkeley, CA.

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) is detoxified by a denitrosation reaction catalyzed by glutathione-dependent enzymes in rat liver cytosol (Talcott, R E and Levin, V A. Drug Metab. Disp. 11: 151-156, 1982). Using a modification of their procedure, we have measured the ability of different purified glutathione transferase isozymes to denitrosate BCNU. Apparent Km and Vmax values were derived from Wolf-F-Augustinsson-Hofsteet plots. The Vmax values obtained for isozymes 1-2, 3-3, 3-4, and 4-4 were 1.6, 3.3, 54.5, and 94.5 nmol mg prot. min⁻¹, respectively. The specificities of the isozymes for the reaction given by the ratio of Vmax to Km are as follows: (isozyme, Vmax/Km): 1-2, 2.3; 3-3, 12.2; 3-4, 29.2; and 4-4, 26.1. The k-type transferases containing subunit 4 are by far the best catalysts of the BCNU denitrosation reaction. The k-1-2 isozyme and the 7-7 k-type isozyme are relatively poor catalysts. These results suggest that cells with high levels of subunits 4 and 6 may show resistance to BCNU through increased detoxification of the drug. Studies are underway to determine if the modulation of glutathione transferase levels affects the cytotoxicity of BCNU in 9L rat brain tumor cells.

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CHARACTERIZATION OF RODENT EMBRYONIC GLUTATHIONE S-TRANSFERASE ACTIVITY TOWARD VARIOUS SUBSTRATES. E. M. Faustman, P L. Stapleton, and D L. Eaton. Dept. of Environmental Health, Univ. of Washington, Seattle, WA.

Glutathione S-transferases (GST) are a family of enzymes responsible for the detoxification of a variety of chemicals. Characterization of GST activity in embryonic tissue is critical to the assessment of potential embryonic susceptibility to toxic insult. The in vitro activity of embryonic GST toward 1-chloro-2,4-dinitrobenzene (CDNB), benz(a)pyrene-4,5-oxide (BP0), p-nitroso-7,8-oxide (PNSO), and aflatoxin-B9-oxide (AFBO) was characterized in day 10 Sprague-Dawley rat embryos. HPLC assays were developed to measure the GST activity toward the substrates BP0, PNSO and AFBO. Conventional spectrophotometric assay methods were used to assay GST activity toward CDNB. Homogenates which included pooled embryos, embryonic yolk sacs, and Reicharts and amnionic membranes exhibited measurable activity toward the four substrates tested. GST activities toward CDNB, BP0, PNSO and AFBO were 590±618, 50±5, 157±9, and 15±4 (mean ± SE) pmol per mg protein per min, respectively. The activities in embryonic tissues were approximately 1/200th, 1/170, 1/109th and 1/34th the activity of adult male rat liver homogenates, respectively. These studies demonstrate that rat embryonic tissues contain measurable GST activity toward a variety of substrates and suggest that assessment of potential embryonic susceptibility to toxic insult should be based on specific substrate determinations. These studies were supported by NIH Grant No. ES03157 and the Dana Foundation.
2-Chloroethanol, a widely used solvent and an intermediate in the production of organic chemicals, is also a major metabolite of several compounds and drugs such as 1,2-dichloroethane, 2,2-dichloroethyl ether, and 2-chloroethylthioacetate as well as present in several medical supplies and food treated with ethylene oxide. The toxicity of 2-chloroethanol is mainly attributed to the inhibition of fatty acid elongation in the mitochondria. Present study was undertaken to investigate whether 2-chloroethanol esterifies various fatty acids of liver under in vivo conditions. A single oral dose of 2-chloroethanol in mineral oil (50 mg/kg) was given to male rats while controls were given mineral oil only. Subsequently both groups of rats were maintained on the normal diet. A decrease in body weight of about 10% (p < 0.01) was found on the second day of the 2-chloroethanol treatment and the trend continued until the day of sacrifice. Rats were killed on the fifth day of the treatment and microsomes were prepared from the liver. The lipids were extracted and subjected to preparative thin layer chromatography. The area corresponding to 2-chloroethylstearate was scraped off, eluted and subjected to reverse phase high performance liquid chromatographic analysis. The fractions corresponding to 2-chloroethylstearate were collected and analyzed by desorption chemical ionization mass spectrometry using ammonia as the reagent gas. A pseudomolecular ion at M/Z 364/366 (M+H), base peak, 31) confirmed the structure of 2-chloroethylstearate in the treated rat liver. Esterification of fatty acids may be responsible for the observed toxicity of 2-chloroethanol.


Oral administration of unleaded gasoline to male rats produces phagolysosomes (hyaline droplet) accumulation in epithelial cells of the renal proximal convoluted tubules (PCT) similar to that observed with Decalin, isoparaffinic solvents and numerous other volatile hydrocarbons. Transmission electron microscopy was used to study dose- and time-dependent alterations of renal PCT phagolysosomes in male rats treated with gasoline (0.4-2.0 ml/kg, po, once daily, 1-9 d). A progressive increase in the number and size of phagolysosomes in cells of the PCT was observed. Many of the enlarged (0.05-9 um), angular phagolysosomes in treated rats contained electron-dense, crystalline inclusions and occupied a considerable area of the cytoplasm in contrast to the relatively small (0.05-2.5 um) round phagolysosomes of controls. These morphological observations parallel biochemical determination of dose- and time-dependent accumulation of α2-globulin after gasoline exposure. Furthermore, studies with gold-tagged antibody to α2-globulin have demonstrated α2-globulin localization exclusively in the phagolysosomes. These results suggest that a common defect in phagolysosome turnover induced by hydrocarbons is responsible for the unique sensitivity of male rats to hydrocarbon-induced nephropathy.


This study was designed to examine the morphology of glomeruli and glomerular filtration of male rat kidneys after exposure to decanal and JP-10. Male F-344 rats were dosed with either decanal or JP-10 (2 ml/kg body weight twice; 1 ml/kg, thereafter) by gavage three times a week for 1, 2, 3, or 4 weeks. Urine was collected for 24 hours prior to dosing and prior to sacrifice. Although total volume of urine was significantly elevated for both hydrocarbon groups at each time point, specific gravity and creatinine clearance were not statistically different at any time. Histological parameters indicative of hydrocarbon nephropathy were significantly different from control rats; hyaline droplets at 1 through 4 weeks (only decanal at 4 weeks); basophilic tubules at 1 through 4 weeks; and casts at 2 through 4 weeks. Scanning electron microscopy of glomeruli showed no differences between control and exposed groups at any week as previously seen in glomeruli from rats exposed to 2,3,4 trimethylpentane for 4 weeks.

ASSESSMENT OF THE ABILITY OF SEVERAL IN VITRO MODELS TO PREDICT THE NEPHROTOXICITY OF β-LACTAM ANTIBIOTICS. S M Flan, F A Lask and P D Williams, Bristol-Myers Co., Syracuse, NY.

These studies were initiated to compare cultured cells with kidney slices in ranking the nephrotoxic potential of cephalosporin (CSP) and carbapenem (CBN) antibiotics. The in vivo liability of CSP for rabbits is cephaloridine (CPH)»cefazo- zolin (CFZ)»cephalothin (CLT), and that of CBN is imipenem (IMI)»BMY-25174 (B74)»BMY-26225 (B25). CSP or CBN were dissolved (0.1-25 mM) in culture medium (± a rabbit kidney microsomal fraction = KS9) and applied to confluent monolayers of rabbit proximal tubule cultures (RPC) or the rabbit kidney cell line LLC-RK, (RK). After 48 hr, cell viability was assessed by dye exclusion. The integrity of renal cortical slices was determined by measuring oxygen consumption (QO,) and active uptake of PAH and TEA after coinubcation with the drugs (0.1-100 mM) for 90 min. Both culture systems ranked the cytotoxicity of CSP as CPH>CFPZ>CLT and of CBN as IMI>B74>B25. Supplementation of the cultures with KS9 was required to properly rank CLT (RK,RFC) and B25 (RK). Depression of slice PAH uptake was dose-dependent (CFPZ>CLT>CPh; IMI>B74>B25), as was that of TEA (CPh>CLT>CFPZ; IMI>B74>B25). Inhibition of slice QO, reflected in vivo toxicity in all cases. Support that rabbit renal cells and kidney slices can provide useful models to predict in vivo nephrotoxicity of antibiotics with careful selection of toxic endpoints and inclusion of metabolic capabilities.

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ROLE OF OXIDATIVE STRESS IN CEPHALORIDINE-INDUCED NEPHROTOXICITY. R S Goldstein, R S Sozio, J B Tarloff and J B Hook. Smith Kline & French Laboratories, Dept. of Investigative Toxicology, King of Prussia, PA.

Lipid peroxidation precedes the onset of cephaloridine (CPh) nephrotoxicity in vitro and may represent an important mechanism of CPh toxicity. These experiments were designed to test the hypothesis that CPh toxicity is dependent on generation of oxygen-free radicals via iron-catalyzed reactions and that detoxification of these oxygen species is dependent on glutathione (GSSG) redox cycle. Cortical slices from naive male F-344 rats were preincubated with the iron chelating agent, deferoxamine (20 μM) prior to CPh treatment (0-5 mM). Deferoxamine completely prevented CPh-induced malondialdehyde production and toxicity, reflected by leakage of lactate dehydrogenase (LDH).

To evaluate the role of CPh-induced CPh toxicity, renal cortical slices were preincubated with an inhibitor of GSSG reductase, 1,3 bis-(2-chloroethyl)-1-nitrosurea (BCNU), or a glutathione depleting agent, diethylmaleate (DEM). Both BCNU and DEM potentiated CPh-induced LDH leakage. Furthermore, dithiothreitol completely prevented CPh toxicity. These data suggest that induction of CPh toxicity is dependent on iron-catalyzed reactions and that intracellular thiol status and activity of the glutathione redox cycle are critical defense mechanisms against CPh-induced oxidative stress.

DIFFERENTIAL SENSITIVITY OF RENAL PLASMA MEMBRANES TO MERCURY (Hg) AND CHROMATE (Cr) ION TOXICITY. E J Jensen and W G Berndt. Dept. of Pharmacology, Univ. NE Med. Ctr., Omaha, NE.

Previous work from this laboratory indicated that Cr potentiated the nephrotoxic effects of Hg. As the plasma membrane is considered to be a potential site for the toxic actions of Hg, we examined the effects of Hg and Cr (alone or in combination) on transport processes characteristic of the basolateral (BL) or brush border (BB) plasma membranes. BL and BB plasma membranes were isolated from rat renal cortex and used in assays of p-aminohippurate (PAH) and glucose uptake into BL and BB vesicles, respectively. Significant inhibition of PAH uptake into BL vesicles was observed at 10-100 nM concentrations of Hg or Cr. Glucose uptake into BB vesicles was inhibited by Hg alone at concentrations of 10-100 μM while Cr alone had no effect. Treatment of BL vesicles with a combination of Hg and Cr (10 nM each) inhibited PAH uptake to the same extent as that observed with a single concentration (10 μM). Glucose transport into BB vesicles was unaffected by a combination of Hg (100 nM) and various Cr concentrations (1-100 μM). These data suggest that the plasma membrane is a possible site for the interactive effects of Hg and Cr and that the BL plasma membrane may be especially sensitive to the toxic actions of Hg and Cr ions.

COMPLEXING ACTIVITY OF 2,3-DIMERCAPTO-1-PROPANE SULFONATE (DMPS) AND ITS DISULFIDE OXIDATION PRODUCT (DMPS) IN RAT KIDNEY. G L Diamond, J M Klotschab, and J R Stewart. University of Rochester School of Medicine and Dentistry, Rochester, NY.

Activity of DMPS as a mercury complexing agent is generally attributed to formation of a highly stable and readily excreted complex between the sulphydryl form, DMPSH, and divalent mercury. However, in vivo, DMPSH rapidly oxidizes to a disulfide metabolite (DMPS) in extracellular fluid. The objectives of this work were to examine the effect of administered DMPS on renal excretion of mercury and to determine if DMPSH could be generated from DMPS in kidney. Infusion of DMPS or DMPSH into rats or isolated perfused rat kidneys (IPKR) from rats pretreated with mercurochloride increased urinary excretion and decreased renal retention of mercury. Prolonged infusion of DMPSH and reduction to DMPSH occurred in IPKR and in the chicken, in vivo. Net reduction of DMPSH was demonstrated in isolated rat kidney cytosol supplemented with reduced glutathione (GS) and in Tris buffer (pH 9.0) containing glutathione reductase, NADPH and GSSG, suggesting the possibility of an exchange reaction between GSH and DMPSH to yield DMPSH. Transport of DMPSH in the kidney, coupled with intracellular reduction to generate DMPSH may facilitate enhanced excretion of mercury that is observed after administration of DMPSH to animals. (Supported by NIH grant ES 02148).


Rats administered large doses of MA, a known nephrotoxin, develop a Fanconi-like syndrome characterized by aminoaciduria, glycosuria, phosphaturia and acidosis. DCM is one of the halogenated compounds that appear as a by-product of water purification. Because simultaneous exposure to these contaminants may occur, the possible interaction of DCM with MA on renal function was examined. Rats were housed in stainless steel cages with urine collected at 24hr intervals. MA (150 mg/kg, s.c.) had no effect on all parameters of renal function tested. Male rats administered DCM (300 mg/kg, i.p.) showed a significant decrease in lactate-stimulated p-aminohippurate uptake at 24hr which was unaffected with coadministration of MA. However, tetraethylammonium uptake, though unaltered in the male rat, showed a potentiated decrease in the female rat exposed to MA + DCM. Moreover, potentiated increases in urine glucose and blood urea nitrogen were also observed in the female rat but not the male. These results suggest that MA enhances DCM-mediated renal toxicity in the female rat. (Supported by USAFOSRF49620-B6-C-0096)

Water purification generates many chlorinated compounds one of which is DCMA. Exposure to this compound is likely to occur in combination with other drinking water pollutants, some of which are hepatotoxic. This study was designed to address the interactions of a known hepatotoxin (CCl₄) with DCMA on liver and kidney function in the Sprague-Dawley rat. Animals were housed in stainless steel metabolism cages in which urine was collected at 24hr intervals. Blood samples were analyzed for the parameters of renal and hepatic function. DCMA (200-400 mg/kg, i.p.) caused modest dose-dependent increases in GPT and GOT. Animals receiving CCl₄ (1 ml/kg, i.p.) showed significant increases in GPT and GOT, which were antagonized approximately 30% with coadministration of DCMA. DCMA caused moderate increases in blood urea nitrogen as well as glutathione depletion in the liver but not the kidney. Both effects were antagonized by CCl₄. Renal alinear experiments indicated that DCMA depressed p-aminophthpirurate uptake, while having no effect on tetraethylammonium. DCMA + CCl₄ produced only an uptake. These results suggest a common component in the hepatic metabolism of each compound. (Supported by USAFOSRF49620-86-C-0096)


Established endothelial (FBHE, CPAE) and renal epithelial (PK1, MDCK, MK2, PK13) cell lines are being examined for a sensitivity to CS to study the vascular and nephro toxic potential of CS in vitro. Both cell types display suggestive morphological alterations after CS treatment: multiple vesicles, fat inclusions, enlarged or degenerated mitochondria as observed from clinical biopsies. CS inhibited renal epithelial (6-12 U/ml) and endothelial (12-25 U/ml) cell growth and viability as assessed by 3H-thymidine incorporation, neutral red uptake and MTT. Higher concentrations of CS, a less nephro toxic CS, were required to produce similar effects. Additional parameters examined to evaluate specie differences and a direct organ specific toxicity of CS include changes in the activities of gamma-glutamyltransferase, N-acetylglucosaminidase, and alkaline phosphatase. The renal epithelial cell lines demonstrate little CS metabolism. Only the CPAE line extensively metabolizes CS. The extent of CS uptake may affect cell line sensitivity. The cell lines are being ranked according to specificity to CS.


Renal arteriosclerosis in man is one of the adverse effects caused by CSA. Endothelial cell-platelet cocultures were established to study the effect of CS on the basal adherence of platelets to endothelial cells. Following a 24 hr pre-treatment of endothelial monolayers (FBHE, CPAE, CBAE) at 6.25, 12.5 and 25 U/ml a concentration dependent increase in basal platelet adherence was observed. No effect or a slight inhibition of platelet adherence was observed following pretreatment with CSH, a less nephro toxic CS. A medium change prior to the addition of platelets resulted in a decreased platelet adhesion except for the high dose CSA preexposed monolayers. Both CSA and H caused decreased levels of endothelial cellular 6-keto-PGFlα, thrombocyan B2 and factor VIII-related antigen. Agonist induced platelet aggregation was enhanced by CSA in a concentration dependent manner. An increased platelet adherence and aggregability by CSA could lead to disturbed capillary microcirculation, microinfarction and fibrotic changes.

RELATIVE IMPORTANCE OF GLUTATHIONE PEROXIDASE AND CATALASE FOR PREVENTION OF PEROXIDATION OF GLOBULIN S and T W Simons, Toxicology Program and Biological Sciences, St. John's University, NY.

The relative in vivo importance of catalase (CAT) and glutathione peroxidase (GSH-Px), for protection against peroxidation was assessed in the rat heart. These enzymes were modulated by feeding animals a low selenium (Se) diet either unsupplemented or supplemented with 0.5 ppm Se, with or without the CAT inhibitor, 3-amino-1,2,4-triazole (3AT), in their drinking water. After 8 weeks, Se-deficient rats exhibited reductions of 88% in cytosolic and mitochondrial GSH-Px activities. These reductions were associated with increased peroxidation in heart homogenates and in CS microsomal suspensions. Since increased peroxidation only occurred when both the cytosolic and mitochondrial GSH-Px activities were compromised, these selenoenzymes appear to work in tandem and reductions in both are a prerequisite for peroxidative injury. Peroxidation did not occur in 3AT-treated rats even though cytosolic CAT activity was inhibited by 65% to 80%. In the Se-deficient rats treated with 3AT, cytosolic GSH-Px activity was elevated by 250%, while mitochondrial GSH-Px activity was unchanged. This differential effect of 3AT suggests that the two selenoenzymes are regulated independently. Because increased peroxidation was only associated with reductions in GSH-Px activity irrespective of CAT activity, the selenium appears to be more important for detoxification of H₂O₂ in the heart.
ENERGY-DEPENDENT ENZYME RELEASE FROM HYPOXIC HEART TISSUE PERFUSED WITH CALCIUM-FREE MEDIUM. Y Park and J P Kehr. Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.

There is massive ultrastructural damage and enzyme release upon reoxygenation of isolated-perfused heart tissue following 1 hr of hypoxia. This damage can be prevented if the heart is infused with inhibitors or uncouplers of oxidative phosphorylation. Similar damage and enzyme release is obtained without reoxygenation when calcium is removed from the perfusion medium after the same period of hypoxia. We evaluated the potential role of energy in this calcium-free effect. Rat hearts were perfused at 37° by the method of Langendorff with Krebs-Henseleit medium containing 10 mM glucose and 2.5 mM CaCl₂. Hearts were equilibrated for 30 min followed by 90 min of hypoxia. CaCl₂ was removed from the medium after 50 minutes of hypoxia. This resulted in the release of 208 units/g tissue dry wt of lactate dehydrogenase (LDH) from 85-95 minutes of perfusion. Control hearts perfused with calcium released 6 units during this time. ATP and creatine phosphate (PCr) contents were measured by HPLC with uv detection in freeze-clamped heart tissue. Immediately before hypoxia, ATP and PCr levels were 23 ± 1 and 38 ± 4 mumol/g protein, respectively. After 50 min of hypoxia, the levels were decreased to 2.5 and 4.8. These levels increased to 7.4 and 26 after perfusion for 5 min with calcium-free medium, increases which were comparable to those seen upon reoxygenation (7.7 and 31). Increases in ATP and PCr upon perfusion with calcium-free medium were prevented by infusion of 10 mM 2-deoxyglucose (replacing glucose) or 0.5 mM dinitrophenol. These results suggest that energy, from both glycolysis and oxidative phosphorylation in hypoxic (not anoxic) heart tissue, is required for the lysis of cells damaged during hypoxia. (JPK is the recipient of Research Career Development Award HL 01435.)

CARDOVASCULAR STUDIES ON THE SAFETY OF PHENILPROPIOLANOLINE (PPA) IN DOGS. L R Weisg and J A Vick, Biotox Assoc., Wheaton, MD, and FDA, Drug Biolog Divv., Washington, DC

Extensive investigations previously reported by us showed that at 25-50 mg/kg, p.o. doses PPA stimulates the cardiovascular system (CVS) in rats (a non-vagal species) and has appetite inhibiting actions comparable to its congener, ephedrine (EPH) rather than any dextran sulfate-lower (DAM)-like properties. In the dog (a vagal inhibitory animal similar to humans), PPA lacks appetite depressing effects, has intense actions on sympathetic tone, and causes no signs of central adrenergic excitation as does DAM. PPA, in doses approximating human usage (1-3 mg/kg, p.o.) caused CVS changes indicating significant adverse actions on this target system and model species. Using anesthetized and un-anesthetized dogs, PPA was compared with EPH and DAM on appetite, adrenergic tone, arterial pressures, and heart rates. With surgically monitors, PPA increases systolic and diastolic pressures with a pronounced bradycardia -ia at 3-25 mg/kg doses, p.o. Similar responses were produced in unanesthetized tail-cuff monitor -ed dogs on pressure and heart rates. The longer the dose, the longer the duration, persisting for 4-6 hrs. Tachyphylaxis possibly occurs on repeated doses within this period but is absent at 24 hrs. DAM, but not EPH inhibits food intake and causes excitation and both cause CVS changes like PPA. These data suggest that PPA is like EPH and both lack the food inhibiting action of DAM.

RETENTION OF CALCIUM (Cd) IN RODENT, CANINE AND PRIMATE LUNG: IMPLICATIONS FOR EXTRAPOLATION MODELLING OF CHRONIC EFFECTS. G Oberdörster and C Cox. Environmental Health Sciences Center, University of Rochester, Rochester, NY. Risk extrapolation to humans of pulmonary carcinogenic effects of inhaled Cd found in rats is difficult to perform without knowing pulmonary retention half-times of Cd in both experimental animals and humans. In order to determine these half-times, 30 rats, 5 dogs, and 2 monkeys were exposed to 109CdCl₂ aerosols for 45 min. Pulmonary retention of 109Cd was measured non-invasively up to 650 days after inhalation by extrathoracic counting with a collimated detector system focussed on the lung. Fitting the data by exponential curves gave the following biological half-times for pulmonary retention of inhaled CdCl₂: rats -85 days (mono-exponential); dogs -153 days (mono-exponential plus 12% background); monkeys -835 days (mono-exponential). These large differences between species are reflective of differences in the metabolism of Cd in their lungs, possibly involving metallothionein. The findings have significant implications for predicting pulmonary accumulation rates of inhaled Cd in different species using lung dosimetry models. More generally, the results indicate that knowledge of species specific pulmonary retention of inhaled chemicals is required to extrapolate results of chronic animal inhalation studies to humans.

PHYSIOLOGICALLY-BASED TOXICOGENIC MODEL OF THE GROWING RAT SKELETON. E J O'Flaherty. Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

Physiologically-based toxicokinetic models for bone-seeking elements such as lead, which persist in the body for extended periods, must take into account not only the duration of exposure but also the metabolic state of the bone during exposure. The model of the growing rat skeleton presented here is a composite of rapidly-exchanging surface bone, slowly-exchanging diffuse distribution into deeper bone, and focal deposition of new bone. This last process is especially prominent during growth to maturity. The model is based on measurements of bone and skeletal volume during growth, and on three assessments also based on published observations. The assumptions are that the amount of calcium in the body as a function of age determines the shape of the skeletal density curve, that mineralization during maturation proceeds by replacement of water with an equal volume of mineral having three times the density of water, and that regional bone blood flow is proportional to bone accretion rate. The model accounts for skeletal growth and for the rapid exchangeability of a fraction of bone mineral. The diffuse distribution is capable of accounting for the power-function characteristics of slow loss of tracers, and of lead, from bone. (Supported by USPHS Grant ES04125.)
AN EXPLANATION FOR SEX AND STRAIN DIFFERENCES IN RENAL UPTAKE OF METHYLMERCURY IN MOUSE. M. Iwura, T. Tanaka, K. Kobayashi and A. Naganuma. Dept. of Public Health, Sch. of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo, Japan

We have already suggested that mercuric compounds are carried in blood stream to the kidney as mercury-glutathione (Hg-GSH) complex and incorporated into the kidney by a γ-glutamyltranspeptidase (γ-GTP) dependent system. In the present study the contributions of tissue GSH level and renal γ-GTP to the sex and strain differences in the renal Hg accumulation in mice were investigated using five strain of 4 week-old mice. Renal accumulation of Hg at 1 hr after CH.HgCl (1µmol/kg, s.c.) injection significantly correlated with hepatic and plasma GSH levels, and particularly with renal γ-GTP activity of each strain. Renal γ-GTP activity in males, which was about 2 fold higher than that in females, fell to the level of females by castration, but recovered by testosterone administration to normal level of male mouse. The concomitant changes in renal Hg accumulation by the hormonal treatment were observed. These results suggest that the strain and sex differences in renal accumulation of Hg may result from the discrepancy in renal γ-GTP level among the strains used, which is regulated at least partly by sex hormone.

LEAD TOXICITY IN RAT BRAIN SYNAPTOSOMES. M. Boykin, M. Hobson, S. Rajamani, and B. Rajamani. Division of Natural and Applied Sciences, Selma University, Selma, AL. Sponsor: K. P. Rao

Uptake of neurotransmitters in the brain synaptosomes is energy related function. Some metals inhibit ATP synthesis by inhibiting mitochondrial ATPase. The objective of this research is to determine the effects of lead toxicity on several cellular processes involved in energy transduction. Brains of male Sprague Dawley rats were removed immediately after sacrifice and used to prepare synaptosomal fractions by ficoll-sucrose gradient method. Both in vivo and in vitro lead of varying concentrations were used. The uptake of γ-H-Dopamine (3H-DA) and γ-H-Norepinephrine (3H-NE) was determined by filtration method and Na+/K+ ATPase by enzymatic method. Lipid peroxidation in brain synaptosomes was measured by thiobarbituric acid color reaction. Respiration of brain homogenate was measured as oxygen consumed and carbon-dioxide released. Both in vitro and in vivo lead showed parallel, dose-dependent inhibitory effects on H3-DA and H3-NE uptake, and the Na+/K+ and mitochondrial ATPases activities and tissue respiration. In vivo and in vitro lead increased the formation of lipid peroxidation in brain synaptosomes. The results suggest that lead inhibits cellular mechanisms that are energy related.

The different properties of each of the therapeutically important water-soluble dimercapto chelating agents, dimercapto succinic acid (DMSA), 2,3-dimercapto-1-propanesulfonic acid (DMPPS) and N-(2,3-dimercaptopropyl)-phthalamic acid (DMPA) are beginning to emerge. In the rat only DMPA increases biliary excretion of Cd and decomporates kidney Cd, Po, DMPA increases biliary GSH. DMSA does not. DMSA has twice the cupruretic activity of DMSA or DMPA in normal rats. The EDS50 for each of the three dimer captans as Na arsenite antibodies in mice do not differ. For mice the LD50 of DMSA (13.7nmol/kg) is the largest and that of DMPA the smallest (0.82nmol/kg). For humans DMSA is the drug of choice for treating Pb intoxication. When DMSA is given po to humans, a mixed disulfide consisting of one DMSA and two cysteine residues is the major metabolite found in the urine. Less than 2.5% of the administered DMSA is excreted in an unaltered form. For DMPS, urinary acyclic and cyclic polymeric disulfides are found. Structures of DMSA metabolites have been determined and for Pb or Cd involve S and O as donors but for Hg or Ni the two S atoms are donors. (Supported in part by ES03356 and OH02185).

ACUTE DEPLETION OF PULMONARY LAVAGE CELLS, INHIBITION OF 5'-NUCLEOTIDASE ACTIVITY, AND ENHANCED LIPID PEROXIDATION IN ALVEOLAR MACROPHAGES OF RATS FOLLOWING PARENTERAL INJECTION OF NICKEL CHLORIDE. P W Sunderman Jr, LL An, O Zaharia, SHY Wong, and SM Hopfer, University of Connecticut Med. School, Farmington, CT.

Previous studies in our laboratory demonstrated pulmonary histopathology and enhanced lipid peroxidation in lungs of rats following parenteral administration of nickel compounds. In the present study, administration (sc) of NiCl2 to male F-344 rats (a) greatly increased Ni concentrations in pulmonary alveolar macrophages (PAM) (at dosages > 62 µmol/kg; 4-72 h post-injection), (b) inhibited 5'-nucleotidase activity in PAM's (at dosages > 62 µmol/kg, 24-48 h), (c) enhanced lipid peroxidation in PAM's (at dosages > 125 µmol/kg, 48-72 h), and (d) diminished the yield of PAM's in lavage fluid (at dosages > 375 µmol/kg, 48-72 h). This study identifies PAM's as a cellular target of acute toxicity following parenteral administration of NiCl2 to rats. Moreover, 5'-nucleotidase activity is inhibited before lipid peroxidation becomes evident in PAM's of NiCl2-treated rats.


The toxicity of nickel has been evaluated in a number of studies. However, most of these studies have shortcomings, which makes the risk assessment of nickel difficult. Hence, a subchronic and a multiple-dose acute intraperitoneal studies were conducted. In the subchronic study, NiCl2 (0.5, 3.5 and 100 mg/kg/day) was administered by gavage to male and female (30/sex/group) CD rats 7 days a week for 90 days. At the highest dose, there was 100% mortality. Body weight and food consumption were significantly lower for the two higher dose groups. Adverse clinical effects were seen in the high dose group; at the mid-dose group these signs were less severe. In the 2-generation reproduction study in rats, NiCl2 was administered in drinking water to both sexes of CD rats (30/sex/group) at dose levels of 0.50, 2.50 and 5.00 ppm (0.7, 3.0 and 15.6 mg/kg/day) for 90 days prior to breeding and throughout the breeding period. At the highest dose, there was a significant decrease in the P0 maternal weight and in the absolute and relative liver weights. The number of live pups/litter and the average pup weight were significantly reduced. The pup mortality was significantly increased at the high dose for all the litters in the two generations. No adverse effects were observed at the two lower doses.

REVERSIBLE CYTOSKELLETAL INJURY INDUCED BY Hf(II) COMPOUNDS. J H Chou, Dept. of Microbiology, Boston University School of Medicine, Boston, MA. Sponsor: C T Walsh.

To understand the mechanism of the carcinogenic effect of Hf(II) compounds, we have studied the Ni2+-induced cytoskeletal injury. Exposure of 3T3 cells to NiCl2 or NiSO4 results in time and dose-dependent dramatic perturbations to the organization of microtubules (MT) and microfilaments (MF), two major components of the cytoskeleton, as exemplified by severe aggregation of MT forming large bundles in the cell center or along the long cellular axis. In contrast, the cell periphery, which is largely deficient in MT distribution, is loaded with fine MF often appearing in patchy bundles. Thus, Ni2+ induces MT and MF to redistribute to mutually exclusive regions of the cell. Upon removal of Ni2+, MF distribution is restored to the cell center at 2-4 h, whereas large MT bundles still remain in many cells after 8 h. Exposure of 3T3 cells to Ni2+ in a salt/glucose medium (SGM) of Abrachol et al resulted in a very different pattern of cytoskeletal injury characterized by the loss of MF in many cells. In addition, MT aggregation and bundling occur predominantly at the periphery, especially at the tips of processes. Upon removal of Ni2+, heavy and often patchy bundles of fine MF reappear in the cytoplasm within 2 h. These results suggest that cytoskeletal damage may be important in the Ni2+-induced cellular injury and toxicity development.
676 INHIBITORY EFFECT OF ION ON THE CARCINOGENICITY OF NICKEL SUBSULFIDE IN F344/NCR RATS.
K S Kasprzak, Laboratory of Comparative Carcinogenesis, National Cancer Institute, FCRP, Frederick, MD.

This study was undertaken to determine the effect of iron on nickel carcinogenesis since these two metals usually accompany each other in the environment. Male F344/Ncr rats, 60 - 100 g, 20 rats/group, received single i.m. injections of 2.5 mg (30 μmol Ni) nickel sulfide (NiS2) alone, or combined with different molar proportions of metallic iron (FeO) or ferric sulfate (FeIII) and were observed for 1 yr. Local administration of FeO or Fe(III) up to the Fe/Ni molar ratio of 2.0 inhibited the carcinogenicity of NiS2 in a dose-related manner. Final incidence of local sarcomas decreased from 90% for NiS2 alone to 50% at FeO/Ni = 0.5, 30% at FeO/Ni = 1.0, and 5% at FeO/Ni = 2.0, or to 80% at Fe(III)/Ni = 0.5, 35% at Fe(III)/Ni = 1.0, and 25% at Fe(III)/Ni = 2.0. Im injection of NiS2 accompanied by a distant sc injection of FeO or Fe(III) at FeO/Ni = 2.0 did not inhibit the carcinogenicity of NiS2. FeO and Fe(III) alone, or the injection vehicle, water, did not produce any tumors. Thus, iron appears to be a very effective local inhibitor of nickel carcinogenesis. This concurs with relatively low incidence of sarcomas observed at sites of internal prostheses made of nickel-iron alloys in man and animals compared to the incidence of tumors produced by other derivatives of nickel.

679 INDUCTION OF MUTATION AND ANCHORAGE INDEPENDENCE IN HUMAN FIBROBLASTS BY CHROMIUM(VI) AND CHROMIUM(III) COMPOUNDS.
K Biedermann and J R Landolph, Univ. of Southern Calif, School of Medicine, Los Angeles, CA.

We previously showed that carcinogenic metal salts induce anchorage independence (AI) in diploid human fibroblasts (HFC)(Cancer Res 47:3643). To elucidate the role of the valence state of chromium in the mechanism of induction of AI in Cr(III) and Cr(VI) compounds, we tested the ability to induce AI in HFC. Cr(VI) compounds (PbCrO4, CaCrO4, Na2CrO4, and CrO3; average µM 50 = 0.5 uM) were generally 1000-fold more cytotoxic to HFC than Cr(III) compounds (Cr2O3, CrCl3, Cr2S3; LD50 = 500 uM). Cellular uptake of CrO3 as Na2CrO4 was 100-fold greater than CrCl3. Using sera that support a high plating efficiency of HFC, found that both Cr(VI) and Cr(III) compounds induced mutations to the 6-thioguanine locus in HFC at equitoxic concentrations. Additionally, chromium in both the +6 and +3 valence states induced equivalent frequencies of AI in human cells (100-200x10^-6); interestingly, CrCl3 induced AI at 500-fold lower concentrations than those that induced cytotoxicity or mutagenicity. Cr(III) and Cr(VI) induced colonies isolated from soft agar stably retained their anchorage independent phenotype after several passages in culture. These data indicate that both Cr(VI) and insoluble Cr(III) compounds can produce genotoxic effects in human fibroblasts. With Cr(VI) AI and mutagenicity appear dissociable, but with Cr(III), induction of AI and mutagenicity occur over the same concentration ranges and may be linked.

678 INTEGRATION OF PHARMACOKINETICS AND GENOTOXICITY DAMAGE TO ASSESSMENT OF NICKEL EXPOSURE RISKS.

Most cancer risk estimates ignore the pharmacokinetics of the chemical in the body while most in vitro genotoxicity assays ignore the relative sensitivity of cells from different species. To test the applicability of combining a mathematical model of the pharmacokinetics of a toxicant with the genotoxic and cytotoxic effects of the chemical, a physiologically based pharmacokinetic (PB-PK) model of the removal and translocation of Ni2+ from the lung to the organs and subsequent excretion from the body was developed. The kinetics of Ni2+ uptake were determined in the cultured cells used for genotoxicity to estimate the intracellular Ni2+ concentration causing DNA damage and cytotoxicity. Human lung cells (A549) exhibited non-linear uptake while rat cells (12) had essentially linear uptake up to 24 h over the concentration range of 0 to 11 µM Ni2+. Cytotoxicity of Ni2+ was comparable for human and rat lung cells when expressed as the intracellular Ni2+ concentration (IC50 of 330 and 815 µM, respectively). Single strand DNA breaks were found and quantitated based on an equivalent ionizing radiation dose. The PB-PK model was used to estimate the rad-equivalent damage produced by occupational exposure, cigarette smoking, and urban aerosol inhalation. (Supported by NIEH Grants ES07031, CA14236 and RR01693 and the ISRI Risk Institute.)

679 EFFECTS OF Cu(II)(3,5-DIPS)2 ON SOLID EHRlich CELL TUMOR IN MICE.
L W Chung, D Torregrosa, S L Kasemeier, and J R J Sorenson, Univ. of Arkansas for Medical Sciences, Little Rock, AR.

Cu(II)(3,5-disopropyl salicylate), or CuDIPS has been shown to suppress tumor growth when injected directly into solid Ehrlich cell tumor in mice. Our present investigation was designed to study the systemic effects of CuDIPS on solid Ehrlich cell tumors. The overall histopathological change, and alterations in the metastatic patterns were noted. Solid tumors were induced by injecting Ehrlich ascite cells into the thigh muscle of female C57BL/6J mice. Animals were injected subcutaneously on their backs once or twice daily with 25 mg/kg b.w. of CuDIPS for 76 days. Control animals, either tumor bearing or non-tumor bearing, were injected similarly with either vehicle or CuDIPS respectively. A significant reduction in tumor size was observed in animals treated with higher doses of CuDIPS. Histopathological examination revealed that CuDIPS treated tumors were less necrotic, with a reduction of renal metastasis and high mitotic arrest at metaphase. Although CuDIPS treated animals showed higher pulmonary metastatic incidences, high doses of CuDIPS treatment had less pulmonary metastasis than those of lower doses. Besides an induction of Kupffer cells proliferation, no toxic damage was found in the liver by CuDIPS. Data from our present study suggest that CuDIPS, while suppressing primary tumor growth, alters metastatic characteristics of their metastasis.
Disrupted gap junctional intercellular communication may result in toxic, carcinogenic, and teratogenic responses. It is hypothesized that various chemicals will modulate gap junction function in cells from tissues or organs sensitive to these same chemicals. Several different cell types were used to assess intercellular communication after treatment with modulators of gap junction function which act by changing levels of cAMP, Ca++, or H+ ions; generating free radicals; or activating protein kinase C. Also, cell sensitivity to several toxicants was examined (PBB, Dieldrin, and a tobacco smoke condensate). Intercellular communication was measured using FRAP analysis and a scrape-loading/dye transfer assay. Results indicate that factors such as pH, cAMP, protein kinase C, etc., modulate gap junction function to varying degrees in different cells. Therefore, the different factors exhibiting greatest effect on a particular cell type may be critical in controlling cell to cell communication in a tissue comprised of these cells. Some toxicants inhibited intercellular communication only in some cell types while other toxicants affected all cell types. Determining cell types sensitive to altered intercellular communication by toxic agents may identify potential mechanisms underlying toxicity. (Supported by R.J. Reynolds/Nabisco).

Tumor promoters appear to have large action thresholds and to be non-additive in their effects. Many promoters also inhibit gap-junctional communication between cells, which is a proposed mechanism of tumor promotion. The concentrations of chemicals required to inhibit gap junctional communication vary greatly, suggesting that such chemicals block communication through different pathways. Interactions between such inhibitors could be additive, synergistic, or antagonistic. This hypothesis was tested by evaluating the effects of phorbol myristate acetate (ng/ml), aldrin (ug/ml) and cyclohexylamine (mg/ml) on communication between co-cultured 6-thioguanine resistant and 6-thioguanine sensitive V79 cells. Combinations of these chemicals were tested at effective and ineffective concentrations. Additive effects were obtained with most combinations and concentrations. Synergistic effects were observed with mixtures of phorbol myristate acetate and aldrin tested at effective concentrations. Assuming inhibition of gap-junctional communication is a mechanism for tumor promotion, these results suggest that mixtures of promoters inducing inhibition of communication through different pathways will act additively to exceed action thresholds.

The role of barbiturate metabolism in the inhibition of intercellular communication between cultured hepatocytes C W Meghors and J E Klaunig. Dep. of Pathology, Medical College of Ohio, Toledo, OH

A number of barbiturates have been shown to be liver tumor promoters in the rat and mouse. The mechanisms of tumor promotion whether or not metabolic activation is required for promotion have not been clearly defined. One potential mechanism may involve the inhibition of intercellular communication (IC) via gap junctions. The isolation of initiated cells from cytotoxic growth regulators may potentiate proliferation of this preneoplastic cell type. This study evaluated the effects of barbiturates on IC in cultured hepatocytes and what influence an inhibitor of barbiturate metabolism (SKF 525-A) has on these effects. Cultured hepatocytes (24 h) from male Fisher 344 rats were exposed to either 1) phenobarbital (PB), sodium barbital (BB), amobarbital (AB) or barbituric acid (BA) for 1 and 4 h or 2) SKF 525-A for 1 h. Subsequent to 1 and 4 h treatments with either PB, BB, AB or BA, IC was assessed by the passage of the fluorescent dye lucifer yellow from microinjected “donor” cells into adjacent “recipient” cells (dye coupling). PB and BB significantly decreased IC while AB and BA had no effect. SKF 525-A prevented the inhibition of IC by PB; however it had no effect on the IC inhibited by BB. These data support long term in vivo studies that have demonstrated PB and BB to be liver tumor promoters in the rat and mouse. Our findings also suggest that the metabolism of PB by P-450 is necessary for tumor promotion.

Inhibition of intercellular communication in preneoplastic rat hepatocytes induced by the Solt-Farber model. S G Lilly and J E Klaunig. Dept. of Pathology, Medical College of Ohio, Toledo, OH.

Tumor promoters have been shown to decrease gap junction-related intercellular communication (IC) in rodent hepatocytes. Our hypothesis is that tumor promoters inhibit IC in initiated, preneoplastic cells, thus isolating these cells from intercellular growth control, and allowing for proliferation and eventual neoplastic transformation. In the present study preneoplastic hepatocytes from male Wistar rats, previously initiated with diethylnitrosamine (DENH) via the Solt-Farber protocol were isolated and cultured. Cultured hepatocytes (after 24 h) were examined for IC ionophoretic microinjection of Lucifer Yellow (dye coupling). The effect of phenobarbital (PB) treatment on IC was studied in hepatocytes from male Wistar rats treated with DENA only, DENA and carbon tetrachloride hepatocytic (CCL4), DENA and 2-acetylaminofluorene (2AAF), CCL4 and 2AAF, CCL4 only, 2AAF only, or no treatment were compared to hepatocytes from rats receiving the complete Solt-Farber protocol. Prenoneoplastic hepatocytes from rats treated with the Solt-Farber protocol displayed decreased coupling with and without PB treatment compared to hepatocytes from untreated rats and rats receiving an incomplete Solt-Farber protocol. These results suggest that preneoplastic hepatocytes may have greater susceptibility to inhibition of intercellular communication by tumor promoters.
The Effect of Several Inhibitors of Skin Tumor Promotion on Protein Kinase C Activity in Vitro.
C.L. Crawford and R.C. Smart. Toxicology Program, North Carolina State University, Raleigh, NC.

Various dietary constituents that are known inhibitors of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced epidermal tumor promotion were examined for their ability to inhibit protein kinase C (PKC) activity in vitro. PKC is a major cellular receptor for TPA and is thought to play a critical role in skin tumor promotion. TPA stimulated PKC activity was measured in the 10^5g supernatant fraction of CD-1 mouse epidermis and brain using l-glutamic acid histone as a phosphate acceptor substrate and the maximum velocities were 2 and 25 nmol P[gamma-32P]histone/mg protein/5 min respectively. These values were similar whether the enzyme was stimulated with 100 nM TPA or 100 uM sn-1,2-dioctanoylglycerol (1-Oa). The addition of ascorbic acid (AA), ascorbyl palmitate (AP), t-retinoic acid (RA), alpha-tocopherol (TOC), quercetin (QU) or curcumin (CC) at concentrations of 83, 250 and 750 uM were examined for their effects on TPA or 1-Oa stimulated PKC activity. AP, QU, and CC inhibited TPA or 1-Oa stimulated epidermal and brain PKC activity while the addition of AA, RA or TOC did not inhibit TPA or 1-Oa stimulated PKC activity. These results indicate that the mechanism of inhibition of tumor promotion by AP, QU and CC may involve PKC.


Thirty chemicals, most of which had been tested previously for carcinogenicity in the rodent bioassay and genetic toxicity in Salmonella (Tennent et al. Science, 236 933, 1987), were assayed in the V79 metabolic cooperation (MC) system. Of the 18 carcinogens tested, 8 inhibited MC and 10 did not. However, 5 of 8 chemicals for which there was no evidence of carcinogenicity were positive in the MC assay. Chemicals which were equivocal in either the bioassay or MC assay accounted for the remainder of responses. Of 12 carcinogens which were Salmonella negative, only 6 were positive in the MC assay. Therefore, the number of noncarcinogens detected as positive in the MC assay combined with the detection of only half of the nongenotoxic carcinogens, make it doubtful that the current V79 MC assay will supplement Salmonella in identifying rodent carcinogens.


DCA and partial heptectomy (PH) promote 7-glutamyltransferase-positive (GTT') foci in the livers of rats initiated with diethylnitrosamine (DEN; Gann, 72, p.635, 1981). The present study was conducted to further evaluate DCA promotion. Four groups of 15 male F344 rats were as follows: 1) control, 2) DEN (200 mg/kg, i.p.), 3) DCA diet (9.3%), 4) DEN + DCA diet. DCA diet was started 2 weeks after DEN initiation. Five rats/group were killed after 1, 7, and 13 weeks of DCA feeding. All animals had osmotic minipumps containing 5-H-thymidine implanted 1 wk prior to sacrifice. Sections of liver were prepared for autoradiographic analysis after histochemical staining for GTT'. DEN + DCA resulted in a 5-fold increase in the number of GTT' foci after 7 and 13 weeks compared to DEN alone. Foci in Gp. 4 were 7x larger than in Gp. 2 after 7 weeks of DCA feeding and grew significantly larger after 13 weeks of DCA feeding. Foci in Gp. 2 did not increase in size. In Gp. 4 rats, foci were ~4x more numerous (no cm3) in the right lobe than the left. No foci were detected in animals receiving the DCA diet alone. DEN + DCA significantly increased cell proliferation after 1 wk of feeding (49% compared to 31% in groups receiving DEN or DCA alone). This study demonstrated that DCA-promoted foci were induced without the use of PH, were variable between liver lobes and were associated with an early DCA enhancement of cell proliferation.


Phenobarbital (PB) and barbital (BB) promote hepatocarcinogenesis in the rat, and BB promotes the formation of kidney tumors. We have examined the effects of the decarboxylated hydrolysis products of PB and BB, ethylphenylacetlylurea (EPAU) and diethylacetlylurea (EEAU) on tumor formation in the liver and kidney in DEN-initiated male F344 rats (75 mg/kg; once ip). PB, at 500 ppm in the diet, significantly increased the incidence and multiplicity of DEN-initiated hepatocellular neoplasms. As equimolar doses, EPAU and EEAU increased the incidence of hepatocellular adenomas, but had no effect on either incidence or multiplicity of hepatocellular carcinomas. EEAU, but not PB or EEAU, increased the incidence and multiplicity of renal adenomas and carcinomas. PB, and to a lesser extent, EPAU, caused significant liver weight increases and induction of hepatic cytochrome P450-mediated activities. EEAU was less effective. Thus, the ring hydrolysis products of PB and BB do not contribute significantly to the liver tumor promoting ability of the parent compounds, but EEAU may be responsible in part for the kidney tumor promoting activity of BB.
sn-1,2-Diacylglycerol (DAG) have been demonstrated to mimic several of the biochemical effects of the skin tumor promoter TPA on mouse skin. These effects are mediated through protein kinase C which is a cellular receptor for TPA and DAG (the endogenous ligand). In order to determine if diC_{10}, a short chain DAG, can mimic the morphological changes induced by TPA on mouse skin, CD-1 mice were treated topically with one of the following in acetone: 1 nmol TPA, 2 nmol TPA, 5 nmol TPA, 2.5 μmol diC_{10}, 10 μmol diC_{10} or acetone alone. The effect of a single application or multiple applications (2x/week for 4 weeks) were examined. Eighteen hours after the last topical application, dorsal skin was excised and fixed for microscopy. Single and multiple applications of TPA produced epidermal hyperplasia in a dose related manner. However, neither single or multiple application of diC_{10} produced epidermal hyperplasia. In addition, the epidermis from diC_{10} treated mice was indistinguishable from vehicle treated mice. These data indicate that under these conditions diC_{10} does not mimic the morphological changes induced by the skin tumor promoter TPA.

Exposure to ozone is associated with inflammation and the development of fibrotic lung diseases. Release of IL-1 by macrophages plays a role in the regulation of inflammatory and fibrotic reactions. In the present studies we determined if ozone alters AM production of IL-1. AM were isolated from female SD rats 0, 24 and 48 hr following exposure to ozone (3 ppm, 3 hr). IL-1 activity was quantified in culture supernatants of lipopolysaccharide stimulated AM using a thymocyte proliferation assay. Immediately following ozone exposure, there was a 50-60% decrease in IL-1 production by AM, when compared to control cells. However, AM isolated 24 and 48 hr after ozone exposure exhibited a 50% and 100% increase in IL-1 production, respectively. Using flow cytometry, the increase in IL-1 activity was found to be associated with an influx of small, dense mononuclear cells into the lungs that produced elevated levels of H_{2}O_{2}. These studies suggest that inflammatory mediators may play a role in ozone induced lung injury.

**MORPHOLOGICAL CHANGES INDUCED BY 12-O-TETRAYLPHORBOL-13-ACETATE (TPA) AND SN-1,2-DIACYLGLYCEROL (DIC_{10}) ON CD-1 MOUSE SKIN IN VIVO. N A Montelaro-Riviere and R C Smart. Toxicology Program and School Vet. Med., North Carolina State University, Raleigh, NC.**

**ALTERATIONS IN RAT ALVEOLAR MACROPHAGE (AM) PRODUCTION OF INTERLEUKIN-1 (IL-1) FOLLOWING INHALATION OF OZONE. M A Amoroso, D L Laskin, J B Liesch, J Joselevitz-Goldman, B D Goldstein and F.M. Robertson. UMDNJ-RW Johnson Medical School and Rutgers Univ., Piscataway, NJ.**

**TUMOR-PROMOTING ACTIVITY OF FURNACE OIL FRACTIONS IN CD-1 MICE. W D Johnson, N S Hatoun, S L Schmitt, T M Warne, J K Yermakov, and P J Garvin. III Research Institute and Amoco Corporation, Chicago, IL.**

Furnace oil, a complex mixture of petroleum hydrocarbons, was previously shown to be carcinogenic in lifetime dermal tumorigenesis studies and a tumor promoter, but not an initiator, in initiation/promotion (I/P) bioassays. In order to define the active component(s) which acted as tumor promoters, furnace oil was separated into low-boiling, aromatic, iso/cyclo paraffin and n-paraffin fractions and tested in an I/P bioassay to evaluate their promotional activity. Groups of 30 male mice each received a single dermal application of either 50 μl of acetone (5 groups) or 30 μl of a 1 mg/ml solution of DMBA in acetone (6 groups). The initiated mice were rested for 2 weeks and then treated dermally with 30 μl of furnace oil or the appropriate fraction twice weekly for 25 weeks. One group initiated with DMBA served as a sham control and received no promoter. Application site skin masses occurred at incidences of 14/30, 2/30, 3/30, 19/30 and 22/30 in the DMBA-initiated groups promoted with furnace oil, n-paraffin, iso/cyclo paraffin, low-boiling and aromatic fractions respectively. No masses were present in any of the acetone-initiated or sham control groups. The mass incidence in the furnace oil and aromatic and low-boiling fraction groups was significantly increased compared to their respective control groups. Results indicate that the low-boiling and aromatic fractions are responsible for the tumor-promoting activity of furnace oil.

**THE EFFECTS OF IN VIVO SILICA AND TITANIUM DIOXIDE (TiO_{2}) EXPOSURE ON INTERLEUKIN-1 (IL-1) AND TUMOR NECROSIS FACTOR α (TNFα) SECRETION BY RAT ALVEOLAR MACROPHAGES (AM). K E Driscoll, R C Lindenschmidt, J Higgins, and M Perkins. Procter & Gamble, Cincinnati, OH.**

The effect of silica and TiO_{2} on the release of IL-1 and TNF-α by rat AM was investigated. F344 rats were instilled with saline (control) or 5 mg/100g body weight of silica or TiO_{2}. Groups of 17 rats/treatment were killed on day 7 and 28 days post exposure, their lungs lavaged, and the AM cultured at 10^{5}/ml for 24 hr. Secreted IL-1 was analyzed using the thymocyte proliferation assay, and TNFα activity was determined by its cytotoxic action on L-929 fibroblasts. Silica exposure significantly increased IL-1 release on day 7 (150X control) and day 28 (33X control). Exposure to TiO_{2} stimulated IL-1 secretion, however, the TiO_{2} response (day 17:27X control; day 28:16X control) was significantly less than that observed with silica. A significant increase in AM TNFα secretion was detected after exposure to both dusts. Silica treatment resulted in a 2.5 fold stimulation of TNFα secretion on day 7 and both silica and TiO_{2} exposure resulted in ~3.5 fold increases in TNFα secretion on day 28. These results indicate that: 1) high doses of silica or TiO_{2} stimulate AM to release IL-1 and, to a lesser extent, TNFα; and 2) differences exist between the two dusts in the degree and/or pattern of this response.
SECRETION OF HYDROGEN PEROXIDE (H₂O₂), INTERLEUKIN 1 (IL-1) AND TUMOR NECROSIS FACTOR (TNF) BY RAT ALVEOLAR MACROPHAGES FOLLOWING ASBESTOS EXPOSURE. M M Fort, R K Kumar, R Bennett, A R Brody, M L Lustiger and G J Rosenthal. NIEHS/NH, Research Triangle Park, NC

In addition to its carcinogenic and fibrotic potential, asbestos exposure has also been associated with altered immune function. Alveolar macrophage (AM) dysfunction may account, in part, for some of the pathogenicity of asbestos. We examined the role of asbestos in rat AM activation and have monitored three secretory capacities of these cells, IL-1, TNF and H₂O₂. AM exposed to asbestos in vitro exhibited increased activities of the enzyme leucine aminopeptidase (44.4 vs 29.9 nmol/mg protein/min) and acid phosphatase (5,919 vs 1,695 nmol/mg protein/min), both markers of activation. Increased AM activation was expressed functionally by an increased capacity to secrete H₂O₂ following a brief exposure (6 hr) to asbestos. Increased secretion of H₂O₂ was observed with a pharmacologic stimulus (PMA, 22.8 vs 7.6 nM H₂O₂/mg prot.) as well as a phenotypic stimulus (zymosan: 339 vs 287 nM H₂O₂/mg prot.). Primed AM appeared to be less sensitive to asbestos in this regard than resident or responsive AM. In vitro addition of asbestos to AM also resulted in increased basal and LPS induced TNF secretion. Following a single 5 hr inhalation exposure of asbestos, AM lavaged from animals 2 and 7 days post exposure demonstrated increased TNF and H₂O₂ production. Significantly, increased IL-1 secretion was also observed in AM from rats lavaged 7 days post asbestos exposure. These studies demonstrate that asbestos exposure alters the functional status of rat AM, evidenced by increased activation and production of potential inflammatory mediators.

OZONE-INDUCED PROLIFERATION OF ALVEOLAR MACROPHAGES. J A Hotchkiss, S R Harkema, and R F Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

This study was designed to assess the in vivo, post-exposure, effects of acute ozone exposure on the proliferation and morphology of pulmonary alveolar macrophages (PAM). Rats Inhalation 0.0, 0.1, 0.8, and 3.0 ppm O₃ for 1 h. PAM were killed 0, 3, 16, 42, or 66 hr after exposure, and their lungs were lavaged. PAM recovered by lavage were pulse-labeled, in vitro, with ³H-Thy to determine their labeling index (LI). Exposure to 0.1 ppm had no measurable effect on total number, LI, or morphology of PAM. There were increased numbers of neutrophils 3, 18, and 42 hr after exposure to 1.5 ppm and 42 hr after exposure to 0.8 ppm. The number of PAM was -2x that of controls 42 and 66 hr after exposure to 0.8 and 1.5 ppm. PAM LI was greater than that of controls 18 and 42 hr after exposure but similar to that of controls 66 hr after exposure. There was a transient increase in the mean cytoplasmic area of PAM from rats exposed to 1.5 ppm 18 and 42 hr after exposure. Comparisons of PAM doubling time and cell cycle time suggest that proliferation of macrophages within alveoli contributed significantly to the increase in PAM. PAM proliferation may be a useful indicator of pulmonary damage following inhalation of an irritant oxidant. (Research sponsored by NIH Grant ES04282 and by the U.S. DOE/ORDER under Contract No. DE-AC04-76EV01013.)

FIBROGENIC AND PROLIFERATIVE RESPONSE TO ASBESTOS INHALATION IN LUNG PARENCHYMA OF NORMAL AND COPPER-DEFICIENT (C57BL/6J) MICE. P D McGeever, C Butcher, L H Overby and A R Brody. NIEHS, R & NC and UMC. Sponsor: G H Lucier.

Asbestos inhalation in mice induces complement activation and macrophage (PAM) accumulation which we believe may be required for subsequent epithelial cell hyperplasia and development of the interstitial (IS) lesion observed at alveolar bifurcations. We correlated the increased PAM response in C5- mice with measurements of cell proliferation and the progression of an IS lesion using autoradiography and morphometry of lung tissue from normal (C5+) mice and C5- congeners exposed to chrysotile asbestos. Bronchial and type I/II EP cells of both C5+ and C5- mice showed significant increases in ³H-TdR incorporation 19 to 48 hrs after exposure. The response returned to control levels of less than 1% labeling by one month. C5+ mice developed a fibrotic lesion one month postexposure. However, tissue vol in C5- mice was not different from C5+ controls. The failure of asbestos to cause a lesion in C5- mice may be due to the decreased numbers of PAMs responding. We also observed ³H-TdR incorporation by epithelial smooth muscle and IS cells of the small vasculature in asbestos exposed mice. No labeling of vascular cells was seen in sham-exposed animals. C5- mice provide a useful model for the development of a model of PAMs in asbestos-induced fibrogenesis, angiogenesis and EP cell proliferation. (PDM ST32ES07126)
Laboratory animals exposed to ozone (O₃) exhibit an increased susceptibility to pulmonary bacterial infection which has been associated with decreased oxidant generation by alveolar macrophages (AMs). On the other hand, O₃ exposure can also result in an influx of polymorphonuclear leukocytes (PMNs) into the lung. In this study, we have characterized the oxidant-generating capability of cells lavaged from rats 24 hrs after a 4 hr exposure to 2 ppm O₃ utilizing luminol-amplified chemiluminescence (LACL) and superoxide anion generation. Exposure to O₃ increased the percentage of PMNs in the lavage from 0.4 to 32 which was accompanied by a 70-fold increase in myeloperoxidase activity in the lavaged cells. Additionally, a 10-fold increase in peroxidase-dependent LACL was noted in the cells lavaged from O₃-exposed rats upon addition of either TPA or opsonized zymosan. Superoxide anion generation, however, was suppressed by approximately 50% in the cells lavaged from O₃-exposed rats which is attributed to a decrease in the activity of AMs. These results demonstrate that PMNs recruited to O₃-exposed lungs are capable of generating oxidants which could further contribute to lung injury or to the metabolism of inhaled xenobiotics. (Supported by ES 03760 and 03505, HL 34674 and Amer. Cancer Soc. SIG-3).

Exposure to glass fibers (GF) by intratracheal injection (IT) induces a marked acute inflammatory response which resolves with time. Mechanisms responsible for this resolution compared to mechanisms responsible for a progressive response, as seen with silica (SI) or asbestos (AF) exposure, were investigated in mice exposed once or multiple times. Mice (8 wk C57Bl/6) were exposed by (IT) to either 3 doses (0.25 mg/mouse) at 5 day intervals or 1 dose (0.75 mg/mouse) of either GF, AF, or SI. At 5 days post final exposure, animals were killed and the lungs lavaged with 1 ml saline for analysis of the total number of inflammatory cells and the different cell types. Multiple injections of GF did not increase the total number of inflammatory cells whereas multiple injections of either AF or SI elicited a single injection of GF caused a marked influx of eosinophils into the alveolar space which was enhanced by multiple exposures. Exposure to AF caused a moderate influx of eosinophils whereas SI elicited no eosinophilic response. Exposure to either of the 3 particles also caused a significant increase in both neutrophils and macrophages. The presence of eosinophils may play a role in determining the outcome of a particle-induced inflammatory response. (NIH HL33754)


Changes in lymphoid cells of the lung and mediastinal lymph nodes during the mouse's response to 0.7 ppm ozone were compared at 4 and 14 days of exposure. Using immunofluorescence staining of the lung we determined that T-cells continuously infiltrate the lesions and that a time as many as present on day 14 as on day 4. In contrast, very few B lymphocytes were seen at either time point. Cell numbers increased in mediastinal lymph nodes at both time points. Counts of immune cell subpopulations showed that the decrease in T-suppressor cell percentage first seen at day 4 changed so that by day 14 all subpopulations were present in proportions comparable to control values. This data supplements previous observations which together demonstrates that changes occur in the lung and lymphoid cells in the following temporal sequence up to day 14 of ozone exposure: initiation of lung damage and beginning of lung wet weight increase; proliferation of lymphoid lymphocytes; beginning of T-cell infiltration into the lung and stabilization of lung wet weight; cessation of excess cell proliferation in the node; continuing infiltration of cells into the lung; and restabilization of subpopulation percentages in the node. Current Address: Parke-Davis Pharmaceutical Research, Ann Arbor, MI.

Injury to isolated rat lungs perfused with phorbol myristate acetate (PMA) and neutrophils is attenuated by pretreatment of either lung cells or neutrophils with aspirin. L.J. Carpenter and R.A. Roth. Dept. of Pharmacol./Toxicol., Ctr. for Environ. Toxicol., Michigan State Univ., E. Lansing, MI.

Results from previous studies indicate that injury in isolated rat lungs perfused with buffer containing PMA and neutrophils (PMNs) is dependent on the production of the eicosanoid TxA₂. The purpose of this study was to determine whether the lung or the PMN was the source of TxA₂ required to produce lung injury in this model. TxA₂ synthesis by either rat lungs or PMNs was inhibited selectively by pretreatment of either rats or isolated PMNs with aspirin (100 mg/kg p.o., or 100 μM, respectively). Untreated lungs perfused with PMA and untreated PMNs exhibited increases in weight, lavage fluid albumin and TxA₂ synthesis with respect to lungs perfused only with PMA. In contrast, increases in these markers did not occur in untreated lungs perfused with PMA and aspirin-pretreated PMNs or in aspirin-pretreated lungs perfused with PMA and untreated PMNs. These results suggest that TxA₂ is produced both by PMNs and by lung cells in this preparation, and that TxA₂ production by both of these sources is required for the manifestation of edema. (Supported by NIH grants HL32244 and ES04139.)
The development of silica-induced fibrosis involves secretion of reactive compounds by AM. This study characterized the activation of rat AM by silica and tests the effectiveness of tetrandrine, a Chinese anti-fibrotic drug, in preventing silica-induced activation of AM. In vitro exposure of rat AM to silica increases O2 consumption by 2.7 fold at 1.8 mg/ml silica, enhances O2 release by 2.6 fold at 2 mg/ml silica, activates H2O2 secretion by 30 fold at 1 mg/ml silica, and stimulates chemiluminescence by 12 fold at 0.5 mg/ml silica. Activation of AM declines above these silica levels as cytotoxicity begins. In vitro treatment of AM with tetrandrine (70 µg/ml) does not alter resting activity of AM but does inhibit particle-induced activation of AM; i.e., 95% inhibition of silica-induced O2 consumption (ID50=18 µg/ml), 87% inhibition of zymosan-induced O2 release (ID50=24 µg/ml), 90% inhibition of zymosan-induced H2O2 release (ID50=18 µg/ml), and 87% inhibition of zymosan-induced O2 release (ID50=8 µg/ml). Oral administration of tetrandrine (7.5 mg/day for 30 days) following intratracheal instillation of silica (40 mg/rat) also prevented the silica-induced increase in zymosan-stimulated H2O2 secretion by AM. Therefore, silica activates alveolar macrophages while tetrandrine prevents this activation.

Modification of bovine phase I and phase II metabolism by trans-stilbene oxide.

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Trans-stilbene oxide (TSO) is a proven inducer of hepatic phase II metabolism in rats. The present experiments were undertaken as part of a study of xenobiotic metabolism in various food animal species. Since there are few if any guidelines for the dosage of an experimental drug such as TSO in cattle, a preliminary experiment was done to evaluate the toxicity of TSO in this species. Individual cattle were given 2, 20, 200, and 400 mg/kg TSO in corn oil ip. for 4 days. There were no significant changes in temperature, pulse, respiration, plasma urea nitrogen or alanine amino transferase, but at the two highest doses, there was transient depression and anorexia. In the second experiment five mature, lactating, grade, Holstein cattle were given 50 mg/kg TSO ip. qid. in a corn oil vehicle. This regimen was repeated four consecutive days followed by three days without treatment for 6 wk. Hepatic cytochrome P-450, glutathione S-transferase, and UDP-glucuronyl transferase activities were determined and compared with activity in hepatic biopsies taken before TSO and with activity in control animals. There was no significant change in UDP-glucuronyltransferase activity, however, P-450 and glutathione-S-transferase activities were decreased with respect to controls.


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Recent work in this laboratory indicated that BE causes acute hemolytic anemia and activation of BE to butoxyacetaldehyde (BAL) intermediate, is a pre-requisite for development of hemotoxicity. In the current studies we have compared the effects of BE, BAL, and BAL on rat blood in vitro. Incubation of BE with blood from F344 male rats caused no hemolysis of RBCs and no metabolic alteration of BE. In contrast, incubation with BAL or BAA caused time- and concentration-dependent swelling of RBCs followed by hemolysis, however, BAA was significantly more efficacious than BAL. Investigation of the mechanisms of this effect revealed that BAA and BAL caused a decrease in blood ATP concentration with BAA being significantly more potent than BAL; BE has no effect on blood ATP concentration. Assessment of the human risk by incubation of human blood with BAA showed minimal swelling or hemolysis of RBCs with minimal decline in blood ATP at BAA concentrations several folds higher than required to cause 100% hemolysis of rat RBCs. In summary, the current studies indicate that, a) the hemolytic effect of BE in vivo can be attributed primarily to its metabolite BAA; b) hemolysis of rat RBCs by BAA or BAL is preceded by swelling and ATP depletion, c) humans are comparatively insensitive to the hemolytic effects of BAA in vitro.

Effect of microsomal enzyme inducers on the in vitro glucuronidation of thyroxine.
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At least three distinct forms of UDP-glucuronosyltransferase (UDP-GT) are known to exist. These isozymes demonstrate different substrate specificities and induction profiles in agents such as phenobarbital (PB), polyaromatic hydrocarbons (PAH) and pregnenolone-16α-carbonitrile (PCN). The hormone thyroxine (T4) is glucuronidated by UDP-GT in liver and excreted into bile. Administration of microsomal enzyme inducers has been shown to decrease circulating T4 levels possibly by enhancing its glucuronidation and biliary excretion. The purpose of this study was to determine which microsomal enzyme inducers increase T4 glucuronidation. Liver microsomes were prepared from male, Sprague-Dawley rats (200-225 grams) pretreated ip with PB (75 mg/kg), isosafrole (ISF; 150 mg/kg), 3α-naphthoflavone (BNF; 100 mg/kg), PCN (100 mg/kg), daily for four days, or tetrachlorodibenzo-p-dioxin (TCDD; 10 µg/kg), once 10 days prior to sample collection. Microsomal UDP-GT activity toward 3α-T4 was determined by measuring the appearance of T4-glucuronide from T4 by thin-layer chromatography and quantitated using gamma-scanitillation spectrometry. Our results indicate that PAH-type inducers (TCDD, BNF) significantly increase T4 glucuronidation which parallel the increase in microsomal UDP-GT activity toward α-naphthol. PCN pretreatment also significantly increased UDP-GT activity of PAH-type inducers (PB, ISF), however, did not increase T4 glucuronidation. In conclusion, PAH- and PCN-type inducers of UDP-GT increased the glucuronidation of T4 while PB-type inducers did not. (Supported by USPHS Grants ES-03192 and ES-07079)
PARATHION AND EPN ARE ACTIVATED TO THEIR OXON ANALOGS, PARAoxON AND EPN-OXON, BY MICROSOMAL MONOOXYGENASES. THIS ACTIVATION PRODUCES A POTENT ANTICholinesterase DECREASING THE I50 MORE THAN 600 FOLD. PHOSPHOROCHINONATE POISONING RESULTS FROM RESPIRATORY PARALYSIS DUE TO A FAILURE OF THE RESPIRATORY CONTROL CENTER IN THE BRAIN. MICROSONES FROM MALE AND FEMALE RAT BRAIN REGIONS (CEREBRAL CORTEX, CORPUS STRIATUM, CEREBELLUM, AND MEDULLA/pons) AND LIVER WERE INCUBATED WITH THE PHOSPHOROCHINONATE AND AN NADPH-GENERATING SYSTEM, AND OXON PRODUCTION WAS QUANTIFIED BY THE AMOUNT OF INHIBITION TO AN EXOGENOUS SOURCE OF ACETYLCOLINESTERASE. LIVER DEMONSTRATED MORE THAN 25 TIMES MORE ACTIVITY THAN BRAIN FOR BOTH PARATHION AND EPN. THE MITOCHONDRIAL FRACTION OF BRAIN, AS ISOLATED BY DIFFERENTIAL CENTRIFUGATION, POSSESSED AN ACTIVATION ACTIVITY SIMILAR IN LEVEL TO THE MICROSOMAL FRACTION. WHOLE HOMOGENATES WERE TESTED FOR THEIR ABILITY TO DEGRADE PARATHION AND EPN-OXON, QUANTIFIED BY p-NITROPHENOL PRODUCTION. LIVER, BUT NOT BRAIN, DEGRADED A greater AMOUNT OF OXON THAN BRAIN, OVERALL THE BRAIN AND LIVER DEGRADED AN AVERAGE OF 16 TIMES MORE OXON THAN THEY ACTIVATED. Thus THE BRAIN POSSESSED BOTH ACTIVATION AND DEGRADATION ABILITY, WHICH MAY BE OF SIGNIFICANCE DURING INTOXICATION.

METABOLISM OF 3,4-METHYLEDIENEDIOXYMETHANEMPHETAMINE (MDMA) BY RAT LIVER MICROSOMES. R Gollanudi, M Lopez, J Leakey, P Webb and W Slikker, Jr. U. Tenn., Memphis, TN and NCTR, Jefferson, AR.

Recent data suggest that 1 wk after sc administration, S(+)MDMA is more neurotoxic than its R(-) enantiomer in the male rat. To determine if steric factors play a role in the biotransformation of MDMA, rat liver microsomes were incubated with S(+) or R(-) MDMA (285 nmoles) and a NADPH-generating system. The reaction was terminated with NaHCO3 and the EtOAc extracts were assayed by HPLC (C18, gradient elution with 5:1 MeOH:phosphate buffer, pH 3.0, 0.001 M, 0.1 M). The only metabolite peak identified was that of the N-desmethyl compound NDA. MDA formed (nmoles/mg protein/hr) from S(+) and R(-)MDMA respectively were (n=3-8) untreated female - 2.41, 1.04; untreated male - 1.46, 1.62; phenobarbital pretreated (PB) female - 2.08, 2.34, PB male - 2.32, 2.35; 3-methylcholanthrene pretreated (3MC) female - 1.62, 0.85; 3MC male - 1.01, 0.76. Based on the differential distribution of cytochrome P-450 isozymes, it appears that the enantioselectivity in the N-demethylation of S(+)MDMA is associated with the female (f), but not the male (h) specific isozymes. The results suggest that the PB-inducible (+f) forms lack stereospecificity and the 3-MC inducible (+d) forms are less active in MDMA N-demethylation. The neurotoxic potencies of S(+) and R(-) MDMA in the male rat do not appear to be related to their N-demethylation.

METABOLISM OF ORGANONITRILES AND CYANIDE BY RAT NASAL TISSUE ENZYMES. A R Dahl and B A Waruszewski, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Organonitriles include many compounds of sufficient volatility for substantial vapor inhalation. Most are highly water-soluble and thus, their inhaled vapors are absorbed to a large extent in the nasal mucosa. Liver enzymes metabolize organonitriles to intermediates that release cyanide. Liver also contains Rhodanese which metabolizes cyanide to thiocyanate. Because of the potential for exposure of nasal tissues to organonitriles and hydrogen cyanide, we compared rat liver tissue cyanide-releasing organonitrile monooxygenase and Rhodanese activities to the same activities in the nose. For aceto-, propio-, butyro-, isobutyro-, acrylo-, benzyl-, and succinonitriles the nasal tissue activity was greater than that of liver on a per mg protein basis. The olfactory tissue generally had more activity than the liver or the nasal respiratory microsomes. Rhodanese activity in the olfactory mitochondria was 3-fold higher than for liver mitochondria, whereas nasal respiratory tissue Rhodanese activity was over twice that of liver. The total nasal Rhodanese activity exceeded 10% of the total liver activity. These data suggest that the nasal cavity is an important depot for metabolism of inhaled organonitriles and cyanide. (Research sponsored by the U.S. DOE Office of Health and Environmental Research under Contract No. DE-AC04-76EV-01013.)

THE OXIDATION OF PHORATE BY HEPATIC, RENAL AND PULMONARY MICROSONES FROM MICE FOLLOWING IN-VIVO TREATMENT WITH XENOBIOTICS. S Kinsler, P E Levi, and E Hodgeson, Toxicology Program, North Carolina State University, Raleigh, NC.

Oxidation of phorate, an organophosphate insecticide, was studied in hepatic, renal, and pulmonary microsomes. Phorate is a substrate for the cytochrome P-450 monooxygenase (P-450) system and the flavin-containing monooxygenase (FMO), with phorate sulfoxide being the major metabolite. In the liver of untreated mice, the percent oxidation by P-450 is greater than by FMO; in renal and pulmonary microsomes, the contribution by FMO is greater than by P-450. Recent work has examined the roles of these two enzymes in the microsomal oxidation of phorate after in-vivo administration of phenobarbital (PB), acetone, and hydrocortisone. In PB treated liver microsomes, the rate of phorate sulfoxide formation increased more than 3 times over control, with P-450 being responsible for greater than 90% of the activity; acetone pre-treatment, however, caused no significant increase in phorate sulfoxidase activity in either liver or kidney microsomes. While no increase in phorate sulfoxidase activity was seen in either lung or liver microsomes from mice pretreated with hydrocortisone, additional oxidation to p. oxon and p. oxon sulfoxone was seen in liver microsomes.
Di (2-ethylhexyl) adipate (DEHA) is a widely used plasticizer in food wrapping. According to previous studies, this compound is a peroxisome proliferator and we have demonstrated recently that 2-ethyl hexanoic acid (EHA) is the most potent compound issued from DEHA in terms of peroxisome proliferation. In-vivo and in-vitro metabolism studies of DEHA and its metabolites were carried out in order to appreciate the importance of the different pathways involved in the instantaneous concentration of 2-EHA in rat liver. After hydrolysis of the two ester bonds, 2-ethyl hexanol (2-EH) is conjugated or rapidly converted in the corresponding acid (2-EHA) by cyt. P-450 non-dependent 2 steps oxidation. Subsequently, 2-EHA is metabolized by cyt. P-450-mediated oxidations and lead to the compounds described previously by Abro (1979). The present experiments, in-vivo and in-vitro, show firstly that 2-EHA itself is a substrate for the induced peroxisomal β-oxidation after a β-oxidation involving cytochrome P-450. Secondly, 2-EH and 2-EHA are subjected to glucuronidation. In-vivo, glucuronidation of 2-EHA appears to be dose- and time-dependent. The formed glucuronide becomes the major metabolite with DEHA dosages upper to 10 g/kg b.w./day. In-vitro, conjugations of 2-EH and 2-EHA have equal velocities during the three first hours of experiment. Such a discrepancy must be correlated with 2-EHA glucuronide level in mice appearing as non-time-dependent. According to these results, 2-EHA phase 2 reaction needs to be investigate more carefully in order to obtain a better knowledge of this pathway probably involved in species differences.

We have investigated gestation-dependent variations in the microsomal-mediated activation of aflatoxin B1. Microsomes were prepared from pregnant Sprague-Dawley rats on days 10 and 20 of gestation, and the enzymatic competence of each microsomal isolate was monitored via incubation of microsomes and DNA with AFBI in an NADPH-mediated activation system. AFBI activation on days 10 and 20 of gestation were comparable, and microsomes prepared on both days evidenced greater AFBI microsomal activation than either male and female controls. Microsomes prepared on days 10 and 20 of gestation activated AFBI to greater levels as compared to liver microsomes from male controls by 18-20% and from female controls by 20-22%. These findings support previous in vivo studies documenting gestation-induced increase in AFBI activation in the rat and strongly suggesting the pregnant rat to be preferentially sensitive to the toxic and tumorigenic effects of AFBI.

Cytochrome P-450 is important in metabolism of chloromethanes and chloroethanes. The purpose of this study was to examine the role of cytochrome P-450 in metabolism of chloropropanes (CP). Metabolism rates of CP were determined in microsomal incubations by measuring production of chloride ion (Cl) with a Cl-selective electrode. Liver microsomes were prepared from phenobarbital-induced male F344 rats (180-195 g) using 50 mM HEPES/15 mM K2SO4. Incubation mixtures prepared with these microsomes (1.0 mg/mL) contained 0.15 ± 0.006 mM Cl (mean ± SE). Incubations under air and nitrogen were conducted with the following CP (Numbers indicate Cl positions; DCP, TCP; di-, trichloropropane): 1CP, 2CP, 12DCP, 13DCP, 22DCP, and 123TCP. Nonenzymatic Cl production was less than 0.01 mM/min for all CP, except 22DCP (0.14 mM/min). Under air, Cl production rate (less nonenzymatic Cl production) of 2CP was 0.0057 mM/min. Rates relative to 2CP (=1) were: 1CP, 1.3; 12DCP, 2.5; 13DCP, 1.2; 22DCP, 2.9; 123TCP, 2.0. Under nitrogen, only 13DCP (0.010) and 22DCP (0.17) yielded more than 0.003 mM Cl/min. General conclusions include 1) CP with chlorine on a secondary carbon are metabolized faster than CP with chlorine on a primary carbon, and 2) reductive metabolism is important for some lower chlorinated CP. (Supported by NIH ES03911.)

ACN, a rat carcinogen (brain, stomach, Ysambat's gland), is metabolized to a reactive epoxide (ANO) by rat liver microsomes and hepatocytes. This study examined ANO formation in lung and liver by colorimetric analysis of ANO derivatized with 4-(p-nitrobenzyl)pyridine. In F-344 rat liver microsomes, the rate of metabolism (Vmax) was 2.4 nmol ANO/min/mg 1CP. In rat lung microsomes, ANO formation deviated from linearity at 5 min when activity peaked at 30 nmol ANO/min/mg 1CP. The content of liver and lung microsomes was not altered by ACN. In incubations with [2,3-3H]-ACN (2.1 mCi/mmol, 3.2 μCi), radio-labeled material was irreversibly bound to lung and liver microsomes (0.9 and 1.8 nmol ACN equivalents/mg protein, respectively). The binding was independent of NADPH, suggesting that oxidative metabolism was not necessary for binding. ANO formation was also identified in lung cell fractions enriched for Clara, alveolar type II, and endothelial cells, as well as a suspension of all cell types. Activity in sonicated cell preparations was in the range of 30 to 100 nmol ANO/min/mg 1CP. Inhalation is a major route of exposure to ACN. Therefore, epoxide formation in lung may have important implications for the understanding of ACN metabolism and toxicity.
ENZYMATIC BASIS OF THE ACTIVATION OF 3,3'-DICHLOROBENZIDINE BY LIVER MICROSOMES TO MUTAGENS AND LIPID-BINDING DERIVATIVES. M. W. Iba, B. Lang, and P. E. Thomas, Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, and Roche Institute of Molecular Biology, Nutley, NJ.

3,3'-Dichlorobenzidine (DCB), a precursor of industrial dyes and pigments and a carcinogen in several animal species, is activated by liver microsomes to intermediates that (I) are mutagenic to Salmonella tester strains in the Ames test and (II) bind to polyunsaturated fatty acids. The present studies were undertaken to determine and compare the enzymic basis of (I) and (II), using specific antibodies against liver microsomal cytochromes P-450 (P-450) and chemical inhibitors of P-450 and liver microsomal flavin-containing monooxygenase (FMO). A polyclonal antibody against P-450d (anti-P-450d(4c)) inhibited I and II 65% and 70%, respectively, whereas a monoclonal antibody against P-450c or P-450b had no effect on either I or II. Methaemalbumin and 2-naphthylthiourea, both inhibitors of FMO, inhibited I but had no effect on II. The results suggest that more than one type of reactive intermediate may mediate the mutagenicity by DCB, one contributed by P-450d and another by the FMO; however, only the products formed by P-450d may bind to polyunsaturated fatty acids. (Supported by N.I.H. No. NS-0812459)

THE METABOLISM OF CHLOROBENZENE, THE DICHLOROBENZENES AND BIPHENYL BY RAT AND HUMAN LIVER SLICES IN DYNAMIC ORGAN CULTURE. A. J. Wein, J. Bar, K. Brandel, and I. Spies, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.

The use of a novel liver slice technique has allowed us to determine the metabolism of environmental pollutants using rat, and more importantly, human tissue. We have determined that chlorobenzene, ortho-dichlorobenzene, para-dichlorobenzene and biphenyl, all at a concentration of 0.5mM, are biotransformed by this system. Metabolism has been shown to proceed in a time-dependent manner for up to six hours, as assessed by aqueous soluble metabolite production. All of these substrates are relatively poorly metabolised (<5%), although the results indicate that these rates of metabolism are greater than those observed using other in vitro techniques. Using rat liver slices, no sex differences could be observed using all five substrates. Additionally, the versatility of this method allows us to vary incubation conditions such that metabolism can be increased.

Both rat and human liver slices metabolise these substrates to similar extents, although these compounds distribute into human liver slices to a greater extent than rat liver slices. In all cases, the majority of metabolites were found in the incubation medium.

Overall, this liver slice technique may offer an alternative to existing in vitro cellular methods for the assessment of hepatic drug metabolism. (Supported by N.I.H. No. NS-08135512).

COMPARATIVE METABOLISM OF 4-VINYLCYCLOHEXENE (VCH) IN FEMALE RAT AND MOUSE HEPATIC MICROSONES. B. E. Smith and I. G. Spies, University of Arizona, Department of Pharmacology, Tucson, AZ.

Studies performed by the National Toxicology Program have shown an increased incidence of ovarian tumors in B6C3F1 mice but not Fischer 344 rats chronically administered VCH. Our hypothesis is that a species difference in the metabolism of VCH may provide a partial explanation for the differences observed in ovarian tumor formation. Therefore, we measured the rate of metabolism of VCH (1mM) to VCH-1,2-epoxide in hepatic microsomes. The rate of microsomal epoxidation of VCH was 7 fold higher (p<0.002) in mice (10.6±0.7 nmol/min/mg protein) compared to rats (1.6±0.1 nmol/min/mg protein). The difference in epoxidation rates was not due to a species difference in microsomal cytochrome P-450 content or microsomal hydrolysis of the epoxide since the addition of the epoxide hydrolase inhibitor trichloropropene oxide (2mM) did not alter the amount of epoxide formed. In separate experiments the rate of hydrolysis of VCH-1,2-epoxide (1mM) to VCH-1,2-diol was determined. VCH-1,2-epoxide hydrolysis was more rapid (p<0.003) in rat compared to mouse microsomes with rates of 1.9±2 and 8±1 nmol/min/mg protein, respectively. Epoxide hydrolysis was not detected in hepatic cytosol of either species. We conclude that the microsomal epoxidation of 4-VCH occurs at a faster rate in the mouse and the epoxide is hydrolyzed slower when compared to the rat. This finding does not prove the above stated hypothesis, however, it does provide clues for further investigations. (Supported by NIEHS No. ES-5-5031)

METABOLISM OF 4-ISOPROPYLBIPHENYL IN PRECISION-CUT LIVER SLICES. J. M. Eirriot, K. Brandel, and D. E. Carter, Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

The hydrocarbon 4-isopropylbiphenyl (IPB) has been proposed for use as a replacement for polychlorinated biphenyls in electrical equipment. It has been reported to have low potential toxicity. An in vitro procedure has been developed to examine the routes of metabolism of IPB in rat liver. Precision-cut liver slices obtained from phenobarbitone-induced or control rats were incubated with substrates and homogenates of the tissue slices with methylene chloride/methanol, metabolites were separated by HPLC. Statistically significant differences in tissue potassium levels were obtained between the induced and non-induced groups. The phenobarbitone-induced animals metabolized IPB at a faster rate and the major metabolites were acids. These results demonstrated that this system may be used to study the hepatic metabolism of IPB in vitro. (Supported by Electric Power Research Institute.)
Vitrification is a novel procedure which allows for the transfer of aqueous media to low temperatures (-196°C) without the formation of ice crystals. It is therefore attractive for the cryopreservation of biological systems. Rat, pig and human precision cut liver slices have been vitrified in the presence of a physiological buffer and 35% 1,2-propanediol. Viability of such slices as measured by their ability to maintain intracellular K+ is retained. The ability to biotransform 7-ethoxycoumarin to the hydroxy derivative and the glucuronidation and sulfation of the latter substrate were assessed in liver slices. Biotransformation was measured in fresh, exposed to the cryoprotectant solution and vitrified (thawed) slices. While metabolism in vitrified (thawed) rat slices was severely depressed, significant retention (65%) of metabolic activity was observed both in human and pig tissue. Also, phase I and phase II reactions remained coupled. These results indicate that vitrification of porcine liver slices may serve as a suitable model for attempts to establish a tissue bank for human liver tissue. (NIEH NOIES 56112)

**THE METABOLISM OF N-NITROSOTHIAZOLIDINE BY ISOLATED RAT HEPATOCYTES.** D. Cragin and T. Shibamoto. Department of Environmental Toxicology, University of California at Davis, Davis, CA. Sponsor: A. Buckport

Hydroxylation of the alpha carbon of nitrosamines may be important in the formation of genotoxic metabolites. Hydroxythiazolidine has been recovered from the urine of rats given N-nitrosothiazolidine (NTHZ). The sequence of steps in the formation of hydroxythiazolidine is not known and may be important in determining whether the compound is bioactivated (hydroxylation prior to denitrosation) or detoxified (denitrosation first). In order to determine the intermediary steps in the metabolism of NTHZ, the metabolism of the compound by isolated rat hepatocytes was studied. Metabolites were isolated by an ammonium carbonate - ethyl acetate extraction procedure and analyzed by GC-MS. Two metabolites were tentatively identified - hydroxy-nitrosothiazolidine and thiazoline. However, because of high background these could not be conclusively identified. If the finding of hydroxyinirosothiazolidine proves to be correct, this would be an indication that bioactivation of the NTHZ occurs.

This work was supported in part by a training grant from the NIEHS (5-T32 ES007059).


The metabolism of CAF was evaluated in isolated hepatocytes and S9 to determine the suitability of these preparations for comparative metabolism studies. Hepatocytes were isolated from young adult, male Sprague-Dawley rats and liver specimens from human organ transplant donors by biphasic perfusion with collagenase. Incubations with CAF (10 μM) were conducted with heptocyte suspension (up to 4 hr) and monolayer cultures (up to 60 hr) in hormone-supplemented Waymouth's 752/1 medium and S9 with an NADPH-generating system and appropriate cofactors. At most 6% metabolism occurred in either hepatocyte system regardless of the species. With both human hepatocytes and S9, paraxanthine (1,7-dimethylxanthine) was the principal metabolite, in agreement with in vivo data. With rat hepatocytes significant quantities of 1,3,7-dimethylxanthine and 1,3,7-trimethyluric acid were also formed, as reported in vivo and in the perfused rat liver. In contrast, rat liver S9 produced principally the latter. Known urinary uracil metabolites in both man and rat were not clearly identified in preliminary studies. (Sponsored by NIEHS Contract ES-55109.)


WR 6026, 8-(6-diethylaminohexyl-amino)-6-methoxy-4-methyl quinoline dihydrochloride, is a candidate antileishmanial drug currently in Phase I clinical trials. It has demonstrated in vivo efficacy in the hamster (H), dog (D), and rhesus monkey (M) but active metabolite(s) have not been identified. Metabolic profiles of 14C-WR6026 were therefore studied in isolated hepatocytes from H, D, and M using HPLC with radiochemical detection. Metabolites of WR 6026 have been tentatively identified with authentic standards by HPLC analysis. The predominant metabolite of WR 6026 in the H were the 4-hydroxyxyl (4-CH2OH) and desethyl (DES) derivatives with less active formation of the desethyl (DIDES), carboxylic acid (COOH), and N-oxide (NOX) derivatives. Similarly, D hepatocytes formed the DES, NOX, COOH, and DIDES derivatives. The 8-amino (8-NH2) derivative was also found in smaller quantities. The major metabolites in M hepatocytes were the DES and 4-CH2OH derivatives. Minor metabolites formed in the M cells were the Dides and 8-NH2 derivatives as well as an unidentified, low polar metabolite. One conjugated metabolite, the 6-hydroxy sulfate derivative, was also identified in the monkey hepatocytes. Thus, both qualitative and quantitative differences have been observed between all three species.
Oxidation of phosphorothioates like parathion by mixed-function oxidases rapidly slows down in vitro due to the suicidal reaction yielding reactive metabolite(s). Typically half the initial activity is lost in 1 to 5 min. In contrast, little inactivation is observed in vivo at sublethal doses. To better simulate in vivo biotransformation using cell-free systems or isolated hepatocyte suspensions, "partitioning" of parathion between the medium and the enzyme (or indirectly the enzyme within the cell) was altered by buffering the available free substrate molecules with autologous blood or bovine serum albumin (BSA). With the autologous blood as the medium, linear reaction time courses over 20 min were obtained when the substrate concentration was $10^{-6}$ M or less. BSA at 7% was nearly as effective in preventing the inactivation in the hepatocytes whereas 5% BSA could be used in cell-free systems. Lower BSA concentrations produced curvilinear time courses in all systems. Analysis of reaction rates indicates that the enzyme inactivation depends upon the metabolic rate itself and that the substrate buffer mitigates the "suicidal effect" by maintaining a continuous, low substrate concentration. While a suicidal reaction such as this accentuates the effect of proper substrate delivery, substrate buffers should also be important in reproducing biotransformation of all lipophilic xenobiotics in vitro as it occurs in vivo. (Supported by NIEHS grant ES01018)

**EVALUATION OF HPLC WITH RADIOMETRIC DETECTION FOR PHARMACOKINETIC STUDIES.**

L E Bates, K T McManus and J D deBethizy, R J. Reynolds Tobacco Co, Winston-Salem, NC.

The Ramona LS-4 radiometric HPLC detector was tested for accuracy for plasma analysis of radiolabeled analytes in pharmacokinetic studies. Adult Sprague-Dawley rats were given an IV dose of 14-C nicotine (2.22 umoles/kg). Plasma samples were collected at selected times up to 24 hours. The LS-4 detector was connected in series with a fraction collector which permitted the collection of radioactivity data using both the LS-4 and the fraction collector on each sample run. The amount of 14-C in the nicotine fractions was determined using liquid scintillation counting. These data were compared with the amount of radioactivity for nicotine generated by integrating the radiochromatograms produced by the LS-4. Recovery of injected total radioactivity from the HPLC system was 82% +/- 8.2. The efficiency of the LS-4 solid flow cell was 69.4% for dose standards. However, the efficiency varied with the amount of 14-C applied to the column below 400 dpn. The minimum level of detection for the solid cell ranged from 150 to 200 dpn. There was good agreement for the IV nicotine pharmacokinetic parameters AUC and Ke between the LS-4 and fraction collection.

**HIGH PERFORMANCE LIQUID RADIOCHROMATOGRAPHIC ASSAY OF LIVER MICROSMAL N,N-DIMETHYLAMINE MONOOXYGENASE ACTIVITY.**

M Chen and M M Iba, Dept. of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.

An HPLC-radiometric assay was developed for the rapid analysis of products of NADPH-dependent microsomal oxidation of N,N-dimethylaniline (DMA) following a 15 min incubation at 37°C, a NADPH-fortified microsomal reaction mixture containing 14-C-DMA as substrate was deproteinized by the addition of methanol followed by centrifugation. The resulting supernatant was chromatographed on a Partisil ODS 3 RAC II column (10 cm x 4.6 mm) with methanol:water (62:38, v/v) as the mobile phase at a flow rate of 0.5 ml/min. Fractions were monitored by both uv absorbance (216 nm) and radioactivity. Three radioactive fractions, A, B, and C, with retention times of 4 min, 6 min, and 11 min, respectively, were identified. A, B, and C were confirmed to contain N,N-dimethylaniline N-oxide (DMANO), N-methylaniline (NMA), and DMA, respectively, by mass spectrometry, colorimetric analysis, and uv-vis spectra. Alkalization of the microsomal reaction mixture followed by extraction with diethyl ether led to > 90% recovery of A in the aqueous fraction (free of detectable levels of B or C), concomitant with increased absorbance of A at 216 nm. These methods afford a rapid and sensitive alternative to the standard colorimetric assay of microsomal DMA N-oxigenase. (Supported by US EPA R-812459)

**MODELING HALOCARBON METABOLISM RATES (VMAX) USING QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSAR).**

*S L Dixon, M L Gargas, and M E Anderson. Wright State University, and AARMS/TH, Wright-Patterson AFB, OH.

Vmax values were obtained in vivo with male rats for chlorinated methanes, ethanes and ethenes by gas uptake methods. A QSAR study of Vmax was done with electronic and steric chemical descriptors. Partial atomic charges served as electronic parameters. Chlorine substitution patterns were used to imply steric information. For 10 well-metabolized chemicals, the best 2-term fit ($r^2=0.981$) involved the sum of all hydrogen charges in the molecule and the difference between the numbers of chlorines and hydrogens on the least substituted carbon. Two poorly metabolized chemicals (CCl4 and CH3CCl3) were added to the data set and a more qualitative approach taken. For methanes and ethanes, metabolism required a chlorine and hydrogen on the same carbon; in methanes with two or more chlorines, the effect of replacing a chlorine with a methyl group is slight and predictable; for ethanes and ethenes, larger Vmax occur for chemicals with two nonequivalent carbons. These structural features were combined with a composite index and quantitated in a 3 parameter fit ($r^2=0.934$).

How a cigarette is smoked can influence the chemistry of the delivered smoke and consequently the yield of smoke components. We tested a panel of 19 smokers on two occasions; once smoking their regular brand of cigarettes and once smoking a new cigarette which heats rather than burns tobacco. Human smoking patterns were measured, and then reproduced on a 10-port programmable smoking machine. The particulate phase of the cigarette smoke was captured for analysis. Averaged smoking parameters across all regular brands: total puffs = 12.5, puff volume = 44.1 ml, puff duration = 1.84 s, puff 'frequency' = 33.2 s. For 5 subjects who all smoked the same cigarette, a full-flavor low-'tar' brand, nicotine (NIC) yields ranged from 0.45 to 1.15 mg, compared to the FTC value of 0.52 mg. Yields of nicotine free dry solids ranged from 4.54 to 11.3 mg, compared to an FTC 'tar' value of 8.50 mg. For the test cigarette, NIC yields were similar to those described above. Precise measurement and replications of smoking behaviors are necessary to determine actual smoke yields. Attempts to infer smoke yields without these measurements could be inaccurate.

NICOTINE YIELDS AND PLASMA CONCENTRATIONS DURING HUMAN SMOKING. J H Robinson, J D deBethizy, R A Davis, D W Griffith, J H Reynolds and A W Hayes. R.J. Reynolds Tobacco, Winston-Salem, NC.

The 'tar' and nicotine (NIC) values that are determined by the Federal Trade Commission (FTC) method do not always reflect the NIC yield of a cigarette or NIC absorbed during human smoking. We have integrated a series of research techniques that enables us to determine NIC yields during human smoking and to apply these techniques to the study of NIC pharmacokinetics. We have also developed a programmable smoking machine that reproduces the measured human puffing patterns, allowing subsequent replication of these patterns and chemical analyses of the smoke generated. We studied 4 males who each smoked 7 commercially available cigarettes. The NIC area under the curve (AUC) ranged from 339 to 941 ng-min/ml, and was positively correlated with both number of puffs and average time of smoke inhalation. AUC was negatively correlated with averaged puff volume and puff intensity. NIC yields ranged from 0.96 to 2.45 mg, compared with an FTC determined value of 0.71 mg. These techniques are essential to the determination of the pharmacokinetic properties of NIC derived from cigarette smoking.

BIOAVAILABILITY OF NICOTINE FROM A NEW CIGARETTE THAT DOES NOT BURN TOBACCO. J D deBethizy, J H Robinson, R A Davis, D W Griffith, J H Reynolds and A W Hayes. R.J. Reynolds Tobacco Co., Winston-Salem, NC.

The bioavailability of nicotine from the smoke aerosol of a new cigarette (test) was compared to a conventional cigarette (reference) in a panel of 12 smokers on 3 occasions using identical smoking conditions. These conditions were: once smoking the reference, once smoking the test, and once smoking the test after smoking only this product for 39 days. On each occasion the subjects were required to smoke one cigarette every 30 min for a total of 7 cigarettes to achieve plasma steady-state levels of nicotine. Blood samples were analyzed for nicotine and cotinine, using GC. Human smoking patterns were measured and then reproduced on a smoking machine to compare nicotine yields with absorbed nicotine. The amount of nicotine absorbed from test cigarettes (0.618 +/- 0.233 mg) was half that absorbed from reference (1.144 +/- 0.533 mg). The amount of nicotine absorbed was proportional to the amount yielded by the cigarette, indicating that the bioavailability of nicotine has not been altered by the design of the new cigarette.


In order to obtain data on the in vivo metabolism and disposition of the three-carbon nitrosamines, which is a crucial step for an understanding of their carcinogenicity, the toxicokinetics of 14C-NMHA was studied in 8-week-old male F-344 rats by HPLC assay of serial 50 uL retro-orbital blood samples. An i.v. bolus dose of 0.6 umol/kg revealed biphasic first order elimination with a terminal half-life of 37.4 +/- 1.7 min (mean +/- SEM, n=4) for NMHA and 101 +/- 6 min for total radioactivity, indicating extensive conversion to polar metabolites. The systemic blood clearance and steady-state volume of distribution for NMHA were 13.1 +/- 0.9 mL/min/kg and 685 +/- 31 mL/kg, respectively. Plasma protein binding of NMHA was found to be negligible, using the equilibrium dialysis method. Doses of 10 umol/kg given by gavage (n=4 rats) indicated a systemic bioavailability for NMHA of 71 +/- 10%. The major NMHA metabolite, occurred extensively by metabolism, the rate of hepatic biotransformation was not sufficient to cause a large first-pass effect. (Supported in part by Contract NO1-CO-23910 to Program Resources, Inc.)
Attempts have been made to calculate lethal doses of cyanides, including inhaled hydrogen cyanide. These have employed constant rates of cyanide detoxification, independent of blood levels. We have investigated the rate of fall of blood cyanide concentrations, after intravenous administration to Beagle bitches. Potassium cyanide (0.82 mg/kg) was injected into the cephalic vein of each of 4 animals. Blood was taken for cyanide analysis by the method of Faedelstein and Klenkhoj 5 min and then quarter-hourly till 90 min. A similar study with a 5 min and subsequent half-hourly analyses for 245 min was also done. The short study showed first order kinetics up to about 80 min (Vd, 0.185-0.220 l/kg; t1/2, 21-25 min; Ke, 0.028-0.033 min⁻¹; range, n=4). The last point in this study, and the semi-logarithmic plot of the longer study, showed that there was a second phase of much slower elimination (t1/2 = 5h).

It was concluded that in acute cyanide poisoning first order kinetics could be assumed, but that such an assumption could not be made over longer periods.

Male F344 rats were treated with TDI by gavage (6 or 60 mg/kg) or by inhalation (head-only) at 0.5 or 2.0 ppm for 4 hr. Excreta, blood, and tissues were sampled during a 96-hr period. 14C appeared in blood rapidly; 1% of the low dose was recovered at 1-2 hr. Recovery after the high dose was limited to 0.5%. Urinary excretion was rapid but limited to a total of 20% of dose in 96 hr; the remainder (80%) was recovered in feces. 14C in faces and GI tract was likely due to biliary excretion of absorbed material. Tissue levels were low at blood, liver, and kidney. After inhalation, blood levels were highest immediately following exposure (4 and 2% of the low and high dose, respectively). Urinary elimination of 14C was slower than after oral dosing, though similar amounts (22%) were recovered. Fecal excretion was also slower than after oral dosing and lower amounts (53%) were recovered. The remainder of dose (23%) was found in the GI and carcass. 14C levels in nasal tissue and lungs were high, while levels in other tissues were similar to those following gavage. Urine contained large amounts of polar products; feces, GI and liver contained more nonpolar components. No significant differences in HPLC profiles due to route of exposure or dose were noted (supported by International Isocyanate Institute, Inc.).

FAL is used to synthesize furfuryl alcohol (FOL). FAL, but not FOL, has undergone chronic toxicity and carcinogenicity studies. The disposition and metabolism of FAL, and in a parallel study FOL, were investigated in rats to help determine whether chronic studies on FOL are necessary.

$^{14}$C-FAL was administered by gavage in corn oil to male Fischer 344 rats at 0.127, 1.15 or 12.5 mg/kg, ca. 0.001, 0.01 and 0.1 of LD50. Radioactivity was determined in urine, feces, expired air (high dose only) and in tissues. Urine at the low and high doses was analyzed by HPLC. At all doses 85% of the dose was excreted in urine, primarily in the first 24 hr, and 22% in feces. At 12.5 mg/kg 7% was exhaled as CO2. These data show FAL was well absorbed. At 72 hr 0.6% or less was found in tissues. Highest concentrations of $^{14}$C were in liver and kidney. Concentrations in tissues were proportional to dose. Furoylglycine was the major urinary metabolite, ca. 76-80% of the dose, and furfurylic acid and fumaramic acid were 1 and 3-4%, respectively. Over the dose range, absorption, extent of metabolism, relative amounts of metabolites and rate of excretion were linear. Also, the disposition and metabolism characteristics of FAL in rats were quantitatively similar to those of FOL.

(Supported by Contract NOI-ES-66138).

PHARMACOKINETICS OF ACRYLAMIDE AFTER MULTIPLE DOSES IN RATS. C E Drager and D E Carter. College of Pharmacy, University of Arizona, Tucson, AZ.

The widely used industrial chemical acrylamide (ACR) produces a cumulative peripheral neuropathy in man and animals. Kinetic studies of the toxic monomer were performed in 5 and 11 week old male Fischer-344 rats (n=9) following multiple exposure. Multiply dosed rats received 50 mg/kg ACR i.p. for 5 consecutive days and $^{14}$C-labeled (13.55 mg/kg, 98 uCi/kg) i.v. on day 6. The rats given the single dose received the radiolabeled dose only. Rats were anesthetized and the femoral vein was cannulated for introduction of the ACR and subsequent removal of blood samples over 4 hours. Blood was extracted with 0.1% TAH in methanol to quantify parent ACR with additional verification by HPLC. After multiple exposures to ACR, the blood levels decrease rapidly from about half the dose at 15 minutes to 10% at 1 hour in both age groups. Later time points exhibit a sustained level of approximately 10% dosed ACR. Single dose studies did not display this trend at later time points, blood levels decreased rapidly. Pharmacokinetic differences in the terminal elimination phase of cumulative and single exposures to ACR may relate to specific tissue accumulation.


SK&F 104353 is a novel leukotriene receptor antagonist currently under development for the treatment of asthma and other leukotriene related diseases. Studies with [14C]-SK&F 104353 in male(M) and Female(F) rats showed that >90% of the administered dose was recovered in the 24 hr excreta after po (100mg/Kg) and iv (25mg/Kg) dosing. Urinary excretion of 14C was 3-4% (iv) and 0.1-0.4% (po) and fecal elimination was in excess of 80%. In bile duct cannulated rats, 70.36±10.11% (M) and 41.25±5.26% (F) of the dose was recovered in bile, 6 hrs after iv administration. In contrast, after po dosing, biliary excretion was only 12.89±3.21% (M) and 7.81±1.37% (F).

Unchanged SK&F 104353 accounted for 10.3±1.5% (M) and 19.0±3.8% (F) of the biliary 14C following iv administration. At least 7 biliary metabolites were present; the phenolic sulfate conjugate was identified as the major metabolite. These studies demonstrated that an oral dose of SK&F 104353 was only partially absorbed and substantial sex differences were noted in the metabolic fate of this compound in rats.

PHARMACOKINETICS OF ACRYLAMIDE AFTER MULTIPLE DOSES IN RATS. C E Drager and D E Carter. College of Pharmacy, University of Arizona, Tucson, AZ.

The widely used industrial chemical acrylamide (ACR) produces a cumulative peripheral neuropathy in man and animals. Kinetic studies of the toxic monomer were performed in 5 and 11 week old male Fischer-344 rats (n=9) following multiple exposure. Multiply dosed rats received 50 mg/kg ACR i.p. for 5 consecutive days and [14C]-labeled (95 mg/kg, 98 uCi/kg) i.v. on day 6. The rats given the single dose received the radiolabeled dose only. Rats were anesthetized and the femoral vein was cannulated for introduction of the ACR and subsequent removal of blood samples over 4 hours. Blood was extracted with 0.1% TAH in methanol to quantify parent ACR with additional verification by HPLC. After multiple exposures to ACR, the blood levels decrease rapidly from about half the dose at 15 minutes to 10% at 1 hour in both age groups. Later time points exhibit a sustained level of approximately 10% dosed ACR. Single dose studies did not display this trend at later time points, blood levels decreased rapidly. Pharmacokinetic differences in the terminal elimination phase of cumulative and single exposures to ACR may relate to specific tissue accumulation.

METABOLIC FATE AND PHARMACOKINETICS (PK) OF SULOTROBAN, 4-(2-PHENYLSULPHONYLAMINODETHYL) PHENOXACETIC ACID (BM 13.177) IN SPRAGUE-DAWLEY RATS. J Kao, R Gagnon, M Carbonare, G Joseph, P Levandoski and G Rhodes. Dept. Drug Metabolism, SK&F Labs, King of Prussia, PA.

Sulotroban(S) is a novel, non-prostanoid thromboxane(1xA) receptor antagonist developed by Boehringer Mannheim GmbH for the treatment of 1xA related cardiovascular and renal diseases. Studies with [14C]-S in male rats (4/group) showed that >75% of the administered dose (100mg/Kg) was found in the 24 hr excreta following po and iv dosing. Only unchanged S was found in the urine and urinary excretion was 68% (iv), 34% (po). Fecal elimination of 14C was 13% (iv), 45% po. S was also the major component in bile, and a taurine conjugate was identified as the major biliary metabolite. Biliary excretion of 14C in 24 hr was 13% of dose for both iv and po routes. PK of S S (100mg/Kg) showed multiphasic elimination with an initial plasma half-life (t1/2) of 6.7±0.5 mins and terminal t1/2 of 155.7±31.4 mins. tmax and terminal t1/2 for po S (100mg/Kg) was 30.2±6.2 μg/ml, 21.6±3.7 mins and 149.7±36.8 mins respectively. Bioavailability of S was found to be 72% and clearance was 25.8±1.7 (iv) and 35.5±3.0 (po) ml/min/Kg. These results indicate that in rats, S was rapidly absorbed, and it was excreted essentially unchanged, as the parent compound, by renal excretion.

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The distribution and binding properties of $^{14}C$-Gossypol were studied by whole-body autoradiography. $^{14}C$-Gossypol was administered intravenously (10 mg, 50 μCi/Kg). Animals were killed 30 min, 1, 6, 12, and 24 hours after injection. The rats were embedded in 2% carboxymethyl cellulose, frozen in liquid nitrogen and sectioned at 50μ. Frozen whole rat sections were freeze-dried overnight at -20°C. Sections were either placed directly on X-ray film or placed on film following a 30 sec. wash in ethanol-ether (5:2) to extract non-bound radioactivity. Liver contained the highest level of radioactivity (3.29 μCi/cm²) followed by blood, bone marrow, kidney, fat, and testes (3.11, 2.05, 1.87, 1.75, 0.84, and 0.59 μCi/cm², respectively). Gossypol related radioactivity was moderately extractable from most tissues within the first hour following administration, while it was bound in all tissues particularly in fat, testis, and muscle between 6 and 24 hours. This study demonstrated binding of gossypol to a variety of tissues followed by its slow subsequent release. (Supported WHO Grant #84040, Rockefeller Found. Grant #GAPSB42).

Low levels (200-300 ppm) of long-chain ketones are normally formed during the alkali-catalyzed rearrangement of triglyceride (TG) oils. These are thought to be derived from an intermediate in which there is a Cl to C2 attachment of adjacent fatty acids of the TG. In order to determine the fate of these commonly ingested materials, the ADE of radiolabel was determined after oral dosing of $^{14}C$-linoleate (cis-9,12,13-tridecatriene ketone) and $^{14}C$-oléate (cis-9,10,12-tridecatriene ketone) at 50 mg/kg to male Sprague-Dawley rats. Radiolabel was rapidly eliminated (majority within 24 h) in urine and CO₂ as follows: 4.8% and 5.4% of dose for L and 1.2% and 4.6% of dose for O. Intravenous dosing with 0 showed minimal fecal excretion (3.5% of dose), suggesting that biliary excretion of these ketones is minimal. Approximately 4.4% and 2.0% of the dose were retained for L and O, respectively, after 72 hours; this radiolabel was not localized in any particular tissue, and was most likely the result of the incorporation of oxidation products into carbohydrates, lipids, and proteins. It is concluded that absorption of these ketones is low (16% for L, and 8.5% for O), and that absorbed radiolabel is readily metabolized and rapidly eliminated.
831 VEHICLE AND pH EFFECTS ON THE DERMAL PENETRATION

In vitro percutaneous penetration studies using excised human cadaver skin indicated that acrylic acid absorption can vary significantly as a function of pH (phosphate buffer) and delivery vehicle. In vitro flux estimated after a 1 mg dose varied 600-fold within the treatments studied and decreased in the order acetone > pH 6.0 > ethylene glycol > pH 7.4. The decrease in flux associated with an increase in pH was consistent with the decrease in octanol-water partition coefficient of acrylic acid.

In vivo dermal absorption studies were conducted in the rat at 2 mg/kg dose with pH 6.0 and 7.4, gpd with acetone. The rate of appearance of 14C-CO2 in exhaled breath was used as measure of the rate of absorption. A similar pattern of absorption was seen as the rate of absorption decreased in the order acetone > pH 6.0 > pH 7.4. These results suggest that for acrylic acid, in vitro data can be used to estimate in vivo dermal absorption. The results also suggest that caution should be exercised in interpreting toxicity end-points from oral exposure studies with acrylic acid where different delivery vehicles are used.

833 NITRYPYRIN: KINETICS AND METABOLISM IN THE
FISCHER 344 RAT. C Timchalk, M D Dryzga and
R A Campbell. HES, The Dow Chemical Co.,
Midland, MI. Sponsor: A M Schnu

Nitrpyrin (2-chloro-6-trichloromethyl pyridine) is the active component of N-SERVE* brand nitrogen stabilizer. The 1 C plasma time-course was determined in male rats at 1 and 60 mg/kg. The 1 mg/kg dose was absorbed at a faster rate than the 60 mg/kg dose (t1/2 = 1.2 and 3.2 hr, respectively). Both doses were cleared from the plasma in a biexponential manner [t1/2 = 2.2 (a) and 14.6 hr (b)]. Five 14C nitropyrim 2 orally
dosed with 1 or 60 mg/kg 14C-nitrpyrin or 14 daily 1 mg/kg doses (unlabeled) followed by 1 mg 14C-nitropyrim/kg on day 15 and sacrificed 72 hrs post-dosing. Over 94% of the dose was recovered. Urine accounted for >80% and feces >11% of the dose. No radioactivity was found in the expired air. Less than 1% of the dose was found in the liver, kidney, lung, and blood 72 hrs post-dosing. Bone, brain, carcass, fat, gonads, heart, muscle, skin and spleen did not contain sufficient 14C to quantify. Urinary metabolites were identified as 6-chloropicolinic acid (6-CPA) and the glycine conjugate of 6-CPA. The overall disposition of 14C-nitrpyrin was independent of sex, dose level, or prior exposure. These data indicate that the rat rapidly metabolizes and eliminates nitropyrim in the urine as 6-CPA and the glycine conjugate of 6-CPA.

*Trademark of The Dow Chemical Company.

832 PHARMACOKINETICS AND METABOLISM OF [IR, CIS]-
AND [IR, TRANS]- ISOMERS OF TETRAMETHRIN IN
RATS. L S Silver and W C Daumerman. Toxicology Program, North Carolina State University, Raleigh, NC.

The pharmacokinetics of [cis, and trans]-isomers of the insecticide tetramethrin was investigated in vivo in the unanesthetized rat following rapid intravenous injection of a 0.25 mg/kg dose, and in the isolated perfused rat liver after a bolus dose of 25 µg to the reservoir of a recirculating system. A two compartmental pharmacokinetic model fit the experimental data for the time course of tetramethrin concentration in plasma. The in vivo metabolism and excretion of tetramethrin-isomers was described in relationship to the pharmacokinetic model. The recirculating isolated perfused rat liver system was described by non-compartmental analysis. The metabolism and biliary elimination was described for the tetramethrin-isomers in relationship to their pharmacokinetic parameters. Curve fitting for the two compartmental model was initially performed with a computer program JANA, and was refined by the computer program NONLIN with a weighting procedure(1/c).

834 EXCRETION BALANCE AND PHARMACOKINETIC
EVALUATION OF 14C-DIPHENYLDIOXONIUM
HEXAFLUOROARSENITE (PIFA) AFTER INTRAVENOUS,
ORAL AND INTRATRACHEAL ADMINISTRATION IN RATS.
L W Smith, J L Eisenman, A K Thakur and
S L Yurasevcew. General Electric Company, Pittsfield, MA and Hazleton Laboratories, Vienna, VA.

Young adult male and female Sprague-Dawley rats were used to determine the patterns and rates of excretion and tissue distribution of PIFA. Single doses were: intravenous, 5 mg/kg; oral, 20 mg/kg; intratracheal, 10 mg/kg. Diphenyldioxonium cation was analyzed by 14C-liquid scintillation; arsenite anion was measured by As atomic absorption. PIFA was rapidly absorbed after oral administration; 64% was bioavailable. Oral administration revealed a triexponential function -- absorption (half-life = 0.65 hr), distribution to peripheral tissues (half-life = 2 hr) and elimination/disposition (half-life = 13 hr). Extravascular sequestration was suggested from extremely large estimates of central compartment and distribution volumes. Urinary and fecal excretion values, respectively, after 48 hr were: intravenous, 70% and 14%; oral, 70% and 22%; intratracheal, 60% and 35%. Expired 14CO2 was minimal. Cationic and anionic moieties were handled differently. Diphenyldioxonium ion localized in the heart, liver and kidneys, which were target organs in subacute toxicity studies.
The percutaneous pharmacokinetics of single doses of DMAE containing 14C-DMAE were evaluated by measuring plasma 14C levels for 48 hr after the dose. DMAE was applied to occluded skin of male and female rats and rabbits. Initial i.v. studies using two dose levels (200 and 2 mg/kg in rats, 100 and 1 mg/kg in rabbits) indicated that transfer and elimination were first-order and that elimination was not capacity-limited at the higher dose level. DMAE applied to the skin (200 mg/kg in rats, 100 mg/kg in rabbits) was rapidly absorbed and maximal plasma 14C levels were observed at approx. 2 hr post-dose. Absorption and elimination were apparently first-order by this route in both species, with excretion occurring primarily via urine. The data are consistent with an open two-compartment pharmacokinetic model. Of interest, marked accumulation of 14C label was observed 48 hr post-dose in rabbit kidney relative to plasma, but not in rat kidneys or other rabbit or rat tissues. These findings indicate that, in both species tested, DMAE or a metabolite readily diffuses across skin, rapidly attains significant plasma levels and is largely excreted in the urine.

IN VIVO METABOLISM OF METHACRYLONITRILE TO CYANIDE IN RATS. R Cavaux Jr., M Y H Farooqui, and W W Day. Dept. of Biology, Pan American University, Edinburg, TX

Methacrylonitrile (MeAN) is an industrial monomer in the production of plastic elastomers and coatings. It is a potent lacrimator, neurotoxin and a dermal, eye, and nasal irritant. Previous studies in our laboratory have shown that MeAN reacts with glutathione (GSH) both in vivo and in vitro causing significant GSH depletion in many organs of rats. Now we have looked at metabolism of MeAN to cyanide (CN). MeAN (0.75 mL, 0.15 mmol/Kg) was given orally to male Sprague Dawley rats and the animals were sacrificed at designated times. Concentrations of CN were determined in various organs and those of thiocyanate (SCN) in plasma and urine. In all the organs studied we found significant (p<0.05) levels of CN. In the liver the CN concentration was highest at 25-35 mmol/g for initial 6 hr and returned gradually to normal levels. Similar trend was observed in other organs including kidney, lung, heart and brain. In plasma the SCN concentrations increased significantly (p<0.05) 26.3 mmol/L at 1 hr and 81.2 mmol/L at 6 hr after dosing. The SCN did not appear in urine to significant amounts during this period. These findings indicate that MeAN is metabolised to CN and SCN in the rats which may be partially responsible for its toxicity.
EFFECT OF XYLENE ISOMERS ON RAT BRAIN MICROSONAL MEMBRANES AND GLUTATHIONE LEVELS. T. Auceda, C. Furman, C. Lebel, A. Roberts and R. Schatz. Toxicology Program, Northeastern Univ., Boston, MA.

Previous studies in our laboratory have demonstrated that the organic solvent p-xylene (1g/kg, i.p., 1h) affects pulmonary microsomal membrane composition. Total phospholipid (PL) content of these membranes was decreased 28% while total cholesterol (CL) remained unchanged. The PL/CL ratio, an indirect measure of membrane fluidity, was decreased 34% and direct measurement of membrane fluidity by fluorescence polarization revealed a 5% decrease. Phospholipid methylation (PLM), an index of membrane function, was increased 25-50%. Glutathione (GSH) levels were unchanged. Because p-xylene is highly lipoiphilic and alters CNS function, we investigated the actions of p-xylene and its isomers on cerebral microsomal membranes. p-Xylene administration did not alter either total PL or CL content; PL/CL ratio and membrane fluidity were also unaltered as were GSH levels. PLM was unchanged. PL, CL, PL/CL and fluidity parameters also remained unaltered in microsomes after m-xylene and o-xylene. These isomers, however, decreased GSH levels by 31% and 29%, respectively. While xylene isomers had no effect on microsomal membrane parameters, their ability to decrease GSH paralleled the integral role of GSH in membrane homeostasis, may indicate that these isomers elicit perturbations at other subcellular loci in brain. Supported in part by GE Plastics.


p-Xylene (1g/kg, i.p., 1h) has been shown to inhibit pulmonary microsomal metabolism of benzof(A)pyrene (BaP), possibly mediated by a decrease in cytochrome P450. Alterations in membrane lipids, especially phosphatidylcholine, have been proposed to be in part responsible for these effects (Toxicologist 7(1),988-987). The current study describes the effects of individual xylene isomers on BaP metabolism (expressed as aryl hydrocarbon hydroxylase (AHH) activity), cytochrome P450, microsomal total phospholipid (PL), cholesterol (CL) and the PL/CL ratio, an index of fluidity. p-Xylene was shown to decrease AHH activity 47% (p<.01), reduce cytochrome P450 to nondetectable levels (p<.05) and decrease the PL and the PL/CL 28% and 34%, respectively (p<.05). Alterations in the lipid microenvironment of cytochrome P450 may be responsible for the observed inhibition of AHH activity. Conversely, m-xylene produced a 62% increase in AHH activity (p<.05), but no change in cytochrome P450, PL/CL or the PL/CL ratio, suggesting that the increased AHH activity is not mediated by change in the lipid environment. o-Xylene treatment produced no change in AHH activity, cytochrome P450 or PL but did increase CL content (10%) and decrease the PL/CL ratio (15%). Taken together these data suggest that individual isomers of xylene have different effects on BaP metabolism, cytochrome P450 and endoplasmic reticulum lipid content.

TOLUENE INDUCED DECREASE OF PHOSPHATIDYLETHANOLAMINE IN SYNAPTOSOMAL LIPIDS. C. Lebel and R. Schatz. Toxicology Program, Northeastern Univ., Boston, MA.

Organic solvent containing products are in widespread use in both industry and in the home. Toluene and xylene are two solvents that have respiratory and CNS effects. This laboratory previously reported that p-xylene decreases rat lung microsomal membrane phospholipids (PL). The purpose of the study was to investigate whether the CNS effects of toluene (i.e. decreased reaction times, memory loss) result from altered neuronal membrane (synaptosomal, microsomal) composition and function. Toluene (1g/kg, i.p., 1h) did not alter rat brain microsomal PL or cholesterol (CL) content. Synaptosomal PL was found to be decreased (21%), while CL levels were unaltered. Phospholipid speciation studies showed that the decrease in synaptosomal PL was due to a decrease in phosphatidylethanolamine (PE) (32%), a major membrane component. The increase in the PL/CL and phosphatidylcholine (PC)/PE ratios, indices of membrane fluidity, suggested an increase in synaptosomal fluidity. However, no alterations in fluidity were detected when this parameter was measured directly, via fluorescence polarization. These data suggest that toluene induced alterations in rat synaptosomal PE may alter normal synaptic function, which may help further elucidate the mechanisms of organic solvent induced neurotoxicity. Supported by GE Plastics Division and BRSG 05830-08.

HPLC DETECTION OF TRANS, TRANS-MUCONIC ACID IN RATS EXPOSED TO BENZENE. J. M. Mitchell, B. D. Goldstein and W. J. Hitz. UMNJ-R W. Johnson Med Sch/Rutgers Univ, Joint Grad Prog in Toxicology, Piscataway, NJ.

Previously our laboratory showed that trans-muconaldehyde (MUC) is formed from benzene and that it caused a hematoxicity similar to that observed for benzene. In vivo MUC is metabolized to trans, trans-muconic acid (MA) and excreted in the urine. The present study was conducted to determine whether MA could serve as a marker for low levels of benzene exposure. For this purpose we adapted an HPLC procedure for detecting MA in urine. In this system, MA has a retention time of 11 minutes and a detection limit of 500 picograms. Male Sprague-Dawley rats were injected i.p. with benzene at .5, 1, 5, 10 and 20 mg/kg, two rats per dose. The rats were housed individually in metabolism cages for 48 hours. The initial 24 hr total MA output in the urine ranged from 0.3% to 6.6% of the administered dose. For the second 24 hr period the MA excreted ranged from 0.1% to 0.3% for the 10 and 20 mg/kg dose. Little to no MA was detected during this period for the other doses administered. The results show that this method is sensitive for determining MA after low dose benzene exposure. Supported by NIH grant ES02555 and a grant from the Advanced Technology Center in Hazardous and Toxic Substance Management.
In order to examine the consequences of long-term exposure to styrene (STY) on nervous system functioning, rats were exposed by inhalation to STY at 0, 350, 700 or 1400 ppm for 16 hrs/day, 5 days/week for 18 weeks. Animals were evaluated at periodic intervals using a battery of neurobehavioral tests including: spontaneous motor activity, grip strength, automated analysis of hindlimb movement, visual discrimination performance and peripheral nerve conduction velocity. Compared to Controls, STY-treated rats demonstrated mild reductions in spontaneous activity and grip strength. Coordinated movement and peripheral nerve conduction velocity were unaffected. With respect to discrimination performance, STY produced a marked reduction in response speed and accuracy on Day 1 of exposure which was followed by the rapid development of tolerance. Further, post-exposure functional evaluations failed to provide evidence for a persistence of STY-induced deficits beyond the exposure period. Results from subsequent studies measuring brain and blood STY levels indicated that changes in styrene kinetics may account for the rapidly developing tolerance to the deficits in discrimination performance produced by STY during the early stages of exposure.

We have characterized the embryotoxicity of the glycol ethers 2-methoxy (2-ME) and 2-ethoxyethanol (2-EE) via postimplantation rodent embryo culture systems. Sprague Dawley rat embryos, explanted on day 10 of gestation, were cocultured with each compound in 50% Waymouths 752/1 media and 50% rat serum supplemented with antibiotics for 24 hours. Over a 10-fold dose range, 2-ME was found to exhibit greater embryotoxicity as compared to 2-EE supporting decreasing embryotoxicity with increasing substitution of the ethyl moiety. Embryotoxic effects monitored for both compounds included decreased growth (crownto-rump length) and decreased nerve structure development (somatic count). Addition of rat hepatic S-9 fractions were found to decrease significantly the toxicity of 2-ME while not altering the prenatal toxicity of 2-EE. These findings support epidemiological studies implicating both solvents as reproductive toxins in manufacturing industries.

This program assesses the issue of the formation and toxicity of brominated dioxins and/or furans when formed as part of the soot and char in real fire environments. Analytical and comparative toxicity studies (rat oral LD50 and rabbit ear comedogenicity) were conducted on soot and char samples generated by combustion conditions designed to simulate real fires for HIPS with and without DBDPO/Sb2O3 as the flame retardant. The same types of toxicity studies were conducted on pure samples of 2,3,7,8-TeBDD and 2,3,7,8-TeDFP.

The results can be summarized as follows: 1) Brominated dioxins were not formed from the combustion of HIPS/DBDPO/Sb2O3, but at levels lower than reported from previous studies. The mono- to tri-brominated isomers accounted for approximately 95% of all polybrominated dibenzofurans found. 2) An absence of toxicity was noted in dermal (rabbit) and oral (rat) exposure to the soot/char samples generated from combustion of HIPS or HIPS/DBDPO/Sb2O3, indicating the presence of the brominated flammable retardant did not contribute to discernible toxicity. The extracts with the pure samples of 2,3,7,8-TeBDD and 2,3,7,8-TeDFP showed qualitatively similar toxicity historically associated with exposed dioxins/furans, but with quantitative indicators of one to two orders of magnitude of a lesser degree of toxicity relative to 2,3,7,8-TeCDF.

ECHP (CAS No. 112-25-4) is an alkyl glycol ether used as a solvent in paints and varnishes and it is structurally related to lower molecular weight homologs which are reproductive and developmental toxins. Genotoxic potential of ECHP was studied using four in vitro assays to determine capability to produce gene mutations, chromosome aberrations and DNA damage. All tests were conducted using concurrent positive and negative controls both with and without addition of an Aroclor 1254-induced, rat-liver, S9 homogenate activation system. ECHP did not produce significant mutagenic effects in the Ames test or in a forward gene mutation assay with Chinese hamster ovary (CHO) cells. No significant or dose-related increases in sister chromatid exchanges or chromosome aberrations were produced in CHO cells within a range of cytotoxic to noncytotoxic doses. Cytotoxicity of ECHP to CHO cells and Salmonella was similar with and without S9 activation. The lack of genotoxicity in this series of sensitive screening tests suggests that ECHP is unlikely to be active in vivo as a mutagen or as a carcinogenic initiating agent.

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A TERATOLOGY SCREENING STUDY IN RATS WITH CYCLOPENTANONE. G M Rusch, D E Rodwell, M D Nemec and E J Tasker. WIL Research Laboratories, Inc., Ashland, OH. 2Springborn Life Sciences, Inc., Spencerville, OH. 3Allied Corporation, Morristown, NJ.

Potential maternal, embryotoxic and teratologic effects of Cyclopentanone were evaluated. The compound was mixed with Mazola® corn oil and administered by oral gavage at doses of 50 and 300 mg/kg/day. Each dose and vehicle control group consisted of 25 bred female COBS® CD® rats. The animals received 10 consecutive daily doses (gestation days 6-15) at a dose volume of 5 ml/kg. Appearance and behavior were evaluated and body weights were recorded. A gestation day 20 Cesarean section was performed to evaluate intrauterine survival and fetal developmental indices including fetal body weights and external, skeletal and visceral morphology. No maternal, embryotoxic or teratologic effects were expressed at either dose level. However, the 300 mg/kg/day dose was selected to produce maternal toxicity as expressed by reduced body weight gain in a range-finding study. The only potential compound-related effect in the study was a slightly decreased mean fetal body weight value at the 300 mg/kg/day dose level although this value was within the range of the WIL historical data. An increase in the number of litters with the fetal variant malaligned sternabrae occurred at the 50 mg/kg/day dose level, but the incidence at the higher dose level was comparable to the control group. The 50 mg/kg/day dose level was considered a "no-effect" level.


Butoxypropanol (BP), a glycol ether, was evaluated for dermal and systemic toxicity following repeated application to the intact skin of New Zealand albino rabbits over a period of 91 days. Four groups of 5 males and 5 females/group were treated with 50/50 (w/v) ethanol/distilled water (vehicle control), 0.57% (w/v), 5.7% (w/v), or 57% (w/v) BP at a dose of 2 ml/kg body weight. Animals were exposed to the test solutions for 7 hours/day, 5 days/week. During the exposure period animals were restrained to prevent oral ingestion. Evaluation of in-life signs, in-life body weight changes, terminal organ weights, hematology, and gross and microscopic pathology data did not reveal any BP-related effects other than mild to moderate irritation at the site of application. Examination of test site skin demonstrated erythema (57% and 5% groups), edema, atonia, desquamation and fissuring (57% group). Microscopic evaluation of treated skin (57% group) exhibited acenosis and hyperkeratosis. These results demonstrate that subchronic percutaneous exposure to BP does not produce systemic toxicity.


Potential maternal, embryotoxic and teratologic effects of N-hexanol were evaluated. The compound was mixed with Mazola® corn oil and administered by oral gavage at doses of 200 and 1000 mg/kg/day. Each dose and vehicle control group consisted of 25 bred female COBS® CD® rats. The animals received 10 consecutive daily doses (gestation days 6-15) at a dose volume of 5 ml/kg. Throughout gestation, appearance and behavior were evaluated and body weights were recorded. A gestation day 20 Cesarean section was performed to evaluate intrauterine survival and fetal developmental indices including fetal body weights and external, skeletal and visceral morphology. No embryotoxic or teratologic effects were observed at either dose level. Maternal toxicity occurred only at the 1000 mg/kg/day dose level as indicated by clinical signs and decreased maternal body weight gain during the treatment period. Mean fetal body weight at this dose level was slightly decreased but within the WIL historical range. An increase in the number of litters with the fetal variant malaligned sternabrae occurred at the 200 mg/kg/day dose level although the incidence at the higher dose level was similar to the control group. The 200 mg/kg/day dose level was considered a "no-effect" level.

BUTOXYPROPANOL IS NOT A DEVELOPMENTAL TOXIN IN RABBITS EXPOSED BY THE DERMAL ROUTE. W B Gibson, G A Nolen, Procter & Gamble, Cincinnati, OH and M S Christian, Argus Research Labs, Inc., Horsham, PA.

Butoxypropanol was evaluated for developmental toxicity (embryofetotoxicity and teratogenicity) potential and maternal toxicity in New Zealand White rabbits. Dosages of 0 (water), 10, 40 or 100 mg/kg/day, derived from a pilot study, were administered percutaneously to groups of artificially-inseminated females, 17 control animals and 19 in each test group, on days 7 through 18 of presumed gestation using a dose volume of 2 ml/kg. Does were observed for signs of test substance effect, abortion or delivery. Body weights and food consumption were recorded daily. On day 29, females were sacrificed and examined for pregnancy, number and placement of implantations, early and late resorptions and live fetuses. Pregnancy occurred in 15 control does (88%) and in 16 does in each test group (84%). One low dosage group rabbit aborted. There were no statistically significant differences between test and control groups for maternal body weight gain, food consumption, number of corpora lutea per ovary, implantations, live fetuses, early and late resorptions, fetal body weights, gender, or gross external changes. There were no developmental visceral or skeletal alterations at any dose tested. No signs of maternal toxicity were observed, other than for mild erythema at the site of application at the 100 mg/kg/day dose level. The developmental no-effect level was >100 mg/kg/day.
CS₂ is hepatotoxic following its P450-mediated metabolism. It is also neurotoxic, an effect which is associated with inhibition of the conversion of dopamine to nor-epinephrine in the brain. Pretreatment (PT) with alliphatic alcohols (isopropyl alcohol (IPA) being most potent) can enhance the hepatotoxicity of CS₂ in rats by inducing a specific P450 involved in CS₂ activation. We found that markedly higher doses of i.p. CS₂ were required to alter brain catecholamine metabolism (CM) than to inhibit hepatic P450 activity (amine oxidase (AO)) in C57BL/6 mice. APT PT with IPA, which markedly enhances CS₂ hepatotoxicity (loss of AO activity), had no further effect on the ability of i.p. CS₂ (1250 mg/kg) to alter brain CM. Ten ppm CS₂ in air (the current TLV) for 4 hrs caused a significant decrease in hepatic AO activity. This effect was markedly enhanced by IPA PT. In contrast, the brain was relatively resistant to CS₂, levels of 700 ppm for 4 hrs being required before significant alterations in brain CM were seen. As above, IPA PT had no further effect. Thus, hepatic P450 function is markedly more sensitive to CS₂ than is brain CM regardless of the site of administration. Furthermore, at levels of CS₂ that are toxic to the CNS, the hepatic capacity to metabolize CS₂ is vastly exceeded and any increase in this capacity (e.g., IPA PT) has essentially no effect on the amount of unchanged CS₂ that will reach the brain.

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THE MECHANISM OF ORGANIC SOLVENT TRANSPORT IN THE BLOOD. C.W. Lam,* T J Galén,* J F Boyd,* and D L Pfrierson. NASA Biomedical Laboratories Branch and KRUS International, Johnson Space Center, Houston, TX.

A previous study on the transport mechanism of CS₂ in the blood indicated that about 90% of the blood CS₂ in the exposed rats was present in the RBCs and that a majority of this was carried by hemoglobin (J. Appl. Toxicol. 6, 81-86, 1986). The present study extended the investigation to several typical organic solvents. Rats were exposed to 500 ppm of n-hexane, chloroform, toluene, and methyl isobutyl ketone vapor for 2 hr; 93.6%, 89.5%, 62.7%, and 51.2%, respectively, of these solvents in blood were found in the RBCs. The partitioning of these solvents into the RBCs is roughly parallel to the hydrophobicity of these solvents. Similar results were obtained in vitro when these solvents were added to rat DTGnd. In vitro studies were also conducted on human blood, and the extent to which these solvents partitioned into the RBCs was significantly less than that into the rat RBCs. When solutions of these solvents were added to human plasma or RBCs, large fractions (64-94%) of the solvents were recovered from the plasma proteins or hemoglobin, respectively. Less than 10% of the solvents added to the RBCs was recovered from the membrane fractions. These results indicate that RBCs are the major carriers for hydrophobic solvents in the blood and that organic solvents are mainly transported by proteins. An investigation on diethyl ether is in progress.
TOXICOGENETICS OF ACETONITRILE. II. AUTORADIOGRAPHIC DISTRIBUTION AND BINDING OF 2-14C-ACETONITRILE IN MICE.
J.-P. Loh, G I Hussein, and A E Ahmed. Department of Pathology, University of Texas Medical Branch, Galveston, TX

Acetonitrile, a commonly used solvent in various industries, is known to cause central nervous system toxicity. The objective of this study is to characterize the distribution and molecular interactions of 2-14C-acetonitrile in mice. Male mice were treated (0.05, 0.06 mCi/Kg, 11.4 mCi/mole) with tracer dose of 2-14C-acetonitrile. At various time intervals (0.08, 0.5, 1, 4, 8, 24, and 48 hr) after treatment, mice were anesthetized with ether and frozen by immersion in dry ice/acetone mixture or dissected for collection of organs. The mice were processed for whole body autoradiography (WBA) by Udberg's method. Nonaqueous metabolites are detected at their sites of accumulation by this method. Covalent binding was determined using trichloroacetic acid/ethanol/ether extraction method. The WBA revealed heavy localization of acetonitrile metabolites in the gastroenterointestinal tract and bile. At early times (5 min) liver and kidney contained the highest levels of radioactivity which declined by time. Covalent binding studies indicated that most of the radioactivity present in the liver was incorporated in the macromolecular fraction. The radioactivity contents of other organs were mostly present in the acid soluble fraction of the tissue. These studies suggest that the incorporation of radioactivity derived from 14C-acetonitrile may be due to a) covalent binding of a reactive metabolite formed; b) the incorporation of a single carbon into the synthesis of macromolecular fraction. (Supported by NIH Grant No. ES 01871).

CHARACTERIZATION OF 14C-BUTADIENE ADDUCT FORMATION TO HEMOGLOBIN IN MICE AND RATS. J D Sun, A R Dahl, J A Bond, R F Henderson and L S Birnbaum. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; NIEHS, Research Triangle Park, NC

1,3-Butadiene (BD) is a major intermediate in rubber manufacturing. Rodent studies showed that inhaled BD is metabolized to mutagenic epoxides detectable in blood. Our goals were to see if BD metabolites form adducts with hemoglobin (Hb) and if such adducts are linearly related to dose. 14C-BD dissolved in corn oil was injected IP into male BCGSF mice or Sprague-Dawley rats at 1 to 1000 μg/mole. Animals were killed at 1 day, globin isolated from blood and analyzed for 14C. For mice and rats, the dose-response was linear up to 100 μmole/kg and within this range, adduct formation was 1.75±0.03 and 1.24±0.06 pmoles/mg Hb/μmole dose (kgSE; n=9), respectively. Repeated dosings (1/day for 3 days) with 100 μmole/kg showed adduct accumulation was linear with dose. Retention of adducts in blood correlated with the life-time of erythrocytes in mice and rats (24 and 55 days, respectively). Thus, adducts formed in a dose related manner, accumulated upon repeated dosing and were relatively long-lived. Development of chemical methods to measure BD adducts on Hb may be a useful marker for occupational exposure to inhaled BD. (Research supported under U.S. DOE Contract DE-AC04-76EV-01013 through IAA 222-Y04-ES-20092 with NIEHS.)


Inspite of lack of adequate toxicological data for pyridine, there was continuing concern for reported neurotoxic symptoms associated with short-term occupational exposures. This prompted U.S. EPA to evaluate pyridine for its potential toxicity as a part of the toxicity program. S-D rats (10/dose/sex) were gavaged daily with 0, 0.25, 1.0, 10, 25 and 50 mg/kg/day pyridine for 90 days. In order to assess potential neurotoxic effects of pyridine, rats were perfused at the time of sacrifice; histopathological examinations of brain, liver, and other target organs were conducted. A significant dose-related increase in female liver: body weight ratios were observed in the 10 mg/kg groups. Histological examination revealed no treatment-related lesions of the CNS. Incidence of non neoplastic hepatic lesions, 30% and 70%, respectively in females and males of 50 mg group was observed compared with 10% incidence in controls, 0.25 and 1 mg groups; no such lesions were observed in the 10 or 25 mg groups. The rationale for this apparent anomaly is unresolved. Based on these findings and other supportive literature, 1 mg/kg/day pyridine represents the NOAEL. Application of an uncertainty factor of 1000 (10x10x10x10) to this NOAEL results in a reference dose of 1×10^-3 mg/kg/day.


The toxicity of N,N-dimethylthanolamine (DMEA), 3-dimethylamino-N,N-dimethylpropionamide (DDPA), and 2-(2-dimethylamino)ethoxy)ethanol (DMEA) from repeated cutaneous exposures was assessed. Zero, 50, 250, or 500 mg of test material/kg body wt/day were applied continuously to New Zealand White rabbits (5/sex/group) for 9 applications (occluded, 6 hours/day) over 11 days. Test materials produced local edema, erythema, necrosis, and ecchymoses. Microscopic changes included necrotizing dermatitis and anacanthosis. Attributed to the necrotizing dermatitis were increases in total leukocyte count and segmented heterophilia, and alterations in serum albumin, calcium, and globulin concentrations in DMEA and DDPA treated animals. Evidence of possible systemic toxicity from DMEA included loss of body weight, hypokalemia, decreased blood urea nitrogen, decreased hemoglobin and hematocrit, and increased liver, kidney, and adrenal weights. However, no microscopic lesions were observed in liver, kidneys, or adrenals. Based on this study, DMEA, DDPA, and DME are considered to be severe skin irritants at doses of 50 mg/kg/day and above in rabbits. Further, DMEA may cause significant systemic toxicity at 50 mg/kg/day and above.
SUSTAINED INTRAPERITONEAL DELIVERY OF 1,1,1-TRICHLOROETHANE BY A CERAMIC DELIVERY SYSTEM. D E Hollembach*, P K Bajpai*, L M Morris*, M L Cargas, R R Drawbaugh, and M E Andersen, AAMRL/TH, Wright-Patterson AFB, OH.

A simple ALCAP (aluminum oxide, calcium oxide and phosphorus pentoxide) ceramic containing 1,1,1-trichloroethane (TCE) was prepared and evaluated for the purpose of developing a sustained release solvent delivery system. The ALCAPs filled with TCE and 5% mineral oil were evaluated in vitro and in vivo. The capacity of the ceramic delivery system (CDS) was 35 ul (45 mg), and it provided a delivery rate of 3.6 mg/hr for 8 hr in vitro. In the in vivo study, analysis of blood from Sprague-Dawley rats implanted with a CDS containing 45 mg TCE exhibited a constant level (7.2 ug/ml) for 20 hr. Analysis of exhaled air from these rats showed a sustained ceramic release rate of 4 mg/hr. The data obtained in this investigation suggests that this ceramic system may be useful for toxicokinetic studies with a variety of volatile compounds.

* UES Participants under Contract F49620-85-C-0013

INDUCTION OF PEROXISOMAL β-OXIDATION IN CULTURED RAT, DOG, AND MONKEY HEPATOCYTES BY BEZAFIBRATE, CPIROFIBRATE, AND LIY171883. P S Foxworthy and P L Escho, Lilly Research Laboratories, Toxicology Division, Greenfield, IN.

Interspecies differences in sensitivity to peroxisome proliferation have been reported, with dog, monkey, and human being much less sensitive than rodents. A method has been developed evaluating the induction of peroxisomal β-oxidation (8-OX) in cultured rat, dog and monkey hepatocytes in order to facilitate interspecies comparisons. Treatment of cells with bezafibrate, cipofibrate and LIY171883 (i-2-[2-hydroxy-3-propyl]-4-[4-(18-tetrasol-5-y1)butoxy]phenyl]-ethanone) was begun after 20 hr of culture and continued for 72 hr. Peroxisomal 8-0X was measured as the cyanide-insensitive reduction of NAD+ in the presence of 50 μM palmitoyl CoA. Maximum induction (% of control) of 8-0X in hepatocytes from rat, dog, and monkey were as follows: bezafibrate (0.2mM), 869, 176, and 158; cipofibrate (0.2mM), 1342, 188, and 190; LIY171883 (0.1mM in rat; 0.2mM in dog and monkey) 550, 120, and 141. Glucagon (10^{-6}M) induced the activity of tyrosine amino transferase and phoshoenolpyruvate carboxykinase in hepatocytes of all species, demonstrating the capacity for enzyme induction in vitro. The results demonstrate that the in vitro model accurately reflects the interspecies differences in sensitivity observed in vivo and may provide a means for evaluating human responsiveness to peroxisome proliferating agents.


Activated neutrophils (PMNs) may cause tissue injury by releasing oxygen radicals and other products. We have observed recently that certain bile acids potentiate the release of superoxide anion (O_{2}^{•−}) from activated PMNs, suggesting that bile acids might exacerbate PMN-dependent tissue injury. PMNs preincubated with lithocholate (10-100 μM) and activated with phorbol myristate acetate (PMA) released much greater amounts of O_{2}^{•−} than controls exposed to PMA alone, illustrating a priming effect. O_{2}^{•−} release from lithocholate-primed PMNs rose sharply between 5 and 10 minutes after PMA addition and then ceased between 10 and 30 minutes. The cessation of O_{2}^{•−} release corresponded to increased release of lactate dehydrogenase from PMNs. PMNs washed after lithocholate preincubation and then activated with PMA released approximately half as much O_{2}^{•−}. Lithocholate's priming effect was not dependent on extracellular Ca^{2+}. Lithocholate also primed PMNs for O_{2}^{•−} release when F-met-leu-phe was used as the stimulus. These data indicate that lithocholate can prime PMNs such that subsequent stimulation results in a much enhanced release of O_{2}^{•−}, and that this effect is independent of extracellular Ca^{2+}. It seems possible that bile acids may prime PMNs in toxicoses or other disease states characterized by elevated serum bile acids. (Supported by NIH grant ES04138.)

Hepatotoxicity Due to 2-Ethylhexanol is O_{2}^{•−}-dependent in the Perfused Rat Liver. R Keller and R Thurman. Dept. of Pharmacol., U. of N.C., Chapel Hill, NC.

2-Ethylhexanol (EH) is a primary metabolite of the plasticizer diethylhexyl phthalate, a known carcinogen in rodents. Toxicity of EH was assessed in the perfused rat liver. Livers from fasted rats were perfused with EH (3 mM) dissolved in Krebs-Henseleit buffer (pH 7.4; 37 C, satu-rated with 95% O_{2} : 5% CO_{2}) in both the antrograde and retrograde direction. Cell damage was detected by the appearance of lactate dehydrogenase (LDH) in the effluent perfusate within 20 min. of EH infusion which increased to maximal values around 1100 U/l in 40 min. Trypan blue was then infused from the antrograde side of the liver and the lobule regions of the liver lobule were damaged by EH. Only O_{2}^{•−}-rich periportal regions of the lobule took up dye when EH was infused in the antrograde direction. In contrast, when the liver was perfused in the retrograde direction, only O_{2}^{•−}-rich upstream periportal regions were damaged. When O_{2}^{•−} tension was lowered both by reducing the flow rate to around one-quarter of normal or by mixing gases, LDH release was diminished dramatically. Therefore, it is concluded that the toxicity of ethylhexanol in the liver is O_{2}^{•−} dependent (85-04325).
Hepatotoxicity due to Menadione in Potentiated by Ethanol in Perfused Rat Liver. P E Caney and R G Thurman, Dept. of Pharmacol., Univ. of North Carolina at Chapel Hill.

Menadione (MQ) is an oxygen-dependent hepatotoxin that damages perportal regions of perfused liver presumably due to redox cycling, a process that requires reducing equivalents. The metabolism of ethanol generates reducing equivalents; therefore, the effect of ethanol on MQ hepatotoxicity was examined. Redox cycling due to MQ was assessed in perfused rat liver from increases in oxygen uptake, and toxicity was evaluated from LDH release. Oxygen uptake increased 20-30 μmol/g/h due to MQ alone. LDH was released into the effluent by 50 minutes of perfusion and reached 200-300 U/g/h. In the presence of ethanol, pyridine nucleotide fluorescence increased, and the maximal increase in oxygen uptake due to MQ was 2-3 fold greater. The MQ-stimulated oxygen uptake also persisted longer, so that the area under the curve was increased 10-fold by ethanol. LDH release occurred earlier (at 40 minutes after addition of MQ) and reached higher values (400-500 U/g/h). These data indicate that ethanol enhances redox cycling and hepatotoxicity due to MQ (ES02759, ES05431 and A.L.F.).

Correlation of Morphological (Apoptosis) and Biochemical (Serum Alanine Transaminase-ALT) Indices of Hepatotoxicity in Fasted Female Mice Treated Intraperitoneally with 1,1-Dichloroethylene (DCE). G P Bond, J C Garrison and E N Uyeki. University of Kansas Medical Center, Kansas City, KS.

Apoptosis as an indicator of toxicity has not been widely used. Reynolds et al (1980) reported that apoptosis precedes increases in ALT in fasted rats. We correlated morphological and biochemical events induced by DCE treatment over a dose and time course. DCE was administered ip in corn oil to five fasted female mice per group at doses of 12.5, 40 & 125 mg/kg. The mice were bled and sacrificed at 0.5, 1, 2, 4 & 8 hours after dosing. Liver sections were stained using the Feulgen reaction for DNA. One entire section was scored for apoptotic figures (bodies and nuclei) and mitotic figures. Serum ALT activity was determined. The morphological and biochemical responses were quite variable for each group. Significant increases in apoptosis and ALT were clearly observed at 4 hours after dosing with 40 & 125 mg/kg DCE. The relationship between apoptosis and ALT was less clear at 12.5 mg/kg after 4 hours, as apoptosis was slightly increased and ALT was unaffected. Increases in apoptosis over time were evident at 125 mg/kg but less so at the other doses. An increase in apoptosis occurs at a lower dose and sooner than an increase in ALT, the standard indicator of necrosis, suggesting that apoptosis is a sensitive indicator of pending hepatotoxicity. (Supported by NSF grants ES-07079 & CH-01130)

In Vitro LC₅₀ Determination of Solubilized 2,3,4-Trimethylpentane Using Primary Rat Hepatocytes. N J DelRaso and D R Mattie. Northrop Services, Inc. Dayton, OH. "AAMRL/ITP", Wright-Patterson AFB, OH. Sponsor: R S Kitzman

2,3,4-Trimethylpentane (TMP) has been shown to produce a nephropathy in male rats. While the effects of TMP on the kidney have been studied, the effects on the liver have not. Primary adult rat hepatocytes were exposed to TMP. The study consisted of two experiments with duplicate 4 h suspension cultures lacking albumin. TMP was solubilized in acetone. Acetone concentrations remained at 0.5%, while the TMP concentrations varied. Viability and lactate dehydrogenase (LDH) leakage data were statistically analyzed by SAS probit analysis (n=4). The LC₅₀ for TMP was found to be 1.49 x 10⁻² M and resulted in a ~50% increase in LDH leakage. When the study was repeated with albumin in the media, the LC₅₀ for TMP was 2.8 x 10⁻³ M (an increase of almost 2-fold). Ultrastructurally, control cells were normal with a slight increase in smooth endoplasmic reticulum (SER) in the 0.5% acetone controls. Exposure of hepatocytes to TMP produced vacuoles in the cytoplasm, increased amounts of SER, damaged nuclei, smooth plasma membranes, slight swelling of mitochondria in some cells at higher concentrations of TMP and progressively induced cell lysis with increasing concentrations of TMP.

Effects of Sesquiterpene Lactones on Mitochondrial Oxidative Phosphorylation. T R Narasimhan, H L Kim, and S H Safe, Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

The effects of three toxic sesquiterpene lactones namely helenalin, hymenoxon, and tenulin on the isolated mouse liver mitochondria are investigated. These lactones stimulated the State 4 respiration; 1.5-fold by helenalin, 1.3-fold by tenulin, and 1.27-fold by hymenoxon, of the control. They also decreased the respiratory control; 41% by helenalin, 59% by tenuulin, and 64.6% by hymenoxon. In addition, the State 3 respiration was inhibited; 68% by helenalin, 76% by tenuulin, and 81% by hymenoxon. These results suggest that the toxic sesquiterpene lactones uncouple the oxidative phosphorylation in isolated hepatic mitochondria. In addition, the dietary administration of ethoxyquin hydrochloride, an antioxidant and hepatic glutathione inducer, provided significant protection from the effects of sesquiterpene lactones on mitochondrial oxidative phosphorylation. (Supported by U.S. Department of Agriculture and the Texas Agricultural Experiment Station.)

Exposure of isolated rat hepatocytes to TEPAu (>250μM) results in rapid loss of mitochondrial function, disturbances in adenine nucleotide metabolism, and cell death. The purpose of these investigations was to determine if TEPAu caused disturbances in intracellular Ca²⁺ homeostasis. TEPAu induced "Ca²⁺-cycling" in isolated rat liver mitochondria and thus caused a release of sequestered mitochondrial Ca²⁺ (EC₅₀ = 20). Likewise, TEPAu also caused a release of Ca²⁺ sequestered by isolated rat liver microsomes. In the intact cell, TEPAu (10μM) caused a rapid loss of mitochondrial calcium (FCCP-releasable pool) followed by a loss of extramitochondrial calcium (A23187-releasable pool). This loss of Ca²⁺ was not associated with any changes in cytosolic free Ca²⁺ utilizing Indo-loaded hepatocytes nor was any cell death observed. Elevation of hepatocytes with 10μM TEPAu for 90 min does not cause any changes in cellular, antimycin-sensitive respiration and ATP content despite a complete loss of mobilizable Ca²⁺ from the mitochondrial and extra-mitochondrial pools. Therefore, TEPAu-induced cell injury may not be directly linked to the mobilization of free Ca²⁺ from these subcellular compartments.

MECHANISM OF TRIETHYLPHOSPHINEGOLD CHLORIDE TOXICITY TO ISOLATED RAT LIVER MITOCHONDRIA: INDUCTION OF Ca²⁺-CYCLING. D W Hoke, G K Mirabelli, and G F Rush. Smith Kline & French Laboratories, King of Prussia, PA

Triethylphosphinegold chloride, TEPAu, is a potent cytotoxicant to isolated rat hepatocytes. Biochemically, TEPAu causes a rapid drop in cellular ATP and respiration and inhibits the mitochondrial electron transport chain. Isolated mitochondria exposed to TEPAu exhibited a concentration-dependent collapse of the inner mitochondrial membrane potential (IC₅₀ = 24.67 ± 2.49 μM). In Ca²⁺-preloaded mitochondria (40 mMole CaCl₂) the TEPAu-induced decrease in membrane potential was potentiated (IC₅₀ = 11.33 ± 3.77 μM). TEPAu-induced Ca²⁺ efflux from mitochondria was not sensitive to ruthenium red. Collapse of mitochondrial membrane potential induced by TEPAu could be partially prevented by addition of ruthenium red or EGTA. Oxidation/hydrolysis of endogenous mitochondrial pyridine nucleotides induced by TEPAu could not be attenuated via addition of ATP. TEPAu causes the swelling of mitochondria and an increase in the permeability of the inner mitochondrial membrane to oxaloacetate. These data suggest that TEPAu induces "Ca²⁺ cycling" in isolated rat liver mitochondria, which contributes to the dissipation of the mitochondrial membrane potential and release Ca²⁺ sequestered by mitochondria.


Triethylphosphinegold chloride (TEPAu) is highly cytotoxic to suspensions of isolated rat hepatocytes. The purpose of these investigations was to determine if disruption of cellular thiols is an important mechanism in TEPAu-induced cell injury. TEPAu (500μM) caused rapid intracellular ATP depletion and cell death (lactate dehydrogenase leakage) by 60 min. of incubation. Addition of dithiothreitol (DTT) following 15 min. of incubation with TEPAu resulted in a partial reversal of the loss of intracellular ATP and an attenuation of lethal cell injury. TEPAu-induced inhibition of both state 3 respiration and FCCP-stimulated respiration in isolated rat liver mitochondria was reversed by the addition of 2 mM DTT. This reversal was not complete as ADP did not reestablish state 3 respiration in TEPAu/DTT-exposed mitochondria. In isolated rat liver mitochondria, TEPAu caused concentrations-dependent decrease in inner mitochondrial membrane potential and caused an efflux of sequested calcium. Addition of 1 mM DTT inhibited or reversed these responses. TEPAu-induced microsomal calcium efflux was also inhibited by DTT. These data suggest that many of the biochemical changes that were observed in hepatocytes following TEPAu exposure may be mediated via interactions with cellular sulfhydryls.

ATTENUATION OF THE IN VITRO CYTOTOXICITY OF SK&F 104524 IN ISOLATED RAT HEPATOCYTES BY FRUCTOSE. P F Smith*, G D Hoke, D W Alberts, C K Mirabelli, and G F Rush. Dept. of Investigative Toxicology, Smith Kline & French Labs, King of Prussia, PA and **Mark, Sharp and Dohme Research Labs, West Point, PA

SK&F 104524 (bis-[1,2-bis(di phenylphosphino)ethane]gold(II)lactate) is an experimental anti-neoplastic agent which has been shown to be hepatotoxic. The toxicity of this agent appears to be related to its potent mitochondrial uncoupling actions. The uptake of [¹⁴C]-labeled SK&F 104524 by isolated rat hepatocytes in suspension was rapid (maximal uptake by 30 min) and concentration-dependent. Exposure of hepatocytes to SK&F 104524 resulted in plasma membrane blebbing, increased O₂ consumption, ATP depletion, and eventually, LDH leakage. When fructose (50mM) was added to the hepatocyte suspensions 5 min prior to SK&F 104524 (20μM), a marked attenuation of toxicity was observed based upon LDH leakage. By 90 min, LDH leakage was 30% greater in the cells exposed to SK&F 104524 alone. After 120 min, cell lethality was 90% in cells exposed to SK&F 104524 in comparison with 54% in the cells pretreated with fructose. Fructose did not interfere with [¹⁴C] SK&F 104524 uptake by isolated hepatocytes. Maintenance of the cellular energy charge via fructose-induced activation of AMP metabolic pathways was associated with the observed cytoprotection.
SEQUENTIAL ULTRASTRUCTURAL HEPATIC, PULMONARY AND RENAL CHANGES DUE TO MICROCYSTIS AERUGINOSA HEPATOTOXIN IN THE RAT. J F Hooser, E J Basgal, V R Reasley, W M Haschek. College of Vet. Medicine, Univ. of Illinois, Urbana, IL.

The cyanobacteria, M. aeruginosa, cause hepatotoxicity and death in animals worldwide. To determine the cellular and subcellular target(s) of the purified heptapeptide toxin, microcystin-A (cyanoginosin-LE), at a lethal dose of 160 µg/kg was injected ip in male HSD rats. At 0, 5, 10, 20, 30, and 60 min, rats were anesthetized and whole body perfusion and fixation with Tyrodes solution and 2.5% glutaraldehyde was performed. Hepatic changes began centrilobularly and progressed in extent and severity over time. By 20 min, hepatocytes had invaginations of the plasma membrane and numerous cytoplasmic vacuoles as well as blebbing and loss of microvilli along the sinusoidal face. At 60 min, there was widening of sinusoidal endothelial fenestra and the space of Disse. At 60 min, centrilobular areas contained necrotic cells and free floating, intact organelles together with RBC's and platelets. At 60 min the pulmonary vasculature contained intact hepatocytes and cellular debris. In the renal cortex, capillaries contained moderate amounts of cellular debris. These changes were compatible with primary hepatocyte cytoskeletal or cell membrane effects.

THE EFFECTS OF METHYLENEDIHYDROPHYL (MDP) COMPOUNDS ON HEPATIC MICROSONAL PROTEIN OF CS78/SMICE (III), Y C Chui, M Lowandowski, P Levi, and E Hodgson. Toxicology Program, North Carolina State University, Raleigh, NC.

The effects of butylbenzoxidole (n-BBD), tert-butylenzoxidole (t-BBD), methylbenzoxidole (MBD), nitrobenzoxidole (NBD) and n-bromobenzoxidole (BrBBD) on hepatic microsomal enzymes were examined in mice after ip administration of 200 mg/Kg/day for 3 days. MBD, NBD and BrBBD did not induce hepatic microsomal proteins and mixed-function oxidase activities. In contrast, n-BBD and t-BBD significantly induced cytochrome P-450 content and ethylmorphine N-demethylation and ethoxyresorufin O-deethylation activities. Benz(a)pyrene hydroxylase activity was not induced by either the n-BBD or t-BBD; however, acetanilide hydroxylase activity was induced by t-BBD but not n-BBD. SDS-PAGE and immunoquantification with 3-methylcholanthrene (3MC) and phenobarbital (PB) antibodies indicated that n-BBD and t-BBD were 3MC- and PB-type inducers, respectively. However, the MFO activities of n-BBD and t-BBD induced microsomes did not fit the inductive pattern of a typical 3MC- or PB-type inducer. This observation may be due to the formation of a stable type III MFP metabolite-cytochrome P-450 complex; both n-BBD and t-BBD pretreatments formed this complex which was maintained during microsomal preparation and is known to result in enzyme inhibition.

L-2-CYTOSINE-LILIDINE-4-CARBOXYLIC ACID (OTZ) PROTECTION AGAINST 1,1-DICHLOROETHYLENE (DCE) HEPATOTOXICITY IS ASSOCIATED WITH DECREASES IN TOXIN METABOLISM AND LIVER CYTOCHROME P450. M T Moslen, R F Whitehead, A E Ferguson and M F Kann. Chemical Pathology Laboratory, University of Texas Medical Branch, Galveston, TX.

Our objective was to determine if OTZ protection against DCE hepatotoxicity was associated with alterations in circulating levels, urinary clearance, or covalent binding of label derived from 14C-DCE. The working hypothesis was that OTZ, by its reported effect as an intracellular precursor of cystine and thus of GSH (Williamson, et al., PNAS, 75:6256, 1982), might hasten hepatic formation and body clearance of dechlorinated GSH-conjugated metabolites of DCE. Fasted male rats were pretreated at -1 hr with 10 umol OTZ/kg in saline or saline and at 0 hr were given 50 mg 14C-DCE/kg p.o. in mineral oil. Serum blood sampling indicated that OTZ animals consistently had lower circulating levels of DCE label. OTZ animals also had lower amounts of total and covalently bound DCE label in liver, lung, and kidneys at 24 hr which would be consistent with their diminished liver injury. However, urinary clearance of DCE label (presumably chiefly GSH-conjugate) by the OTZ animals was not faster and actually resulted in 30% less label. OTZ alone rapidly decreased hepatic GSH and P450 contents. DCE is activated by a P450-dependent reaction. This unexpected OTZ-induced decrease in hepatic P450 could contribute to both the decrease in urinary metabolites and the protection against liver injury. (Supported by NIH ES 03396 and ES 34896).

DOGRASIC PHOSPHATE (Pi) AS AN INDOMENOUS INDICATOR OF 1,1-DICHLOROETHYLENE (DCE)-HEPATOTOXICITY INJURY. L Kaphalia, M F Kann, and M T Moslen. Chemical Pathology Laboratory, University of Texas Medical Branch, Galveston, TX.

Our objective was to determine if Pi would be a useful indicator of altered hepatobiliary function in vivo. We developed a method to measure Pi in 25 to 50 µl bile using malachite green Powerwave Spectrophotometer and with a previously exterminated bile duct to duodenal fistula and a jugular cannula were used to collect serial bile samples before and after 50 mg DCE/kg p.o. in mineral oil. Bile flow was maintained by transected in infusion. Some rats were fasted overnight to enhance DCE-induced alterations of cannula integrity and hepatotoxicity. Concentrations of Pi in control bile were 100 times lower than serum Pi concentrations which indicates Pi exclusion from bile. Hepatobiliary function was also monitored in bile by exclusion markers (phenolphthalaimine glucuronide [PG] and homovanillic peracidase [HPF]) given i.v. DCE did not appreciably alter biliary Pi concentrations in fed rats which developed little hepatic necrosis but showed alterations in biliary clearance of exclusion markers. In contrast, biliary Pi concentrations gradually increased up to 10 times in fasted rats which showed pronounced early changes in biliary secretion of exclusion markers and developed moderate hepatic necrosis within four hr of DCE administration. These findings indicate that biliary Pi is a useful endogenous indicator of hepatobiliary dysfunction associated with hepatic necrosis. (Supported by NCI grant AM-24806).

A number of compounds have been shown to produce liver enlargement and hepatic peroxisome proliferation in rats and mice but controversy exists as to the response of man and other primates. We have investigated the hepatic effects of napropalin (2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl) phenoxo] proplionic acid) in male Sprague-Dawley rats, Syrian hamsters, Dunkin-Hartley guinea pigs and marmosets. Napropalin was administered orally for 21 days at dose levels of 0, 5, 10, 15 and 50 mg/kg/day (rat), 5, 25 and 250 mg/kg/day (hamster) and 50 and 250 mg/kg/day (guinea pig and marmoset).

With the rat, and to a lesser extent in the hamster, napropalin treatment produced dose related increases in liver size and peroxisome numbers and induction of peroxisomal (palmitoyl-CoA oxidation) and mitochondrial (lauric acid 12-hydroxylation) fatty acid oxidising enzyme activities. In contrast, in the guinea pig and marmoset, there was no effect on liver size and only comparatively small changes were observed in enzyme activities. These results demonstrate marked species differences in response to napropalin, a known potent peroxisome proliferator in the rat, and suggest that primates may be less susceptible than the rat to peroxisome proliferators. (Supported by the UK Ministry of Agriculture, Fisheries and Food)

877 THE INDUCTIVE EFFECT OF MIREX AND CHLORODECON ON THE CYTOCHROME P-450 MIXED-OXYGENASE SYSTEM. M Lewandowski, P Levi and E Hodgson. Toxicology Program, North Carolina State University, Raleigh, NC.

The effect of the insecticides, mirex and chlorodecon (Kepone) on the cytochrome P-450 monooxygenase system in C57BL/6J C57 mouse liver microsomes was studied in vivo. Mice received 1 p.p.m. with low (6 mg/kg) and high (30 mg/kg) doses of mirex and Kepone in corn oil. For comparison, mice were also treated with phenobarbital (PB) and 3-methylcholanthrene (3-MC). The cytochrome P-450 content, ethoxyresorufin O-deethylase, benzo(a)pyrene hydroxylase and acacetanilide hydroxylase activities were determined and P-450 isozyme induction was determined by immunoquantitation using monoclonal antibodies. In high doses, both mirex and Kepone significantly induced the cytochrome P-450 content and the enzyme activities over that of control. Similar results were also observed in the low dose group but the amount of induction was less. This data shows that the induction of enzyme activities by mirex and Kepone on mouse liver is similar to the induction by PB. These results were confirmed by SDS polyacrylamide gel electrophoresis and immunoquantitation with monoclonal antibodies.


Sponsor: Dr F A de la Iglesia

PD 119819 (7-[3-[4-(2-pyridinyl)-1-piperazinyl] propoxy]-4H-1-benzopyran-4-one) has been identified as a dopamine auto receptor agonist and has shown excellent antipsychotic profiles in pharmacology screenings. An exploratory rising dose study was completed in male and female cynomolgus monkeys with the protocol design including doses of 5, 10, 20, 40, and 80 mg/kg given for a minimum of 2 days over a 13-day period. Both animals appeared depressed after doses 50 and 80 mg/kg with doses of 80 mg/kg also resulting in tremors and dehydration. Biochemical parameters indicating hepatic cellular damage (elevated ALT, SDH, and OCT) were noted after doses of 5 mg/kg and greater. BUN and creatinine levels were increased after doses of 40 and 80 mg/kg. Structural changes in the liver consisted of multifocal unicellular hepatocyte necrosis associated with needle-like crystals in yosomes and bile canaliculi. The kidneys showed proximal tubular degeneration and inflammation associated with the presence of crystals in the lumen and cells of the proximal tubules. The localization of drug crystals in the proximal part of the nephron contrasted with other poorly soluble drugs such as sulphonamides and purine analogues which form crystals mainly in the distal nephron where most concentrated urine is formed.


To determine the appropriateness of the liver/spleen scan, a human hepatic functional assay, for measuring irreversible liver damage in animals, the assay was modified and tested in rats. Seventy-day-old Fischer 344 rats were exposed to carbon tetrachloride (CC14) in corn oil by gavage at 0, 20 or 40 mg CC14/kg five days a week for 12 weeks and sacrificed 1, 8, and 22 days later. The rats were anesthetized and injected iv with 10 uCi (3.7x10^6 Bq) of technetium 99m as the sulfur colloid. Ten minutes after the animals were perfused for 5 minutes with a 0.9% NaCl solution. The liver, spleen, lung and kidneys were removed, samples taken and counted in a gamma counter. Tissue samples from the liver were also analyzed microscopically for damage. Damaged tissue was examined after the exposure to the liver/spleen ratio (counts/g tissue) was lower in the highest dose group, while the liver/tongue and the liver/kidney ratios showed no change. The liver/ spleen ratio returned to that of control levels by day 8. This recovery corresponds with the return of the serum enzymes to control values (see poster by Allis et al.). (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

Hepatotoxicity and recovery of male F344 rats from subchronic gavage with carbon tetrachloride (CCl4) has been assessed. Rats were dosed 5 days per week for 12 weeks with CCl4 at 0, 20 or 40 mg/kg body weight and sacrificed 1, 3, 8, and 15 days later. On day 1 post-exposure, liver-to-body-weight ratio, alanine aminotransferase (ALT), aspartate aminotransferase, and lactate dehydrogenase increased and cytochrome P450 decreased in a dose-dependent manner. Alkaline phosphatase and cholesterol showed significant increase only at 40 mg/kg. On day 3 post-exposure, each of these endpoints was significantly different for the high dose group compared to controls, but only ALT differed at 20 mg/kg. By days 8 and 15, all endpoints returned to control levels except for liver-to-body-weight ratio, which remained significantly elevated. This elevation persisted through day 22 post-exposure (see accompanying paper by Ward et al. showing a measure of irreversible liver damage). These results indicate that CCl4 is hepatotoxic when administered orally at low doses over an extended period; however, based on our parameters hepatocyte damage disappears within one week after cessation of exposure. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

ACUTE TOXICITY OF MICROCYSTIN-LR IN THE RAT: A COMPARATIVE DOSE-RESPONSE STUDY USING SERUM CHEMISTRIES AND MORTALITY AS INDICES. R D LoClare, W B Lawrence, K A Bostian and K A Mereish. United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD. Sponsor: R W Wannemacher.

The purpose of this study was to investigate the dose-response relationship between the acute hepatic injury produced by microcystin-LR and temporal alterations in select serum chemistries. Groups of 6 male, Fischer rats were given intraperitoneal (I.A.) doses of microcystin-LR at 0, 12.5, 25, 50, 100, or 200 μg/Kg body weight. Serum was harvested from 250 μl blood samples at 0, 0.5, 1, 2, 4, 8, 16, 24, 32, 48, and 72 hours post dosing. Core body temperature was monitored continuously and tissue samples were collected at time of death or at 72 hours post dose. Histological lesions noted included: hepatic centrolobular hemorrhagic necrosis; pulmonary emboli; myocardiotic degeneration and necrosis; and renal nephrosis. The 72 hour I.A. LD50 was 50 μg/Kg with a 95% confidence interval of 36 to 68 μg/Kg. Mean time to death at the LD50 was 30.4 hours with a SEM of ± 6.4 hours. Serum enzyme elevations indicated hepatocellular injury as early as 0.5 hours. Serum idiol dehydrogenase (SID) activity elevations proved to be the most sensitive index of hepatic injury, with a median effective dose ED50 of 25 μg/Kg and toxicity ratio of 2.00. Serum alanine amino transferase (SALT) activity increased with a ED50 of 31.5 μg/Kg and toxicity ratio of 1.59. The slope of the dose-response curve for SID was twice as steep as that of SALT. The incidence and severity of toxic responses observed were dose-related.


The administration of either ETH or Cd produce toxic effects in liver. The present study examines the interaction between these hepatotoxicants. Rats received 7g ETH or 10 ml H2O/kg po 24 hr prior to the administration of 4.5 mg Cd/kg iv. Within 24 hr of receiving Cd, all control rats (n=6) died whereas all ETH-pretreated rats (n=6) survived. The reduced lethality has been proposed to result from liver injury, therefore the effect of ETH on Cd-induced hepatotoxicity was studied. ETH pretreatment decreased Cd-induced serum transaminase activities by about 90%. The protective effect of ETH cannot be attributed to a decreased hepatic content of Cd, as the biliary excretion of Cd (1 mg/kg, iv) was decreased 98% and total hepatic Cd content increased 31% in ETH-pretreated rats. ETH, however, altered the subcellular distribution of Cd as its concentration in nuclear, mitochondrial and microsomal fractions was decreased about 60% while a 90% increase was noted in cytosol of ETH-pretreated rats. ETH also altered the distribution of Cd in the cytosol. In hepatic cytosol, ETH pretreatment decreased the percentage of Cd bound to high molecular weight proteins (65 to 5%) and increased Cd bound to metallothionen (MT, 34 to 95%), a cysteine-rich, metal-binding protein. The concentration of MT in liver was elevated 11 fold by ETH. In conclusion, ETH protects against the hepatotoxicity of Cd. The mechanism of protection appears to be due to induction of MT which sequesters Cd and decreases its accumulation in critical organelles and proteins of the liver. (Supported by USPHS Grant ES-01142 and ES-07079)
THE DIFFERENTIAL EFFECTS OF HEPATOXICANTS ON THE SULFATION PATHWAY IN RAT LIVER. T J Maziasz, J Liu, C Madhu and C D Klages. University of Kansas Medical Center, Kansas City, KS.

The purpose of this study was to further examine the effects of xenobiotic-induced liver damage on the various components of the sulfation pathway in rats. Acute liver injury was produced in male Sprague-Dawley rats by the administration of hepatotoxic doses of carbon tetrachloride (CCL₄), dichloroethylene (DCE), α-naphthylisothiocyanate (AIN'T), aflatoxin B₁ (ATX), allyl alcohol (AA), bromobenzene (BB), cadmium chloride (Cd), or thioacetamide (TA). Liver damage was confirmed by measuring serum sorbitol dehydrogenase and alanine aminotransferase (ALT) activities as well as by histopathological examination. Adenosine 3'-phosphate 5'-phosphosulfate (PAPS) concentrations were generally decreased by hepatotoxicants (35-80% of control), although BB and AA were without effect and ANIT increased PAPS levels to 117% of control. The decrease in PAPS levels appeared to vary directly with serum ALT activity. Activities of the PAPS synthetic enzymes, ATP sulfurylase and adenosine 5'-phosphosulfate (APS) kinase, were decreased selectively by the hepatotoxicants studied. ATP sulfurylase activity was significantly decreased by Cd and TA (55 and 62% of control, respectively), whereas APS kinase activity was significantly decreased by Cd, TA, BB and DCE (65-77% of control). In conclusion, not all xenobiotic-induced liver injury results in decreased hepatic PAPS concentrations. Moreover, the decrease in hepatic PAPS concentrations produced by some hepatotoxicants appears to occur by mechanisms other than through decreased co-substrate synthesis. (Supported by USPHS Grants ES-03192 and ES-07079).


It has been shown that after treatment with some long-acting gastric antisecretory agents, there is an increase in neuroendocrine cell numbers and mucosal height in rat oxyntic stomach. To ensure accurate evaluation of mucosal changes, it is necessary to sample representative areas of the stomach.

Neuroendocrine cells were identified using the immunocytochemical demonstration of Chromogranin A and the areas of positivity and mucosal height were quantified using an image analysis system (Quantimet 920). By sampling across the whole fundic and pyloric mucosa, it was possible to construct a 3-D graphical representation of Chromogranin positive and mucosal height for each stomach studied (n=4).

Results showed a difference between fundic and pyloric mucosa in both parameters, although the variation within these areas was not marked. The cardiac region showed less neuroendocrine cell positivity.

Therefore to quantify increases in neuroendocrine cell numbers and mucosal height it is necessary to consistently sample areas of stomach from animal to animal.

THE EFFECTS OF ALPHA,BETA-UNSATURATED ALDEHYDES (ABUA) ON THE LIVER. G Witz, K O Cooper, K R Cooper and C Mitmer. The Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ.

In vitro studies suggest that ABUA may be mediators of hepatotoxicity in vivo. Male rats were given a single dose of muconaldehyde (MUC) (36umole/kg), acrolein (ACR) (89umole/kg), crotonaldehyde (CRO) (450umole/kg) or the saturated aldehyde, propionaldehyde (PRO) (85umole/kg), and sacrificed 30 min, 4 hrs or 24 hrs later. At 4 hrs after dosing with either MUC, ACR or CRO, hepatocrits were 128, 144 and 128% of control, respectively, and returned towards control values at 24 hrs. There were slight increases in serum ALT (127-259% of control) and AST (170-350% of control) at 24 hrs following dosing with ABUAs but not at 30 min or 4 hrs. PRO had no effect on hepatocrit or these serum enzymes at any time. Livers from animals treated with ABUAs and sacrificed 24 hr later were stained with hematoxylin and eosin and then examined by light microscopy. In these livers there were minor focal hepatocellular necrosis and congestion of the sinusoids with red blood cells. In addition all the hepatocytes had clumped cellular organelles and stained basophilic. No significant lesions were observed in either control or PRO treated animals.

EFFECTS OF LONG-TERM DIETARY RESTRICTION ON HEPATIC DRUG METABOLIZING ENZYMES (DME) IN RATS. K Phiggs, E Gralchen, R Goldstein and T Leonard, Smith Kline & French Laboratories, Swedeland, PA.

Dietary restriction increases life-span and decreases age-related disease in rodents. The effects of long-term reduced food or protein intake on hepatic DME were investigated in male Fischer-344 (F) and Sprague-Dawley (SD) rats. Rats (2.5 months of age) were fed Purina 5002 rat chow (20% protein) (P) or an isocaloric reduced protein (12%) chow (RP), ad libitum, or Purina 5002 rat chow for 6.5 lighted hours daily (RE). Hepatic microsomes were isolated at 6,12 or 18 mo of age. At 6 mo cytochrome P450 (P450) content was similar in all diet groups in both strains of rats. At 12 mo, P450 content in microsomes from F rats had decreased to 61%(P), 59%(RP), and 78%(RE) of the 6 mo value. By 18 mo, P450 decreased to 67%(P), 45%(RP), and 61%(RE) of the 6 mo values. P450 content remained constant at 12 mo in SD rats; however, by 18 mo levels decreased to 63%(P), 82%(RP) and 71%(RE) of the 6 mo value. Ethoxycoumarin and benzphetamine metabolism mirrored this trend. No significant differences between diet groups were noted in hepatic epoxide hydrase activity or glutathione content at 12 or 18 mo. These data demonstrated that hepatic P450-dependent metabolism was reduced in aging F and SD rats, while dietary restriction had minimal effects on hepatic DME activities.
Our previous studies revealed that CCl4 is less acutely hepatotoxic in rats when given in corn oil than as an aqueous emulsion or in water, due to delayed and diminished GI absorption from the corn oil. In the current investigation, male Harlan S-D rats were given 100 mg CCl4/kg by gavage in either corn oil or a 1% Emulphor aqueous emulsion once daily for 1, 2 or 4 weeks. Blood was collected 24 hr after the last dose for measurement of serum enzymes, and liver samples taken for measurement of lipid peroxidation and triglyceride (TG) levels in liver homogenates and covalent binding of C-CCl4 and enzyme levels in microsomes. Effects on levels of serum and hepatic microsomal enzymes were quite similar in the two vehicle groups after 1 week of dosing, and slightly greater in groups given CCl4 in corn oil than in aqueous emulsion after 2 and 4 weeks. Hepatic TG levels were higher in the control group given corn oil than in other controls. Hepatic TG levels were significantly higher in groups given CCl4 in corn oil than in aqueous emulsion at 1, 2 and 4 weeks. Our findings suggest that differences in lipid accumulation in the liver could cause altered tissue deposition of CCl4, and thereby responsible in part for the apparent increase over time of exposure in potency of CCl4 given in corn oil. (EPA CR812267)

The pharmacokinetics of molsidomine (M) and its pharmacologically active metabolites 3-morphfolinosydoninone (SIM 1) in nine patients with decompensated hepatic cirrhosis were compared with a control group consisting of four healthy patients with coronary heart disease. The antipyrin clearance (CL(Ant)/app) was chosen as a measure for the microsomal activity of the liver and correlated with clinical chemical parameters, including cholinesterase activity (OE). In the cirrhosis patients (n = 9), maximum plasma M levels were attained between 0.75 to 2 hours after application (23.8 to 82.8 ng/ml). The terminal halflife of M (t 1/2 tau) is greatly raised (7.18 and 11.68 hours respectively) in only two patients with high-grade cirrhosis (CL(Ant)/app = 0.5 and 1.4 mL/min; OE = 1.51 and 0.81 kU/l). The corresponding values (t 1/2 tau = 1.93 to 5.07 hours) of the remaining cirrhosis patients (CL(Ant)/app = 12.3 to 24.1 mL/min; OE = 4.71 to 24.2 kU/l) are comparable to those of the control group (t 1/2 tau = 1.20 to 3.88 hours). Patients with a CCI4/CH5-induced toxic liver damage of moderate severity did not show any significant impairment of metabolism of M and thus no effective underdoeage of the metabolite SIM 1.

The aim of the study was to determine the hepatic zonation of GS and various enzymes. Digi-tonin and other sugars can selectively permeabilize the plasma membrane of the cell without affecting the ultrastructure of the cell. In addition, understanding and the validation of the result could be appreciably extended by tracer experiments using 14C glycocholate. The data indicates that cytoplasmic enzymes can be investigated just as efficiently as in microdissection studies. The intracellular compartmentation can be determined of the comparison of the different releases of cytosolic and mitochondrial marker enzymes. Periplasmic (gp) and penicilcon (pv) hepatocytes are distinguished. GS plays a major role as a cytozyme in numerous cellular processes. The importance of reduced glutathione (GSH) in preventing accumulation and toxic effects is known, and it even detoxifies reactive intermediates formed intracellularly, either spontaneously or enzymatically. The enzyme pattern of GSH, GSH 5-transferases, ALAT, SHG, and glucoritride has found great significance for metabolism and biotransformation in the liver. By comparing the distribution of GSH to known zonal markers, we determined that GSH has a proportional portal distribution, and we termed this zone I.
SERUM AND HEPATIC RETINOID REDUCTION AND MORPHOLOGIC CHANGES INDUCED BY 3,4,3'-4'-TETRACHLOROBIPHENYL (TCB) IN THE RAT LIVER. A. Brouwer* and S K Durham,* Univ. of Wageningen, The Netherlands and Hoffmann-La Roche, Nutley, NJ 

Sponsor: W H Haliwell

The distribution of TCB and induced effects on serum and hepatic retinoid content, and liver morphology was studied by LSC, HPLC, light microscopic autoradiography and transmission electron microscopy. Adult female WAG/Rij rats received either 15 mg TCB or 200 mg H-TCB (2 mCi) / Kg BW by IP injection and were studied at 1, 3, 7 and 14 days. There was a persistent increase over time in the amount of H-TCB present in the liver and > 90% was parent compound. The parenchymal cell had the largest percentage grain distribution in autoradiographic studies accompanied by a gradual increase in the grain distribution in sinusoidal cells over time. Serum and hepatic retinol contents were significantly decreased by day 3. These biochemical changes were accompanied by morphological changes in the mitochondria and endoplasmic reticulum of parenchymal cells, and the distribution and quantity of lipid droplets in liver cell populations. The results of this study indicates that TCB treatment induces a marked reduction in serum and hepatic retinoid content and that minimal shifting of TCB between hepatic cell populations occurs over time.

EFFECT OF 2-NAPHTHYL ISOTHIOCYANATE AND CCL4 INTERACTION ON HEPATOCYTE DAMAGE. W D Zinerman, V Prakash and A Agarwal. Toxicology Research and Training Center, John Jay College of CUNY, New York, NY. 

Sponsor: H H Hemberg

Male Sprague-Dawley rats were administered α-naphthyl isothiocyanate (ANIT) at doses from 50 to 400 mg/kg ip in corn oil vehicle. The animals were sacrificed 24 hr after ANIT administration. Mitochondrial and microsomal 45Ca uptake, ATPase and cytochrome P-450 were measured. SGPT and serum bilirubin were also determined. Significant decreases in microsomal 45Ca uptake and cytochrome P-450 were observed. ATPase in mitochondria increased significantly. Serum bilirubin and SGPT increased significantly at all doses of ANIT. Groups of animals also received 200 mg/kg ANIT and 24 hr later were given CCl4 ip at doses of 0.5, 1.0 and 1.5 ml/kg. Potentiating effect of ANIT on CCl4 induced liver damage was evident in the form of 50% lethality in ANIT 1.5 ml CCl4 group. Only 2.5% lethality was observed in the group receiving only 1.5 ml CCl4/kg. Intrahepatic cholestasis produced by ANIT makes the liver increasingly susceptible to CCl4 toxicity.

CELLULAR FUNCTIONS OF PRIMARY HEPATOCYTE CULTURE FROM RATS FED HIGH SELENIUM DIETS. L A Doody, L R Shull. Department of Environmental Toxicology, University of California, Davis, CA.

Since the liver is a target organ of selenium (Se) intoxication, primary cultures of hepatocytes were chosen as an experimental tool for assessing biochemical indices of toxicity for both organic and inorganic forms. Fisher 344 male rats were maintained on 4 ppm Se as selenomethionine, selenocysteine, or selenite in a Torula yeast basal diet. Chow and basal diet controls received a nutritional level of selenite (0.1 ppm). Hepatocytes isolated from animals of each treatment group were 85-95% viable as determined by trypan blue exclusion. Hepatocyte attachment to the matrix, as determined by DNA content, was highest in the selenite group after 24 hours in culture. Other measures of cell viability over time in culture were ATP production and lactate dehydrogenase release. The latter was significantly increased for all high Se diets at 15 hours, however levels became similar at later times. The most significant effect was a 50% reduction in protein synthesis in all Se treatment groups. 7-OH coumarin glucuronidation, but not sulfation, was increased 2-4 fold during ethynocoumarin metabolism in the high selenite group only.

VALPROIC ACID HEPATOTOXICITY IN RAT, PIG AND HUMAN LIVER SLICES. R Fisher, A J Gandoiff, H Nau, and K Bredeel. Department of Pharmacology, University of Arizona, Tucson, AZ.

Valproic acid (VPA) is an anticonvulsant used in the treatment of epilepsy. Its use results in severe, sometimes fatal, hepatic injury in a small portion of the patients. In order to characterize this toxicity, we incubated 100, 300 and 500 μM of VPA with precision-cut liver slices obtained from Sprague-Dawley rats, 4 wk-old domestic pigs and human livers aquired from the Arizona Organ Donor Bank. After the liver slices were incubated for 2, 4, 6, 12 and 24 hrs, viability was analyzed by intracellular K+ content and LDH leakage. Only slight toxicity was observed with Sprague-Dawley rat liver slices i.e. only 500 μM VPA at 12 and 24 hrs showed significant differences from control. In slices obtained from 4 wk-old domestic pigs, a more severe toxicity was observed, in that the 100 μM dose of VPA was significantly different from control at 5 hrs. When the three doses of VPA were incubated with human liver slices, two different VPA hepatotoxicity profiles were observed. 60% of the human livers we tested gave VPA hepatotoxicity profiles like that of the rat. The remaining human livers (40%) gave toxicity profiles comparable to the 4 wk-old pig. This seems to indicate a predisposition to valproate hepatotoxicity in certain patients.
Pretreatment of rats with ketones and their metabolites potentiate the cholestatic responses of taurocholate (TLC) and a combination of manganese-bilirubin (Mn-BK). Experiments show that an hepatic protein(s) is probably implicated. Pretreatment of rats with MBK, TMP, p-dichlorobenzene (DCB) was investigated. TMP and DCB were included because they affect alpha-2-globulin, a male-specific protein in the rat. Male and female rats were given MBK, TMP or corn oil (vehicle) for 3 days; male rats received DCB for 3 days. On day 4 the rats were anesthetized with urethane (1 g/kg), the bile duct and a femoral vein cannulated. Mn (4.5 mg/kg), BR (10 or 15 mg/kg) or TLC (10 or 15 mg/kg) were injected iv and bile collected at intervals of 15 or 30 min up to 150 min. MBK and TMP potentiated the cholestatic effect of TLC in males but not in females. TMP did not affect the reduced bile flow caused by Mn-BR. DCB had no effect on TLC cholestasis. These results indicate that the substance(s) protein implicated in the potentiation of cholestasis is more prominent in male rats. The involvement of alpha-2-globulin, however, is unclear since TMP and DCB exerted dissimilar effects. (Supported by MRC and FCAR).

Ketoconazole (KC), an effective systemic imidazole antifungal agent, has been associated with symptomatic hepatotoxicity with an incidence of between 1:10,000 and 1:15,000. These results describe the biochemical and pathological effects of KC (150 or 250 mg/kg po x 21 days) administered to male Swiss Webster mice as a 0.2% gum tragacanth suspension at 10 mL/kg. At 24 h after the last dose of KC hepatic biochemical and pathological effects were examined. Liver weight, microsomal protein, cytochrome P-450 and total heme increased, whereas p-nitroanisole-O-dealkylation and glucuronol transferase decreased. Light microscopy revealed a perivascular distribution of numerous swollen cells with increased granular cytoplasm. Granules were uneven in size and irregularly distributed throughout hepatocytes. Electron microscopic studies exposed osmiophilic lysosomal inclusions of varying density. These structures were laminated through concentric arrangements of membranous matter forming a finger print pattern. This pathology, characterized as lipodosis, was attributed to enzyme inhibition by KC.

To compare the effect of fenbendazole on the liver and liver microsomal monooxygenases of goats, quail and rats, an oral dose of 25 mg/kg was administered to the animals daily for 9 consecutive days. On the 10th day, the control and the treated animals were sacrificed, and blood samples and livers collected for preparation of serum and microsomes, respectively. Determination of the activities of SDH, ALT and AST in the serum samples showed that there was no significant increase in the activities of these enzymes in the treated animals as compared to their corresponding controls suggesting no liver damage. Similarly, no significant difference in the amount of microsomal cytochrome P-450 was found between the control and the treated animals of the same species. Compared to their respective controls, the activities of microsomal benzphetamine N-demethylase and aniline hydroxylase were almost unchanged in the treated goats and rats. However, fenbendazole treatment appears to increase the activity of these two microsomal enzymes in quail. The results indicate that fenbendazole is not liver toxic to goats, quail and rats at 25 mg/kg dosage level. (Supported by PD-U-000064-03).

Occupational health standards have not been established for sulfur mustard (bis(2-chlorethyl)-sulfide), a strong alkylating agent with known mutagenic properties. Seventy-two Sprague-Dawley rats of each sex, 6-7 weeks old, were divided into six groups (12/group/sex) and gavaged with either 0, 3.3, 11, 33, 100 or 300 µg/kg of sulfur mustard in sesame oil 5 days/week for 13 weeks. No dose-related mortality was observed. A significant decrease (P < 0.05) in body weight was observed only in the 300 µg/kg group; both sexes were affected. Hematological evaluations and clinical chemistry measurements found no consistent treatment-related effects at the doses studied. The only treatment-related lesion associated with gavage exposure upon histopathologic evaluation was epithelial hyperplasia of the forestomach of both sexes at 300 µg/kg and males at 100 µg/kg. The hyperplastic change was minimal and characterized by cellular disorganization of the basilar layer, an apparent increase in mitotic activity of the basal epithelial cells, and thickening of the epithelial layer due to the apparent increase in cellularity. Estimations of dose ranges for NOEL were >100 and >300 µg/kg. Supported by the U.S. Army Medical Research and Development Command contract 84PP4865.
REDUCTION OF DICETOXYSCIRPENOL (DAS) TOXICITY IN RATS BY ATROPINE AND METHYLATROPINE. D E MacIver, B H Conner and A W Rogers. Pathology Dep't. Boston Univ. Sch. of Medicine, Tufts Univ. Sch. of Veterinary Medicine, Boston, MA.

Lethality of DAS appears to be the result of cardiovascular collapse and possibly related to necrosis of intestinal and other tissues. Evaluation was made of the effects of atropine and methylatropine on DAS-induced lethality and gut necrosis. Male Fischer rats, 100-150 g, 10-20 rats per treatment group, were given an LD50 (96 hr) of DAS ip followed immediately or at 15 or 30 min and again at 4 hr by sc atropine sulfate (2.5-10 mg/kg) or methylatropine nitrate (0.1-20 mg/kg). In all 5 trials using atropine, mortality was reduced from an average of 57% in DAS-treated rats (N = 89) to an average of 25% in DAS-atropine-treated rats (N = 159; P < 0.01). In 2 trials using methylatropine, mortality was reduced to 36% (N = 160; P < 0.01). There was no consistent dose response to either form of atropine. Semi-quantitative histological evaluation was made of the small intestine from rats given an LD50 of DAS and examined at 1, 4, 8, 24 or 96 hrs. Necrosis of crypt epithelium and gut-associated lymphoid tissue, edema and inflammation of lamina propria were seen at 1 hr, progressed to greatest severity at 8 hr and had resolved at 96 hr in rats that survived. Atropine-treated (5 mg/kg sc at 0, 4 hr) rats showed less severe damage with resolution by 24 hr. The results suggest that atropine protects against cardiovascular effects of DAS that are responsible for its lethality and for the gut necrosis. (Supported in part by contract DAMD 75-C-2235, U.S. Army Med. Res. and Development Command).

TOLUENE NEUROTOXICITY IN RATS AFTER SIMULATION OF HUMAN SOLVENT ABUSE FOR 14 WEEKS. S J Gorzinski, J L Mattson, T S Geshow and H A Zimmer*. The Dow Chemical Company, Midland, MI and *Dow Corning Corp., Midland, MI.

Fischer 344 rats were epididymally exposed to 8000 ppm toluene for 14 weeks to determine the validity of rodents as animal models in solvent neurotoxicity studies. Exposure were multiple and short (15 to 35 min), adjusted according to tolerance. Rats were tested on non-exposure days. Flash evoked potentials (FEPs) over the three months became progressively slower and topographically disorganized. Mifrequency (10 kHz) tone pip auditory brainstem responses (ABR-T) showed severe loss of power and waveform detail. Click ABRs and 30 kHz ABR-Ts were much less affected. Mild but statistically significant changes also occurred in the shape of the somatosensory evoked potential (SEP). Although body weight was reduced by 23% from controls, the treated rats had clean haircoats and appeared to be healthy. No neuropathologic changes attributed to toluene exposure were seen by light microscopy. The significant visual, auditory and somatosensory changes seen in this toluene study indicates that rats are a suitable animal model for many aspects of solvent neurotoxicity studies.

CNS EXCITABILITY CHANGES PRODUCED BY ACUTE EXPOSURE TO SOLVENTS. R Dyer. Neurophysiology Branch, USEPA Research Triangle Park, NC.

Exposure to some solvents produces changes in CNS excitability in humans. Depending upon the solvent, excitability may be either increased, as defined by seizures, irritability or euphoria, or decreased, as defined by depression, disconsolant activity, coma or anesthesia. It is not clear whether subtle excitability changes may occur at exposures below those required to produce seizures, depression or other behaviorally evident signs. Further, most tests of CNS excitability require production of seizures, and therefore cannot be performed in humans. We report here a comparison of one traditional measure of excitability, pentyleneetraezol (PTZ) seizure sensitivity, with one novel measure of excitability, amplitude of peak N3 of the flash evoked potential (FEP). Decreased PTZ seizure sensitivity was detected following oral administration of toluene (2000 mg/kg), p-xylene (500 mg/kg) and styrene (2000 mg/kg). These same compounds produced decreased amplitude of FEP peak N3 at dosages of 250 mg and above for toluene & p-xylene and 2000 mg/kg for styrene. Increased seizure sensitivity was detected following i.p. administration of sulfolane (400 mg/kg), a treatment which also increased FEP peak N3 amplitude. The FEP may be a more useful method for detecting excitability changes produced by exposure to solvents, since it is at least as sensitive as PTZ seizures and may be performed in humans.

EFFECTS OF ACETYLCHOLINESTERASE INHIBITION ON SPATIAL VISION IN RATS. V K Boyes and H K Rudnell. USEPA, Research Triangle Park, NC.

Organophosphate and carbamate pesticides, acetylcholinesterase inhibitors, affect vision (Plaster and Plestar, CRC Crit Rev Tox, 6:1-23, 1978). The reported effects of cholinergic agents on spatial vision appear contradictory. In cats, acetylcholinesterase inhibitors primarily disrupt vision at low spatial frequencies (i.e., large visual patterns; Harding et al., Science 221:1076-1078, 1983), whereas in rats the cholinergic blocker, scopolamine, disrupts vision at high spatial frequencies (>0.1 cph; Fox et al., Neurobehav Tox Ter, 4:689-693, 1982). This discrepancy could result from differences in species, agents or measurement techniques. Therefore, the effects of a cholinesterase inhibitor (physostigmine, 0.4 to 0.6 mg/kg, ip) were investigated in rats over a range of spatial frequencies (0.02, 0.05, 0.1, 0.2, 0.4, 0.8 cph). Spectral amplitude of patterns on/off evoked potentials at 1 and 2 Hz stimulus rate (1P and 2F) was measured. Preliminary results showed a dose-dependent reduction of 2F, with a concomitant increase in 1P, at both low and high spatial frequencies. This evidence suggests that cholinergic action in the visual systems of the two species may be similar.
EFFECT OF VERAPAMIL ON ORGANOPHOSPHATE-INDUCED DELAYED NEUROPATHY (OPIDN) IN HENS. H A N El-Pawel, B S Jortner and N Ehrich. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

To test if calcium is implicated in OPIDN, the calcium channel blocker verapamil (7 mg/kg/day) was given to 14 adult white leghorn hens for 4 days. Phenyl saligenin phosphate (PSP, 2.5 mg/kg/day) was administered on the second day to 7 of these hens. PSP was also given to 7 hens not previously treated. Hens given neither verapamil or PSP comprised the control group. Ataxia was more pronounced in hens treated with PSP alone than in those treated with verapamil + PSP (scoring 0-5, unaffected to those with both leg and wing paralysis). Strength-duration curves of biventer cervicis nerve-muscle preparations at 28 days showed a significant increase in rheobase values for hens given PSP only (2.76 ± .36 volts, x ± SE, n=5) than for hens given verapamil + PSP, verapamil only, or controls (1.45 ± .19; 1.12 ± .05; 1.36 ± .06 volts, respectively). Muscle from PSP-treated hens was more sensitive to stimulation with acetylcholine than muscle from verapamil + PSP, verapamil alone, or control hens (threshold 10⁻⁷, 10⁻⁵, 10⁻⁴, and 10⁻³ M, respectively). Gastrocnemius muscle acetylcholinesterase levels were lowest in muscle of hens given PSP alone. This preliminary data indicates that verapamil interacts with an organophosphate inducing delayed neuropathy. (Supported by NIEHS 03364).

STEREOSPECIFIC ACTION OF THE PYRETHRIDE DELTAMETHRIN ON SODIUM CHANNELS. L D Brown and I Narahashi. Department of Pharmacology, Northwestern University Medical School, Chicago, IL.

Deltamethrin prolongs the nerve membrane sodium current in a highly stereospecific manner. This preliminary study was undertaken to determine the nature of stereospecificity and the site of action of deltamethrin in the sodium channel of neuroblastoma cells (NIE-115) using the patch clamp technique. The sodium channel tail current was greatly increased and prolonged after exposure to 10 μM deltamethrin, and was taken as a measure of pyrethroid activity. Unlike deltamethrin which has the 1R,3R,αS configuration, RU 40767, an isomer with the 1S,3S,αR configuration, caused no increase in tail current even at 50 or 100 μM. However, RU 40767 antagonized deltamethrin in a non-competitive manner, indicating that the inactive isomer binds to the sodium channel at a site different from that of deltamethrin. The separate binding sites were also implicated by the dose-response curve for the tetrodotoxin block of sodium current. Whereas deltamethrin did not change the dose-response curve with a Kd of 3 nM, RU 40767 shifted the curve to increase the Kd to 20 nM in a competitive manner. It appears that the stereospecificity is accounted for by the inability of the inactive isomer to bind to the target site of the active isomer. Supported by NIH grant NS14143.

FORMAMIDINES, LIDOCAINE AND CLONIDINE DIFFERENTIALLY ALTER AMYGDALOID KINDLING. ME Gilbert and C Mack, Northrop Environmental Sciences, RTP, NC. Sponsor: FJ Bushnell.

Electrical kindling of the amygdala and hippocampus in the rat is facilitated by the formamidines, chloridormine (CDF) and amitraz (AMZ) (50G, 1986). Local anesthetic and α-2 actions of the formamidines were compared to determine their relative contributions to formamidine enhancement of kindling. Male Long Evans rats were implanted with electrodes in the amygdala and administered lidocaine (20 or 40 mg/kg), clonidine (0.01 or 0.1 mg/kg), or vehicle, 30 min prior to testing. Afterdischarge (AD) thresholds were determined and animals stimulated once daily at a standard 200 μA stimulus intensity. Both CDF and AMZ facilitate amygdaloid kindling rate and increase AD durations. Similar effects were seen with the local anesthetic lidocaine.

Unlike the formamidines, however, lidocaine produced spontaneous seizures in 26% of treated animals. In contrast, treatment with the α-2 agonist clonidine retarded kindling development and reduced AD durations. It appears that local anesthetic rather than α-2-adrenergic properties of the formamidines contribute to enhanced seizure susceptibility in the kindling model. These data stand in contrast to the effects of AMZ on hippocampal field potentials, where excitability increases in the dentate gyrus can be mimicked by clonidine, but not lidocaine.


Pregnant Fischer 344 rats were administered 0.00, 0.01, 0.03, 0.10 mg methimazole/ml drinking water, from gestational day 17 through lactation. Pups were evaluated for physical measures of maturation, thermoregulation, flash evoked potential (FEP), motor activity and selected morphology. The same rats, as adults, were given an extensive neurological test battery (body weight, observation battery, grip strength, body temperature, FEP, auditory brainstem response to tone pips (ABR-T) and clicks (ABR-C), somatosensory evoked potentials recorded from the somatosensory cortex (SEP) and the cerebellum (SEP-C), and caudal nerve action potentials (CNAP)). Positive findings in pups included slightly decreased body weight, delayed incisor eruption, delayed thermoregulation, and FEP changes. Although a pup NOEL was not determined, effects at 0.01 were minimal. Late components of the adult FEP had increased power. Adult ABR-C, SEP, and SEP-C exhibited reduced power, increased latency and altered shape. The SEP also showed an overall smoothing. Effects were detected in adults at all doses. Thus, this developmental battery detected delayed physical maturation, alterations in neonatal nervous system function and the persistence of neonatal effects in adults.
CARBOXYHEMOGLOBIN AND HUMAN VISUAL FUNCTION.
H. K. Rudnall and V. A. Benignus. USEPA, and The University of North Carolina, Chapel Hill, NC.

Conflicting results have been reported concerning the effects of Carboxyhemoglobin (COHB) levels below 20% on absolute thresholds, discrimination thresholds, pattern detection, critical flicker frequency and visual evoked potentials. Differences in luminance levels, levels of light adaptation, COHB levels, measurement techniques, sample sizes and analyses may account for the discrepancies.

A battery of tests was designed to assess visual function at photopic and scotopic luminances for the current study. First, photopic contrast thresholds were measured for both pattern and motion detection with sinusoidal gratings. Second, photopic, pattern-onset evoked potentials were recorded. Third, the absolute luminance threshold for detecting a flash was recorded throughout the course of dark adaptation. Fourth, the contrast threshold and evoked potential measurements were repeated at a scotopic luminance level with dark adapted subjects.

Ten control and eleven exposed (19% COHB) males were tested before and after an exposure period in a double-blind study. Analysis of variance indicated that carbon monoxide exposure had no effect on contrast thresholds or absolute thresholds. Pattern-onset evoked potential amplitudes, however, were apparently enhanced by exposure at both luminance levels. The exposure effect seems to have countered an opposite effect of time-of-day or fatigue that was seen with the controls.

INTRACELLULAR RECORDING OF CA1 PYRAMIDAL CELL RESPONSES FOLLOWING EXPOSURE TO TRIMETHYLLITIN. A. R. Garber, D. L. Armstrong, M. J. Wayner, and F. Montemayor. Brain Research Laboratory, Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX.

The mechanism underlying the degeneration of hippocampal pyramidal cells in animals that have been exposed to the neurotoxin, trimethyllum (TMT), is not known. To determine the effects of TMT on membrane properties intracellular recordings of CA1 pyramidal cells in rat hippocampal slices were made before, during, and after application of various doses of the toxin. Three groups of cells (10 cells/group) were tested with either 10, 50, or 100 μM TMT. At the lowest dose tested significant changes were not observed with exposure periods of up to one hour. At the higher doses a reduction of input resistance and increased spike threshold was observed following exposure periods of 15 to 45 min. In a small percentage of cells (15%) these changes were accompanied by membrane depolarization and reduced action potential amplitude. Following a 30 min wash period partial recovery of input resistance and membrane potential was observed, but in most trials the effects were irreversible. The decrease in input resistance provides an explanation for the reduction of extracellularly recorded population spike amplitude that has been reported by several laboratories. Supported by NIH Grant RR08194.


Small daily doses of dithiobuuret (DTB) cause a delayed onset muscle paresis and a decrease in neuromuscular transmission. A single large dose of DTB, in excess of the total amount given in the small daily doses, does not cause paresis. Acute bath application of DTB, in concentrations approximating those attained in plasma with a single ip injection, decreases neuromuscular transmission. The object of this study was to assess whether acute administration of DTB, which does not cause paresis, does decrease neuromuscular transmission. Rats were treated with 25 mg/kg ip of DTB. At intervals of 1, 4, 8 and 10 hr after DTB the hemidiaphragm was removed and endplate potentials (EPPs) and miniature endplate potentials (MEPPs) were recorded using conventional microelectrode recording techniques. A significant decrease in MEPP frequency and in amplitude of EPPs evoked at a stimulation frequency of 0.5/sec was observed 1 hr after DTB; these values gradually returned to control levels by 24 hr. MEPP amplitude appeared to be reduced 1 hr after DTB, but the decrease was not significant. These results indicate that acute administration of DTB is associated with subtle changes in neuromuscular transmission at early time periods. These changes, which reverse with time as blood levels of DTB progress, may progress to more generalized observable weakness with continued administration of DTB. (Supported by NIH grant NS20683.)


Previous studies show that pretreatment of mice with CPH, a drug that inhibits secretion and synthesis of insulin (I), protects against AL-induced damage to pancreatic B-cells. At the time of AL-administration (3 h after CPH), drug-induced hyperglycemia (HG) along with CPH and its metabolite desmethylicyproheptadine (DMCPH) were present in the animals. To determine whether glucose (GL) and/or CPH and DMCPH might be responsible for protection against AL, in vitro experiments were performed using isolated mouse islets. Cultured (16 h) islets were pretreated (10 min or 3 h) with either CPH (1 or 10 μM), 10 μM DMCPH or equal mixture of the two (final conc. 10 μM) and/or GL (0, 100, 200, 300 and 400 mg/dl); followed by a 5 min exposure to AL (0.2 mg/ml). Washed islets were further cultured (20 h) then tested (1 h incubation) for GL-stimulated I release, a measure of islet cell function. Pretreatment with increasing concentrations of GL afforded a graded protection against AL-induced loss of I-release capability. The presence of CPH or DMCPH alone or in combination failed to protect islets from AL or to potentiate the protective effect of GL. The results indicate that the protective action of CPH when given to mice before AL is due to drug-induced HG and not to a direct effect of CPH or its metabolite.
DIFERENTIAL RESPONSES OF GENETICALLY IDENTICAL MICE TO STREPTOZOTOCIN TREATMENT. C L Wolfel, D L Greenman, and L C Frigerio. National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR, and Whittier Institute, La Jolla, CA.

Are obese yellow YV/- mice more susceptible than their lean non-A Littermates to induction of responses, other than hypoglycemia, by toxic insults? Female yellow A'/A (BALB/c X YV) F1 hybrid mice were compared to their agouti A/a littermates. Groups of 20 yellow(Y) and 20 agouti(A) mice were injected i.p. with 0, 150 or 200 mg/kg streptozotocin (STZ) at 4 wks of age. Induction of diabetes was assumed if blood glucose was more than 200 mg/dl. Control Y mice gained 149% and A mice 58% in body weight (BW) by 20 wks of age. Control blood glucose did not differ between the genotypes during this period. 25% of Y mice in the 150 mg/kg group were diabetic after 22 wks. None of the A mice in the 150 mg/kg group became diabetic. Of Y mice treated with 200 mg/kg, 80% were diabetic at 18 wks; all were diabetic after 22 wks. Among the A mice in this group, 55% became diabetic. BW of non-diabetic treated A mice increased similarly to untreated controls. BW of non-diabetic treated Y mice were dose-dependent. Diabetic Y mice treated with 150 mg/kg STZ gained 56% in BW, while those treated with 200 mg/kg gained only 10% and developed polyuria. Diabetic A mice treated with 200 mg/kg STZ lost 4% BW during the hold period. Thus, Y mice are more sensitive than A mice to non-carcinogenic effects of STZ.

ADRENALECTOMY ABATES SelenIUM-INDUCED HYPERGLYCEMIA. Z Mallory, J L Early, M J Mclean, and V K Nonavinakere. Florida A&M University, College of Pharmacy, Tallahassee, FL. Sponsor: R C Schnell.

This study was undertaken to investigate the significance of adrenal gland in selenium (Se) induced hyperglycemia. Male Sprague-Dawley derived rats (200-300g) were segregated into two groups. Bilateral adrenalectomy was performed on rats in group I and rats in group II underwent sham surgery. Each group consisted of 6 subgroups (N=5) which received one of the following treatments intraperitoneally: saline, saline and Dexamethasone (DMX) 2 mg/kg, Se 1.6 mg/kg, Dexamethasone 2 mg/kg, Se 3.8 mg/kg, Se 1.6 mg/kg, and Dexamethasone 2 mg/kg. Blood samples were collected before, 15, 30, 60, 120, and 180 min after treatment and plasma glucose was measured using the glucose analyzer. Selenium (3.8 mg/kg) increased plasma glucose (p<0.05) and simultaneous administration of DMX enhanced the hyperglycemic response to 1.6 & 3.8 mg/kg Se during the first hr in adrenal-intact rats. In adrenalectomized rats, simultaneous administration of Se (3.3 mg/kg) and DMX induced a transient increase in plasma glucose at 30 min. Results indicate that though an intact adrenal is indispensable for a pronounced Se-induced hyperglycemia, a supplemental mechanism involving the action of glucocorticoid could be contributing to hyperglycemia at least initially. (Supported by NIH Grant R01B110.)


Ibopamine, a synthetic dopamine analog, is under investigation for use as a cardiotoxic agent. In man, a single injection of ibopamine lowered serum aldosterone while in rats, chronic administration prevented age-related atrophy of the zona glomerulosa (ZG) which produces aldosterone. Since atrophy is associated with decreased hormone release, it was of interest to differentiate the effects of single and repeated doses of ibopamine on aldosterone secretion. Dose response and time course changes in serum aldosterone were measured in rats by RIA following a single oral dose of ibopamine. By 1 hour after dosing, 10-200mg/kg ibopamine maximally suppressed serum aldosterone compared to controls (260 ± 36 vs 111 ± 36pg/ml). Two hours after dosing, aldosterone levels returned to baseline. The effects of repeated ibopamine administration at a dose which previously prevented ZG atrophy (180 mg/kg/day) were then tested. One hour after the eleventh daily dose of ibopamine serum aldosterone levels were significantly elevated (269 ± 46 vs 401 ± 30pg/ml) compared to controls. Reversal of the initial inhibitory effect of ibopamine on serum aldosterone suggests that repeated doses of the drug facilitate function of the ZG.


The administration of 62.5 mg/kg of SRI 200-110, a calcium channel blocker, to Sprague-Dawley rats for 2 years produced a 2.5 fold increase in benign Leydig cell tumors. Doses of 2.5 and 12.5 mg/kg had no effect. The drug was not mutagenic and did not induce tumors in female rats or mice of both sexes. In another study the tumor induction was repeated and the 62.5 mg/kg dose increased FSH and LH blood levels two-fold beginning at 52 and 66 weeks after drug administration. Decreases in prolactin and testosterone to 60% of concurrent controls occurred at 52 and 78 weeks. A dose of 6.25 mg/kg did not alter blood hormone levels or induce tumors. The 62.5 mg/kg dose decreased testicular LH receptors at 104 weeks to 60% of concurrent controls with testosterone levels not being altered. A single massive dose of the drug decreased serum testosterone to 10% of controls without altering LH. This single dose decreased testosterone production by ex vivo isolated Leydig cells as induced by LH, progesterone, dibutyl cAMP and forskolin indicating a direct inhibition of testosterone synthesis acutely. In conclusion, SRI 200-110 increased serum gonadotropins which is correlated with an induction of benign Leydig cell tumors.
EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON THE REGULATION OF PLASMA LUTEINIZING HORMONE (LH) CONCENTRATION IN MALE RATS. R C Bookstaff, R W Moore, and R E Peterson. School of Pharmacy, University of Wisc., Madison, WI

The normal response to a decrease in plasma testosterone (T) is an increase in plasma LH. Yet treatment of sexually mature male rats with TCDD (50 μg/kg) lowered plasma T concentrations 3-fold by Day 7 with no change in plasma LH. To determine if TCDD-treated rats are inherently unable to increase plasma LH, rats were castrated 2 hours after dosing. In the absence of gonadal steroids, TCDD treatment did not allow the increase in LH, which was 2-fold by Day 7. To determine if T is more potent at suppressing LH in TCDD-treated rats than in control rats, rats were dosed (100 μg/kg), castrated, and implanted with sustained release preparations of T. Seven days later, plasma T, dihydrotestosterone, and 17β-estradiol (E2) concentrations were similar in treated and control rats. However, TCDD-treated rats with subphysiological concentrations of T had plasma LH 6-fold lower than did control rats. To determine if this effect is specific for T, another group of rats was castrated and implanted with E2. While TCDD treatment had no effect on plasma E2, plasma LH was suppressed 10-fold. It appears that TCDD treatment markedly increases the potency of both androgenic and estrogenic steroids as feedback inhibitors of LH secretion. (Supported by NIH ES01332).

EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON TESTOSTERONE (T) PRODUCTION BY ISOLATED PERFUSED TESTES. J M Kleeman, R W Moore, and R E Peterson. Env. Toxicol. Center & School of Pharmacy, Univ. of Wisc., Madison, WI

Previously, we showed TCDD decreased hCG-stimulated T production in decapsulated testes and suggested this was largely due to impaired LH availability. However, decapsulated testes yield low steroidogenic rates and maximal T production cannot be estimated. Therefore, testes were removed from rats 7 days after 100 μg TCDD/kg and perfused with artificial blood containing graded concentrations of the LH analog, hCG. Testes from TCDD-treated rats secreted up to 5000 ng T/hr, 50% as much as controls and feed-restricted rats, yet 5-10X more than is needed to maintain normal plasma androgens. Intratesticular T was similarly reduced as was maximum dibutyl cAMP stimulated T secretion, suggesting no increased T storage and no receptor related defects. Pregnenolone (PREG) supported T secretion was only decreased at supraphysiological T secretion rates suggesting that TCDD-induced effects in post PREG enzymes do not decrease T production in the intact rat. However, TCDD treatment lowered OH-cholesterol supported T secretion at a slightly above normal T production rate. Thus, results with perfused testes are in qualitative agreement with those in decapsulated testes and further suggest that if LH were elevated in response to low plasma T, normal plasma androgens could be maintained. (T32 ES07015 and NIH ES01332).

EFFECTS OF SPIRONOLACTONE INGESTION ON SERUM THYROTTROPIN AND THYROID HORMONES IN THE MALE RAT. D E Semler and T M Radziszowski. Product Safety Assessment, G.D. Searle Research and Development, Skokie, IL. Spons: S C End

To determine a possible mechanism for thyroid changes induced by spironolactone, male rats were fed spironolactone admixed with diet at doses of 6, 50, and 200 mg/kg/day. At 2, 4, and 13 weeks, groups of 10 rats were sacrificed, serum levels of thyrotropin (TSH), thyroxine (T4), and triiodothyronine (T3) were determined, and thyroids were collected for histologic examination. At 50 and 200 mg/kg, serum TSH levels were significantly increased throughout the 13 week treatment period. At the same doses, serum T4 was significantly decreased at weeks 2 and 4, but returned to control levels by week 13. Serum T3 levels were occasionally statistically different from controls, however, no consistent dose-related pattern was apparent. There was a dose-related increase in thyroid gland weight. Histopathologic examination revealed the presence of dose-related thyroid hyperplasia and hyper trophy. It is hypothesized that spironolactone reduced serum T3 levels, indirectly stimulating TSH production, and that homeostatic mechanisms produced functional thyroid hyperplasia, re-establishing a normal level of serum T3 by the end of the thirteenth week of the study.

Previous studies (Mistry et al. J.P.T. 232:462, 1985) have shown that the rat kidney contains several lead-binding proteins (PbBP) which share a number of properties with receptors for other biologically active molecules. Western blots with rabbit polyclonal antibodies to the purified 12,000 dalton PbBP were used to evaluate tissue, intracellular, sex and age differences in this PbBP. Results of this study demonstrated this PbBP in only kidney and urine. PbBP was present in kidney of 19 day fetal, newborn, and adult rats with the highest apparent concentration in adult males on a per gram of tissue basis. Renal nuclear extracts from control rats demonstrated the presence of PbBP suggesting an association of this molecule with chromatin binding sites. The data confirm the kidney specific nature of PbBP and its possible association with chromatin from purified nuclei thus providing further support for the receptor-like nature of these proteins. Age and sex differences in the apparent tissue content of these molecules may aid in explaining individual differences in sensitivity to Pb. PbBP may also provide the basis for a new class of biological indicator for Pb toxicity once relationships between PbBP in urine and toxic potential are delineated.

Isolation and initial characterization of a high affinity lead-binding protein (PbBP) from rat brain. G. E. DuVal, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The cytosolic receptor for lead in rat brain has been partially purified and characterized. Sephadex fractions of rat brain cytosol containing this protein have previously been shown to attenuate lead inhibition of aminolevulinic acid dehydratase (Goering et al., J.P.E.T. 237: 220-225, 1986). Thus, the protein is known to be metallothionein by the fact that its elution volume of Sephadex DEAE ion-exchange chromatography at pH 8.6 has been used to obtain enriched samples of the protein. It is shown not to be metallothionein by the fact that its elution volume off Sephadex DEAE columns is greater than metallothionein standards, and its molecular weight and amino acid composition are different from metallothionein. These preparations were 9 mole percent cysteine and glutamate and 10 mole percent aspartate respectively with lower levels of other amino acids. SDS polyacrylamide gel electrophoresis showed the protein to have a molecular weight of approximately 19,000 daltons. N.Western blot analysis of brain cytosol using antibody to the recently purified rat kidney lead-binding protein failed to detect the protein purified from kidney in the brain indicating different antigenic determinants between these two lead-binding proteins.


The pattern and magnitude of MT mRNA accumulation in liver after Cd administration were examined in C57BL/6J mice of various ages. In adult mice, MT mRNA levels in liver depended on the dosage level of Cd and the concentration of this metal in the liver. In 7 day-old mice, administration of 2 mg of Cd/kg ip increased MT mRNA levels in liver 2 to 3-fold over those found in age matched saline-treated mice. In 28 day-old and adult mice, this Cd dosage level resulted in a 12 to 19-fold increase in the MT mRNA level in liver. Peak MT mRNA levels in these age groups occurred at 3 to 4 hrs after Cd treatment. Patterns of Cd accumulation in liver were similar in 7 and 28 day-old and adult mice; hence, ontogenic differences in MT mRNA induction in liver were not directly attributable to differences in the accumulation of the inducer in the target organ. The estimated LD50 for Cd correlated significantly with the degree of liver mRNA induction in liver after Cd treatment. Taken together, these data suggest that tissue-specific factors may influence MT gene expression and that developmental variation in Cd lethality in mice may depend on variation in MT gene expression. (Supported in part by NIH GM 32606)


As part of a study to evaluate the ability of interferon-inducing agents to also induce the synthesis of metallothionein, male CD-1 mice were treated with the synthetic polynucleotide, poly (rI-rC). Metallothionein (MT) was measured using the Cd-heme binding method described by Eaton and Toal (Toxicol. Appl. Pharmacol. 1982 66:134-142). Following a single ip injection of poly (rI-rC) at a dose of 10 mg/kg, hepatic MT increased to a maximum concentration of 80 ug/g at 24 hr and then decreased. A dose-response study revealed that maximal induction occurred at a dose of 5 mg/kg where MT levels were 7-fold higher than control levels. Pretreatment of the mice with actinomycin-D completely blocked the induction of MT. Sephadex G-75 gel filtration of hepatic cytosol from treated mice yielded a Zn peak with a relative elution volume consistent with that of MT. The bound Zn could be replaced by Cd following incubation of the cytosol with Cd. Anion-exchange chromatography yielded two additional peaks. A combination of heat-treatment and selective acetone precipitation produced material which absorbed at 260 nm but not at 280 nm. The study indicates that treatment of mice with poly (rI-rC) results in the induction of a material in hepatic cytosol characteristic of In-metallothionein.
ZINC (Zn) INTERACTION WITH ALA DEHYDRATASE (ALAD) IS REGULATED BY METALLOTHIONEIN (ZnMT) AND APOTHEIN. B A Fowler, P L Coering, and G E DuVal. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

ZnMT has been postulated to function in essential metal homeostasis. This study was undertaken to evaluate the potential of liver ZnMT and apothein to mediate the interaction of Zn with the Zn-metalloenzymes, ALAD. Purified rat liver ZnMT increased purified bovine liver ALAD activity 2-fold higher than control. Equimolar ZnCl₂ activated ALAD 3.5-fold higher than control. Fractionation via gel filtration of incubates containing ZnMT demonstrated that Zn is transferred from ZnMT to ALAD. Addition of apothein prepared under argon atmosphere to ALAD incubates containing 0.3 μM glutathione (GSH) attenuated enzyme activity to 36% of control. Following fractionation, a change in the distribution of Zn was observed; Zn eluted bound to ALAD and GSH in control incubates but in the presence of apothein, eluted as re-constituted ZnMT. In the presence of 3 μM GSH, however, Zn eluted primarily in the GSH fraction. Thus, these data suggest that 1) GSH may play a role as an intermediate in metal exchange reactions between ZnMT and ALAD, and 2) ZnMT appears to function as a regulatory protein which controls activity of Zn-metalloenzymes (e.g., ALAD) by donating or sequestering Zn to meet metal cofactor requirements of these enzymes.

THE ROLE OF TESTICULAR METAL-BINDING PROTEINS IN STRAIN-DEPENDENT RESISTANCE TO CADMIUM IN THE MOUSE. M P Waalkes, A Perantoni, M R Bhave, and S Rehm. Laboratory of Comparative Carcinogenesis National Cancer Institute-FCRF, Frederick, MD.

The nature of cadmium (Cd) binding proteins in mouse testes is unknown, though studies do imply metallothionein (MT) accounts for the high strain-dependent lesions found in the tests. The role of MT in Cd resistance was thus studied with NFS (N; susceptible) and BALB/c (B; resistant) mice. The testicular proteins were compared to liver MT isolated after zinc (Zn) treatment. A low Mt Zn, Cd-binding protein was seen in testicular and hepatic cytosol of both strains by. When these proteins were purified with reverse phase HPLC, the presence of two forms of MT in N or B liver was confirmed by amino acid analysis. However, of two forms seen with HPLC from N or B testes, neither were MT based on amino acid analysis. MT-I gene methylation was also studied. Unlike the liver gene, the testes gene of both N and B mice was highly methylated, a state often linked to quiescence. Zn exposure had no effect on testicular MT-I gene methylation in either N or B mice, though making N mice resistant to Cd. Zn did not alter levels of the testicular protein in either strain while causing marked increases in hepatic MT levels. These results indicate the low-Mt Cd-binding testicular proteins in resistant and susceptible mice are not MTs and that the MT gene may not be expressed in either strain.

DOSE-RESPONSE RELATIONSHIP BETWEEN URINARY CADMIUM AND METALLOTHIONEIN IN A JAPANESE POPULATION. T Kido, E A Shalik, H Kito, R Honda*, and K Nogata*. University of Rhode Island, Kingston, RI and *Kanazawa Medical University, Kanazawa, Japan.

The dose-response relationship between cadmium (Cd) exposure and renal dysfunction, as measured by urinary Cd and metallothionein (MT), was evaluated in a population living in the Kakashi River basin, a Cd-polluted area. Morning urine specimens were collected from 3,168 inhabitants (1,418 men and 1,750 women) who were 50 years or older. In addition, 240 specimens (110 men and 130 women) were collected from a control population. The 95% upper limit for MT in the control population was 645 and 738 μg/g creatinine for men and women, respectively. Using these values, the prevalence rates for renal dysfunction at various Cd concentrations were calculated and probit analysis was performed for the dose-response relationships. In the control population, prevalence rates for men and women were 2.3 and 3.1%, respectively. The maximum allowable urinary Cd concentration in the exposed population, at these prevalence rates, were 4.6 and 4.8 μgCd/g creatinine in the men and women, respectively. These data are similar to those reported earlier using B₄-microglobulin, which is a commonly used indicator of renal dysfunction. (Supported in part by a U.S. Public Health Service Grant No. ES03187)


The distribution of Cd was studied in adult female guinea pigs 2, 7, 15, 25 and 35 days following initiation of Cd treatment (80 ppm as CdCl₂, in drinking water). Hepatic Cd and MT levels increased during the first 25 days of treatment but thereafter remained relatively unchanged while renal Cd and MT levels increased linearly during the entire exposure period. Moreover, Cd and MT levels between day 2 and day 35 of exposure were higher in the kidney than the liver (Cd, 5-18 fold; MT, 3-8 fold). Cd in the hepatic and renal cytosol was mostly bound to the MT fraction. Cd and MT levels were also elevated in other tissues (intestine, pancreas, spleen, heart, lung) but were lower than those of the liver. Plasma MT levels, which were detected as early as day 2, were highest at day 35 of treatment. However, serum GPT levels were not significantly elevated during the first 15 days of treatment. Urinary excretion of Cd increased beginning at day 9 whereas fecal excretion of Cd was increased between day 1 to 2 but thereafter remained relatively constant. These data suggest that Cd accumulates preferentially in the kidney of subchronically-exposed guinea pigs and the high concentrations of Cd and MT attained in the kidney during the first 2 weeks of treatment is not due to their translocation from hepatic tissues following Cd-induced hepatic injury.
INCREASED METALLOTHIONEIN LEVELS DURING LACTATION. D Soleman, Chemistry Department, Duquesne University, Pittsburgh, PA. M H Bhattacharyya, Biological and Medical Research Division, Argonne National Laboratory, Argonne, Ill. J S Garvey, Biology Department, Syracuse University, Syracuse, NY.

Previous studies indicated that the absorption and retention of trace-level 109Cd by the duodenum, kidney, and mammary tissue increased markedly during lactation. This abstract reports our study to determine the possible involvement of the metallothionein (MT) in this milksecretion-enhanced Cd absorption/retention. Tissue homogenates were prepared from the liver, kidney, duodenum, and jejunum of the nonpregnant (NP) BEC7/1 ANL female mice, the mouse dams on lactation days 13 and 20 (L13 & L20), and the mice on postlactation day 5 (W5). After heat treatment to precipitate out the majority of the unwanted proteins, the concentration of the heat-stable MT in the supernatant was determined by the cadmium/hemoglobin (Cd/Hb) assay and the radioimmunoassay. The measurement of MT in the Cd/Hb assay was verified by Sephadex G-75 gel filtration. The results showed that the liver, kidney, and duodenum of the L13 and L20 mice contained from 2- to 24-folds higher MT levels than their NP and W5 counterparts. The MT levels of the jejunum did not seem to increase in milksecretion. We conclude that the MT levels of specific organs increase significantly during milksecretion and return to the basal levels after the lactation period.

METALLOTHIONEIN (MT) PROTECTS AGAINST METAL TOXICITY IN RAT PRIMARY HEPATOCYTE CULTURES. J Liu, W C Kershaw and C D Klassen, Univ. of Kansas Med. Ctr, Kansas City, KS.

MT, a low molecular weight, cysteine-rich, metal-binding protein, has been implicated in the detoxification of Cd. However, whether MT protects against the cellular toxicity of other metals has not been examined thoroughly. This study was designed to determine the effects of MT on the toxicity, uptake and subcellular distribution of 7 metals in rat primary hepatocyte cultures. Hepatocytes were grown in monolayer culture for 22 hr and subsequently treated with ZnCl2 (100 μM) for 24 hr, which produced an approximate 10-fold increase in MT concentration. Follow ing Zn treatment, hepatocytes were exposed to various concentrations of Cd, Cu, Pb, Hg, Ag, Ni or Co for 24 hr. Cytotoxicity was assessed by enzyme leakage and loss of intracellular K+ The toxicity of all 7 metals was significantly less in Zn-pretreated cells. Zn pretreatment had no appreciable effect on the hepatocellular uptake (1-24 hr) of 109Cd, 112Ag and 203Hg but markedly altered their subcellular distribution. Specifically, these three metals accumulated more in cytosol and less in the nucleus, mitochondrion and microsomal fractions of Zn-treated hepatocytes. In the cytosol of Zn-pretreated cells the metals were associated mainly with MT, while in control cells the metals were associated with non-MT fractions. In summary, Zn-induced elevation of MT in rat hepatocyte cultures protected against the cytotoxicity produced by Cd, Cu, Pb, Ag, Hg, Ni and Co. This protection appears to be due to increased binding to MT and a reduction of the amount of Cd associated with critical organelles and proteins. (Supported by USPHS Grants ES-0142 and ES-07079).

ACUTE PARENTERAL EXPOSURE TO FORMALDEHYDE (HCHO) INDUCES HEPATIC METALLOTHIONEIN (MT) SYNTHESIS IN MICE. P L Goering, Center for Devices and Radiological Health, Food and Drug Administration, Rockville, MD.

Diabetes patients are potentially at risk following inadvertent parenteral exposure to HCHO, a common dialyzer membrane disinfectant. This study examined the effect of acute parenteral administration of HCHO on induction of hepatic MT synthesis. Adult male C57bl/6J mice were administered HCHO intraperitoneally and hepatic MT was quantified by the Cd-radioassay method. HCHO (50 mg/kg) increased hepatic MT an early as 6 hr after dosing with maximal levels (25-fold increase) occurring at 72 hr. MT concentrations were elevated (15-fold) 24 hr after 50 or 100 mg HCHO/kg but not at lower dosages. Coconitants elevations in hepatic Zn and Cu content were observed. Induction of MT by HCHO 1) may reflect direct de novo synthesis since the response was abolished by pretreatment with the RNA synthesis inhibitor, actinomycin D, and 2) does not appear to be mediated by stress-induced release of corticosteroids or catecholamines from the adrenal since the response was unaltered in adrenalectomized mice. Confirmation of MT synthesis was obtained following spectral and chromatographic analysis. Thus, HCHO appears to be a direct inducer of hepatic MT and alters hepatic Zn and Cu homeostasis. These responses may have utility as biological indicators of HCHO exposure/toxicity.

Cd-METALLOTHIONEIN NEPHROTOXICITY IN INBRED STRAINS OF MICE. L E Sendelbach, W C Kershaw and C D Klassen, Univ. of Kansas Medical Center, Kansas City, KS.

Acute exposure to Cd produces hepatic and testicular injury. However, tolerance to liver injury occurs on repeated exposure as a result of induction of metallothionein (MT) which binds Cd and renders it nontoxic. In contrast, the CdMT complex is released from hepatic tissue during chronic exposure and redistributes to kidney, where renal damage is manifested. Genetic variation in the acute hepatic and testicular toxicities of Cd has been shown among different strains of mice. However, whether there exists a corresponding genetic variation in susceptibility to CdMT-induced renal damage is not known. Therefore, mice of the C3H/HeJ, C57/B1 10, B6/C3Ca and DBA/2J strains, previously shown to differ in susceptibility to Cd-induced hepatic and testicular injury, were administered 100CdMT (0.2, 0.4, 0.8 or 1.6 mg/kg, sc). Urine was collected over a 2 hr period beginning 22 hr after treatment and assayed for creatinine and glucose. Renal, hepatic and testicular tissues were examined for Cd content. Kidneys accumulated 2-3 times more Cd than liver while no detectable amounts of Cd was noted in testes. Strain differences in Cd content were not observed. Elevated urinary glucose levels and histopathological manifestations of kidney injury were apparent at the three highest dosages of CdMT, however, no differences between strains were noted. In conclusion, in contrast to the Cd-induced hepatic and testicular injury previously demonstrated in these strains of mice, CdMT-induced nephrotoxicity shows no apparent genetic variation. (Supported by USPHS Grants ES-01142 and ES-07079)

Menadione treated phenobarbital(PB)-induced hepatocytes have been used as a tool to elucidate factors involved in quinone metabolism and oxidative stress. We have noted that PB treatment increase NADPH cytochrome P-450 reductase(NR), with no increase in menadione-induced cytotoxicity. We hypothesized that PB pretreatment leads to the induction of cytoprotective mechanisms that offset the increase in NR. Microsomal superoxide anion (O2-) production and NR activity in PB pretreated vs naive male SD rats were increased over 2-fold. However, no increase in O2- production was observed in whole or sonicated hepatocytes, suggesting a cytosolic origin for the cytoprotective mechanisms. Glutathione(GSH) levels, glutathione reductase(GR) and DT-diaphorase(DT) activities were increased more than 2-fold by PB treatment. BCNU treatment led to >75% inhibition of GR activity and a subsequent decrease in the GSH/ GSSG ratio, yet it did not increase the menadione-mediated cytotoxicity as measured by LDE release. Release of DT and to a lesser extent GSH and GR may be sufficient to overcome the increase in NR activity, thus making the assumed superiority of the PB-induced hepatocyte model questionable. (Supported by ES-07045 and EPA R-811072).

DEPRESSION OF HEPATIC GLUTATHIONE BY OPIOID ANALGESIC DRUGS IN MICE. N P Soulia, R C James, R D Harbison, and S M Roberts. University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Previous studies have shown that morphine administration results in a depletion of hepatic glutathione (GSH) concentration and administration of morphine to mice potentiates the hepatotoxicity of compounds dependent upon GSH for detoxification such as acetaminophen and cocaine. In the present study, the intracerebroventricular (icv) administration of 100 μg of the opioid receptor antagonist naloxone completely abolished the GSH depression associated with a 100 mg/kg dose of morphine administered ip. This observation indicates a role for central opioid receptor stimulation in morphine-induced hepatic GSH depletion in mice. Other opioid analogues were examined for their ability to depress hepatic GSH. Intraportal administration of mu opioid receptor agonists hydromorphone (100 mg/kg), methadone (25 mg/kg), ethyldiamorphine (75 mg/kg), and 1-alpha acetylmethadol (75 mg/kg) produced a 20-35% depression of hepatic GSH. This is similar in magnitude to the hepatic GSH depletion produced by doses of morphine which potentiate hepatotoxicity. Opoid analogues with agonist effects at kappa receptors (pentazocine, 90 mg/kg) or kappa and sigma opioid receptors (nalbuphine, 100 mg/kg; and butorphanol, 100 mg/kg) had no effect on hepatic GSH when administered in maximally-tolerated doses. These observations indicate that opioid analogues with mu, but not kappa or sigma agonist effects produce hepatic GSH depression and may consequently increase liver injury resulting from exposure to hepatotoxic drugs.


Increased peroxisomal NADPH production has been implicated in the rodent hepatocarcinogenicity of non-mutagenic PPS. Since direct evidence of early oxidative stress is lacking, we investigated the effect of PPS on glutathione redox status in primary cultures of rat hepatocytes. Hepatocytes cultured for 70 hr with 100 μM cipofibrate showed a 20-fold increase in palmitoyl-CoA oxidation but little or no increase in the ratio of oxidised (GSSG) to reduced (GSH) glutathione. However, in the presence of decanoic acid (1 mM), a substrate for the NADH-generating peroxisomal 3-oxidation system, cipofibrate-treated hepatocytes showed a 3 to 4-fold increase in GSSG with no change in GSH. Increases in GSSG were proportional to the concentration of decanoic acid added and were potentiated by the GSH reductase inhibitor, BCNU. Similar results were obtained for cultures treated for 70 hr with clofibric acid (500 μM), BR-931 (50 μM) and nafenopin (50 μM). No changes were observed in conjugated diene formation or in the activities of GSH reductase, GSH peroxidase and GSH S-transferase. The observed fatty acid-dependent increases in GSSG provide evidence of early oxidative stress associated with peroxisome proliferation. (Supported by the UK MAPPF)


DNA damage following exposure to 1,2-dibromo-3-chloropropane (DBCP), several methylated analogs of DBCP and their BDCP-DNA adducts was measured by alkaline elution in isolated rat testicular cells. Of the methylated analogs studied, only the C-methyl analog caused DNA damage (10-50 μM), but it was less potent than DBCP. In both time- (0-60 min; 10 μM) and concentration- (0-10 μM; 60 min) dependent experiments, the testicular cell DNA damage produced by D1-DBCP was almost identical to the damage resulting from DBCP. In contrast, preincubation (1 hr) of testicular cells with diethylmaleate (DEM: 0.05-1 μM) prior to DBCP exposure resulted in an inhibition of the DNA damage. The decrease in DBCP produced DNA damage following DEM pretreatment was proportional to the relative decrease in cellular glutathione (GSH) induced by DEM. The ability of several methylated and deuterated analogs of DBCP to cause DNA damage in testicular cells in vitro correlates with their induction of testicular necrosis in vivo. DBCP is activated within testicular cells to DNA damaging products. Depletion of cellular GSH levels with DEM blocked this damage. Thus, it could be that DNA damage is an early event in DBCP induced cell death in vivo and that glutathione conjugation leading to formation of an episulfonium ion may be the major pathway in the in vivo testicular DNA damage and cell death produced by DBCP.
We have previously shown that conjugation of 2-bromohydroquinone (2-BHQ) with glutathione (GSH) results in the formation of several isomeric mono-substituted and disubstituted GSH adducts, the latter (2-BH-digSyl-HQ) of which is a potent and selective nephrotoxicant. As an initial attempt to determine whether or not a general mechanism may exist by which other quinones conjugated with GSH may elicit toxicity, we have synthesized the GSH adducts (s) of benzoquinone (BQ-GSH), 1,4-naphthoquinone (NQ-GSH) and menadione (MD-GSH). Administration of BQ-GSH (0.07 to 0.35 mmol/kg, i.v.) to rats caused a dose- and time-dependent elevation in blood urea nitrogen (BUN) and extensive renal necrosis with histological alterations similar to those caused by 2-BH-digSyl-HQ. In contrast, administration of the unsubstituted hydroquinone (0.2 mmol/kg, 1.2 mmol/kg, i.p.) had no effect on BUN after 24, 48 or 72 hrs. Administration of NQ-GSH (0.21 mmol/kg, i.v.) to rats also caused elevations in BUN and pathological changes in kidney identical to those of 2-BH-digSyl-HQ and BQ-GSH. However, MD-GSH (0.15 mmol/kg, i.v.) had no apparent effect on the kidney. None of these conjugates caused any alterations in SGPT concentrations and liver histology was normal. These results demonstrate that the renal toxicity caused by 2-BH-digSyl-HQ is not unique to this substrate and other, although not all, quinone-GSH conjugates are capable of producing a similar toxicity. (Supported in part by NIH grant ES 04662)


Hemoglobin (Hb) binding has been used as a biomarker for monitoring exposure to the carcinogen 4-aminobiphenyl (4-ABP). It is believed that oxidative hepatic metabolism of 4-ABP yields the N-hydroxy arylamine which enters the blood and is cooxidized with Hb, generating 4-nitrosobiphenyl (4-NOB). It has been postulated that 4-NOB may then react with Hb sulfhydryl groups forming cysteine sulfonamide adducts. We have investigated the role of glutathione (GSH) as a modulator of 4-NOB metabolism and its effect on Hb binding. HPLC analyses of incubations of GSH with [3H]-4-NOB at a ratio of 10:1, respectively, revealed one major covalent product. Further studies in which unlabelled 4-NOB was reacted with [3H]-GSH indicated the product to be a GSH conjugate. Analysis of the isolated product by FAB/MS yielded data consistent with a N-(glutathione-Syl)-4-ABP S-oxide structure. Incubation of [3H]-4-NOB with Hb resulted in protein binding. The ratio of Hb at 2.5:1 and 0.11:1 relative to Hb sulfhydryl groups decreased binding by 91, 65 and 112%, respectively. These data suggest GSH may be an important determinant in the binding of 4-NOB to Hb and, hence, a factor to be considered in the use of Hb adducts as indicators of 4-ABP exposure.

EFFECTS OF GLUTATHIONE (GSH) DEPLETION ON MODULATING CYTOSKELETAL PERTURBATION BY 1-CHLOR-2,4-DINITROBENZENE (CDBN). M F Leung and T N Chou. Dept. of Microbiology, Boston University School of Medicine, Boston, MA. Sponsor: C T Walsh

We have shown that CDBN induces microtubule (MT) disassembly in mouse 3T3 cells. Since CDBN is an excellent substrate for glutathione-S-transferase, we have investigated the role of cellular GSH content in modulating CDBN-induced perturbation to the organization of MT and microfilaments (MF) as visualized by fluorescence microscopy. Cellular GSH content was manipulated by treatment with CDBN and/or buthionine sulfoximine (BSO), an effective irreversibly inhibitor of GSH synthesis. Incubation of 3T3 cells with 2.5 μM CDBN and 250 μM BSO for 3 hr results in complete depletion of total GSH content accompanied by severe MT disassembly. BSO increases the extent of cellular GSH depletion and MT disassembly of 3T3 cells treated with CDBN alone. BSO also prevents the restoration of cellular GSH content and MT reassembly 5 hr after CDBN treatment of 3T3 cells. Exposure of 3T3 cells to 50 μM cyclohexemone which depletes GSH by conjugation with GSH, for 3 hr resulted in complete depletion of cellular GSH content without altering the MT organization. These results suggest that GSH content is important in the recovery from CDBN-mediated cellular injury and that depletion of cellular GSH alone is not enough to cause MT disassembly in CDBN-treated cells.

IMMUNOCYTOCHEMICAL LOCALIZATION OF GLUTARIMIDE TRANSAMINASE K A RAT KIDNEY CYSTEINE CONJUGATE 8-LYASE. T L Jones, C Qin, and J L Stevens. University of Maryland School of Medicine, Baltimore, MD and W. Alton Jones Cell Science Center, Lake Placid, NY.

8-Lyase has been implicated in the toxicity of a number of nephrotoxic glutathione and cysteine conjugates. The toxicity of these conjugates is largely restricted to the pars recta or S3 segment of the proximal tubule. The purpose of the present study was to determine if this segment specificity reflects the distribution of the enzyme within the nephron. A goat anti-rat kidney 8-lyase antibody was prepared and affinity purified. The specificity of the antibody was characterized by immunoprecipitation and western blot analysis. The antibody was found to cross react with the cytosolic and mitochondrial enzymes. Immunocytochemistry, using CDBN-fixed rat kidney, revealed uniform staining throughout the proximal tubule. Glomeruli, distal tubules, collecting ducts, and vascular elements were negative. Immunostaining was blocked by prior addition of purified antigen to the primary antibody. These results clearly indicate that the segment specificity of the proximal tubular injury associated with 8-lyase activation of nephrotoxic conjugates is not a result of enzyme distribution but must involve other factors.
MDR is associated with the emergence of a pattern of cross-resistance to the cytotoxic action of a wide variety of structurally and functionally unrelated antineoplastic agents. The purpose of this study was to determine if the doxorubicin (DOX)-induced MDR 8226 human myeloma cell line (R) had an increase in GSH and its associated enzymes, a pattern observed in other MDR lines, which might confer an increased ability to detoxify drugs as compared to the drug sensitive line (S). Nonprotein sulfhydryl (NPSH) content was significantly elevated in R (19.2±0.1 nmol/10^6 cells) as compared to S (12.6±0.5 nmol/10^6 cells). GST activity was assayed using CDNB and GSH as substrates and found not to be elevated in R. When GSH-px activity was measured, again no difference between R and S was found. However, when R was removed from the presence of DOX there was a steady decline in NPSH values until levels were identical to that of S and yet there was no change in the level of resistance to DOX. Thus, it appears that the GSH system is not involved in the maintenance of the MDR phenotype in 8226 myeloma cells but may be an epiphenomenon associated with the selection procedure.

*1987-88 Sterling-Winthrop fellow in Pharmacology/Toxicology. (Supported by NIH CA 17094 and CA 23078).

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**Reproductive Profiles of Sprague-Dawley Male Rats from Two Animal Suppliers.**

K. R. Bodnar, S. L. Kerstetter, and M. H. Feuston.

Nobil Oil Corporation, Princeton, NJ.

The reproductive profiles of Sprague-Dawley rats from Charles River Breeding Laboratories [Rat/Crl:COBS CD(SD)BR] and Taconic Farms [Rat/Tac: N(SD)FBR] were evaluated on postpartum days 28, 50, 75, 100, and 125 (postpartum day 0 = day of birth). Parameters evaluated included: caudal epididymal sperm count and morphology, testicular spermatid count, and reproductive organ weights (testes, epididymides, seminal vesicles, prostate, pituitary gland). Preliminary results indicate that Crl:COBS CD(SD)BR rats and Tac: N(SD)FBR rats have similar reproductive profiles. In general, organ weights increased until postpartum day 75, and then leveled off. The presence of spermatids in the testes was first noted on postpartum day 50. Spermatozoa were apparent in the cauda epididymides of all male rats on postpartum day 75; one Tac: N(SD)FBR rat had spermatozoa on postpartum day 50. Besides providing us with reference values for our historical data bank of control information, the results of this study have provided us with a greater understanding of the reproductive development of male rats. This information will assist in the interpretation of results from 90-day and reproductive toxicology studies should a test material alter the development of reproductive processes in the male rat.

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**Glutathione Depletion by Acrylate and Methacrylate Esters in Vitro: Structure Activity Relationships.**


Acrylate and methacrylate esters are α, β-unsaturated esters whose toxicity may involve in part their ability to react with tissue nucleophiles via Michael addition. Structure-activity relationships for reactivity of acrylate and methacrylate esters with glutathione (GSH), a prototype nucleophile, were investigated in the present study. Esters were incubated for up to 1 hr at 37°C and pH 7.4 with either GSH or rat red cells in PBS. The concentration of unreacted thiols was measured using Ellman's reagent. In PBS acrylate esters reacted 200 times faster than their methacrylate homologs. In the red cell system acrylate esters were 20 times more potent than their methacrylate homologs. Multifunctional esters of either series were at least twice as reactive as monofunctional esters. In a limited series of esters the chain length of the alkyl group had little effect. The nature and number of acid moieties appear to be major determinants of reactivity.

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**Methoxychlor Blocks Implantation in Rats.**

A. N. Cummings. USEPA, HERRL, DCDT, RTP, Research Triangle Park, NC. Sponsor: R. Chadwick.

The pesticide methoxychlor (MXC) blocks pregnancy if given prior to mating and is fetotoxic if given days 7-16 of pregnancy in rats. Also, MXC inhibits uterine decidualization in pseudopregnant rats suggesting a uterine effect. To determine effects on implantation, rats were dosed with MXC during days 1-8 of pregnancy and killed on day 9. While 100 mg/kg of MXC had no effect, doses of 200, 300, 400, and 500 mg/kg progressively reduced the number of implantations (# sites) and uterine weight (UW) with no effect on ovarian weight (OW). The number of corpora lutea (CL #), or BW. All doses reduced serum progesterone conc. (P). MXC administration during days 1-3 (preimplantation) at 200 or 500 mg/kg reduced the # sites and UW with no effect on CL #, OW, or serum P. Postimplantation MXC dosing (days 4-8) reduced UW (with evidence of resorptions) only at 300 mg/kg. Reduced serum P at both doses, and failed to affect the # sites, CL #, or OW. These data show that preimplantation dosing with MXC blocks implantation and that postimplantation dosing produces fetal resorptions. These effects may be attributed to MXC's estrogenic influence on uterine decidualization and a failure of maternal placentaion due to imbalanced hormonal support.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
LINDANE AFFECTS FEMALE REPRODUCTIVE FUNCTION BY IMPAIRING ESTROGEN RECEPTORS. R L Cooper, R W Chadwick, J M Goldman, G Rehnherg, K C Booth, J Hein and W K McElroy. USEPA, HEKL, KTB & GSB, Research Triangle Park, NC

The effect of the organochlorine insecticide, lindane (L1n), on female reproductive function was investigated. Fischer-344 rats were gavaged with 5, 10, 20 or 40 mg L1n/kg/day beginning at 28 days and continuing until approximately 100 days of age. Lin delayed vaginal opening and produced irregular ovarian cycles (prolonged diestrus) until approximately 80 days. By 90-100 days, most females showed regular vaginal cycles. Females were decapitated on vaginal proestrus (13-1400h). There was a dose-dependent decrease in uterine wt. & LH, FSH & prolactin conc. in the blood & pituitary. These data suggested that Lin caused a decrease in serum estradiol (E2). But, serum E2 measures did not support this and progesterone and thyroid hormone concs. were the same in all groups. Combined, these results suggested that E2 receptors were altered by Lin treatment. To test this, we treated pups from days 21-9 with Lin only or Lin and E2 (10 µg/pup, on day 28) and weighed the uterus 30 h later. Lin reduced the ability of E2 to increase uterine weight. These data suggest that Lin reduces the animal's response to E2 and that this reduced response results from altered E2Rs.

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

EVIDENCE FOR THE FORMATION OF SUPPLEMENTARY CORPORA-LUTEA IN PREGNANT AND PSEUDOPREGNANT RATS. D A Garstide* & G R Foxcroft. *SKF Research Ltd., Welwyn, UK, & Nottingham University, Sutton Bonnington, UK. Sponsor: J D Hook

In a series of experiments investigating a post-coitum contraceptive, it was observed that numbers of corpora-lutea (CL) of pregnant Sprague-Dawley (CD) rats were greater than in cyclic, unmatned control females (P<0.001).

The ontogeny of these supplementary CL (SCL) was established by recording the mean number of CL in pregnant Sprague-Dawley rats on days 1 to 9 post-coitum (day 1 being the first day post-coitum) using the gross morphological dissection of the ovary. Compared to cyclic controls, an initial significant increase in mean CL numbers was recorded on day 1 post-coitum (P<0.05) with a further significant increase between days 5 & 7 (P<0.001). This phenomena was neither Sprague-Dawley strain, nor pregnancy specific, as a similar increase was also recorded for Wistar rats and in pseudopregnant rats of both strains. Histological studies of ovarian tissue confirmed the presence of newly formed CL on day 5 and 7 post-coitum. Follicular development was also present in early pregnancy with Graafian follicles evident on day 4 post-coitum. The formation of SCL is probably initiated by a consistent physiological event and obviously has an impact on the estimation of embryonic mortality studies in this species.


During chronic peroral treatment of weanling female, Fisher 344 rats with daily injections of 0.099 µmol/kg of either 1, 1-(2,2,2-trichloroethylidene) bis (f-4-chlorobenzenetol (p,p'-DNT), (7,4-dichlorophenoxyl) acetic acid (2,4-D), or γ-hexachlorocyclohexane (Lindane, γ-HCH), the lindane treatment induced a significant 29% increase in body weight after 110 days of treatment. Further investigation with 0.5, 10, 20, and 40 mg/kg lindane confirmed a significant increase in average body weight gain at the two highest treatment levels after 10 weeks of treatment. Significantly greater food consumption was observed and the Lee Index [Body wt (g)] 0.33 x 10^n/naso-anal length (mm) indicated that rats in the highest two treatment groups were obese. A lindane-induced delay in vaginal opening, more diestrous days in the treated rats, and dose-related reductions in the weight of the uterus suggested that the difference in body weight might be due to altered ovarian estrogen secretion or to altered estrogen receptor sites in the brain. This is an abstract for a presentation and does not necessarily reflect EPA policy.

BROPİRİMİNE INDUCED NECROSIS OF UTERINE DECIDUA DURING GESTATION. D G Branstetter, T A Marks, D L Black, S M Poppe, and R D Terry. The Upjohn Company, Kalamazoo, MI

Bropirimine is an immunomodulator and interferon inducer with antiviral and anticancer activities. Oral rat gestation has caused embryotoxicity at doses which are also maternally toxic. This effect is evident after a single dose of 200-400 mg/kg on days 3-5, 7-11, and 18-19 of gestation. In order to better understand the nature of the embryotoxicity observed, pregnant rats were treated orally with doses of 200 or 400 mg/kg on days 5-19 of gestation and necropsied 24 hours later. Selected maternal organs, including uterus, implantation sites and placenta, as well as developing fetus were examined histologically. Necrosis of the uterine decidua at the implantation site was commonly observed in rats treated on days 5-11 of gestation and was thought to have played a role in the pathogenesis of the embryotoxicity observed. Recent reports indicate that the decidua plays an important immunoregulatory role in the maternal response to alloreactive fetal antigens. In addition, some decidual cells are reported to be of bone marrow origin and share some surface antigens characteristic of immunologically active cells. These findings suggest that the toxic effect of bropirimine on the decidua may be related to its immunomodulatory activity.
This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

This study evaluated the effects of inhaled TELONE II soil fumigant (1,3-dichloropropene) on reproduction and neonatal growth and survival. Groups of 30 male and female F-344 rats, approximately 6 weeks of age, were exposed via inhalation to 0, 10, 30 or 90 ppm TELONE II soil fumigant for 6 hours/day, 5 days/week, for two generations. Parental effects were limited to rats exposed to 30 ppm TELONE II soil fumigant and included decreases in body weights and histopathologic effects on the nasal mucosa of adult male and female rats. The histopathologic effects included a slight, focal hyperplasia of the respiratory epithelium and/or focal degenerative changes in the olfactory epithelium. No adverse effects on reproductive parameters for neonatal growth or survival were noted in the F1, F2, or F3 litter sizes or on adult rats. Based on these results, it is concluded that inhalation exposure of rats up to 90 ppm TELONE II soil fumigant for two successive generations did not adversely affect the reproductive and neonatal parameters evaluated.

*Trademark of The Dow Chemical Company.

THE EFFECT OF DIET AND LITTER SIZE ON THE DIFFERENTIAL ELIMINATION OF 14C-2,4,5,2',4',5'-HEXACHLOROBIPHENYL (6-CPB) FROM LACTATING MICE. B J Ring, K R Seitz, L A Gallenberg and M J Vodicnik. Medical College of Wis., Milwaukee, WI.

It has been shown that 6-CPB administered to adult female mice was eliminated to offspring more rapidly than a dose administered to weaning mice. To examine the influence of diet and litter size, female ICR mice were treated with 4 mg/kg 6-CPB as 13g weanings (W) or as adults (A). Animals were mated and placed on a control or high fat diet on d1 of pregnancy. On the day of birth, litters were culled to 2 (A2,W2) or 8 (A8,W8). Elimination of maternal 6-CPB was calculated by assessing radioactivity in offspring on d1, 3, 5, 10, and 15 postpartum (n=4-5/group). 6-CPB was eliminated more rapidly from A8 (t1/2=1.8d) than W8 (4.6d) in spite of similar tissue concentrations of the PCB. A high fat diet resulted in a slower rate of elimination from both A8 and W8, but A8 t1/2 (3.0d) remained significantly more rapid than W8 t1/2 (6.8d). On the control diet, t1/2 was increased in both A2 (12.0d) and W2 (7.3d). The high fat diet further extended the t1/2 in A2 (15.4d) and W2 (12.4d). Reducing litter size to 2 eliminated the differences in t1/2 between A and W. Exposure to a high fat diet extended the t1/2 of elimination of 6-CPB from maternal animals. Reduction in litter size had a greater influence. Differential mobilization of 6-CPB relative to its time of administration may only be observed under conditions of massive lipid depletion. (ES04008).


Caffeine is regularly consumed by pregnant women. In a study originally designed to assess the neurotoxic potential of prenatal caffeine exposure, forty female monkeys (Macaca fascicularis) were divided into three groups and administered caffeine in their drinking water at concentrations equivalent to 0, 10-15 or 25-35 mg/kg/day of caffeine seven days a week. After a period of adaptation to caffeine these monkeys were mated with untreated males. Reproductive failure in the form of stillbirths and miscarriages was observed in the treated groups. Necropsy of stillborn infants revealed that the infants' lungs were not inflated and there were indications of intrauterine stress. Although the precise cause of death of the stillborn infants could not be determined, the absence of autolytic changes suggested that they most probably died during labor or delivery. Maternal weight gain and infant birth weights decreased in a dose-related manner. These results indicate that in utero exposure to methylxanthines (caffeine and/or its major metabolite theophylline) adversely affects pregnancy outcome in the monkey.

THE BEHAVIORAL EFFECTS OF PERINATAL ORAL EXPOSURE TO PYRetroIDS ON RAT PUPS. L Sylwanowski, M C Wilson and M J Kallman. Departments of Psychology and Pharmacology, School of Pharmacy, University of Mississippi, University, MS.

This investigation was undertaken to examine the behavioral consequences of pre/postnatal exposure to a Type 1 (AmbushR) and Type 2 (PydrinR) pyrethroid. Thirty female rats were mated. Following delivery, the litters were culled to 8 pups/litter. Pups were exposed by maternal gavage pre and postnatally (until day 21) to one of the following treatments: the LD50/100 (40 mg/kg) AmbushR, the LD50/1000 (4.0 mg/kg) of AmbushR, the LD50/100 (12.5 mg/kg) PydrinR, the LD50/1000 (1.25 mg/kg) PydrinR or to the corn oil control (6 litters/treatment). Behavioral testing was done on different days of development as follows: eye-opening, righting, forepaw grasping, forelimb placing, rooting, cliff drop aversion, bar holding and auditory startle on days 1-15; locomotor activity on days 16-18; inverted screen test from day 19; radial arm maze performance days 20-24 and passive avoidance testing on days 24-25. Both AmbushR and PydrinR exposure reduced reflex capabilities and altered the sequence of normal reflex development. Motor ability was reduced as indicated by several measures and passive avoidance learning was also disrupted. These data suggest that PydrinR is more potent than AmbushR. A safe no-effect level was not observed for either formulation.
EVALUATION OF THE DEVELOPMENTAL TOXICITY OF
NO-DEHTEPOANICO ACID (NHA) IN RATS. J H Smith, P J
Wier, R W Bles and R A Scala. Exxon Biomedical
Sciences, Inc., East Millstone, NJ

NHA is composed of branched, tertiary monocylo-
xylic acid isomers which contains 70% 7-carbon
isomers (range C5-C7). The potential for
developmental toxicity of NHA was evaluated
because of structural similarities to valproic acid.
NHA was dosed to mated Sprague-Dawley rats
by gastric intubation on gestational days 6-15 at
0, 50, 250, 600 and 800 mg/kg. Damms were eutha-
nized on day 20, and fetuses examined for exter-
nal, visceral and skeletal malformations. NHA
produced concurrent dose-related maternal and
developmental toxicity at 600 and 800 mg/kg.
Maternal toxicity was evidenced by 20% mortality,
reduced body weight gain, a 50% decline in mean
food consumption and other signs of toxicity at
800 mg/kg. This dose produced severe embryo leth-
ality (80%), delayed development and 25% fetal
malformations. Maternal toxicity was associated
with 600 mg/kg consisted of decreased body weight gain and a 30% reduction of food consumption.
Developmental toxicity at 600 mg/kg was observed as a
16% incidence of embryo lethality, an increased inci-
dence of major malformations, but few signs of
delayed development or running. No statisti-
cally significant maternal nor developmental
toxicity was observed at dose levels of 250 or 50
mg/kg. Thus, NHA treatment during organogenesis
produced no evidence of developmental toxicity at
doses which were not maternally toxic.

DEVELOPMENTAL EFFECTS OF CODEINE IN LGV HAMSTERS
AND CD-1 MICE. C A Kimmel, 4C J Price, 4R B
Sleet, 4J D George, 4W C Marr, 4R E Morrissey
AND 4M A Schwartz, Research Triangle Institute, Research
Triangle Park, NC and 4NTP/NIEHS, Research
triangle Park, NC.

Codeine (COD), a widely used narcotic analgesic,
was evaluated for developmental toxicity. COD
was given bid by gavage on gestation days (gd)
5-13 to LGV hamsters (0, 10, 50, 150 mg/kg) or
on gd 6-15 to CD-1 mice (0, 75, 150, 300 mg/kg). 
Hamsters were killed on gd 14, mice on gd 17,
and fetuses were examined for external, visceral
and skeletal defects. Maternal toxicity (reduced
body weight gain) was seen at 150 mg/kg bid in
hamsters, and at 150 and 300 mg/kg bid in mice.
Developmental effects were seen in both species,
primarily expressed as increased resorptions
(150 mg/kg bid in hamsters, 300 mg/kg bid in
mice) and reduced fetal weight (50 and 150 mg/kg
bid in hamsters, 150 and 300 mg/kg bid in mice).
No significant increase was seen in the incidence
of malformations in either study, although five
cases of meningomyelocele were found in
hamsters at 150 mg/kg bid versus none in any
other other dose group. Thus, the primary
developmental effects of COD were increased
prenatal death and growth retardation. Supported
by NTP/NIEHS Contract NO-1-ES-55080.

ON NEONATAL MORTALITY OF RAT OFFSPRING WHOSE
SIRES RECEIVED METHADONE BEFORE MATING. F R
Alleva, S Takagi, and T Balazs. Food and Drug
Administration, Washington, DC.

An increase in mortality of rat offspring whose
sires were treated with methadone before mating
has been reported (Smith and Joffe, Nature 253:
202, 1975; Suyka et al., TAP 45: 797, 1978).
These workers gave male Charles River CD rats
methadone HCl at 1 to 12 days before mating
and observed in the pups an increased frequency
of small litters, decreased body weights at
birth and weaning and increased mortality from
birth to 21 days of age. The present study was
aimed at investigating this mechanism of this
mortality. We injected 29 4-month-old male CD
rats with methadone HCl at 10 mg base/kg sc
once daily for 5 days. Twelve controls
received vehicle. Thirteen treated and 10
controls mated with females at least once
during treatment (evening of 2nd day) and again
either during treatment (evening of 4th or 5th
day) and/or within 5 days after treatment
ended, resulting in 35 treated and 29 control
births. No significant differences occurred
between treated and control groups in litter
size or body weight at birth and weaning or in
mortality of pups from birth to 22 days of age
for each of the 2-4 matings pooled per male.
The number of deaths for all matings pooled were
25/400 (control) vs 39/474 (treated). These
data reveal no adverse effect in rat offspring
whose sires received methadone before mating.

TERATOLOGICAL EVALUATION OF GELLAN GUM IN
RATS. E E Osborne, H A Birnbaum,
K Robinson, C Thibault, D G Proctor,
J K Luckin, Biehler & Co., Inc.,
Montreal, Quebec, Canada. A Bailliere
Tindal, W Palm Beach, FL and Biehler & Co., Inc.,
San Diego, CA.

Groups of mated Sprague Dawley rats were
administered gellan gum (a microbial
polysaccharide with potential teratogenic
potential. The gum was incorporated in the
diet at levels of 2.5, 3.8 and 5.0% from
day 6 to 15 of gestation inclusive. At
sacrifice on gestation day 20, fetuses were
exposed for external, visceral and
skeletal malformations. No evidence of
maternal toxicity or embryolethality was
noted. No effects of treatment on pregnancy
rate, uterine parameters and overall
incidences of fetal major malformations,
and minor external and visceral anomalies was
found. There were significant intergroup
variations between the control group and the
2.5 and 3.8% gellan gum treated groups in
the incidences of minor skeletal anomalies and
between control and 2.5% group for
common skeletal variants for sternaebrae 1 to
4. These differences were not considered to
be related to treatment. The findings of
this study indicated that gellan gum was not
embryotoxic or teratogenic at dose levels up
to 5.0%.

NF, an antibiotic widely used in human and veterinary medicine, was evaluated for developmental toxicity. NF (0, 5, 10, 15 or 20 mg/kg/day, po) was administered to artificially-inseminated New Zealand White rabbits during major organogenesis (gestational days (gd) 6-19). Dams (22-27/group) were terminated on gd 30: each live fetus was weighed. Each fetus was examined for external, visceral and skeletal malformations. No significant maternal or embryo/fetal toxicity was observed at 5, 10 or 15 mg/kg/day. Exposure at 20 mg NF/kg/day was associated with maternal mortality (8%), reduced maternal wt. gain during treatment, increased maternal liver wt., increased incidence of resorptions/litter (31% for high dose vs. 1% for control) and increased incidence of malformations/litter for female fetuses (17% for high dose vs. 1% for control). Malformations included a variety of skeletal anomalies, but no specific malformation or pattern of malformations were characteristic of NF exposure. Thus, maternal and embryo/fetal toxicity was observed only at 20 mg NF/kg/day. Supported by NTP/NIEHS Contract N01-ES-55090.

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SCOP, a naturally occurring anticholinergic agent, was evaluated for developmental toxicity in timed-mated CD rats and CD-1 mice. SCOP (0, 10, 100, 450 or 900 mg/kg/day, po) was administered during major organogenesis (gestational days (gd) 6-15). Dams were killed on gd 20 (rats, 21-28/group) or gd 17 (mice, 23-32/group) and each live fetus was weighed. Fetuses were examined for structural defects. Maternal mortality occurred at 450 mg/kg in rats, and at 900 mg/kg in both species. Maternal body wt., wt. gain during treatment and gestation, and corrected wt. gain were significantly reduced at ≥100 mg/kg in rats; marginal reductions for these measures were observed at ≥450 mg/kg in mice. Fetal body wt. was not affected in rats, and was marginally reduced at ≥450 mg/kg in mice. In rats (≥450 mg/kg), but not mice, exposure to SCOP was associated with a significant increase in the % malformed fetuses/litter, primarily due to an increased incidence of short rib. Thus, in both species, developmental toxicity was observed only at doses which caused maternal toxicity. Supported by NTP/NIEHS Contract N01-ES-55090.

**EVALUATION OF THE EFFECTS OF SCOPOLAMINE HYDROBROMIDE ON FERTILITY IN RATS.** L D Anderson, J E Shaw, M E Prevo, ALZA Corp, Palo Alto, CA; F E Reno, Hazleton Laboratories America, Inc., Vienna, VA.

This study assessed the effects of scopolamine HBr when administered by subcutaneous injection to female rats prior to and during mating and during gestation and lactation at levels of 0, 1, 70 and 210 µg/kg/day. Criteria evaluated included maternal body weight, prebreeding food consumption, appearance and behavior, survival rates, mating performances, pregnancy rates, gross pathology and reproduction data; and prenatal and postnatal viability, sex and growth. Statistically significantly lower mean body weight gains during gestation (Days 0-20) and lower mean body weights during the prebreeding, gestation and lactation periods were noted in the females treated at a level of 210 µg/kg/day. These findings were considered indicative of a marginal maternal toxic effect from scopolamine HBr administration. No effects attributable to the administration of scopolamine HBr were noted in comparisons of prebreeding food consumption data, survival rates, mating performances, pregnancy rates, gross pathological findings or reproduction data. Also, no compound-related indication of embryotoxicity was noted, nor were any differences attributable to treatment noted in evaluations of the offspring.
This study evaluated the embryotoxic and teratogenic potential of scopolamine HBr when administered by intravenous injection to female rats and rabbits from Day 6 through Day 15 or 18 of gestation at levels of 0.0, 0.1, 7.0 and 21.0 μg/kg/day. Criteria evaluated for compound effect included maternal body weights, weight gains, food consumption, appearance and behavior, survival rates, pregnancy rates, and reproduction data; and offspring viability and development. No effects attributable to the administration of scopolamine HBr were noted in comparisons of maternal body weight and food consumption values, appearance and behavior, survival rates, pregnancy rates, or implantation efficiencies. Also, no compound-related differences were noted in evaluations of fetal size or development. In rabbits the incidence of resorptions was greater and the corresponding incidence of fetal viability lower in the high dose group than in the control group and, though not statistically significant, was considered indicative of a marginal embryotoxic effect. Therefore, scopolamine HBr is considered to be non-teratogenic and non-embryotoxic when administered to rats at levels up to 21 μg/kg/day and rabbits up to 7 μg/kg/day.

MAA, a teratogenic metabolite of 2-methoxyethanol, was administered by gavage to timed-pregnant rats [sperm-positive day = gestational day (gd) 0] on gd 11, 12, 13, 14 or 15 at a single dose of 250 mg/kg. Fetuses were examined for external, visceral and skeletal defects on gd 20. MAA induced no apparent maternal toxicity, yet embryotoxicity was manifested as reduced fetal body weight and increased nonlive implants per litter. The induced anomalies were phase specific with 50% of fetuses malformed after treatment on gd 11, 85% on gd 12, 100% on gd 13, and 0% on gd 14 or gd 15. Malformed paws (microdactyly, ectrodactyly, polydactyly) were the predominant external defect and occurred after treatment on gd 11, 12 and 13. Cardiovascular anomalies were observed after dam exposure on gd 12 and 13. Skeletal defects (missing or shortened fibula/tibia) predominated in fetuses from dams treated on gd 12 and 13. The results indicate critical periods of development and times of MAA exposure for induction and expression of specific malformations in Fischer-344 rat offspring.

The mycotoxin, ochratoxin A (OA), i.e., 7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3R-methyl isocoumarin-7-L-beta-phenylalanine, is a potent teratogen, in vivo. Studies were performed to determine the effects of OA on postimplantation rat embryos, in vitro. Embryos were explanted on day 10 of gestation and were cultured for 45 hr in gassed rat serum containing OA at concentrations between 0 and 300 μg/ml. Gross morphology, histopathology and protein and DNA content of embryos were evaluated. An OA concentration-dependent reduction in yolk sac diameter, crown–rump length, somites number count, and protein and DNA content was observed. OA treatment also resulted in an increase in the frequency of defective embryos. Malformations included: growth retardation, hypoplasia of the telencephalon, poor flexion, stunted limb bud development, underdeveloped sensory primordia and decreased mandibular and maxillary size. Histological examination demonstrated extensive OA-induced necrosis of embryonal mesodermal structures and neuroectoderm. Thus, the rat embryo in culture is a sensitive indicator of OA toxicity and may be useful for predicting developmental hazards associated with this mycotoxin (Supported by USDA Project 84CRSR-2-2434, USAID CRSP 02-50305-2 and AH 8830).

EG (CAS No. 107-21-1), a major industrial chemical, is teratogenic in rats and mice, po, 750-5000 mg/kg/day, and in mice only by WB aerosol exposure at 1000 and 2100 mg/m³. To define the relative importance of different routes (ingestion after grooming, percutaneous absorption, and/or inhalation) from WB exposure, pregnant mice were exposed to EG aerosol by NO (0, 500, 1000 or 2500 mg/m³) or WB (0 or 2100 mg/m³), 6 hr/day, gestational days (gd) 6–15, 30/group. Controls were exposed to water aerosol. Mice were sacrificed on gd 18. At WB–2100, reduced maternal weight gain, increased water consumption, reduced gravid uterine weight and number of live fetuses, and increased resorptions were observed. At NO–1000 and NO–2500, maternal kidney weight was increased. At WB–2100 and NO–2500, fetal weights were reduced. Fetal pooled skeletal and total malformations were increased only at WB–2100. Only rib fusions were seen at NO–2500. Fetal skeletal variations were increased at WB–2100 and NO–2500. The developmental NOEL was NO–1000; the maternal NOEL was NO–500. Previous WB results were confirmed.
The developmental toxicity of 1,3-butadiene (BDE) was assessed in CD-1 mice and Sprague-Dawley rats following inhalation exposures of 6-15 days of gestation (dg). Mice and rats were exposed for 6 h/day to 0, 40, 200 or 1000 ppm BDE. females and males, and the number and status of implants were recorded. Fetuses were examined for external, visceral, and skeletal effects. Mice exhibited an exposure-related reduction in maternal body weight and in extra-gestational weight gain (CWG). Rats did not demonstrate a significant reduction in maternal body weight, although decreases were found in the 200 and 1000 ppm groups. However, a significant increase in the incidence of supernumery ribs and reduced ossification of the skull were observed in mice for the 200 and 1000 ppm groups. This may result from maternal toxicity or stress and not from a direct effect of the chemical. Supported by NIEHS/NTP Contract 1YOIES40131.

This study was intended to assess the perinatal toxicity of n-hexane (HEX) vapors as well as to determine the age at which dams and litters could be introduced into exposure chambers following delivery. Pregnant dams were exposed to 0, 200, 1000 or 5000 ppm HEX for 20 h/day on 6-19 days of gestation (dg). Following parturition, dams with litters were placed into chambers at 1, 3, 5, or 7 days postnatal (DPN) and exposed until weaning at 21 DPN. The number, sex, and weight of live pups was recorded at 1 DPN and the viability and weight change of dams and litters was monitored throughout the exposure. The number of live offspring was not affected by prenatal exposure to HEX. Age at initiation of postnatal exposure did not affect litter cumulative weight gain (CWG) for the 0 ppm group, although CWG was reduced in litters exposed to 5000 ppm regardless of age at entry. Dam CWG was indistinguishable regardless of age at entry for 0 ppm group but CWG for dams litters at 1 or 3 DPN was significantly less than controls in all group concentrations. In summary, we have developed a successful method for inhalation testing of perinatal toxicity and concurrently shown HEX to retard weight gain of litters, but not to cause postnatal mortality. Supported by NIEHS/NTP Contract 1YOIES40131.

Inhalated commercial vinyl acetate (VA) monomer was administered in drinking water, prepared fresh daily, at nominal concentrations of 0 (control), 200, 1000 or 5000 ppm (v/v). The initial parental generation (F0) of 36 female and 18 male Sprague-Dawley rats at each dose level received VA for 10 weeks prior to mating and throughout gestation and lactation. Groups of 25 male and 25 female pups (F1) were selected as the parents of the second generation (F2) and continued to receive VA in drinking water at the same concentrations until study termination. In addition, about half of the F1 males were cross-mated with the F1 females (control females with high-dose males and vice versa). There was no observed effect on the reproductive performance of rats receiving 200 or 1000 ppm VA in drinking water. At 5000 ppm there was an equivocal effect on male fertility. This is suggested since there was a slight, but not statistically significant reduction in the number of F2 litters in the high-dose group. Also in the cross-mating study there was a slight reduction in the number of litters produced by control females mated to high-dose males. However, histopathological examination of F1 male reproductive organs revealed no treatment-related abnormalities.

This study was intended to assess the perinatal toxicity of n-hexane (HEX) vapors as well as to determine the age at which dams and litters could be introduced into exposure chambers following delivery. Pregnant dams were exposed to 0, 200, 1000 or 5000 ppm HEX for 20 h/day on 6-19 days of gestation (dg). Following parturition, dams with litters were placed into chambers at 1, 3, 5, or 7 days postnatal (DPN) and exposed until weaning at 21 DPN. The number, sex, and weight of live pups was recorded at 1 DPN and the viability and weight change of dams and litters was monitored throughout the exposure. The number of live offspring was not affected by prenatal exposure to HEX. Age at initiation of postnatal exposure did not affect litter cumulative weight gain (CWG) for the 0 ppm group, although CWG was reduced in litters exposed to 5000 ppm regardless of age at entry. Dam CWG was indistinguishable regardless of age at entry for 0 ppm group but CWG for dams litters at 1 or 3 DPN was significantly less than controls in all group concentrations. In summary, we have developed a successful method for inhalation testing of perinatal toxicity and concurrently shown HEX to retard weight gain of litters, but not to cause postnatal mortality. Supported by NIEHS/NTP Contract 1YOIES40131.
TREATABILITY EVALUATION OF 3-CHLOROPHENOXYPROPIONIC ACID (CPA) BY GAVAGE TO NZB RABBITS. L C Fisher, R W Tytus, T A Savine and J M Charles. Bushy Run Research Center, Export, PA and Home-Poulenac Company, Research Triangle Park, NC.

CPA (CAS No. 101-10-0) is a plant growth regulator used on pineapples. Timed-pregnant NZW rabbits were gavaged at 0, 25, 1000 or 200 mg/kg/day on gestational days (gd) 6 through 18. At sacrifice on gd 29, fetuses were examined for malformations and variations. At 200 mg/kg/day, maternal weight gain was reduced for gd 6-12 in most does. There were no other maternal effects and no effects on gestational parameters, including implantations or fetal body weight/litter. There were no effects on fetal external, skeletal or total malformations or variations. There was an increase in the incidence of fetal visceral malformations at 200 mg/kg/day involving asymmetric eye and alterations in the anterior cerebral hemisphere and cranial nerves V and/or VII on the same side. Seven fetuses from 5 litters (out of 16 litters total) exhibiting this defect came from does with only marginal weight gain effects at gd 6-12. Therefore, administration of CPA during organogenesis to NZW rabbits resulted in slight maternal toxicity and teratogenicity at 200 mg/kg/day. The NOEL for maternal and developmental toxicity was 100 mg/kg/day. CPA is sold in an 8% formulation that is further diluted for field use. CPA does not pose a significant risk to workers following label precautions.


The organophosphorus insecticide malathion and its metabolite, malaoxon, are teratogenic in Xenopus laevis embryos. Xenopus eggs were exposed to the vehicle or test compounds during the first 4 days, the period of organogenesis. Both compounds produced the following defects in a dose dependent manner: reduced size, bent notochords, enlargement of the atria, abnormal gut, abnormal pigmentation and lowered NAD+. Notochords were bent downward between the third and sixth somites with concurrent compression of the somites. Duodenal diameter was increased with a concurrent reduction in intestine length. Unreated embryos undergoing normal development treated on the fourth day were as severely affected as those exposed the entire 4 days, indicating that the fourth day is the most critical time. When tryptophan was administered along with the insecticide, NAD+ levels were measured at control levels or above, yet severity of defects and their rate of occurrence was not reduced. The teratogenic defects produced by these compounds are similar to the experimental lathyrogen, semicarbazide, the positive control. Thus reduction of NAD+ levels does not seem to be responsible for the defects, as occurs in avian species, and alterations in collagen formation and utilization may be responsible for these defects.

EARLY DEGENERATIVE AND REGENERATIVE CHANGES AT THE NEUROMUSCULAR JUNCTION [NMJ] IN ACRYLAMIDE NEUROPATHY. P L DeGrandchamps and H E Lowndes. Neurotoxicology Laboratories, Rutgers College of Pharmacy, Piscataway, NJ.

Acrylamide neuropathy is clinically characterized by ataxia, dysmetria, and uncoordinated gait. Histopathological studies of clinically impaired animals have revealed pathological changes in sensory and motor axons and terminals; neuronal regeneration in severely affected animals is also impaired or completely lacking. Incipient degenerative and regenerative changes occurring prior to clinical manifestations of neuropathy were assessed using a combined silver-ACME histochemical method to visualize intra-muscular nerves and NMJ of rat soleus muscle. Rats were given acrylamide, 35 mg/kg, i.p., 5 days a week and killed either 7 or 14 days after the first dose. Approximately 70 and 90% of the NMJ in the 7 and 14 day animals, respectively, had degenerating terminals. Significantly, 45 and 23% of the NMJ in the same groups also showed evidence of terminal sprouting. Pathological changes were not seen in the intramuscular nerves. These results suggest that the terminal branches at the endplate may be a primary target for acrylamide. The results also indicate that acrylamide does not prevent regeneration, but may actually initiate it. Supported by NS-23325.


Neurofilamentous giant axonal swellings are associated with axonal degeneration in many distal axonopathies, e.g. acrylamide (AC). However, axonal degeneration is not a necessary sequel to swelling formation since 6-N,N-dimethylpropionyltri- (IDPN) produces massive proximal swellings without axonal degeneration. In addition, a strict spatial relationship is not observed in distal axonopathies demonstrating both alterations. We tested the hypothesis that swelling formation may increase the vulnerability of the axon to a second neuronal insult. Rats were pretreated with IDPN (1.5 mg/kg, i.p.) to induce proximal swellings one week prior to a single injection of AC (75 mg/kg, i.p.), a dose not producing axonal degeneration; rats were perfused with 5% glutaraldehyde 2 weeks following IDPN administration. Axonal degeneration was observed in both ventral (0.10%) and dorsal (0.25%) root fibers. However, degeneration was more prominent in the distal sciatic nerve (0.55%) and its branches, e.g. peroneal nerve (0.87%). Thus, neurofilamentous swellings appear to confer an increased susceptibility upon the axon to further toxic chemical injury even in regions removed from swelling formation. (ES04078)

Carbon disulfide (CD) produces a distal axonopathy, associated with distal neurofilamentous axonal swellings, upon repeated exposures. The mechanism of neurotoxicity remains unknown but it may involve inhibition of copper/zinc-dependent enzymes via formation of dithiocarbamates. We have compared the ultrastructural changes following CD and diethylidithiocarbamate (DTC) intoxications. Rats were given CD (1 mg/kg, i.p., for 1-3 days) or DTC (0.5-1.0 mg/kg, intragastric gavage, 5 days/week for 4 weeks) in 2% DCM and olive oil and perfused with 5% glutaraldehyde. Neurofilamentous axonal swellings were prominent in the dorsal and ventral roots and lumbar spinal cord, supporting a dose-dependency for the location of swelling formation; i.e., more proximal swellings are produced by high dose administrations. Neurofilament densities were significantly increased (>50%) in both models. Swollen fibers demonstrated abnormal mitochondria, often associated with large dense particles; preliminary X-ray microprobe analysis revealed high levels of zinc and possibly copper. These studies support a role for DTC in CD neuropathy and suggest that their pathogenesis may involve a defect in mitochondria. (ES4078)

A Model System for Reversible and Irreversible Responses of the Developing Central Nervous System to Toxic Agents. S. Norton, I. Kotkoskie, and B. F. Kimler. University of Kansas Medical Center, Kansas City, KS

A model for quantifying damage to the fetal nervous system has been developed. Pregnant rats are exposed to toxic agents on gestational day 15, a critical period for cerebral cortical development. Fetal brains are examined from 2 µm plastic sections, 12, 24, or 48 hrs after maternal exposure. Morphometric measurements of the developing cerebral cortex with the light microscope include: size of pial blood vessels and subventricular sinusoïds; cell counts/1000 µ² in the 5 cortical zones (ventricular, subventricular, intermediate, cortical plate and marginal zones); and nuclear area of neuroblasts in the subventricular zone. Gamma radiation is used as a standard agent with known toxicity to the developing nervous system. Irradiation of the pregnant rat with 0.25, 0.5, 0.75, or 1.0 Gy causes dose-related changes within 12 hrs in the morphometric parameters. Recovery may occur within 48 hrs (reversible response) or may persist indefinitely, depending on dose. Other agents under investigation (e.g., ultrasound and ethanol) cause similar but not identical effects. Supported in part by NIH grants NS16694, HD21669, and ES07079.

Acute Response of the Fetal Telencephalon to Maternal Ethanol Exposure in the Rat. L. A. Kotkoskie and S. Norton. University of Kansas Medical Center, Kansas City, KS

Prenatal ethanol exposure causes a broad spectrum of effects termed the fetal alcohol syndrome including brain malformations, mental retardation, and developmental delays. The present study investigated the acute morphologic changes in the developing rat cerebral cortex following short-term maternal exposure to ethanol. Pregnant Charles River (CD) rats were intubated with ethanol (4.5 g/kg) twice daily on gestational days (gd) 14 and 15, a critical period for the development of the cerebral cortex. On gd 16, twenty-four hours after the last dose of ethanol, fetal brains were processed for light or scanning electron microscopy. Light microscope changes were dilated pial and cortical capillaries, swelling of subventricular zone nuclei, and increased thickness of the ventricular zone. Scanning electron microscopy was used to examine morphologic changes on the lateral ventricular surface. Small hemorrhages were numerous on the ventricular surface and marked bleb-like swelling of matrix cells into the ventricular lumen were seen. The degree of reversibility of these changes is under investigation since most fetuses survive acute gestational ethanol exposure at this dose. Supported by NIH grants NS16694 and ES07079.


Due to leaching from waste sites, trichloroethylene (TCE) has been detected in drinking water supplies. Previous studies indicate that the hippocampus may be a brain area susceptible to TCE exposure. In this study we found that TCE exposure caused a decrease in myelin activity in the rat hippocampus. Rats were maternally exposed to 312 ppm or 625 ppm TCE via the dam's drinking water until weaning (21 days) and again at 100 days for 2 weeks via drinking water. Following a histochemical stain for the presence of myelin, frozen sections of the brain were observed to determine changes in myelin activity. A decrease in myelin activity was observed in the hippocampal layer receiving input from the entorhinal cortex (stratum lacunosum-moleculare). Rats exposed to 312 ppm exhibited much larger decrease in myelin than those exposed to 625 ppm. These changes provide an anatomical basis for behavioral dysfunctions previously observed in rats similarly exposed to TCE.
The "unusual" non-protein amino acid, β-N-methylamino-L-alanine (BMAA) when administered to cynomolgus monkeys by gavage for 6 months (100-250 mg/kg) has been found to induce a primate-motor-system disorder (Spencer et al., Science 237:517-522, 1987). A novel high performance liquid chromatographic (HPLC) procedure has been developed for determining serum levels of BMAA in monkeys administered this neurotoxic by gavage (100-250 mg/kg). Serum from treated primates was collected at 0, 2, 4, 6, and 24 hr, deproteinated with TCA (10%), centrifuged, and the filtered supernatant derivatized with 9-fluorenylmethyl chloroformate (FMOC). The FMOC derivatives of serum BMAA and amino acids were separated by gradient reverse-phase HPLC and detected by fluorescence. Serum levels of aspartate, glutamate, glycine, and alanine were 35-55% lower in BMAA-treated monkeys than in controls (saline). Significantly lower (p<0.01) levels of glycine were observed over the entire 24 hr post-intubation period. Serum levels of BMAA increased over a 24 hr period for one monkey which was determined to have motor dysfunction (Ludolph et al., Electroenceph. Clin. Neurophys. 67:53-57, 1987). No changes in primate motor function were observed in a similarly treated monkey whose serum BMAA levels actually decreased over the 24 hr period. The results of these studies suggest: (1) that selective serum amino acid levels are lower in BMAA-treated monkeys than untreated (saline) controls, (2) elevated serum BMAA levels are consistent with the hypothesis that edible plant products may have etiological significance in some motor system disorders. (Supported by NS 19511).

The mechanism by which distal nerve segments degenerate following transection has not been determined. An initial intial of Ca is proposed based on the evidence that have used x-ray microanalysis to measure directly distribution of Na, P, Cl, K and Ca in transsected nerve fibers. Rats (275-300 gm) were anesthetized and either sham operations or unilateral transections of sciatic nerves were performed. At 16 or 48 hrs post-transection, control nerves or distal stumps were frozen in situ. The elemental content (mmol element/kg dry wt) of axoplasm, mitochondria, myelin and ECM was determined by analysis of frozen, thin (<300 nm) unfixed sections of control nerves and distal stumps. At 16 hrs only small diameter myelinated fibers displayed selective changes in elements when compared to controls. The intracellular compartments, axoplasm and mitochondria, exhibited significant increases in Na and decreases in K. Changes in other elements and compartments were observed. At 48 hrs generalized alterations in elemental distribution occurred. Regardless of fiber size, intracellular compartments showed marked increases in Na, Cl and Ca, and decreases in K and P. Myelin exhibited increases in Ca. These temporally-related changes suggest that axotomy-induced nerve degeneration might be mediated by an initial perturbation of intracellular and extracellular K followed later by a generalized rearrangement of elements. Supported by NIH grants ES03830 and NS21455.

Intrathecal or intracerebral injection has been a useful tool for neurotoxic studies of drugs which show low blood-brain barrier (BBB) transfer. Since drugs are locally administered into the brain by these injection methods, it is difficult to detect the local vulnerability and sensitivity to the drugs. After the induction of transient osmotic opening of BBB by the injection of 1.4M mannitol into the carotid artery, the neurotoxic effect of several chemicals on the rat brain was investigated. Vincristine (0.75mg/kg) or kainic acid (25mg/kg) were injected into the right carotid artery after the osmotic opening and the animals were sacrificed at days 1, 3 and 7 after the injection. Cllal mitotic arrest and axonal swelling were observed mainly in the midbrain in the vincristine treated group. Hippocampal neuronal necrosis and necrotic area of the asygdaloid nuclei, the entorhinal and pyriform cortex were noted in the kainic acid treated group. These lesions were observed in the right hemisphere of the brain. These results suggest that the osmotic opening of BBB by hyperosmolar solution of mannitol is a useful technique for the neurotoxic study of chemicals which show low BBB transfer.

Chemical analyses indicate that manganese (Mn) tends to accumulate in basal ganglia structures. Because Mn is also found in areas indicated by electron microscopic studies can be visualized by proton magnetic resonance imaging (MRI), a consequence of the ability of Mn to shorten spin-lattice relaxation times (T1). The extent of T1 shortening depends on Mn dose, but the shape of the dose-effect function varies from tissue to tissue. The time course of Mn clearance from different brain regions and the pituitary was examined by estimating T1 values in these regions before and after iv administration of Mn to Cebus monkeys. After 5 mg/kg Mn, iv, T1 was shortened only in the globus pallidus. In a second monkey, 7 days after a series of doses totalling 30 mg/kg over a 1-month period, T1 decreased throughout the basal ganglia and pituitary, but not in cortex and white matter. 40 days later, T1 in caudate and putamen returned to pre-dosing values, but remained shortened in globus pallidus. Pituitary T1 lengthened, but not to pre-dosing values. Five months later, pallidal T1 remained shorter but, in other areas, had returned to pre-dosing values. (Supported by ES-01247, ES-01248, and AA-01586)
The Y diketone 2,5-hexanediene (HD) exerts its neurotoxic effects by binding to protein lysyl residues and cyclizing to form pyroles. Whether the presence of pyrole residues in neurofilaments causes the Y diketone neuropathy, or whether pyrole oxidation and protein crosslinking must also occur is currently under debate. We synthesized the HD analog 3-acetyl-2,5-hexanediene (AchHD) and assessed its rate of pyrole formation, ease of oxidation of these pyroles, and its ability to crosslink proteins in vitro. AchHD formed pyroles at a rate comparable to that of the potent HD analog 3,4-dimethyl-2,5-hexanediene (DMHD). The AchHD pyrole was more resistant to oxidation than that derived from HD, and AchHD did not crosslink proteins in vitro. Rats receiving 0.1 or 0.25 mmol/kg/day AchHD ip did not become paralyzed, and the axonal swellings seen following exposure to neurotoxic Y diketones were not observed. Pyrole derivatives were demonstrated on globose isolated from rats treated with HD, DMHD or AchHD. Crosslinked spectrin was detected in rats treated with HD or DMHD, but not with AchHD. Given that AchHD, a Y diketone which rapidly forms very stable pyroles and is incapable of protein crosslinking, does not produce the typical Y diketone neuropathy, we propose that pyrole oxidation and protein crosslinking are necessary steps in the pathogenesis of Y diketone neuropathy.

Several neurotoxic compounds cause aggregation of neurofilaments in peripheral axons; it is not known whether this is a primary action or secondary to other cellular damage. We have examined a number of these compounds in a cell culture model system, Ptk2 cells. These cells contain vimentin intermediate filaments (VIF), closely related to neurofilaments. Both AchHD and 2,5-hexanediene (2,5-HD) caused perinuclear aggregation of VIF; other cytoskeletal components (microtubules and microfilaments) were not affected. Crosslinking of vimentin was demonstrated on Western blots of proteins from cells treated with high concentrations of 2,5-HD but not AchHD. Analogs of hexanediene have been used in vivo to alter the location and time course of the neurological lesion. Several of these compounds were tested also in our system. The effectiveness parallels that reported in vivo: 3,4-dimethyl-2,5-HD > 3-methyl-2,5-HD > 2,5-HD; 3,3-dimethyl-2,5-HD did not disrupt VIF distribution.

These data suggest that aggregation of intermediate filaments/neurofilaments may be a common mechanism of damage for certain neurotoxic compounds. Vimentin filaments of cultured cells respond in a manner similar to neurofilaments in affected axons.

**Research on excitotoxic damage to mouse cortical explants.**

Rodents exposed to single doses of trimethyltin (TMT) show signs of central nervous system toxicity including aggressive behavior, tremor, and convulsions. Pathological evaluation of TMT-intoxicated animals revealed bilateral and symmetrical neuronal destruction in the hippocampus, basal cortex, neocortex, and brain stem. Organotypic cultures established from neonatal mouse cortex (2 day-old) were treated with TMT (2.5 μM-2.5 mM) for thirty minutes, fixed and prepared for evaluation of neuronotocytotoxicity by light microscopy. TMT produced widespread vacuolation with greatest concentration in the molecular layer of the cortical explant. Selected neurons at both superficial and intermediate levels of the explant contained an abnormal number of cytoplasmic vacuoles, while others were dark or shrunken. TMT-induced vacuolation was concentration-dependent and appeared indistinguishable from the postsynaptic vacuolation elicited in cortex and spinal cord explants by excitotoxic amino acids and their analogues (e.g., aspartate, N-methyl-D-aspartate). Pretreatment of cortex explants with N-methyl-D-aspartate-receptor complex antagonists (D-2-amino-7-phosphonoheptanate, (+)-S-methyl-10,11-dihydro-5H-dibenzo-cyclohepten-5,10-imine maleate) attenuated TMT-induced vacuolation and dark cell change in molecular and cellular layers. These data support an "excitotoxic" mechanism of action for TMT neuropathy. (Supported by grant NS 19611).

**Effect on auditory function by chemical asphyxiants and noise.**

The cochlea has been identified as being extremely sensitive to oxygen delivery; several ototoxic agents are thought to disrupt function through this mechanism and increased cochlear perfusion has proved efficacious in treating some forms of sudden hearing loss. We demonstrate that hypoxia can potentiate noise-induced hearing loss producing large, permanent impairments primarily at high frequencies. Neither the extent of the loss nor its frequency characteristics are predicted based upon the noise exposure alone. Tests were carried out at a range of CO concentrations and using noise exposures of different frequencies. Auditory thresholds were measured, animals sacrificed and cochleae examined histologically for survival of sensory receptor cells. We report potentiation of noise induced injury with 500ppm CO exposures for 3.5hr. Combined exposures greatly increase the number of hair cells destroyed in the base of the cochlea as compared to noise alone. The base of the cochlea is responsible for encoding high frequency tones and is generally most vulnerable to ototoxic agents. The data point to a specific sensitivty of the base of the cochlea to disruption of oxidative metabolism. (Supported in part by NIH R01 ESO0125, RO/ R02852.)
EFFECT OF METYRAZONE IN CHICKENS AND INTERACTION WITH TRI-ORTHO-TOXYL PHOSPHATE (TOTP). M Ehrich, BS Jortner and WB Gross. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Previous studies (Tox Appl Pharm 70: 269, 1983; Tox Lett 31:9, 1986) showed stress and high concentrations of corticosterone, both of which increased heterophil-to-lymphocyte ratios (H/L), to exacerbate clinical evidence of delayed neuropathy in chickens given TOTP. This study used the adrenal blocker metyrapone to modify delayed neuropathy in chickens given TOTP. For these experiments metyrapone was provided to adult white leghorn chickens beginning 4 days before TOTP 360 mg/kg po and continuing 8 days afterwards. H/L’s measured 24 hours after TOTP were lower in blood samples from birds given metyrapone + TOTP than in those given TOTP alone. Clinical signs and lesions of delayed neuropathy were less in chickens given metyrapone. Ataxia scores were 2.4 ± 0.1 and 2.1 ± 0.1 twelve days after TOTP and 500 or 1500 ppm metyrapone, respectively, mean ± SD, N=9, compared with 3.1 ± 0.2 in chickens given TOTP alone (0-6 = no affected to paralyzed). Quantity of cytochrome P450 was elevated by metyrapone, specifically the activity of steroid-metabolizing cytochrome P450 associated aniline hydroxylase. Increased metabolic capability is, therefore, likely to make an important contribution to the beneficial effect of this adrenal blocker against TOTP-induced delayed neuropathy.

(Supported by NIEHS grant 03384)

EFFECT OF β-NAPHTHOFLAVONE ON TOLYL SALIGENIN PHOSPHATE-INDUCED DELAYED NEUROTOXICITY. S J Burrison, E Lehninig, L Correll and M Ehrich. Department of Animal Science, Michigan State University, East Lansing, MI and Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Previous studies conducted in both laboratories have shown that a mixed-function oxidase enzyme inducer, β-naphthoflavone (BNF), protects against the development of delayed neurotoxicity induced by tri-ortho-tolyl phosphate (TOTP). To determine if BNF would protect against delayed neurotoxicity induced by TOTP’s neuroactive metabolite, tolyl saligenin phosphate (TSP), BNF was administered to 5-7 month old chickens (80 mg/kg) via a single intraperitoneal injection 48 hours prior to administration of TSP via a single subcutaneous injection at doses ranging from 20 to 60 mg/kg. BNF had no effect on whole-brain neurotoxic esterase inhibition by TSP nor on the development of delayed neurotoxicity clinical signs. Liver cholinesterase and carbamoylcholine esterase activities were not affected by BNF administration, but induction of enzymes responsible for the hydroxylation of aniline and the demethylation of p-nitroanisole was demonstrated. This study suggests that the protection offered by BNF against the development of TOTP-induced delayed neuropathy is not due to increased degradation of its neuroactive metabolite, TSP. (Supported in part by NIEHS grant 03384).


Sarin, Type I (800, 600, 400, 200, 100 or 0 ug/kg) was given by oral gavage to groups of five SPF White Leghorn Hens [14-18 months; 900 -2500 grams] protected by atropine (100 mg/kg on days 1-5; 10 mg/kg on days 6-7). Probit analysis determined that the LD10 was 121.5 ug/kg and the defined Maximum Tolerated Dose (MTD=LD10/2) was 60.75 ug/kg. Sarin was given by gavage to three groups of ten atropine protected hens as a single dose on Day 1 (MTD, MTD/2, MTD/4 per kg) or as a divided dose (one-third the single dose groups) on Days 1, 8 and 15. Tri-o-cresyl phosphate (510 mg/kg [single dose] or 170 mg/kg [divided dose]) and water were the positive and vehicle controls, respectively. Hens were examined 5 days per week to assess the degree of clinical ataxia. On Day 43, the hens were killed and the brain, spinal cord, and sciatic nerve were examined microscopically and the severity of the lesions was rated. There were no statistically significant differences between the vehicle control and the Sarin treated groups in ataxia scores or histopathology. Positive control data are comparable with published data. (Supported by US Army Medical Research & Development Command, contract 85PP588).

THE SUBCHRONIC INHALATION TOXICITY OF BISPHENOL A IN FISHER 344 RATS. L G Lomax and K O Mitsche, The Dow Chemical Company, Midland, MI. Sponsor: P G Watanabe

Bisphenol A (2,2-bis 4-hydroxyphenyl) propane, BPA, an intermediate in the production of polycarbonate and epoxy resins, was examined for potential inhalation toxicity. Fischer 344 rats (30/sex/exposure group) were exposed to 0, 10, 50 or 150 mg/m³ BPA for 6 hours/day, 5 days/week for 13 weeks. Rats were sacrificed after 13 weeks of exposure or 4 or 12 weeks post-exposure. The aerosol mass median aerodynamic diameter (MMAD) was 2.2 μm with a geometric standard deviation (σg) of ±2.0. Toxicologically significant findings following 13 weeks of exposure were decreased body weights of rats exposed to 150 mg/m³ and minor morphologic alterations in the anterior portions of the nasal cavity of rats exposed to 50 or 150 mg/m³. There were no morphologic alterations in lungs. Four weeks post-exposure, the body weights of rats exposed to 150 mg/m³ were still decreased; however, significant resolution of the nasal cavity microscopic changes was evident and the nasal tissues of rats exposed to 50 mg/m³ were morphologically normal. The body weight of male rats exposed to 150 mg/m³ was still slightly decreased 12 weeks post-exposure but there were no lesions in nasal tissues indicating full reversibility. No adverse effects were noted following exposure to 10 mg/m³.
DIIMINEs are widely used as industrial intermediates and, in general, behave as strong bases. Groups of Wistar rats were exposed once (4-hr) by inhalation to TMEDA vapor at concentrations of 3058, 1869, or 958 ppm. Mortality occurred in all groups, and signs of irritation, respiratory difficulty, eye opacity, ataxia, and absence of reflex to toe and tail pinch were observed. The 4-hr LC50 value (95% confidence limits) was 1318 (904-1829) ppm. Subsequently, groups of F-344 rats were exposed 6 hr/day for 9 days (over an 11-day period) to 743, 246, 51, or 0 (control) ppm of TMEDA. All rats exposed to 743 ppm died during the first 3 days of exposure. Rats of the 246 ppm group had respiratory difficulties, eye opacity, and lacrimation, while rats of the 51 ppm group had eye opacity (attributed to corneal edema and inflammatory changes). Body weight (BW) loss or a decrease in BW gain was observed in the survivors of the TMEDA exposure regimen. Treatment-related microscopic lesions consisted of keratitis and/or corneal necrosis (743, 246, and 51 ppm), nasal mucosal ulceration (743 and 246 ppm), upper respiratory tract squamous metaplasia (246 and 51 ppm), and thymic atrophy (246 ppm). TMEDA vapor concentrations of 51 ppm and above are highly irritating and toxic to rats. The most overt finding was opacity of the eyes.

TMEDA has chemical reactivity making it useful in various organosilicon synthetic processes. Its acute handling hazards were investigated. The acute rat peroral LD50 was 0.71 (0.51-0.97) ml/kg and rabbit percutaneous LD50 was 0.57 (0.35-0.92) ml/kg. TMEDA was severely irritant to the skin and eyes. The saturated vapor LT50 was 12 min. Using nominal vapor concentrations produced by passing air countercurrent to heated TMEDA liquid, the 4-hr LC50 was 734 (603-893) ppm in female rats. This stoichiometrically accords with toxicity due to dimethylamine (DMA) liberated by TMEDA. In a further study, designed to avoid contamination of the vapor with moisture, nitrogen was passed through a heated tube in which liquid TMEDA was metered. Analytically measured TIMAS vapor concentrations were 395, 127, 62 and 23 ppm; the corresponding DMA vapor concentrations were 112, 31, 10, and 26 ppm. Using 10 male and 10 female rats per group, all died at 62 ppm and above, but none in the 23 ppm group. The 4-hr LC50 for both males was 38 (34-43) ppm. TIMAS is of moderate acute peroral and percutaneous toxicity, a severe primary skin and eye irritant, and of high intrinsic acute inhalation toxicity, but in moist atmospheres DMA vapor may be a significant factor in toxicity.

The acute inhalation toxicity of AC was studied by exposing groups of 5 male and 5 female Sprague-Dawley (SD) rats for 1 or 4 hr. The measured vapor concentrations were 81, 31, 24, 22, and 14 ppm for 1-hr exposures, and 12.3, 21.4, 21.4, 15.1, 7.0, and 4.8 ppm for 4-hr exposures. The LC50 values for combined sexes were 26 (24-27) ppm for 1 hr, and 8.3 (7.0-9.9) ppm for 4 hr. Signs of toxicity included lacrimation, hypoactivity, breathing difficulties, and perioral and perinasal wetness. The influence of AC contamination of MDP (0.037%) on inhalation toxicity was studied by exposing groups of 5 male and 5 female SD rats for 1 hr to vapor generated in various ways. By static generation all animals died, with MDP vapor concentrations in two experiments being 8098 and 8044 ppm; the AC concentration in the latter experiment was 240 ppm. Sparging MDP to remove AC before static exposure resulted in reduced mortality (MDP 9076 ppm) or no deaths (MDP 8613 ppm). Dynamically generated atmospheres, by bubbling (1064 and 7748 ppm) or metering with a heated evaporator (1095 ppm) did not cause deaths (trace AC). These findings indicate that AC accumulation from impure MDP may be a major factor in toxicity under static conditions.
*National Toxicology Program, NIEMS, RTP, NC. **Battelle Pacific Northwest Laboratories, Richland, WA. Sponsor: R. Chhabra.

Male and female F344 rats and B6C3F1 mice were exposed to 0, 100, 200, 400, 800, or 1600 ppm acetonitrile (ACN) 6 hrs/day/5 days/week for 13 weeks. ACN-related mortality was observed in male rats (1/10, 800 ppm; 6/10, 1600 ppm), female rats (3/10, 1600 ppm), male mice (1/10, 800 ppm; 10/10, 1600 ppm), and in female mice (1/10, 400 ppm; 4/10, 800 ppm; 10/10, 1600 ppm). Clinical signs of toxicity, primarily seen the first 2 weeks of exposure, included hypoaactivity, abnormal posture, and ataxia. There was significant reduction in rate of weight gain in rats (1600 ppm) and male mice (800 ppm). There were no significant compound-related histopathological findings in rats. However, ACN exposure induced fore stomach lesions (hyperplasia of the squamous epithelium) in male and female mice (400 ppm and higher in males; 200 ppm and higher in females). These lesions appeared as areas of acanthosis with hyperkerosis of the epithelium taking on a folded appearance. In some instances small ulcerations accompanied the hyperplasia. In summary, exposure to ACN for 13 weeks produced toxic effects in rats and mice with mice being more affected than rats as evidenced by distinct ACN-induced fore stomach lesions and high mortality.

UNLATERAL CHANGES IN OLFACTORY EPITHELIUM OF RATS FOLLOWING INHALATION EXPOSURE TO METHYL BROMIDE. D A Thomas, O Lyght, and K T Morgan.
CIT, RTP, NC. Sponsor: E Gross-Bermudez.

Inhalation exposure of male F344 rats to 175-325 ppm methyl bromide (MeBr) (6 hr/day, for 5 days) causes extensive destruction of the olfactory epithelium. In the present study, MeBr-induced changes in the olfactory epithelium were examined by transmission electron microscopy in rats that were sacrificed immediately following a single exposure to 400 ppm MeBr for 1, 2, 4, or 6 hr. The characteristic ultrastructural features of acute MeBr toxicity indicated sensitivities of two cell populations, nerve sensory cells and sustentacular cells. Early evidence of sustentacular cell damage included blebbing of apical cytoplasm with blebs containing abundant smooth endoplasmic reticulum. Early changes in affected sensory cells were indicated by nuclear degeneration. After 6 hr, swelling and fragmentation of sustentacular cells were associated with shedding of immature sensory cells and gobose basal cells which were otherwise "morphologically healthy". These ultrastructural observations suggest that an important primary lesion involves edema and fragmentation of sustentacular cell cytoplasm, with consequent loss of all cells supported by them. True basal cells, attached to the basement membrane, were unaffected indicating that they are not dependent upon sustentacular cells for their survival. Their independence from sustentacular cell support may account for the resistance of true basal cells to MeBr toxicity.

SUBCHRONIC INHALATION TOXICITY OF ETHYLAMINE (EA) VAPOR IN F-344 RATS. D W Lynch, W J Moorman, T R Lewis, P Stober, R D Hanlin*, and R L Schueler**.
NIOSH, Cincinnati, OH; *Dept. Vet. Physiology and Pharmacology, Ohio State Univ., Columbus, OH; and **Research Pathol. Assoc., Inc., Sykesville, MD.

Male and female F-344 rats were exposed at 0, 10, 100, or 500 ppm EA vapor, 6 hr/day, 5 days/week for 24 weeks in order to assess cardiac and other organ system toxicity. Rats were weighed biweekly and scheduled sacrifices were conducted following 30, 60, and 120 days of exposure. Examination of the nasal cavity of rats exposed to 500 ppm EA for 120 days disclosed moderate-to-marked atrophic rhinitis in 16/16 male and 17/17 female rats. The lesion involved principally the anterior half of the nasal cavity and was characterized by: purulent exudate in the nasal meatuses; chronic, active inflammation that was often ulcerative; necrosis and loss of the cartilaginous nasal septum; loss of bony turbinate; and squamous metaplasia of nasal epithelium. No lesions were detected in the nasal cavities of the controls, 10, or 100 ppm EA rats exposed for the same time period. Body weight gain in rats of both sexes exposed to 500 ppm EA was statistically reduced compared at the controls throughout the 24 weeks of exposure. No treatment-related effects on hematology, clinical chemistry, or ECG indices were observed, and no evidence of cardiotoxicity was seen in rats exposed to EA for up to 120 days.

ULTRASTRUCTURAL CHANGES IN OLFACTORY EPITHELIUM OF RATS FOLLOWING INHALATION EXPOSURE TO METHYL BROMIDE. D A Thomas, O Lyght, and K T Morgan.
CIT, RTP, NC. Sponsor: E Gross-Bermudez.

Inhalation exposure of male F344 rats to 175-325 ppm methyl bromide (MeBr) (6 hr/day, for 5 days) causes extensive destruction of the olfactory epithelium. In the present study, MeBr-induced changes in the olfactory epithelium were examined by transmission electron microscopy in rats that were sacrificed immediately following a single exposure to 400 ppm MeBr for 1, 2, 4, or 6 hr. The characteristic ultrastructural features of acute MeBr toxicity indicated sensitivities of two cell populations, nerve sensory cells and sustentacular cells. Early evidence of sustentacular cell damage included blebbing of apical cytoplasm with blebs containing abundant smooth endoplasmic reticulum. Early changes in affected sensory cells were indicated by nuclear degeneration. After 6 hr, swelling and fragmentation of sustentacular cells were associated with shedding of immature sensory cells and gobose basal cells which were otherwise "morphologically healthy". These ultrastructural observations suggest that an important primary lesion involves edema and fragmentation of sustentacular cell cytoplasm, with consequent loss of all cells supported by them. True basal cells, attached to the basement membrane, were unaffected indicating that they are not dependent upon sustentacular cells for their survival. Their independence from sustentacular cell support may account for the resistance of true basal cells to MeBr toxicity.

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MULTI-WEEK VAPOR INHALATION STUDY OF PROPYLENE GLYCOL MONOPROPOXIL ETHER (PGPE) IN F-344 RATS. P E Loaco, D R Koenne, D E Dodd, C M Troup, and D Ballantyne, Bushy Run Research Center/ Union Carbide Corporation, Export, PA.

PGPE is an industrial solvent with a wide variety of applications. This study evaluated the effects of repeated vapor exposure (240 ppm) during a 2-wk period (9 exposures) to mean vapor concentrations of 0, 503, 983, or 2000 ppm. Ataxia and prostration, decreased body weight (BW) gain, increased absolute and/or relative (to BW) liver and kidney weights, and histopathologic corneal lesions (keratitis, ulceration, necrosis, and atrophy) were evident at 2000 ppm. No histopathologic lesions were observed in the liver or kidneys of these rats. Exposure to 503 or 983 ppm produced increases in absolute and/or relative liver and kidney weights (males only) and corneal lesions similar to those observed at 2000 ppm, but with a decreased incidence and severity. While loss of blinking and tearing reflexes (due to ocular lesions during exposure) may be invoked as an explanation for the ocular lesions occurring at 2000 ppm, it cannot explain the lesions observed at 503 or 983 ppm. It has been noted in this laboratory that untreated F-344 rats are susceptible to atrophyalization. The contribution of this susceptibility to the lesions observed in this study is unknown.
999 TOXICITY OF 2-METHYL-5,6-CYCLOPENTAPYRIMIDINE (MCPF) FOLLOWING ORAL OR INHALATION EXPOSURES IN RATS. G L Kennedy, Jr, E I du Pont de Nemours & Co, Inc, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

2-Methyl-5,6-cyclopentapyrimidine (MCPF, CAS No. 36274-29-0) is a white dusty solid with a powerful lingering odor and is formed as a by-product in polymer synthesis. The acute toxicity following both oral and inhalation exposures and effects of repeated inhalation exposures in rats were determined. The chemical is moderately toxic with a lethal dose following a single oral administration of 90 mg/kg. Doses of 130 mg/kg or greater produced convulsions. Excessive salivation, hyperactivity and twitching was seen at 90 mg/kg and with little seen in surviving rats (50 mg/kg or less). Liver injury was produced at doses as low as 17 (but not at 12) mg/kg. The material was highly toxic by inhalation with the approximate lethal concentration in rats following single 4 hr exposures being 9 ppm. Convulsive-like movements were seen at 9 ppm or greater (not at 2 ppm). Histologic findings suggest that MCPF causes dilation of blood vessels with hyperemia of various organs in rats exposed to 1 ppm (sacrificed 1 or 2 days post-exposure). No evidence of liver or central nervous system damage was seen. Repeated (9 daily 4-hour exposures) inhalation of 2 ppm MCPF failed to produce any signs of a toxic response. The material is considered as a potent acute toxin.

1001 INHALATION SUBCHRONIC TOXICITY STUDY OF N-HEXANE IN B6C3F1 MICE. J K Dunnick1, D G Graham2, R S H Yang3, S B Haber4, BRIERSYNF Research Triangle Park, NC, 2Duke University, Durham, NC, and 3Brookhaven National Laboratory, Upton, NY.

B6C3F1 mice were exposed to n-hexane 5 days per week for 13 weeks at concentrations of 500, 1000, 4000, and 10,000 ppm 6 hrs/day, and at 1000 ppm continuous exposure (1000 C group). All mice survived the exposure. Exposure related toxicity was seen at 1000 C and 10,000 ppm, including clinical signs at the high dose and body weight gain depression at 1000 C and at 10,000 ppm. A complete histopathologic evaluation was performed and the only dose-related effects noted were inflammatory, erosive and regenerative lesions in the olfactory epithelium of the nasal cavity at 1000 C and 10,000 ppm. The spinal cord and facial nerve were examined for evidence of neurotoxicologic damage. Paranodal axonal swellings were observed in the facial nerve at 1000 C and at 10,000 ppm, but not in the control groups. This peripheral nerve lesion was mild in nature and not considered to be a life-threatening lesion. Locomotor activity was decreased in female mice at 1000 C and 10,000 ppm. The nerve damage seen in the mouse was less severe than reported in the rat at 10,000 ppm (Cavender et al, Fundam Appl Toxicol. 4:191, 1984).

1002 ACUTE INHALATION TOXICITY OF ETHYLENE OXIDE/PROPYLENE OXIDE COPOLYMERS. B A Burgess, L A Kinney and G L Kennedy, Jr. E I du Pont de Nemours and Co, Inc, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

A series of ethylene oxide-propylene oxide (EO/PO) copolymers with 50/50 random distributions of EO/PO and molecular weights ranging from 730 to 4000 were tested for acute inhalation toxicity. Male rats were exposed to respirable aerosols (MMAD of 2 microns) for 4-hours and the approximate lethal concentration (ALC) was determined. Two other EO/PO copolymers tested. The 50/50 EO/PO copolymer with a molecular weight of 4000 was found to have an ALC of from 61 to 92 mg/m³ (three replicate experiments). Death occurred from 2 to 6 days postexposure following a relatively symptom-free period. Rats exposed to 230 mg/m³ and sacrificed 2 days later had prominent pulmonary edema and hemorrhage. ALC's for materials in this series with molecular weights of 730, 1700, and 2900 were greater than 5120 mg/m³, 2800 mg/m³, and 390 mg/m³, respectively. The 75/25 EO/PO copolymers did not produce lethality at the highest concentrations which could be generated (approximately 1800 mg/m³). The unexpected higher toxicity of the 50/50 EO/PO copolymer (mw 4000) reemphasizes the need to monitor aerosols in the workplace and to use appropriate controls to minimize human exposure.

1002 TWO-WEEK AEROSEOL INHALATION STUDY ON POLYETHYLENE GLYCOL (PEG) 3350 IN F-344 RATS. C M Troup1, D R Klonne1, D E Dodd1, P E Losco1, and T R Tyler2, Bushy Run Research Center, Export, PA, Union Carbide Corp., Dambury, Ct.

PEGs in the 3000 to 3700 Mw range are used in many products for human application; they produce little ocular or dermal irritation and have extremely low acute and subchronic toxicity by oral and dermal routes of administration. Little information exists, however, on the potential of aerosols of these materials to produce adverse health effects. F-344 rats were exposed to respirable aerosols of PEG 3350 (20% w/w in water) at 0, 109, 567, or 1008 (highest attainable) mg/m³ for 6 hr/day, 5 days/week for 2 wk. No exposure-related toxicity was found with regard to clinical signs, ophthalmology, serum chemistry, urinalysis, or gross pathology. Exposure-related effects included: 50% increase in the neutrophil count (males only) at 1008 mg/m³; decreased body weight gain (17%) for both the 567 and 1008 mg/m³ groups (males only); absolute lung weights of both sexes increased 10% and 18% for the 567 and 1008 mg/m³ groups, respectively. A mild increase in macrophage numbers in the alveoli was the only change observed histologically in all PEG 3350 exposed groups. Therefore, inhalation of aerosols of PEG 3350 at concentrations up to 1008 mg/m³ produced minimal toxicity in rats.
Benomyl is a fungicide and the possibility for inhalation exposure exists for field workers. To assess the toxicity of this compound, groups of 20 male and female CD rats were exposed nose-only 6 hrs/day, 5 days/wk, to concn. of 0, 10, 50 or to 200 mg/m^3 of a benomyl atmosphere. At the midpoint and end of the exposure period, blood and urine samples were collected for clinical evaluation from 10 rats/group/sex and these animals were sacrificed for pathologic examination.

After approx. 45 days on test, compound-related degeneration of the olfactory epithelium was observed in all males and 8 of 10 female rats exposed to 200 mg/m^3 of benomyl. Two male rats exposed to 50 mg/m^3 had similar, although less severe, areas of olfactory degeneration. After approx. 90 days of exposure, all of the males and females exposed to 200 mg/m^3 had olfactory degeneration, along with 3 males exposed to 50 mg/m^3 benomyl. No other observed lesions were interpreted to have been caused by the benomyl exposure. There were no biologically significant hematologic or clinical chemical results observed in rats exposed to any con. of benomyl. Male rats exposed to 200 mg/m^3 of benomyl had depressed body weights which correlated with a reduction in food consumption. Based upon pathologic observations, 10 mg/m^3 represents the no-observable-effect level (NOEL) for the male rats, and 50 mg/m^3 is a NOEL for the female rats.

Synthetic graphite is a pure crystalline form of carbon made from high temperature treatment of petroleum products and contains less than 1% quartz. Exposure to airborne graphite may occur in manufacturing or application processes. Previous acute inhalation studies on synthetic graphite have found it to be nontoxic. In this experiment, groups of male and female rats were exposed by inhalation for 2 hrs/day, 5 days/wk, for 2 wks to 100, 10, 1 mg/m^3 graphite (Asbury Micro 280). Both exposed and air exposed control rats were evaluated for bronchoalveolar (BAL), chemical, clinical, physiological, and pathological changes at 3 days and 3 months post-exposure (PE). Exposure to 100 mg/m^3 graphite resulted in BAL changes, adenomas, hyperplasia, and hystiocytosis at 3 days and 3 months PE. There were no significant changes at the lower concentrations. BAL changes were the most sensitive indicator of damage and were evident in the high concentration groups for both sexes at 3 days PE and were diminishing by 3 months PE. Repeated exposure to graphite dust results in more pulmonary damage than single exposures.

Terephthalic acid (TA) is a monomer component of polyester which is widely used to produce fiber, beverage bottles, tire cord, and adhesives. Male and female rats (10/sex/group) were exposed by inhalation to respirable TA dust 6 hrs/day, 5 days/week for four weeks at 0, 0.52, 1.19, or 3.31 mg/m^3. In addition, 6 rats/sex in the control and high exposure groups were designated for pulmonary function assessment. No deaths or overt clinical signs occurred during the study. No effects of TA exposure were noted at necropsy. No significant differences were observed between treated and control rats in body or organ weights, hematology or clinical chemistry parameters. Histological findings consisted of minimal degeneration of the tracheal epithelium in 19/20 high exposure rats, compared to 1/20 in controls. However, assessment of pulmonary air flow, transpulmonary pressure, tidal volume, dynamic lung compliance, airway resistance, respiratory rate, and minute volume, under normal and stressed breathing conditions, showed no differences between the control and high exposure groups. Therefore, TA is well-tolerated by male and female rats exposed to inhalation concentrations as high as 3.31 mg/m^3 daily for four weeks.

A 90-day inhalation experiment compared cigarettes that contained tobacco which was combusted (reference) with cigarettes where the tobacco was only heated (test). In the test cigarette a carbon heat source in the tip lit in the conventional way, heating tobacco and producing a smoke similar in appearance to that produced by the reference cigarette. Both test and reference cigarettes had conventional filter-tips. The test cigarettes yielded similar amounts of nicotine as the reference cigarettes, but chemically in a much simpler aerosol. To compare the cigarettes, Sprague-Dawley rats were exposed nose-only to diluted smoke, 1 hour per day, 5 days per week, for 13 weeks. Changes induced in low, medium and high dose exposure groups were compared with changes seen in both sham and room controls. The comparisons of test and reference at each of the 3 doses were made on the basis of equal concentrations of nicotine (5, 15 or 30 ug per liter) in the smoke presented to the animals. The study incorporated a reversibility section.
NI Ninety-day inhalation study in rats, comparing smoke from cigarettes which burned or only heated tobacco. 2. Nose-only inhalation system: smoke chemistry. R A James, J T Avalos, A T Mosberg, C R E Coggins and R J Reynolds Tobacco Co., Winston-Salem, NC.

A series of nose-only smoking machines were designed and built, for use in a 90-day inhalation study. During test (tobacco not burned) and reference (tobacco burned) cigarettes. Smoke was generated by automated 30-port carousels, providing a mixing unit with a constant stream of undiluted smoke. Aerosols with comparable concentrations of nicotine (NIC; 5, 15 or 30 ug per liter) were produced by varying flow rates of dilution air into the mixing unit. The smoke exposure system provided each of 64 animal exposure ports with a supply of diluted smoke, each port also having an exhaust line to prevent rebreathing of exhaled smoke. The aerosols produced by test and reference cigarettes matched the targets very closely, with approximately comparable concentrations of total particulate matter (TPM; 110-860 ug/liter) and carbon monoxide (CO; 140-860 ppm). All aerosols had mass median aerodynamic diameters of around 0.8 um. Test and reference cigarettes thus produced aerosols that were similar for particle size distribution and for concentrations of NIC, TPM and CO.

Blood samples were taken at the end of 60-minute daily exposures in animals used in a 90-day inhalation study, using retro-orbital sampling. A collection of blood was also made at necropsy. From the vena cava. Blood carboxyhemoglobin (COHb) concentrations in the low, medium and high dose groups (5, 15 and 30 ug nicotine per liter) at the end of the exposures were approximately 10, 20 and 40 % for both test (tobacco not burned) and reference (tobacco burned) cigarettes. Plasma nicotine concentrations at the end of the exposures were 40, 120 and 240 ng/ml for the test cigarette groups, and 15, 30 and 55 ng/ml for the reference groups. Plasma cotinine concentrations were 45, 100 and 160 ng/ml for the test cigarette groups, and 10, 20 and 30 ng/ml for the reference groups. There was no difference between test and reference responses in the metabolism of nicotine to cotinine. At necropsy (16-24 hours of the last exposure), blood COHb and plasma nicotine / cotinine concentrations were similar in all groups. There were no effects of group on hematology or clinical chemistry.

Measurements were made of breathing frequency (f), tidal volume (TV) and minute ventilation (MV) in animals used in a 90-day inhalation study comparing test (tobacco not burned) and reference (tobacco burned) cigarettes. Measurements were made throughout the 60 minutes of exposure, and were compared with data obtained during the 10 minutes pre-exposure. The smoke from the reference cigarette produced dose-related reductions in both f and MV. At low, medium and high doses respectively f was 80, 45 and 38 %, and MV was 55, 30 and 30 %, of pre-exposure values. The smoke from the test cigarette at all doses, and sham-exposure, all decreased f and MV to 85% of pre-exposure values. TV was not affected by treatment. The reference smoke is apparently an upper airway irritant, whereas the test smoke is not. Because of the changes in f, animals in the test cigarette groups inhaled up to twice as much material during their exposures as did animals in the reference groups.
NINETY-DAY INHALATION STUDY IN RATS, COMPARING SMOKE FROM CIGARETTES WHICH BURNED OR ONLY HEATED TOBACCO. 6. HISTOPATHOLOGY. G T Burger, C R E Coggins, A W Hayes, P H Ayres, A T Mosberg and J W Sagartz, R J Reynolds Tobacco Co., Winston-Salem NC and Veritas Labs, Burlington, NC.

Histopathological examinations were made on all respiratory tract organs of all animals, using H&E and Periodic Acid Schiff Alcian Blue stains. All slides were read without knowledge of treatment group. Responses obtained in the reference (tobacco burned) groups were similar to published observations. In the test (tobacco not burned) groups the following responses were either absent, or were present with substantial decreases in both severity and incidence, when compared with the reference groups: epithelial inflammation, hyperplasia and squamous metaplasia (nasal I, larynx), goblet cell hypertrophy (nasal II), goblet cell numbers (bronchi and bronchioles), pulmonary congestion, and an increase in intra-alveolar macrophages. A novel lesion, atrophy of the olfactory epithelium in nasal II, was only noted in the high dose reference groups. With the exception of the nasal I and laryngeal responses, all of the lesions induced were absent at the end of a 6-week recovery period.


A subchronic inhalation study compared the potential toxicity of two cigarettes. One cigarette (IR4F Univ. of Kentucky), which burned tobacco, provided a reference smoke and another cigarette, which only heated tobacco, created the test smoke. 34 Syrian-Golden hamsters/sex/group were exposed to 0.12, 0.35 or 0.64 mg total particulate matter/L of either reference or test smoke given 1 hr/day, 5 days/week for 13 weeks. 10 hamsters/sex/group were held for 6 more weeks and then sacrificed. Clinical appearance, minute ventilation, mortality, body and organ weights, gross- and histo-pathology, and hematologic and clinical chemistry parameters were examined. The test smoke produced considerably less biologic activity than the reference smoke. Histologic changes were observed in the respiratory tract of hamsters exposed to reference smoke, but were absent in all test smoke exposed groups.

SUBCHRONIC INHALATION STUDY IN RATS, COMPARING SMOKE FROM A CIGARETTE WHICH BURNS AND ONE THAT ONLY HEATS TOBACCO. A P Wehner, R A Renne, B J Greenspan and O R Moss, Battelle Northwest Labs, Richland, WA., A W Hayes, G T Burger and A T Mosberg, R J Reynolds Tobacco Co., Winston-Salem, NC.

A subchronic inhalation study compared the potential toxicity of a reference cigarette (IR4F Univ. of Kentucky), which burned tobacco, with another cigarette, which only heated tobacco. 34 Sprague-Dawley rats/sex/group were exposed by nose-only inhalation to 0, 0.12, 0.35 or 0.64 mg total particulate matter/L of either reference or test smoke given 1 hr/day, 5 days/week for 13 weeks. 10 rats/sex/group were held for 6 more weeks and then sacrificed. Clinical appearance, feed consumption, minute ventilation, mortality, body and organ weights, gross- and histo-pathology, and hematologic and clinical chemistry parameters were examined. The test smoke produced significantly less biologic activity than the reference smoke. Histologic changes were either absent, or less frequent and less pronounced in the test groups. Complete regression of histologic findings occurred for all test groups and completely or partially for IR4F groups after 6 week recovery.

EXTENDED INHALATION EXPOSURES OF RATS TO CIGARETTE SMOKE. L Gerald, P H Ayres, A T Mosberg, A W Hayes, G T Burger, J W Sagartz and C R E Coggins, R J Reynolds Tobacco Co., Winston-Salem, NC and Veritas Labs, Burlington, NC.

Sprague-Dawley rats were exposed nose-only to smoke from IR4F reference cigarettes, for 6 hours per day, for 10 days. The concentrations of total particulate matter (TPM) in the smoke was 0.1 and 0.3 mg per liter for extended duration low dose (EDLD) and extended duration high dose (EDHD) groups respectively. Comparisons were made with a reference high dose group (RHD), exposed to 0.6 mg TPM per liter for only one hour per day. Blood carboxyhemoglobin concentrations in the EDHD group were 16% after 1 hour and 35% after 6 hours; they were 34% at the end of the RHD exposures. Animals in the EDHD group showed marked depressions in body weight and in feed consumption, compared with all other smoke-exposed and control groups. In the EDHD group there were no changes in the weights of heart and lungs, and decreases in the weights of liver and spleen. Histo-pathology in the EDHD group provided evidence of considerably more irritation than seen in other groups. The dose administered in the EDHD group would probably not be tolerated in sub-chronic exposures.
The biological effects of 2-week and 13-week nose-only exposures of Sprague-Dawley rats to glycerol or a glycerol-mixture aerosol were evaluated. The liquids were aerosolized using a nebulizer designed specifically for viscous materials. For the 2-week study, 10 male and 10 female Sprague-Dawley rats were exposed to 1, 2 or 4 mg/L glycerol or glycerol mixture, 6 hours/day, for 10 consecutive workdays. In the 13-week study, 15 rats/sex/group were exposed to 0.03, 0.16 or 0.66 mg/L glycerol, 6 hours/day, 5 days/week, for 13 weeks. Experimental criteria included body weights, diet consumption, clinical pathology parameters, survival, gross- and histo-pathologic (including ultrastructural) examinations, and organ weights. Minimal histopathologic changes were observed. No biologically significant effects on any of the other test parameters were observed in either study.

Diacetoxyxscripenol (DAS) (4s,15-diactoxy-12,13-epoxytrichothece-9-ene), a mycotoxin produced by Fusarium sps, has been implicated in several outbreaks of moldy corn toxicosis in farm animals, but a laboratory description of the toxicosis is not available. DAS (0.5, 1, 2, 4 and 8 mg/g diet) was fed to A A Agemoreno and P B Hamilton, N C State University, Raleigh, NC. broiler chickens per treatment from hatching until 3 weeks of age. Growth rate and feed consumption were reduced significantly (P<0.05) by 4 and 8 mg/g while feed conversion efficiency was unaffected. This relationship implied that growth inhibition was in part a consequence of the feed refusal caused by DAS. The most sensitive indicator of DAS toxicosis was spleen size which was reduced significantly (P<0.05) by all levels of DAS. The size of the bursa of Fabricius was decreased by 2, 4 and 8 g/g, the proventriculus and ventriculus were increased by 4 and 8 g/g, and the pancreas was increased by 8 g/g while the other organs were unaffected. There was a dose-related increase in liver hematomas and oral lesions similar to those in T-2 toxicosis. Serum aspartate aminotransferase and cholesterol increased significantly (P<0.05) at growth inhibitory levels. The similarity of the toxic effects of DAS to those of T-2 toxin, a member of another family of trichothece toxins, imply that differentiation of the two toxicoses requires chemical analyses.

Inherent problems are associated with the use of qualitative methods to evaluate, score, and grade irritation in acute intramuscular irritation testing. The present study was conducted to examine some of these problems and to develop ways to improve the techniques used. Male New Zealand White rabbits were assigned to one of three treatment groups (N = 4). The treatments, which consisted of SC-54871 (a novel enkephalin analog), morphine, and haloperidol, were injected into the M. vastus lateralis (ml/site). Twenty-four hours later, animals were sacrificed, and the M. vastus lateralis and overlying muscle were excised, grossly evaluated, and fixed in formalin for subsequent histopathological examination. Muscle irritation, ranging from mild to marked, was observed for all three compounds. Data analyses indicated that the best correlation between irritation scores and pathology was achieved when the M. vastus lateralis and the overlying muscle were evaluated together (rather than evaluating the M. vastus lateralis alone). Also to some extent, the use of two observers instead of one improved the correlation between endpoints.

DAS, 15-diactoxy-12,13-epoxytrichothece-9-ene, and DAS (4s,15-diactoxy-12,13-epoxytrichothece-9-ene), a mycotoxin produced by Fusarium sps, has been implicated in several outbreaks of moldy corn toxicosis in farm animals, but a laboratory description of the toxicosis is not available. DAS (0.5, 1, 2, 4 and 8 mg/g diet) was fed to 10 A A Agemoreno and P B Hamilton, N C State University, Raleigh, NC. broiler chickens per treatment from hatching until 3 weeks of age. Growth rate and feed consumption were reduced significantly (P<0.05) by 4 and 8 mg/g while feed conversion efficiency was unaffected. This relationship implied that growth inhibition was in part a consequence of the feed refusal caused by DAS. The most sensitive indicator of DAS toxicosis was spleen size which was reduced significantly (P<0.05) by all levels of DAS. The size of the bursa of Fabricius was decreased by 2, 4 and 8 g/g, the proventriculus and ventriculus were increased by 4 and 8 g/g, and the pancreas was increased by 8 g/g while the other organs were unaffected. There was a dose-related increase in liver hematomas and oral lesions similar to those in T-2 toxicosis. Serum aspartate aminotransferase and cholesterol increased significantly (P<0.05) at growth inhibitory levels. The similarity of the toxic effects of DAS to those of T-2 toxin, a member of another family of trichothece toxins, imply that differentiation of the two toxicoses requires chemical analyses.

The rabbit colon model (Fara et al. The Toxicologist 6(1): 307[1986]) was used to evaluate nine controlled-release oral potassium products. In this evaluation, a new controlled-release formulation, GITS (KCI) was compared to the following commercial KCI products: Slow-K 8 mEq, Micro-K 10 Extencaps, K-Tab, Klortrix, Kaon CI-10, Kdur 10 mEq, Kdur 20 mEq and Ten-K. The proximal colon of an anesthetized rabbit, with blood supply intact, formed the floor of a continuously perfused test cell in which the test articles were placed directly on the isolated colonic mucosa for 3 hours. Following the exposure, the mucosal test sites were cleared of debris and photographed. Any area of irritation was assigned a score (0-4) and the area was measured (0-6.95 cm²). The score and area values were multiplied to yield an Irritation Index (0-27.7). The KCI products were tested on five occasions and had the following mean Irritation Indices: K-Tab, 21.0; Slow-K, 16.2; Micro-K, 15.6; Kdur 20 mEq, 15.3; Klortrix, 14.4; Kaon CI-10, 7.4; Ten-K, 6.1; Kdur 10 mEq, 6.6; and GITS (KCI), 0.6. This study demonstrated that GITS (KCI) had a lower Irritation Index than 7 commercial controlled-release KCI products.

The subchronic toxicity of a 1,6-naphthyridine calcium modulator was evaluated in male and female beagle dogs. The compound was administered orally in gelatin capsules to groups consisting of 3 dogs/sex at daily dose levels of 0, 0.5, 1, 5, 10, 20, 30, and 40 mg/kg for 4 weeks. Deaths occurred in the females at doses of 20 mg/kg/day and above and in the males only at 40 mg/kg/day. All deaths occurred within the first week of dosing. Clinical signs, generally seen at dose levels of 20 mg/kg/day or more, included emesis, salivation, anorexia, hypoactivity and prostration. Mean systolic and diastolic blood pressures were reduced in males at doses of 1 mg/kg/day or more and in females at doses of 5 mg/kg/day and greater. Twenty-four hour monitoring of cardiac events indicated prolonged periods of bradycardia and sinoatrial block in several animals prior to death. Elevations in ALT, AST, ALP, bilirubin, creatinine, BUN, and K+ were noted primarily in dogs given 40 mg/kg/day. Gross pathologic findings included edema of several organs, and erosions and hemorrhages in the GI tract. Microscopic lesions, were observed in the lymphoid tissues, bone marrow, liver, stomach and intestines. Doses of 10 mg/kg/day and below were well tolerated.


Acute and subchronic toxicity testing was conducted to determine the toxic potential of ST, a 2-congener detergent ingredient. ST has a low order of toxicity; it had an acute oral toxicity of 2-3 g/kg in rats and was not an eye or skin irritant (rabbita) using the low volume eye test and standard skin irritation test methods, respectively. ST was not mutagenic in 5 mutagenicity tests. A 14-day oral gavage study in rats (dose range 0.05-1.0 g ST/kg) was conducted. Gross and microscopic pathologic changes observed were confined to gastric irritation. The NOEL for gastric irritation in this study was 0.1-0.5 g/kg for males and 0.05-0.1 g/kg for females. A 28-day percutaneous toxicity study in rabbits produced no adverse systemic effects at a high dose of 450 mg/kg/day. Acute AIME studies in male rats showed that 10-15% of the oral dose and 1-3% of the dermal dose were absorbed. Approximately 98% of orally administered ST was eliminated as 14C in urine, feces, or expired CO2 after 72 hours. Approximately 80% of the dermally absorbed 14C dose was eliminated in urine, feces or expired CO2 after 72 hours. In summary, ST has a low acute and subchronic toxicity profile, similar to that of other structurally-related materials used safely in consumer products.


Amsacrine (N-[4-(9-acridinylamino)-3-methoxyphenyl]malondialdehyde) is a synthetic DNA intercalating agent. The present study was conducted preliminary to a tumorigenicity bioassay. Groups of 30 male Wistar rats were administered amsacrine intravenously at doses of 3.0, 1.0 and 0.25 mg/kg daily for five days followed by 23 days without treatment, repeated six times. Five animals per group were sacrificed on the eighth and twenty-sixth day of the first, third and sixth treatment cycles. Hematological, clinical biochemical and pathological parameters were evaluated on each animal at sacrifice. There were no deaths during the study. Body weight gain (24%) and food consumption (50%) occurred in the high dose group and leukopenia and megalocytopenia were observed at each sampling interval in animals administered 3.0 and 1.0 mg/kg. Lymphoid depletion, hypocellularity of bone marrow, segmental degeneration of seminiferous tubules and renal tubular degeneration were observed after dosing at 0.25 mg/kg. With the exception of testicular and renal tubular changes which appeared after the sixth treatment cycle, pathologic lesions were reversible during the recovery period.

1022 PRECHRONIC TOXICITY OF p-NITROBENZOIC ACID IN RATS AND MICE. B S Levine1, K M Abdo2, R Kovatch3, M Elwell4, and L T Mulligan5. Microbiological Assoc. Bethesda, MD; 2 NTP, RTP, NC; 3 Pathology Assoc., Ijamsville, MD.

These studies examined the toxic effects of p-nitrobenzoic acid (p-NBA) in F344 rats and B6C3F1 mice following 13 weeks of treatment. Dose levels (% in diet) were 0, 0.065, 0.125, 0.25, 0.5, and 1.0% for rats, and 0, 0.125, 0.25, 0.5, 1.0 and 2.0% for mice. Toxicologic endpoints included clinical signs, body weights clinical pathology (rats only), organ weights and gross/tissue morphology. Mortality and clinical signs of toxicity were not observed. Reductions in body weight gain were seen at the highest doses for both species. At the high dose level for rats, hemolytic anemia consisted of reduced RBC count, hgb, and hct; reticulocytosis; elevated numbers of RBCs with Heinz bodies; macrocytosis; splenomegaly; and splenic hemosiderosis. Increased SGPT, decreased alk. phos. and methemoglobinemia were also seen at this dose. For both species, testes weights were decreased at the higher dose levels and was accompanied by degeneration of the seminiferous tubules. Additional organ weight changes for rats and mice were not accompanied by histologic alterations.

28-Day repeated dose studies of ortho, meta, para and a meta:para (60:40) mixture of cresols were conducted to determine the comparative toxicity of these isomers and the mixture. Data from these studies were used to select compounds for study and to determine dose levels for subsequent 13-week studies of cresols. All isomers and the mixture were given in dosed feed containing 3.10, 0.3, 0.1, 0.03 or 0 per-cent cresols to F-344 rats and B6C3F1 mice of each sex. Five animals per group were fed 7 days a week for 28 days. Toxicity was evaluated on the basis of mortality, body weight, organ weight, histopathology and clinical signs. No mortality was observed in rats. All high dose mice fed p-cresol died, and 20-40% mortality was observed in the high dose groups of ortho and m-cresol. Significant reduction in final body weights for mice occurred at only the 3% level for ortho, meta and mcp-cresols. Final body weights for rats were significantly reduced at the high dose level for all three isomers and the mixture. Pathological effects were found in bone marrow, uterus, ovary, liver and kidney. The spectrum of pathology varied somewhat for the different isomers; however, toxicity was not markedly different.


The issue of using a "body weight" or a "body surface area" approach in calculating dietary exposure to pesticides in the EPA Tolerance Assessment System (TAS) is discussed. TAS provides the capability for identifying the most highly exposed subpopulations. These are most frequently infants and children, since they eat more food than adults relative to their body weight. The question has arisen whether the currently used exposure estimates expressed in units milligram per kilogram body weight tend to overstate risk for infants and children, especially since clinicians frequently scale dose for different sized individuals on a surface area basis. Yet Allowable Daily Intake (ADIs) are currently derived from animal data on a body weight basis. We discuss how the use of the two different approaches to extrapolate firstly from animal to adult humans and secondly from adults to children may not provide for an adequate margin of safety. Finally, we show by example how the use of either approach may not be health protective because it provides for inconsistent differences in sensitivity between adults and children. In conclusion, exposure estimates in TAS calculated on a body weight basis will be consistent with toxicological procedures used and are more protective of infants and children.


The effects of treatment with C6A-12223 were evaluated in mice and rats in 10- and 24-month diet-dose studies, respectively. Diet levels ranged from 0.1 to 300 ppm. Animals were killed at 12, 18 (mice) or 24 months (rats) and after a 4-week recovery period following 12 months of dosing. Tissues were examined microscopically and brain cholinesterases (CHE) was determined. Clinical pathology, including CHE determinations, was done at 6-month intervals. Dose level-related signs of toxicity, including decreased survival, were typical of CHE inhibition. At doses above 1 ppm, plasma and RBC cholinesterase activities declined until at the highest dose they were about 10% and 26% of control, respectively. However, brain CHE activity was never lower than 65% of control even at the highest dose tested. The percent inhibition of CHE at a given diet level was consistent at all time points, but reverted to control levels after 4 weeks recovery. No treatment-related or single neoplasm or combination of neoplastic and preneoplastic findings of a specific cell type were found to be treatment-related.


A bone replacement material consisting of a copolymer and a bone inductive agent is being developed. This study observed the effects of various degassing times, both in room air and with vacuum drying on the in vitro cytotoxicity of the copolymer in a 51Cr release assay. An initial experiment employing L929 cells used 0.5 mm thick disks of the copolymer and ETO degassing times from one to 14 days. At 24 hr post cell treatment, the disks degassed for up to 14 days caused significantly increased cytotoxicity. A second experiment with L929 cells used copolymer blocks which were cut into 1 mm disks and ETO degassing times of 2, 4, and 6 weeks and a vacuum degassing of 72 hr. Results at 24 hr post cell treatment showed a time-related decrease in cytotoxicity from 2 to 6 weeks degassing, but all times still caused a significant increase over the untreated disks. Vacuum degassing was comparable to 4 weeks in room air. A third experiment observed ETO vacuum degassing times of 1, 7, and 14 days using both L929 and human fibroblast cells. Results with both cell types demonstrated that vacuum degassing up to 14 days did not reduce the cytotoxicity to the levels observed for samples which had not been ETO sterilized.
Consumer safety assessments for dishwashing products (automatic dishwashing granules and liquids, and hand dishwashing liquids) require careful consideration of: 1) the potential toxicity of the product and its ingredients, and 2) the types and extent of potential consumer exposures. To assess the extent of consumer dermal, inhalation, and ingestion exposures to dishwashing products and their ingredients, realistic guidelines have been developed. Key data for dishwashing products include surveys of how the products are used by consumers (e.g., typical tasks, frequency and duration of task, and concentration of product used for task). Key data for ingredients include: 1) analytical determination of the possible levels of dinnerware deposition and subsequent extraction into food, 2) percutaneous flux rate data, and 3) oral absorption data.

An approach to determine the extent of consumer exposure to airborne materials in laundry detergent products which may have health effects is presented using an enzyme, e.g., Subtilisin, as a model material. This enzyme provides a good model since extensive data on enzyme health effects are available and there are strict industrial hygiene guidelines for their use. Also, there have been no confirmed cases of consumer health effects with current enzyme-containing detergents. A comparison can be made between the consumer exposure and that which an employee could safely experience during a working day. The present calculations are based on: (1) exposure via inhalation to total enzyme dust from granular detergent product pouring (either into an automatic washer or into a sink for hand laundering), and (2) total airborne enzyme generated as a result of the use of a clothes dryer which is not vented indoors (the exposure from the clothes dryer includes cleaning of the dryer lint screen).

Rigorous evaluation of the skin sensitization potential of new consumer product ingredients includes analytical characterization, guinea pig and human prophylic testing and, in some cases, controlled product use tests. At each step, results from tests on the new ingredient are compared to those on benchmark materials. The decision to use the new ingredient in a product depends on results and anticipated consumer exposure. Also critical is the follow-up of consumer skin comments, a process that provides on-going confirmation that the new ingredient is safe under conditions of exposure for that particular product. To illustrate this evaluation scheme, data on two chemicals are reported. For alkyltrimethoxysilane, product use tests among sensitized subjects, sensitization patch tests of product formulations, and exposure data demonstrated that this class of ingredients could be used safely in laundry detergents. For alpha olefin sulfonate, clinical dermatitis in two patch test-sensitized individuals upon use of a dishwashing product containing the new ingredient indicated the ingredient should not be used for this particular product application.

Existing methodologies for determining occupational exposure criteria are reviewed to select those most appropriate for application to chemical agents. Among those agents of current interest are the nerve agents VX, GA, GB, and GD, and the chemical vesicants sulfur mustard and lewisite. Although the most likely route of exposure to these chemicals is by inhalation, much of the toxicological information will come from studies in which other exposure routes are used. Therefore, in addition to the inhalation exposure route, methods are presented for threshold toxicants that demonstrate how to calculate inhalation exposure criteria when the toxicological information is from oral and parenteral routes. Other than route-to-route extrapolation, the methods presented also accounts for subpopulation sensitivities and for animal and human response and duration of exposure differences. For carcinogens, (i.e., sulfur mustard) the linear multistage model used by the Environmental Protection Agency is recommended. Supported by US Army Med Research Development Command PP 6838. *Operated by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05- 84021400*.
CONSIDERATION OF TOXICOLOGICAL INTERACTIONS IN THE DEVELOPMENT OF REGULATORY CRITERIA FOR KETONE MIXTURES. S M Dizzio and J J Yong. California Department of Health Services Toxic Substances Control Division, Sacramento, CA. Sponsor: J P Christopher

It is a goal of the State of California in the mitigation of toxic waste sites to develop health-based exposure criteria for the risk manager. These criteria are needed in order to protect humans or other sensitive species at the point of contact. As chemical agents at waste sites often occur in mixtures, possible potentiation of toxic activities are valid considerations. Therefore, the toxico logical interactions between the neurotoxic compounds n-hexane, methyl n-butyl ketone (MBK), and the non neurotoxic compound methylethylketone (MEK) were examined. Noneffective levels of exposure via inhalation or ingestion were first determined for the separate compounds, with consideration of electrophysiological, microscopic, and clinical endpoints. These endpoints vary in sensitivity between experiments, thus no one endpoint alone could be used to designate a dose producing no observable adverse effect. Next, as MEK is known to potentiate the neurotoxicity of n-hexane and MBK, an algorithm was developed to change the predetermined allowable exposure levels of the neurotoxins when MEK was present in an exposure mix containing compounds capable of metabolism into a stable gamma diketone form.

EVALUATION OF NONCANCER HEALTH HAZARDS ASSOCIATED WITH WASTE-TO-ENERGY FACILITIES. M A Hart*, G V Alexeoff, J F Collins, and N Gravitz. California Department of Health Services (CDHS), Berkeley, CA. *consultant to CDHS.

Inclusion of municipal solid waste with concomitant energy production is being proposed as a solution to the problem of waste disposal in the United States and elsewhere. Waste-to-energy facilities may be associated with environmental and public health risks. In order to assess risks from noncancer health hazards posed by these facilities, multipathway exposure assessments were evaluated to determine exposure to chronic toxicants emitted from waste-to-energy facilities in California. Results of the multipathway exposure assessment were compared to NOELs and LOELs for chronic toxicity endpoints. Exposure to acutely toxic compounds was evaluated for the inhalation pathway and a hazard index approach was used to assess the risk posed by acute toxicants. Emissions of lead and mercury from waste-to-energy facilities appear to have the greatest potential for producing chronic adverse health effects. Hydrogen chloride and other respiratory irritants have the greatest potential for producing acute effects.


The National Toxicology Program (NTP), under the auspices of Superfund Act is initiating toxicological studies of chemical mixtures of environmental concern. The first mixture to be studied is a representative of groundwater contaminants derived from hazardous waste disposal. Phase 1 effort, chemistry development work, centered on the formulation of a chemical mixture containing the most frequently detected groundwater contaminants. The work encompassed a separate purity analysis of each of the chemicals as well as dose formulation and analysis of the multiple component system under normal laboratory conditions. Anticipated problems included: analytical method development, sample handling, logistics, limitation of solubility, chemical interactions, solution stability, and extreme volatility of some of the components in the unique matrix. A technically achievable solution containing 25 chemicals (19 organics and 6 inorganics) in deionized water was finally formulated at approximately 90% saturation levels of the components. Based on the analytical data obtained, we believe that this formulation, and its serial dilutions, may be used to assess the subchronic and chronic toxicity of a chemically defined mixture of groundwater contaminants in laboratory animals.


A preliminary method was developed for assessing potential human and ecological risk associated with indirect exposure to contaminants emitted from municipal waste combustors (MWC). Models used to estimate indirect human exposure are: Industrial Source Complex (ISC), Terrestrial Food Chain (TFC), Surface Runoff (SRM), Groundwater Infiltration (GIM), and Dermal Exposure (DEM). The ISC predicts pollutant atmospheric dispersion and ground level concentration of emitted pollutants. TFC and DEM estimate human daily intake of contaminants via food and/or soil. SRM and GIM estimate contaminant water concentrations. Typical worst exposure scenarios are developed for a hypothetical most-exposed individual. Predicted human exposure from ingestion and dermal absorption following pollutant deposition to surface layers of soil, runoff into surface waters, migration to groundwater and bioaccumulation into the food web is compared with EPA-established reference values for systemic toxicants or unit risk levels for carcinogens. An Ecological Model assesses indirect exposure and risk to soil biota, soil biota predators, plants, and aquatic organisms and wildlife aquatic predators.
1035 COMPUTER BASED ANALYSIS OF LEAD TOXICITY ON ATPase KINETICS IN RAT BRAIN SYNAPTOSOMES. S Rajanna, N Abston, and B Rajanna. Department of Computer Science and Mathematics, and Division of Natural and Applied Sciences, Selma University, Selma, AL. Sponsor: S F Rao.

Various computer programs are available to explain the kinetics of a number of enzyme reactions. However, most all the computer programs available in the literature are specific to certain specific reactions. Hence, this study was undertaken to develop a comprehensive computer program to explain the kinetics of lead toxicity, and to provide alternate routes other than Lineweaver-Burk reciprocal plots for presentation of the Michaelis-Menton kinetic data. The experimental procedures consisted of in vitro treatments of freshly prepared rat brain synaptosomes with 0, 50, and 100 μM lead. Various concentrations of ATP, Na+ and K+ ions were used as substrate. The computer on-line control and off-line analyzing programs were written in R BASIC, using IBM PC AT computer with 640K RAM and 20 MB hard disk, DOS 3.2, color graphic card. The main computer program includes Subroutines for two alternate Lineweaver-Burk plots; Hanes-Woolf: [S]/v versus [S] and the Woolf-Augustinson-Hofstee plot: v versus v/S. The results indicated that the computer program was more versatile compared to conventional graphic analysis. Hanes-Woolf, and Woolf-Augustinsson plots were comparable to Lineweaver-Burk plot.


The relationship between asbestos fiber size and carcinogenic potential has not been thoroughly studied, but there is increasing evidence that long asbestos fibers (≥ 8-10 μ) are more carcinogenic than short fibers. For example, Stanton et al. (J. Nat. Cancer Inst. 58:587-607, 1977) administered 17 samples of fiberglass intratracheally to rats, and observed the greatest incidence of tumors for fibers in excess of 8 μ in length. Similar findings in rats were reported by Wagner et al. (Br. J. Cancer 28:173-185, 1973) for several types of asbestos and asbestiform fibers. Most oral studies of asbestos carcinogenicity have been negative, but these have typically involved exposure to short fibers (< 10 μ). The NTP has reported that chronic ingestion of intermediate range chrysotile fibers (6% > 10 μ) results in benign epithelial neoplasms in the intestine in male rats, while no effects were observed for short-range fibers (98% < 10 μ) (NTP Pub. No. 84-2551). Since most asbestiform fibers detected in drinking water are short (an average of 4 μ) (Millette et al., Environ Health Pesp. 34:19-25, 1980), these findings indicate that any regulations of asbestos levels in drinking water must take fiber length into consideration.

1037 SCREENS AND GRAPHICAL METHODS OF ANALYSIS IN TOXICOLOGY. S.C. Gad, Department of Toxicology, G.D. Searle and Co., Skokie, IL.

Screening for (either the presence or absence) of activity constitutes the single largest activity in toxicology. Though most screens are focused on a single end-point (mutagenicity, irritation, lethality, neurotoxicity, etc.), their actual design, conduct and analysis fail to reflect this or to take into account the special characteristics of screens.

The three types and operating characteristics of screens, as the biological analog of exploratory data analysis (EDA) in statistics, are presented and evaluated. Methodology for improving design and operation, for incorporating historical data, and for rapid analysis of results is proposed and evaluated.

Similarly, graphical methods constitute the best known form of EDA in toxicology, yet are minimally understood or utilized. The full range of univariate and multivariate graphical methods as they apply to toxicology will be overviewed, along with their common guiding principles. Graphical analysis of various screening data sets (both single end-point and "shotgun") by multiple methods will be demonstrated and the resulting performance evaluated.

1038 TOXPERT AN EXPERT PRODUCT RISK ASSESSMENT SYSTEM R J Soto, T C Osmitsz, R E Turk and R D Stewart. S C Johnson and Son, Inc., Racine, WI.

TOXPERT is a personal computer based artificial intelligence (AI) advisory system used to simulate the product approval decision processes. The basis of TOXPERT was a decision framework using an "expert system" or "knowledge base." The primary focus of TOXPERT was a goal seeking strategy for product marketing approval. Backward chaining rule control linked the "marketing approval" goal to the type of product, regulatory agency, exposure conditions and toxicity. Marketing risks are primarily a function of the toxic hazard and exposure potential. The method differentiated between regulatory requirements in goal seeking control for various types of products. In addition, TOXPERT produces classifications of toxicity ratings and suggested product labeling. This production rule system uses principles on toxicology, regulations, corporate guidelines and internal "rules of thumb." In summary the AI paradigm provides a structured environment for problem solving. TOXPERT is suitable for this narrow domain as an advisor. An advantage is that it can make routine decisions freeing the toxicologist's time for more complex problem solving. Lastly, this system can also serve as a backup to the toxicologist and as a training aid.
LIMITATIONS OF CHRONIC RODENT BIOASSAYS FOR THE QUANTITATIVE RISK ASSESSMENT OF HUMAN CARCINOGENS. J P Rieh and T B Starr. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

One method of quantifying human carcinogenic risk has been to define a standardized parameter, carcinogenic potency, which could be used to compare the risks associated with exposure to different chemicals. The Environmental Protection Agency uses a more conservative index of carcinogenic risk, an upper-bound risk estimate. The data presently used to calculate such potency and upper-bound estimates are usually derived from chronic rodent bioassays. Others reported that good correlations exist between potency estimates for rats and mice which developed tumors after exposure to the same chemical. This finding was used to justify the extrapolation from these data to human risk. The high correlations, however, are artifacts of the chronic bioassay experimental design. We have demonstrated this by showing that the correlation between the carcinogenic potencies for rats and mice which did not develop tumors is as strong as the correlation for those that did (r = 0.79, n = 82 and r = 0.83, n = 83, respectively). The correlations are also large between rats developing tumors and mice not (r = 0.73, n = 76), and mice developing tumors and rats not (r = 0.76, n = 93). These high correlations are inevitable because the magnitude of the potency estimate is affected more by the maximum dose tested than by the treatment related carcinogenic response.

MINERAL FIBER CYTOTOXICITY TO RAT PLEURAL MESOTHELIAL CELLS. L D Paler, J F Eyre, D G Rocha, and D L Coffin. Northrop Services, Inc. and US EPA, RTP, NC.

We have developed and standardized a clonal cytotoxicity assay with rat pleural mesothelial (RPM) cells that looks promising as a predictor of tumorigenesis of mineral fibers. The RPM cells were exposed to 4 mineral samples which produced mesotheliomas in rats and 7 mineral fibers which were negative in the same system. The cells were incubated in DMEM, 10% FBS and antibiotics at 37°C and 5% CO₂ for 7 days. The number of colonies formed were then counted. Cytotoxicity was measured as the percentage of surviving colonies in exposed dishes relative to controls. The results indicate that mineral fibers fall into one of two categories. Tumorigenic fibers induce >50% cytotoxicity in the dose range of 1-100μg/mL, while non-tumorigenic mineral fibers induce <50% cytotoxicity over the same dose range. Cytotoxicity of erionite, crocidolite and chrysotile were further analyzed by expressing the dose in the number of fibers. The results indicate that the dose required to produce 50% cytotoxicity was in increasing order for erionite, crocidolite and chrysotile. The number of fibers of dimension L×8μm and W×0.25μm was 0.07x10⁶, 0.15x10⁶ and 2.94x10⁶ per ml respectively. This correlates with the tumorigenic potencies of the mineral fibers. Erionite is more tumorigenic than crocidolite and chrysotile.

DEVELOPMENT OF A DIELECTROPHORETIC ASSAY FOR CELLULAR TOXICOLOGY. M B Fatmi and S B Baumann, Northrop Services, Inc.; R J Spiegel, U S Environmental Protection Agency, Research Triangle Park, NC. Sponsor: R W Luebke

Dielectrophoresis is the translational motion that results when nonuniform electric fields act on neutral but polarizable particles. For cellular suspensions this causes the cells to collect in chains (yield) on the electrodes. To determine the utility of dielectrophoresis as a toxicological tool, this study measured the dielectrophoretic spectra (yield vs. frequency) produced by Saccharomyces cerevisiae exposed to the fungicide Funigex (triforine). Spectra were obtained from 1 KHz to 100 MHz for untreated, autoclaved (dead) and chemically treated cells at a variety of doses. Untreated cells showed a peak in yield at 40 KHz, whereas dead cells had a flat curve with yields about 1/2 as large. Chemically treated cells at high doses mimicked the dead cell spectrum, but cells treated at smaller doses had a spectra in between those of untreated and dead cells. These results demonstrate that dielectrophoresis may be a useful assay of cellular toxicity. (This abstract of a proposed presentation does not necessarily reflect EPA policy.)
Argininosuccinate Lyase (ASAL), a cytoplasmic urea cycle enzyme, has recently been demonstrated to be a sensitive marker for centrolobular liver injury in rats. To evaluate whether serum ASAL would be elevated following injury in the perportal zone of the hepatic lobule, male Sprague-Dawley rats (TACN(SD)FBR) were treated with a single oral dose of allyl alcohol (4.0 ml/kg of a 2% solution) and serum and tissues were collected at either 0, 16, 24, 48, 72, or 168 hours post-dose. Histologically, marked perportal necrosis with mild acute inflammation occurred as early as 16 hours post-dose and was generally associated with extreme elevations in SGPT, SGOT, and ASAL as well as GTT, total bilirubin, and cholesterol. Typical baseline values for serum ASAL ranged from 0-4 ASAL Units (umol Arg/hr/dl). In this study, the percent elevations over baselines for ASAL were not as exaggerated at peak levels as the elevations in transaminases; however, serum ASAL values were clearly sensitive to allyl alcohol-induced hepatic injury. These findings further support the potential use of ASAL as a serum enzyme marker for hepatic injury in rats.

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Also present:


To evaluate whether serum Argininosuccinate Lyase (ASAL), a cytoplasmic urea cycle enzyme, would be elevated following acute cholestasis, male Sprague-Dawley rats (TACN(SD)FBR) were given a single oral dose of ANT (150 mg/kg) and serum and tissues collected at either 0, 16, 24, 48, 72, or 168 hr post-dose. Histologically, significant degenerative changes were observed in the portal zone of the liver. Serum ASAL, GPT, GOT, GTT, total bilirubin, and cholesterol were all elevated at 16 hr and typically reached peak levels at 24 or 48 hr post-dose. In general, the elevations in serum liver parameters were associated with morphologic hepatic injury and typically were equivalent to baseline levels by 168 hr. Baseline and control serum ASAL ranged from 0-4 ASAL Units (umol Arg/hr/dl) and values greater than 5 were usually associated with liver lesions. The results of this study indicate that serum ASAL values are elevated following cholestatic injury by ANT in the rat, and further support the potential use of ASAL as a serum marker for liver injury.
The usefulness of routine urine sediment examination in toxicity studies was evaluated using a mild renal dysfunction induced in Beagle dog by administration of a known tubular nephrotoxic agent: HCB D. Twelve dogs randomly allocated into three groups of four animals, (one olive oil control, two HCB D-treated groups, at dosage levels of 2.5 and 5 mg/kg/day) were orally dosed daily for 21 days. Complete urinalyses and serum BUN and creatinine were regularly carried out. While there were no serum BUN and creatinine changes, microscopic examination of urine sediments revealed granular casts: onset of this change was dose-related and severity was dose and time dependent. In addition, increased proteinuria and dose-dependent increased \( \gamma \)-GT/creatinine ratio were observed. Histopathological evaluation of kidney sections confirmed the tubular pathology. Urine sediment analysis provides early, accurate and reliable information for toxicity assessment of a xenobiotic.


Reducing the number of animals used in acute toxicity/irritation tests may affect hazard communication labeling as set forth by the U.S. Occupational Safety and Health Administration (OSHA) and the European Economic Community (EEC). A reanalysis of petrochemical toxicity/irritation data was conducted to evaluate this possibility. Thirty eye and 29 skin irritation studies were randomly selected for reanalysis. From each original test group of 6 rabbits, data from 3 animals were randomly selected for recalculation and comparison to the original results. Twenty oral toxicity studies were reanalyzed: male and female LD50s were individually compared to data from both sexes combined. Correlation coefficients of .975, .979, .937, and .982 were obtained for the eye irritation, skin irritation, male LD50 and female LD50 data comparisons, respectively. Under OSHA, different labeling would have been required using fewer animals in 1 toxicity (males only) and 3 eye irritation studies. Under EEC, different labeling would have been required in 2 eye irritation, 4 skin irritation, and 2 toxicity (1 male vs. combined, 1 female vs. combined) studies. Thus, using a reduced number of animals in safety evaluations of petrochemicals should not greatly impact upon labeling decisions.


In order to identify a low-stress blood sampling method for rats the following techniques were compared: T: whole body warming at 35°C and venepuncture of lateral tail vein; O: ether anesthesia and rupture of the retro-orbital plexus; J: (new method) jugular venepuncture - the rats are held supine in the hand and the external jugular vein is punctured using a 16mm x 21G needle. Six groups of 5 male Wistar rats/group were bled on 3 separate occasions. At each bleeding time, groups were assigned randomly to bleeding method. Plasma corticosterone (CS) concentrations were used as a measure of procedure-induced stress. Mean plasma CS after each method was T: 399ng/ml, O: 363ng/ml, J: 210ng/ml. In addition, blood taken by each method provided comparable haematological and clinical chemical values. Furthermore, preliminary results using mice and guinea-pigs showed that technique J also has utility in these species. Thus, the newly developed jugular venepuncture technique, which is generally applicable to rodents, produce less stress than methods T or O, while yielding samples that produce similar haematological and clinical chemistry results.

Subchronic Evaluation in the Rat to a Microchip Implant Used for Animal Identification. D. J. Ball R. L. Robison, R. E. Stoll, and G. E. Wisscher. Sandos Research Institute, East Hanover, NJ 07936

A new method using electronic microchip implants for animal identification is being evaluated to replace ear tags or tail tattoos in rodents. The microchip is encapsulated in glass, cold sterilized and subcutaneously implanted. Fifty-five male and 55 female rats (Sprague-Dawley) were implanted with a microchip identification number (BioMedic Data Systems, Inc.). The animals were housed individually in a Bioclean® room. Clinical observations, body weights and mass palpations were recorded weekly. Food consumption was monitored once/month. Five animals/sex were sacrificed in Study Weeks 2, 12, and 26 for histopathological evaluation of the implant site. An implant from one animal/sex was evaluated for significant structural changes by scanning electron microscope (SEM). The remaining animals on study will be maintained and evaluated at Study Weeks 52 and 105. No effect was observed on body weight gain and food consumption. Animals appeared normal and palpable masses were not detected at the implant site. Histopathologically, the microchips were surrounded by a very thin rim of fibrous connective tissue with a few inflammatory cells present. No acute or chronic inflammatory reaction or fibrous capsule formation was seen in response to the implant. No alterations to the implant were detected by SEM. For subchronic use, the microchip implants are suitable for animal identification.
The use of microencapsulated chemicals in toxicity studies offers investigators significant advantages of improved stability, longer storage life of dose formulations, reduced study costs vs. gavage, and greater safety in handling. The stringent requirements for purity, stability, digestibility, particle size, and chemical load imposed by these studies, however, severely limit the choices of acceptable encapsulating media and processes which can be used. An encapsulation method was developed which satisfies the constraints of these studies and which uses all food-grade materials for shell systems. A number of chemicals have been encapsulated by the method for use in toxicity studies, which otherwise would not have been amenable for evaluation. Among these were reactive or volatile alcohols, aldehydes, low-boiling chlorinated hydrocarbons, and refined marine oils. Stability studies of the microcapsules indicated greater than 97% retention of chemical after 28 days' exposure to ambient conditions. Studies of microcapsules in diet mixtures have indicated stability which permits storage of formulations for at least 3 weeks.

TCE and 2EH have been microencapsulated in a starch/sucrose or a starch matrix for studying the toxicological properties of these chemicals. Microcapsules containing 47.3% (wt./wt.) 2EH were shown to be stable for at least one year and for at least 3 weeks when mixed with NIH-07 rodent feed. Microcapsules containing 51.3% (wt./wt.) TCE showed similar stability. In separate palatability studies 2EH and TCE microcapsules were mixed with feed at microcapsule concentrations of 0, 0.2, 0.5, 1.2 and 3.0% and provided to groups of 10 male F344 rats for two weeks. Feed consumption was similar among all microcapsule dose fed and control groups. Absorption of the chemical from the microcapsules was compared to the neat material by administering the same dose of either the microencapsulated chemical suspended in corn oil or the neat chemical dissolved in corn oil to rats and comparing blood levels over time. There were no statistical differences in Omam, Tmax or AUC. These results demonstrate the utility of microencapsulation of 2EH and TCE as an alternate means for the study of their toxicological properties of these chemicals.
SORPTION OF AFLATOXINS FROM PEANUT OIL BY ALUMINO-SILICATES. M D Macken, B A Clement, E C Shepherd, A B Sarr, R E Pettit and J D Phillips. Veterinary Public Health and Plant Pathology and Microbiology, Texas A&M University, College Station, TX.

Aflatoxin B₁ (AFL) is a hazardous mutagen and hepatocarcinogen that can occur frequently as a contaminant of peanuts. Methods of detoxification have been reported, but are generally impractical and/or ineffective. Studies were performed to determine the ability of representative clay and zeolitic minerals (i.e., aluminosilicates) to sorb AFL from contaminated peanut oil. Initial studies were conducted with radiolabeled AFL indicated differences in the degree of sorption of AFL by phyllosilicates and zeolites. Hydrated sodium calcium aluminosilicate (HSCAS) removed more than 90% of the total AFL (1.0 ppm) from peanut oil. Extracts from contaminated peanut oil containing concentrations of aflatoxins ranging from 10.0 ppm to 0.01 ppm were tested for mutagenicity by the Ames assay. The mutation response of Salmonella typhimurium (strains TA98 and TA100) to AFL was significantly decreased following sorbent treatment. These results support the conclusion that the sorption of AFL, and possibly other mycotoxins by appropriate aluminosilicates, may represent an effective and practical approach to the detoxification of crude peanut oil (Supported by USAID CRSP 02-50305-2).

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IN VITRO INHIBITION OF MOUSE HEPATIC MIXED FUNCTION OXIDASE (MFO) ACTIVITIES BY METHYLANIDIOXYPHENYL (MIP) COMPOUNDS. P Levi, Y C Chui, M Lemandowski, and E Hodgson. Toxicology Program, North Carolina State University, Raleigh, NC.

The inhibitory effect of 11 MDP compounds on MFO activities and the formation of the type III MFB metabolite complex was examined in untreated, phenobarbital (PB) treated, and 3-methylcholanthrene (3MC) treated mice. Microsomes were incubated with the MDP compounds for 5 min and type III complex formation, acetanilide hydroxylase, ethylmorphine N-demethylase and ethoxyquin/marin O-deethylase activities were determined. In general, safrole, isosafrole, dihydroisafrole and butylisodiole readily formed the type III complex with microsomes and were good inhibitors of MFO activities. Substitution on the methylene carbon prevented the formation of the type III complex and diminished the inhibitory effect. Piperonyl butoxide was a better inhibitor for ethylmorphine N-demethylase than for acetanilide hydroxylase and ethoxyquin/marin O-deethylase activities. Ethylmorphine N-demethylase activity was readily inhibited by the MDP compounds in PB treated but not in 3MC treated microsomes when compared to inhibition in microsomes from untreated livers. In a reconstituted system with purified PB induced isozyme from mouse liver, similar inhibitory effects were observed.
Dietary protein levels of 8, 12, and 22% were fed for 14 days to P344 male weanling rats. Activity rates, in liver and kidney, were compared, using several substrates each, for the following enzyme systems: P450 monooxygenase, P450a, (P450 specific content), aldehyde reductase, esterase, and conjugations with sulfate, glutathione, and glucuronide acid. Activity rates, for the various mechanisms, decreased from 15 to 65% with decreased dietary protein, with the exception of glucuronide conjugation which increased with decreased dietary protein. Protein deprivation (0%) and protein excesses (25%) have been shown to be related to altered enzymic activities. However, little attention has been paid to the differences between nutritionally adequate diets (12%) and a marginally low (8%) or typical (22%) diet. The data suggest that typical lab chows (22-25%) may provide artificially altered rates of biotransformation in rats fed standard lab chows.

Pretreatment of rats with phenobarbital (PB), COCl2, and diethyl maleate has been shown to modulate DBCP-induced renal and testicular necrosis. On the other hand, buthionine sulfoximine was found not to affect DBCP nephrotoxicity. Tissue distribution of DBCP was determined after these various modulator treatments and correlated with their effects on organ damage of DBCP. In untreated rats, peak DBCP tissue levels were in the order kidney > liver > testes. Studies were also performed with deuterated DBCP analogs of DBCP to clarify the involvement of oxidative pathways in DBCP tissue elimination. In untreated animals there were no significant differences in tissue distribution between DBCP and perdeutero-DBC, whereas in PB-pretreated animals a significant isotope effect was observed, suggesting the involvement of oxidative pathways in DBCP elimination under these conditions.

The metabolism of C (Sevin®), a widely used insecticide, was studied using rat liver tissue. Time course studies showed that incubation of C with liver tissue resulted in a decrease in the formation of the oxidative metabolite, dihydrodihydroxy carbaryl, at 6 and 18 hr. This decrease paralleled the decrease in cytochrome P-450 content and associated oxidative enzyme activities. When liver microsomes from controls or PB (75 mg/kg/day x 4)-pretreated rats were added to C, a weak Type I binding spectrum was observed. A much stronger spectral Type I interaction was observed when microsomes from 3-MC (25 mg/kg/day x 4)-pretreated rats were used, indicating a greater affinity of C with the cyt. P-450 induced by 3-MC. When liver tissue from controls or PB-pretreated rats was incubated with C for 4 hr, analysis of the incubation showed no significant difference between controls and PB-pretreated rats with respect to formation of both conjugated and unconjugated metabolites of C. However, when liver tissue from 3-MC-pretreated rats was used, a significant decrease in sulfure conjugation and an increased formation of unconjugated metabolites was observed. These data show that pretreatment of rats with the liver microsomal enzyme inducers PB or 3-MC results in differential effects on C metabolism.

Cytocrome P-450-dependent pathways of testosterone hydroxylations catalyzed by liver microsomes from various laboratory animals (10-week-old males) have been studied with an HPLC system capable of resolving 15 potential metabolites of testosterone. The profile of hydroxylated testosterone metabolites formed by mouse liver microsomes was qualitatively and quantitatively similar to that produced by rat liver microsomes. Overall, liver microsomes from guinea pigs, rabbits and cynomolgus monkeys were less active (1/3 to 1/5) than rat liver microsomes in hydroxylating testosterone, whereas liver microsomes from hamsters were considerably less active (2/3x). Liver microsomes from untreated hamsters catalyzed the 18-, 28-, 68-, and 158-hydroxylation of testosterone at rates comparable to liver microsomes from rats induced with pregnenolone-16α-carbonitrile (PCN). Furthermore, these pathways of testosterone hydroxylation were not induced by treatment of hamsters with PCN. Some pathways of testosterone hydroxylation catalyzed by rat liver microsomes were virtually undetectable in other species. For example, liver microsomes from rabbit and cynomolgus monkey did not catalyze the 7α-hydroxylation of testosterone, whereas 2α-hydroxylation was a major pathway only in rat liver microsomes. These differences presumably reflect species differences in the cytochrome P-450 isozymes present in liver microsomes. Supported by NIH grants ES-03769, ES-00166 and ES-07079.

Adult, male Sprague-Dawley rats were exposed nose-only to diluted smoke produced either from a new cigarette (test) that does not burn tobacco or from a conventional cigarette (reference) for 60 minutes. Pulmonary cytochrome P-450 and P-450-dependent monoxygenase activities were measured at 4 and 24 hr after the exposure. Twelve rats were used in each group. Lungs were perfused in situ, and pulmonary microsomes prepared. Rats placed in restraint tubes and exposed to room air served as sham controls; rats given an i.p. injection of 3-methylcholanthrene served as positive controls. There was no significant difference in the protein, P-450 content and cytochrome C reductase activity in rats exposed to smoke or room air at 4 or 24 hr after exposure. The benzo(a)pyrene hydroxylase activity (AH) was not altered after rats were exposed to test smoke for 60 min. However, the AH activity after exposure to the reference cigarette was 288.8% and 215.6% of control activity at 4 and 24 hr, respectively, indicating a difference between test and reference cigarettes in the chosen parameters.

AGE RELATED DIFFERENCES IN THE METABOLISM OF ALLYL ALCOHOL TO ACROLEIN BY SPRAGUE DAWLEY (SD) RATS. J R Gannon, P J Harrison and C L Lage. Philadelphia College of Pharmacy and Science, Philadelphia, PA.

This study was performed to determine if there exists an age related difference in the metabolism and toxicity of allyl alcohol in male Sprague-Dawley rats. Allyl alcohol was administered at three doses (32, 42 and 52 mg/kg, i.p.) to immature (1 month), mature (3-4 months) and old (13-14 months) rats. Monitoring percent change in body weight, liver morphology and serum amino transferase activity, it was found that immature and old rats exhibited greater hepatotoxicity than mature animals from equivalent doses of allyl alcohol. In vitro studies were conducted to determine if the age related differences in allyl alcohol toxicity could be correlated with differences in the metabolism of allyl alcohol to acrolein. Preliminary results indicate that there are insignificant differences among animals of different age in acrolein production and basal levels of reduced glutathione. Since age related differences in toxicity could be related to differential susceptibility of subcellular components to acrolein mediated damage, mitochondrial cytochrome c reductase and aldehyde dehydrogenase activity were assessed. It was found that there was both dose related and age related depression of cytochrome c reductase and aldehyde dehydrogenase activity upon exposure of animals to allyl alcohol. These data clearly indicate an age related difference in allyl alcohol toxicity and implicate mitochondrial damage as a mediator of allyl alcohol induced hepatic damage.


2,2,2-Trifluoroethanol (TFE) produces bone marrow and small intestine toxicity resulting in leukopenia, loss of intestinal function and consequent lethal septicemia in male Wistar rats. This study investigated the specific metabolite which potentiated the toxicity and the site of its formation. Its metabolic pathway was determined to be: TFE => 2,2,2-trifluoroacetaldehyde (TFAl2) => trifluoroacetic acid (TFAA). Administered TFE and TFAl2 were not toxic per se, since their toxicity and metabolism were inhibited by pyrazole. TFE and TFAl2 were equipotent at equimolar doses thus precluding the oxidative reaction, TFE to TFAl2, from being the toxic step. Since equimolar TFAA exhibited no toxic effects, an oxidative intermediate on the pathway from TFAl2 to TFAA, most likely F-C-C=O(NH), must be the toxic moiety. The Intermediate TFAl2 is stable in serum, and is transported to the target tissues, bone marrow and small intestine, after formation in the liver. Based on the more rapid metabolism of TFE to higher levels of TFAl2 in the small intestine and bone marrow than in the serum, and the decreased toxicity of TFAl2 when administered ic versus ip, the formation of the toxic intermediate of TFE must occur in the target tissues. (Supported by NIH grant GM 23029.)

ETHANOL INHIBITS HEPATIC METABOLISM OF INTRAVENOUSLY ADMINISTERED MORPHINE IN RATS. D R Steup and E R Forney, Sr. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

Ethanol pretreatment has been shown to increase the concentration of free (unmetabolized) morphine present in the livers of rats after a single dose of morphine sulfate. The goal of this study was to determine the mechanism of this effect. Naive, male Sprague-Dawley rats (190-220 g) were administered a single intravenous dose of morphine sulfate (2.5 mg/kg) one hour after an oral gavage of either ethanol (3 g/kg) or an equivalent volume of saline. At various intervals (30, 60, or 120 minutes) following the morphine dose, rats were sacrificed and the livers were removed and frozen in liquid nitrogen. Liver homogenates were later assayed by HPLC for free morphine and also subjected to enzymatic hydrolysis and assayed to determine total morphine (free drug + conjugated metabolites). Free liver morphine was increased by 31-73% (p<0.001) while total liver morphine was not altered in ethanol-pretreated animals. Mean ratios of free to total morphine ranged from 0.11 to 0.24 in control groups and from 0.12 to 0.56 in ethanol-pretreated groups. These findings suggest that the observed changes are a consequence of inhibition of the hepatic metabolism of morphine rather than of enhanced delivery of morphine to the liver.
1067 CROSS-COMPETITION STUDIES WITH THE ETHANOL-INDUCIBLE ANILINE HYDROXYLASE USING SELECTED MONOOXYGENASE SUBSTRATES. S Narayan and G W Winston. Institute for Environmental Studies and Department of Biochemistry, Louisiana State University, Baton Rouge, LA. Sponsor: C R Short

Chronic ethanol (EtOH) ingestion induces at least one hepatic cytochrome P-450 (P-450) isozyme that is distinct from those induced by phenobarbital (PB) and 3-methylcholanthrene (3-MC) type inducers. We have studied the effect of chronic EtOH ingestion on several rat liver monooxygenase activities in relation to high fat (HF) and low fat (LF) diet. Rats were pair-fed for 4 weeks with HF (52% of calories) and LF (12% calories) liquid diets containing 36% of calories as EtOH. Expressed per mg protein, aniline hydroxylase (AH), EROD and ECOD were significantly increased over controls in both LF and HF EtOH groups. Per mmol P-450, ECOD and EROD was increased only in the LF group, whereas AH and aldrin epoxidase activities were lowered in both EtOH-treated groups. In the presence of ER, AH activity was inhibited to the same extent by EtOH as by control microsomes. However, EC inhibited AH in the presence of EtOH microsomes from HF and LF rats whereas stimulation of activity was observed with control microsomes. BP stimulated AH activity when EtOH microsomes from LF rats were used but AH was stimulated by both control and EtOH microsomes from HF rats. These results infer that microsomes from ethanol-fed rats catalyzes EROD, ECOD and AH in a manner that is quite different than that of control, PB, 3-MC and Aroclor microsomes. (Supported by NIAAA grant R3AA96758A)

1068 ETHANOL INDUCED FATTY ACID ETHYL ESTER FORMATION IN VIVO AND IN VITRO BY RAT LUNG. J K Manautou and G P Carlsson. Dept. of Pharmacol. & Toxicol., Sch. of Pharmacy, Purdue Univ., W. Lafayette, IN.

Fatty acid ethyl esters (FAEE) are end products of a nonoxidative pathway for ethanol metabolism in a variety of tissues. Our objective was to determine the significance of this pathway in the rat lung. In vivo, [1-14C] ethyl oleate formation was assayed in rat lungs and compared with other organs. Homogenates were incubated with 50 nM Tris-HCl buffer (pH 8.0), [1-14C] oleic acid (20,000 dpm/mmol) and 0.2M ethanol for 45 min at 37°C. Lipo proteins were extracted with acetone, and [14C] ethyl oleate was isolated and quantitated by TLC and scintillation counting. FAEE synthetic activity in the lungs (in vivo) was found to be intermediate among the organs examined. In vivo, male rats received 3% ethanol in drinking water for 12 days. The 5 organs with the highest in vitro activity for the FAEE synthesis (pancreas, liver and lung) were extracted with acetone and the FAEE were isolated by TLC and separated by GC. The lung had lower activity than the liver or pancreas. Ethyl oleate, ethyl arachidonate and ethyl palmitate were the predominant FAEE. Ethanol induced FAEE may play a significant role in the development of alcohol-related injuries to this organ. (Supported by ES03739 and ES04362)

1069 EFFECT OF ETHANOL ON THE DISTRIBUTION OF FOLATE DERIVATIVES IN THE KIDNEY OF THE RAT. B H Elsenga, T D Collins, and K E McMartin. Dept. of Pharmacology, Section of Toxicology, LSU Medical Center, Shreveport, LA

An acute oral ethanol load to rats caused a significant increase in the excretion of 5-CH3-H-PteGlu but not other forms of folates. When rats are given an acute oral ethanol load and then H-PteGlu, there is no increase in the excretion of the labeled folates. These paradoxical results have led to a study of the distribution and urinary fate of a dose of H-PteGlu in rats pretreated with ethanol. Male Sprague-Dawley rats were treated with ethanol p.o. at doses of 1 g/kg each at 0, 1, 2, and 3 h with controls given glucose. A tracer dose of 5-14C-folic acid was infused i.v., and urine samples were collected via ureteral catheters. Urinary excretion of endogenous folate increased about six fold from 5-6 h after ethanol treatment, but labelled folate excretion did not increase. Labelled folates in the kidney by HPLC analysis showed a broad distribution among 10-HCO-H-PteGlu, Hb-PteGlu, 5-CH3-H-PteGlu, and PteGlu, while endogenous folates were mostly 5-CH3-H-PteGlu. Ethanol had no apparent effect on the distribution of renal folate derivatives. Ethanol may inhibit folate excretion by inhibiting reabsorption rather than by altering renal cellular metabolism of folate. (Supported by U.S.P.H.S. grant AA05308)

1070 FURTHER EVIDENCE FOR THE ROLE OF QUINONE REDUCTASE (QR) IN IN VIVO ETHANOL (EtOH) METABOLISM. J H Chung and R J Rubin. Johns Hopkins Univ., Baltimore, MD.

EtOH metabolism involves the conversion of NAD+ to NADH. QR, in the presence of suitable substrate, can result in the regeneration of NAD+ from NADH, thereby suggesting a role in EtOH metabolism and toxicity. We have previously shown that induction of QR by butyric acid (BHA) and/or administration of quinones lead to enhanced in vivo metabolism of EtOH and decreased hepatotoxicity. In the present study, we have investigated the effect of a quinone, menadione sodium bisulfite (MSB; 80 mg/kg, sc) and/or BHA (0.75% in the diet) on the hepatic redox state in male rats given EtOH (2 gm/kg, iv). Cytosolic (cyto) and mitochondrial (mito) NAD+/NADH ratios were reduced by 50% 4 hrs following EtOH administration. Treatment with MSB, BHA, and MSB/BHA prevented this reduction, such that NAD+/NADH ratios remained at non-EtOH levels. Maintenance of cyto NAD+/NADH ratio was correlated with enhanced EtOH metabolism. Inhibition of QR by dicoumarol (25 mg/kg, po) blocked the MSB-induced establishment of EtOH metabolism. Although MSB, BHA, and MSB/BHA pretreatment enhanced EtOH metabolism, blood acetate (ALD) levels remained unchanged from that seen with EtOH alone. These results support the hypothesis that QR has a role in EtOH and ALD metabolism by controlling the cyto and mito redox states, respectively.

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In vitro evaluation of catalase-mediated methanol (M) oxidation in the eye and liver of folic acid sufficient (FAS) and deficient (FAD) long-evans rats. T B Moore and E Lee. Biomedical Science Dept., General Motors Research Labs., Warren, MI.

In conjunction with the development of a rodent model for M toxicity, in vitro catalase-mediated oxidation to HCHO was investigated in the eye and liver of FAS and FAD long-Evans rats. Fresh homogenate of the two tissues were used as sources of catalase (C). The C activity of the tissue homogenate was completely inhibited by NADH (1 mM), a type C inhibitor. In the eye, exogenous H2O2 was used as a substrate to obtain maximal HCHO production. When the liver C activities of FAS and FAD rats were measured in the presence of 2 mM H2O2, the HCHO production was about the same. However, under the same condition, HCHO production by FAD eyes was reduced by 33, 39, or 28% of what FAS eyes produced at 30, 60, or 120 nM, respectively. K values for both groups of rats were similar while V MAX values in the FAD rats was less than that of the FAS rats (24 and 37 A mol/hr/g of tissue, respectively). These results suggest that M induced ocular toxicity in FAD rats might be due to altered metabolism of M in the eye rather than in the liver. Further studies on the enzymes that catalyze oxidation of HCHO and HCOOH in the eyes and livers of FAS and FAD rats may provide information necessary to understand the mechanism of M induced ocular toxicity.

Human placental peroxidase (HTP): Partial purification, characterization, and in vitro binding study with 2-amino-5-fluorouracil (2-AF). J L Nelson and A P Kulkarni. University of Michigan, Ann Arbor, MI and Florida Toxicology Research Center, University of South Florida, Tampa, FL.

Peroxidases can metabolize xenobiotics to reactive intermediates capable of protein/DNA binding. The role of this enzyme in biotransformation is not well understood. In this study, HTP was partially purified by affinity chromatography from CaCl2 extracts of particulate fraction. HTP appears to be a membrane-bound glycoprotein and metabolizes several model peroxidase substrates. HTP activity was found in all subcellular membranes but not in cytosol. Arachidonate-dependent oxidation of p-methoxyphenol was not observed, suggesting that the activity was not due to prostaglandin synthase. Moreover, HTP preparations were devoid of catalase and spectrally dissimilar to human hemoglobin, cytochrome P-450, eosinophil peroxidase, and myeloperoxidase—suggesting an endogenous origin. A molar equivalent of 119,000 daltons was determined for HTP by gel filtration. Cadmium-slab-PAGE of cetyltrimethylammonium bromide solubilized HTP yielded two peroxidase-staining bands. Preliminary studies indicate that HTP catalyzes the in vitro binding of [3H]2-AF to protein and DNA in the presence of H2O2 at the rates of 104.3 and 8.2 pmoles/min/mg, respectively. Binding studies are in progress with selected xenobiotics. (Supported in part by grant from the Council for Tobacco Research-U.S.A., Inc.)

Neonatal rat skin peroxidase-mediated binding of 7,8-benzo(a)pyrene dihydrodiol and 2-amino-5-fluorouracil to DNA and protein in vito. B H Stromm and A P Kulkarni. Toxicology Program, University of Michigan, Ann Arbor, MI and Florida Toxicology Research Center, University of South Florida, Tampa, FL.

A membrane bound peroxidase activity was extracted from neonatal (3-6 day) rat skin particulate fraction using 0.5 M CaCl2 and partially purified by affinity chromatography on Concanavalin-A sepharose 4B as described in our earlier publication (J. Biochem. Toxicol. 1(4):83-1986). The molecular weight of rat skin peroxidase (RSP), as determined by gel filtration on Bio-gel P-200, was found to be approximately 42,000 daltons. Spectral studies identified absorption maxima of 406 nm (absolute spectrum), 418 nm (dithionite reduced-Co spectrum), and 411 nm (Cr complex spectrum).

In vitro experiments indicated that RSP catalyzes the covalent binding of 7,8-benzo(a)pyrene dihydrodiol and 2-amino-5-fluorouracil to DNA and protein. RSP mediated 7,8-benzo(a)pyrene dihydrodiol binding was found to be approximately 2.5 pmoles diol/mg DNA/min and 114 pmoles diol/mg protein/min. RSP mediated 2-AF binding was 5.2 pmoles 2-AF/mg DNA/min and 133 pmoles 2-AF/mg protein/min. Binding could be inhibited by prior boiling of RSP or preincubation of RSP with Na3.

Peroxyl radical-dependent activation of non-bay region polycyclic aromatic hydrocarbons. G A Reed, M E Layton, and M J Ryan. University of Kansas Medical Center, Kansas City, KS.

The ability of peroxyl radicals to epoxidize polycyclic aromatic hydrocarbons (PAH) to form their ultimate mutagenic and carcinogenic derivatives can occur in both subcellular and cellular systems. All such reactions reported involve epoxidation of bay region double bonds. Cyclopenteno[cd]pyrene (CPP) is a PAH which possesses an isolated double bond but no bay region. Activation of CPP as a mutagen occurs via epoxidation of that double bond. We investigated this reaction in three peroxyl radical generating systems: 1. Oxygenation of aromatic acid by microsomal prostaglandin H synthase, decomposition of lipid hydroperoxides catalyzed by heme, and autoxidation of sulfite. Epoxidation was inferred from the activation of CPP as a mutagen towards Salmonella typhimurium strain TA98. CPP is activated by each of the peroxyl radical systems tested, exhibiting concentration-dependent increases in revertants between 10 and 150 M CPP. The number of revertants observed with the microsomal system is significantly lower than that seen in the model systems. This decrease is partially reversed by epoxide hydrolase inhibitors, further supporting the formation of an epoxide. These data expand the spectrum of potential substrates for peroxyl radical-dependent epoxidation, and underscore the potential importance of this pathway for the toxification of xenobiotics. Supported by NIH grant ES-0492.
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