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Preface

This issue of the *Toxicologist* is devoted to the abstracts of the presentations for the platform and poster sessions of the 26th Annual Meeting of the Society of Toxicology, held at the Washington Hilton Hotel, Washington, D.C., February 24-27, 1987.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 277. The Keyword Index was prepared by Elton R. Homan, Union Carbide Corporation, and Edward Miedel, Conquest Computer Company.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 349.

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TOXICITY OF PARAGUAT IN THE CHICKEN EMBRYO.
B.T. Williams and R.T. Di Giulio. School of Forestry and Environmental Studies, Duke University, Durham, NC. Sponsor: M.B. Abou-Dagga.

Paraguat was tested in the 8-day chicken embryo liver for its ability to redox cycle, an effect associated with lipid peroxidation, glutathione (GSN) depletion and induction of antioxidant enzymes (SOD, GSH-Peroxidase, and catalase) in mammalian. Eight-day embryos were exposed to 0.1 mg/ml by immersion for 3 hours. Paraguat produced no measurable increase in lipid peroxidation (TBARS in the serum or tissue) or in the levels of antioxidants in the liver. The ability of the chick liver to synthesize an antioxidant enzyme, such as glutathione peroxidase, was not enhanced by paraguat exposure. The effects of paraguat on the chick liver and the potential for paraguat to be metabolized in the liver were discussed.

PRESENT research focuses upon the ability of paraguat to inhibit the polymorphonuclear phagocytes, a key component of the immune system, and to induce a decrease in the number of polymorphonuclear phagocytes in the lymph nodes. The study design consisted of three groups of mice: control, paraguat-treated, and paraguat plus antibiotic-treated. The results showed that paraguat caused a significant decrease in the number of polymorphonuclear phagocytes in the lymph nodes of the experimental groups compared to the control group. The addition of antibiotic to the paraguat-treated groups resulted in a further decrease in the number of polymorphonuclear phagocytes. These findings suggest that paraguat may have immunosuppressive effects and that the antibiotic treatment further enhances these effects.


Use of alpha-methyldopa (MD) to reverse essential hypertension during pregnancy is associated with fetal anomalies in humans. MD teratogenicity has not yet been thoroughly evaluated in test species. MD was given to pregnant CD-1 mice (10, 100, 250, 500 & 750 mg/kg) from gestational day 6-20 and rats (0, 50, 100, 250 & 500 mg/kg) from gestational day 6-20. Dam toxicity was manifested in mice (>250 mg/kg) and rats (>200 mg/kg) with mortality at the two highest doses. Dam body weight (wt), wt gain, and liver and gravid uterus wt were reduced in mice at MD doses >250 mg/kg and in rats at doses >100 mg/kg. Prenatal mortality and in utero growth retardation occurred at >250 mg MD/kg in mice and at >100 mg MD/kg in rats along with dam toxicity. In both species, malformed fetuses/litter was only marginally increased at maternally toxic doses of MD. (Support: NTP/NIEHS Contract No. N01-ES-55089).

CHANGES IN SERUM ANDROGEN BINDING PROTEIN (ABP) INDICATE GERMINAL EPITHELIAL DAMAGE.

ABP, produced by Sertoli cells and secreted into seminiferous tubules and blood, was measured in diphenyl phosphatase (DDP) treated rats as a potential index of germinal epithelial damage. A single p.o. dose of DDP (0, 250, 1000, or 2000 mg/kg SW in corn oil) was given to 4 groups of 110 F344 rats; and 10 rats per group were killed weekly for 10 weeks. Changes in serum ABP were then compared with changes in other reproductive endpoints. In high-dose rats, serum ABP values doubled during the first 2 days, then fell to remain below control values from week 4 to 10. Accordingly, 95% of the rats in this group showed >50% degenerated tubules, decreased epididymal sperm density, and up to 97% morphologically abnormal sperm. In mid-dose rats, serum ABP increased during the first week and returned to control values by week 2. Of these rats, 20% showed 20-50% degenerated tubules, decreased sperm density, and up to 23% abnormal sperm morphology. In low-dose rats, serum ABP was similar to controls, and the other parameters also remained unchanged. These results indicate that serum ABP may be useful as a biological marker for toxic effects on rat germinal epithelium.
MONO-(2-ETHYLHEXYL)-PHTHALATE (MEHP), 2,5-HEXANE-DIONE (HD), AND A23187 PRODUCE DIFFERENT SPECTRA OF EFFECTS ON SERTOLI CELLS IN PRIMARY CULTURE. R.E. Chapin & J.L. Phelps. Fertility and Reprod*nt, NTEH, Res. Trl. Park, NC

Previous studies from our lab have indicated that MEHP lowered cellular levels of ATP and increased glycolysis in intact primary Sertoli cell cultures. To better define the effects of MEHP, we examined CO₂ production from 14C-labelled acetate as an index of Krebs cycle activity. At hours 1-6 after the start of exposure, MEHP lowered CO₂ generation by ca. 20%. This decrease was dose-related; the minimal effective dose also had increased lactate output in previous studies. Hexanedione-treated cells also secreted more lactate, though media pyruvate and cellular ATP levels were unchanged. In Sertoli cells exposed to HD, CO₂ production from acetate was increased in a dose- and time-related manner. Though these metabolic changes took days to occur, changes in the distribution of vimentin, but not tubulin, were seen in HD-treated cells at 3, 6, and 9 hrs after the start of exposure. The possibility that the effects of HD or MEHP were due to changes in intracellular Ca²⁺ were studied using the Ca²⁺ ionophore A23187, which increased medium lactate, pyruvate, and incorporation of radiolabelled amino acids into secreted protein, and decreased cellular protein synthesis. These and other data indicate that increased cytosolic Ca²⁺ alone is not a sequela of MEHP or HD treatment.

DIFFERENTIAL RESPONSE OF PRIMARY RAT SERTOLI AND INTERSTITIAL CELLS TO CADMIUM. S.R. Clough, M.J. Welsh, and M.J. Rubenec. Eastern Michigan University, Department of Chemistry, and the University of Michigan, Toxicology Program and the Department of Anatomy, Ann Arbor, MI.

Initial morphologic observations suggested that immature rat Sertoli cells were more susceptible to cadmium than immature rat interstitial cells. This observation was further evaluated by exposing cell cultures to CdCl₂ (90 μM) and comparing the following biochemical endpoints: cadmium uptake and efflux, reduction of the vital tetrazolium dye MTT, incorporation of [H]-leucine, production of heat-stable cadmium binding factor, and production of lactate. Using these parameters, it was observed that the interstitial cell cultures were generally nonresponsive or slightly stimulated by the presence of cadmium, whereas the Sertoli cell cultures were adversely affected in a dose-dependent manner. The results of these in vitro data suggest that a) the interstitial compartment may serve as a protective sink toward heavy metal challenge in the testis b) the Sertoli cell, relative to neighboring cell populations, is particularly sensitive to cadmium. Supported by NIEHS grant #ES04141.


Onosborne-Mendel rats were sacrificed at three time intervals after mating to determine if the prenatal skeletal ossification delays observed following low-level caffeine administration (doses 25-75 mg/kg) are transient or persistent. Caffeine was available ad lib. in distilled water at dose levels of 0, 0.018, 0.036, or 0.072% on days 0-20. In Group A litters (sacrificed on gestation day 20), the number of fetuses with at least one type of sternebral ossification delay was increased significantly in all treated groups. Fetuses from the mid- and high-dose levels also had significantly more defects of other bones such as missing centra. In Group B litters (sacrificed on postnatal (PN) day 0), neonates showed significantly increased incidence of delayed sternebral ossification and neonates from the 0.072% level also showed a significant increase in the incidence of the delayed ossification of other bones such as the metacarpals. In Group C litters (sacrificed on PN day 6), only the pups in the 0.072% level showed a significant increase in incompletely ossified sternebrae. No other skeletal effects were seen in Group C pups. These studies confirmed the skeletal ossification delay in day 20 fetuses and day 0 neonates, but by day 6 much of the ossification problem had reversed.

SRCALONIC ACID D-INDUCED PALATAL cAMP AND cGMP CHANGES IN DEVELOPING MICE. M.M.R. Eldeib and C.S. Reddy. Department of Veterinary Biomedical Sciences, University of Missouri, Columbia, MO.

The mycotoxin, sacalonic acid D (SAD) is known to induce cleft palate in CD-1 mice. In order to study this mechanism both cAMP and cGMP contents were assayed in embryonic palatal extracts (days 13 thru 16) by RIA. Two groups of pregnant females were treated on day 11 with 55 NaHCO₃ or 30 mg per kg of SAD. Cyclic AMP levels in the control embryos peaked between days 14.5 and 15 of development and returned to basal levels by day 15. Although cAMP levels in SAD-treated palates appeared to peak similarly at the same time as controls, significantly lower (p<0.01) cAMP levels were seen 12 hrs before the peak (day 14.5). In addition, fetuses from SAD-treated mothers exhibited a second peak of cAMP on day 16 at which time cAMP in control fetuses was at pre-elevation (basal) levels. This correlated with a 22% incidence of cleft palate in SAD treated to 6% in control fetuses. In contrast, SAD failed to alter cGMP pattern during palate closure. However, the levels in fetuses from SAD-treated mothers were significantly higher than those of controls on days 15 thru 16. These results confirm the hypothesis that cAMP mediated cellular activities are involved in the normal palate development and suggest a mechanistic role for cAMP changes in SAD induced cleft palate.
REDUCTION OF THE INCIDENCE OF 2-METHOXYETHANOL (ME)-INDUCED DIGIT MALFORMATIONS BY D-GLUCOSE. E. Welch, C.I.R.T., Research Triangle Park, NC

ME given by gavage to CD-1 mice on gestation day (gd) 11 causes a dose-dependent incidence of paw dysmorphogenesis in the embryos. Previous studies revealed that the expression of lesions required oxidation of ME to methoxyacetic acid (MAA), and the latter has been invoked as responsible for the teratogenic effects of ME. However, various agents including ethanol, sodium acetate, formic acid and glycine markedly attenuate ME teratogenicity (Pharmacologist 28, A211, 1986). Those observations, pharmacokinetic measurements and the incorporation of label derived from 1,2-3C-ME into diverse macromolecular fractions of the embryo suggest that MAA enters into further biochemical reactions involving carbon and one carbon units. In the present experiments mice received concomitant oral doses of 3.3 mmol ME/kg and 4.3 mmol D-glucose/kg on gd 11. In concurrent controls (given only ME) digit morphogenesis (any digit) was disrupted in 46% of the fetuses or 7% of litters. Among all fetuses 44% had forepaw and 14% hindpaw defects. Concomitant dosing with D-glucose reduced the any digit incidence (p < 0.05) to 26%, while forepaw lesions occurred only in 22% and hindpaw malformations in 7%. This outcome lends further support to the notion that MAA incorporation into macromolecules utilized by the embryo contributes to or determines the teratogenic outcome.

DEVELOPMENT AND CHARACTERIZATION OF A MORPHOLOGICAL SCORING SYSTEM FOR MEDAKA EMBRYO DEVELOPMENT. M. Shi and E.M. Faustman-Watts, Dept. of Environmental Health, University of Washington, Seattle, WA

Interest in non-mammalian alternatives to in vivo animal teratogenicity testing has dramatically increased. However, few of these alternative screening systems have been critically evaluated or validated. This reproducible, standardized and rapid means of monitoring medaka development in culture is an important step in evaluating this alternative test. Medaka embryos were collected within 2 hours post-fertilization and cultured in Petri dishes in buffered saline until hatching (14 days at 25°C). Thirteen different features were selected, and up to six developmental stages of each were defined and assigned scores from 0-5. Control as well as teratogen-exposed (methylthiosourea, 0-10 mM) embryos were assessed. Morphological scores were calculated at four time points during development. Score was significantly related to embryonic age and teratogen-exposed embryos showed dose-dependent increases in mean score even at doses resulting in low malformation percentages. These results suggest that the use of such scoring systems in fish embryo culture will provide a standardized index of embryonic development and aid the detection of growth retardation and dysmorphogenesis. This will allow for quantitative comparisons of growth and development between experimental conditions and possibly to other embryos culture systems (e.g., rodent) which have established morphological scoring systems. Supported by NIH ES-03157 and UW Ind. Hyg. Res. Fund.

2,5-HEXANEDIONE CAUSES IRREVERSIBLE TESTICULAR GERM CELL LOSS AT NEUROTOXIC DOSES.
K. Boekelheide, Brown University, Providence, RI.

The toxic hexacarbon metabolite, 2,5-hexanedione, produces both nervous system dysfunction and testicular injury. In this study, the reversibility of 2,5-hexanedione-induced testicular atrophy was investigated. Charles River CD rats (200 gm) were intoxicated with 12,5-hexanedione in the drinking water for 5 weeks followed by a 17 week recovery period. Clinical neurotoxicity was present from the 4th through 7th weeks and then resolved. Groups of 5 rats were sacrificed at 0, 2, 4, 5, 6, 7, 8, 10, 12, 16, and 22 weeks. Testis weight reached a low point at 7 weeks (0.68 ± 0.04 gm, mean ± S.E., n = 5, 40% of control testis weight) and remained at this low weight throughout the recovery period. Testis histology was examined quantitatively for the presence of viable germ cells. At 12 weeks (5 weeks intoxication followed by 7 weeks recovery) the number of seminiferous tubule cross sections with identifiable germ cells reached a low point (1.3 ± 0.2%, mean ± S.E., n = 3). At the subsequent 16 and 22 week time points, 50% of the rats had seminiferous tubules without detectable germ cells while 50% of the rats demonstrated varying degrees of germ cell re-population. Thus, irreversible testicular injury with complete germ cell loss occurs in 50% of rats exposed to a moderately neurotoxic regimen of 2,5-hexanedione.

DEVELOPMENTAL TOXICITY OF INDUSTRIAL N-NITROSO COMPOUNDS IN VITRO. I. Gilbert-Jones, Z. Kirby and E. Faustman-Watts, Dept. of Env. Health, Univ. of Washington, Seattle, WA.

This research has characterized the developmental toxicity of a variety of indirect acting N-nitroso (NNN) compounds. Day 10 rat embryos were cultured for 24 hours. N-nitrosodimethylamine (NDELA), N-nitrosomorpholine (NMOR) and N-nitrosopyrrolidine (NNPR) were tested for developmental toxicity alone and with monoxygenase systems. Endpoints of developmental toxicity monitored included: lethality, malformations, growth retardation and changes in macromolecular content. NNN produced significant embryolethality and growth retardation at concentrations above 20mM, but not significant increases in malformations. NMOR and NDELA produced cephalic dysmorphism and NDELA exposure produced abnormalities in neurulation, flexure, and the optic system. Embryolethality and growth retardation were also produced by these agents, but at 2-3 fold higher concentrations than NNN. The presence of monoxygenase systems appeared to have minimal effects on the developmental toxicity of NNPR or NMOR but did affect the developmental toxicity of NDELA. In vitro effects mimic the lethality and growth retardation seen in vivo studies with indirect acting NNO compounds; however, in our studies we were also able to detect early dysmorphic events not observed in vivo. Supported by NIH grant ES-03157, Dana Foundation, and the UW Ind. Hyg. Res. Fund.

Previous studies demonstrated that inhalation or oral exposure of pregnant rats to a high-boiling coal-derived complex organic mixture (COM) causes a high incidence of malformations in the pups including lung hypoplasia and cleft palate. To more fully characterize the teratogenicity of this COM, pregnant rats were exposed to the whole mixture, four chemical class fractions obtained from liquid chromatographic separations, and a recombined mixture. The test solutions were dermally applied to shaved backs on days 11-15 of gestation, previously determined to be the sensitive period. Dose levels for the four class fractions-aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH), nitrogen-containing PAHs, and hydroxylated PAHs were equivalent to their respective concentrations in the original and recombined mixtures which were applied at 500 mg/kg. Treatment with the whole and recombined mixtures and the PAH fraction resulted in highly significant incidences of lung hypoplasia, cleft palate, cutaneous syndactyly, edema, and craniofacial dysmorphia in the pups. The male/female sex ratio was decreased significantly by the PAH fraction. The teratogenicity of these mixtures, which appears to bear a strong resemblance to that of some synthetic glucocorticoids, lies entirely with the PAH fraction. Work supported by DOE Contract DE-AC06-76RLD 1830.

RESULTS OF A TWO-GENERATION REPRODUCTION STUDY IN CD® RATS EXPOSED TO CYCLOHEXANONE VIA INHALATION. C.M. Salamon, D.A. Mayhew, W.S. Binehart (consultant). American Biogenics Corp., Decatur, IL.

A reproduction study was conducted to ascertain potential effects of cyclohexanone exposure upon reproductive performance, growth, and development of 2 consecutive generations of Crl:CD® BR albino rats. The first (F0) generation rats were exposed via inhalation to 0, 250, 500, or 1000 ppm. Second (F1) generation rats were exposed via inhalation to 0, 250, 500, or 1400 ppm. Daily time weighted average concentrations were 0, 253.2, 499.2, and 1008.9 ppm (F0 generation) and 0, 249.8, 496.9, and 1387.2 ppm (F1 generation). Exposure to 1000 ppm through 1 generation and exposure to 250 or 500 ppm through 2 generations did not adversely affect growth, development, or reproductive performance. Exposure of F1 animals (progeny of animals exposed to 1000 ppm) to 1400 ppm through 1 generation resulted in exposure-related clinical signs (mainly lethargy), male body weight depressions, reduced male fertility, reduced progeny survival, and progeny body weight depressions. Following cessation of exposure, selected subgroups of males were mated 6 times over an 8-week period with virgin females. There was a reversal of apparent male infertility and a recovery of body weight depression. There was no evidence of a dominant lethal effect and all litter data were normal. A no-observable-effect level of at least 500 ppm cyclohexanone has been confirmed for this study.


Acetaminophen (A) hepatotoxicity correlates with covalent binding of A to protein as 3-(cystein-S-y1)acetaminophen (Cys-A). The acetaminophen-adduct-immunoassay (AAI) utilizes rabbit antisera obtained following immunization with 3-(N-acetyl-L-cystein-S-y1)A (NAC-A) covalently linked to keyhole-limpet hemocyanin. The primary epitope has been extensively characterized by determining the relative efficiency of structurally related molecules to competitively inhibit antibody binding in ELISA assays. Strong inhibitors included NAC-A, glutathione-A, L-Cys-A, 3-S-methyl-A and hepatic microsomal protein-bound-A, which had 50% inhibitory concentrations (IC-50) of 115, 302, 1.5 x 10^-2, 2.3 x 10^-2 and 3.0 x 10^-2 fmol, respectively. Less efficient inhibitors included D-Cys-A, D-Cys-s-a-minophenol, NAC-phydroxynoune, and A with IC-50's of 2.0 x 10^-2, 1.25 x 10^-2, 6.0 x 10^-2, and 7.4 x 10^-2 fmol, respectively. Poor inhibitors included A, dimer, NAC, sulfa- A, glucuronide-A, agglutinin, and phenacetin, all with IC-50s of > 10^-2 fmol. The nature of the epitope, the low affinity of the antibody for free A, the sensitivity of the AAI, and the ability to quantitate non-radio-labeled A-adducts should allow new approaches to the study of acetaminophen toxicity.

AN INTERACTION OF BENZENE METABOLITES REPRODUCES THE MYELOTOXICITY OBSERVED WITH BENZENE EXPOSURE. D.A. Eastmond, M.T. Smith and R.D. Irions, School of Public Health, University of California, Berkeley, CA and Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Benzene-induced myelotoxicity can be reproduced by the co-administration of two principal metabolites, phenol (PH) and hydroquinone (HQ). Co-administration of PH (75 mg/kg) and HQ (25-75 mg/kg) twice daily to B6C3F1 mice for 12 days resulted in a significant loss in bone marrow cellularity in a manner exhibiting a dose-response. One explanation for this potentiation is that PH stimulates the peroxidase-dependent metabolism of HQ. Addition of PH to incubations containing horseradish peroxidase (HRP), H2O2, and HQ resulted in a stimulation of both HQ removal and benzoquinone (BQ) formation. Stimulation occurred with PH as low as 100 μM and with very low concentrations of HRP. When boiled rat liver protein was added to identical incubations containing 14C HQ, the level of radioactivity recovered as protein bound increased by 37% when PH was added. Similar results were observed when 14C HQ was incubated in the presence of activated human leukocytes. HQ binding was increased by approximately 70% in the presence of PH. PH-induced stimulation of HQ metabolism and HQ formation represents the most likely explanation for the bone marrow suppression associated with benzene toxicity.

Thiuram ions formed from the cysteine or glutathione conjugates of 1,2-dihaloethanes are believed to be responsible for the genotoxicity of the parent alkyl halides. The conversions of specifically-deuterated S-(2-hydroxyethyl)-L-cysteine to S-(2-haloethyl)-L-cysteines were studied to provide direct evidence for the involvement of thiuram ions in the reactions of the cysteine conjugates of dihaloethanes. S-(2-hydroxyethyl-1,1-d2)-L-cysteine was converted to an equal mixture of the 1,1-d2- and 2,2-d2 isomers of the corresponding S-(2-haloethyl)-L-cysteines in concentrated hydrochloric, hydrobromic, or hydroiodic acids without detectable formation of the 2,2-d2 isomer of the parent hydroxyethyl derivative. Dissolution of S-(2-hydroxyethyl)-L-cysteine in trifluoromethanesulfonic acid yields a compound with the NMR spectral properties of S-(1-cysteinyl)ethyl thiuram trifluormethanesulfonate. These results demonstrate the facile formation of thiuram ions from S-(2-haloethyl)-L-cysteines.


Conversion of the hepatotoxin 1,1-dichloroethylenedichloroacetylglutathione (GSH, C2H5O) by isolated rat hepatocytes or by GSH-activated microsomes presumably occurs via stepwise acylation and alkylation of two GSH molecules by the microsomal DCE metabolite CICH2CH2CH2O. We synthesized the probable intermediate, S-2-chloroacetylglutathione (CICH2COG), and studied its stability and reactions with model thiol. First order decomposition of CICH2COG, which proceeded with a t1/2 of 35 min at pH 7.5, yielded no GSH. NMR and liquid secondary-ion mass spectrometry analyses of the products indicated that CICH2COG first isomerized to produce N-o-(2-chloroacetyl)-γ-glutamylcysteinylglycine, which then formed a cyclic product. The rates of both the acyl transfer and cyclization reactions increased with increasing pH. CICH2COG reacted with GSH to yield GSCH2COG with N-acetyl-L-cysteine and disulfide-reduced oxytocin to yield S-(2-S-glutathionyl)acetyl derivatives. S-2-Chloroacetyl derivatives were not observed, suggesting that preferential displacement of one chloride by GSH accompanied thiol acylation. Reactive thioesters may modify critical cellular targets in DCE intoxication. (Supported by USPHS Grants ES05309 and ES01978.)


Methimazole (MMI) is a mechanism-based inhibitor of thyroid peroxidase and has been used to inhibit the bioactivation (cooxidation) of certain xenobiotics by the peroxidase activity of prostaglandin H synthase (PHS). The mechanism(s) by which MMI inhibits PHS-mediated cooxidation is not known and was investigated in studies reported here. MMI inhibited the PHS-mediated cooxidation of benzidine (BZD), measured by loss of UV absorbance, and phenylbutazone, measured by O3 incorporation, in a concentration-dependent manner. MMI poorly supported the PHS-mediated reduction of 5-phenyl-4-pentenyl hydroperoxide (PFPHP) to the corresponding alcohol (PPA), suggesting the mechanism by which MMI inhibits PHS is not by serving as a competing reducing cofactor for the peroxidase. MMI did not inhibit BZD-supported reduction of PFS, indicating that MMI is not a mechanism-based inhibitor of PHS. Glutathione (GSH) has previously been shown to reduce the cation free radical formed by cooxidation of aminopyrine to the parent compound. MMI produced the same effect in equimolar concentrations. These data suggest that the mechanism by which MMI inhibits this cooxidation is not through a direct inhibition of the enzyme, but rather by a reductive interaction with a free radical-derived metabolite.


1,2-Dibromo-3-chloropropane (DBCM) was used as a fumigant by virtue of its nematocidal activity until it was determined that this compound was a carcinogen, a mutagen, an acute nephrotoxic and an acute testicular toxicant. The mutagenicity produced by DBCM is dependent on cytochrome P-450 oxidative metabolism and is partially explained by the formation of 2-bromoacrolein. However, the mechanisms of nephrotoxicity and testicular toxicity produced by DBCM have not been well characterized. In an attempt to establish a structure-toxicity relationship, a series of methylated derivatives of DBCM were synthesized and tested. The results showed that methylation decreased mutagenicity in all cases and that only the C2-methyl and the C3-methyl analogs were mutagenic at the amounts studied (0.2-0.4 μmoles/plate). Similarly, methylation decreased the degree of nephrotoxicity produced by DBCM and only the C2-methyl and the 1,2-dibromo-4-chlorobutane analogs were capable of causing any detectable kidney damage at the dose tested (0.17 mmoles/kg). Therefore, it appears that the major mutagenic metabolites of DBCM are probably distinct from those metabolites that cause nephrotoxicity.
The renal bioactivation of estrogens has been suggested to be involved in the formation of renal tumors in hamsters. Bioactivation of diethylstilbestrol (DES) was determined by quantitation of irreversible binding of DES metabolites to protein isolated from male hamster renal cortical slices following incubation with 50 nM (3H)DES. Incubation of cortical slices in medium containing 5 mM glutathione (GSH), decreased the binding of reactive DES metabolites to protein by 17% and increased tissue levels of GSH by 24%. Diethyl maleate (5 mM) enhanced the non-essential binding of DES to cellular protein by 250%, while decreasing tissue GSH levels by 44%. Treatment of hamsters with 1-buthionine sulfoximine (1 mM/kg) for 2 hr decreased renal cortical GSH by 53%. Enhanced irreversible binding of DES metabolites to renal cortical protein was observed using renal cortical slices from 1-buthionine sulfoximine-treated hamsters. No irreversible binding of DES was detected in DNA isolated from the same slices. These results suggest that GSH protected against the irreversible binding of DES metabolites to hamster renal cortical protein. Supported by NIH grant (HD06707) and IEHE Center Grant (ES01247).

We have shown that bromobenzene induced renal necrosis may be mediated by formation of 2-bromo-hydroquinone-glutathione (BHQ-GSH) conjugates. A possible pathway by which these compounds elicit toxicity involves metabolism to the cysteine conjugate and subsequent cleavage by ζ-lyase to give a reactive thiol. We now report the synthesis and toxicity of 6-Br-2,5-dihydroxy-thiophenol (BrDTP) a putative metabolite of BHQ-GSH. BrDTP was prepared by the addition of Na-thiosulfate to 2-Br, 4-benzoquinone followed by the reduction of the S-arylsulfonate to the mercaptan. Structural identification was confirmed by MS and NMR. The major ions in the BrDTP spectrum were m/z 220,222 (M+), 191,193 (M+ - H2O) and 175 (M+ - CO). The 1H-NMR spectrum had two aromatic protons that exhibited an ortho-coupling of J = 8.5Hz. Administration of BrDTP to rats (0.35 mM/kg, i.p.) caused an increase in blood urea nitrogen (BUN) and pathological alterations to the kidney consistent to those seen after BHQ-GSH. In contrast, BrDTP had no effect on liver pathology and SGPT levels were normal. Prilocaine with AT-125, an inhibitor of ζ-glutamyl transpeptidase and renal thiol oxidase inhibited BrDTP mediated elevations in BUN by 50%. The results suggest that BrDTP may contribute to BHQ-GSH nephrotoxicity.

6-BROMO-2,5-DIHYDROXY-THIOPHENOL NEPHROTOXICITY: STRUCTURE ACTIVITY REQUIREMENTS. Serrine S. Lau and Terrence J. Wirth, School of Pharmacy, Univ. of Texas at Austin, TX. and Georgetown Univ., Washington, D.C. Sponsor: Peter J. Wirth.

6-Bromo-2,5-dihydroxy-thiophenol (BrDTP) is a putative metabolite of nephrotoxic 2-bromo-hydroquinone-glutathione conjugates which itself causes renal necrosis in the rat. The mechanism of this toxicity is not known. However, both the quinone moiety and/or the thiol group could contribute to the toxicity of BrDTP. To determine the relative importance of these two functional groups we have examined the toxicity of BrDTP and compared it to that of isomeric Br-thiophenols (BrTP). BrDTP (1p) administration to rats caused a dose-dependent increase in blood urea nitrogen (BUN) which was maximal at 0.35 mM/kg. The debronnitem analog of BrDTP (DTP) was also nephrotoxic but at a dose of 0.6 mM/kg. The activity of renal microsomal S-methyl transferase toward BrDTP and DTP was 1.7 ± 0.1 and 11.9 ± 0.7 nmoles/min/mg of protein respectively. Neither 2-, 3-, nor 4-BrTP had any effect on BUN between 0.2-0.8 mM/kg and no pathological alterations were seen in kidneys from BrTP treated rats. SGPT values were normal. The data suggest that the quinone function of Br-DTP is essential for the expression of toxicity. In this respect, the relatively low activity of DT-diopephorous (NADPH:quinone reductase) in renal cortex (38 u/mg) as compared to the medulla (142 u/mg) and papilla (269 u/mg) may be of toxicological significance.


Previous in vitro and in vitro studies have shown that nitro group reduction of S-nitroimidazole antiparasitic agents is required for covalent binding to protein sulphydryl groups, but the nature of the reactive species is not known. The stoichiometry of the reductive activation of S-nitroimidazoles has been investigated using agarose-immobilized cysteine as a model for protein sulphydryl and dithionite as reductant. The anaerobic covalent binding of [14C]-metronidazole and [15N]-orrasimizole ([1-methyl-5-nitroimidazole-2-y]-methylcarbamate) to agarose immobilized cysteine increased linearly with dithionite concentration to a maximum at 2 moles dithionite per mole drug. These data indicate that 4 electrons are required for maximal covalent binding of S-nitroimidazoles, implicating a hydroxylamine as the reactive intermediate. Studies with variously radiolabeled orrasimizole molecules to characterize the covalently bound cysteine adduct demonstrated that the imidazole ring was intact while the 4-proton and 2-carbomoyl group were lost. This labeling pattern is identical to that obtained for enzyme-catalyzed covalent binding to protein sulphydryl groups in vitro and in vivo. These results suggest that the chemical activation of 5-nitroimidazoles by dithionite occurs by the same mechanism as the enzyme-catalyzed activation in vitro and in vivo.
METABOLIC ACTIVATION OF ETHYLENE GLYCOL HOMO- BUTYL ETHER (2-BUTOXYETHANOL; BE) VIA ALCOHOL/ ALDEHYDE DEHYDROGENASES. B.I. Ghanayem, L.T. Burk, and A.B. Matthews. NIH/NIEHS, Research Triangle Park, NC

Gavage administration of BE to rats has been shown to cause severe acute hemolytic anemia. Butyric acid (BAA) has been identified as a major urinary metabolite of BE. We recently identified two urinary conjugates of BE, namely, BE-glucuronide (BEG) and BE-sulfate (BES). The current studies were undertaken to investigate the metabolic basis of BE-induced toxicity in male F344 rats. Pretreatment of rats with pyrazole (alcohol dehydrogenase inhibitor) significantly inhibited BE metabolism to BAA and prevented BE-induced hemotoxicity. Inhibition of BE metabolism to BAA by pyrazole was accompanied by a parallel increase in BEG and BES. The ratio of BAA/BEG+BES in urine was directly proportional to the hemotoxicity caused by BE. Pretreatment of rats with cyanamide (aldehyde dehydrogenase inhibitor) caused a similar decrease in the BAA/BEG+BES ratio and protected against BE-induced hemotoxicity. Quantitatively, administration of equimolar doses of BE, butyric acid (BAA), or BAA caused similar hemotoxicity. Pretreatment of rats with cyanamide prevented BAL-induced hemotoxicity but had no effect on the hemotoxicity of BAA. It is concluded that metabolic activation of BE via alcohol/aldehyde dehydrogenases is a prerequisite for the development of hemotoxicity.

EVIDENCE FOR REGIOSPECIFICITY IN THE MICROosomal OXIDATION OF NIFEDipINE. J.L. Born and W.M. Hadley. The College of Pharmacy, The University of New Mexico, Albuquerque, NM.

The mechanism for the microsomal mediated oxidation of 4-alkyl-1,4-dihydropyridines has been reported to involve the formation of a radical cation intermediate which liberates an alkyl free radical as the final step in the oxidation. Nifedipine is a 4-ary1-1,4-dihydropyridine which is used as a hypotensive agent. Nifedipine is extensively metabolized via oxidation of the 1,4-dihydropyridine ring to give inactive pyridine metabolites. In order to investigate the mechanism of the oxidation of the dihydropyridine ring of nifedipine we have prepared 4-1H-labelled nifedipine which has high isotopic purity. The Kd and Vmax for the microsomal metabolism of nifedipine were 12.2 x 10^-5 and 13.6 nmoles/mg microsomal protein/min respectively. The Kd and Vmax for the microsomal metabolism of 4-1H nifedipine were determined to be 2.08 x 10^-5 and 1.51 nmoles/mg microsomal protein/min respectively. The Kd/Kd for the microsomal metabolism of nifedipine is 9.0 which is consistent with the formation of a carbon centered free radical as a key intermediate in the oxidation of the dihydropyridine ring.

DEUTERATED 3-METHYLINDOLE IS LESS PNEUMOTOXIC THAN 3-METHYLINDOLE. Jeannette C. Hutzler and Garold S. Yost. Pharmacology/Toxicology, Program, Washington State University, Pullman, WA.

3-Methylindole (3MI) and 3MI deuterated at the methyl position cause pulmonary damage in Swiss Webster mice when administered intraperitoneally. However, deuterated 3MI (D3-MI) is less toxic than 3MI. Histological studies show that the type of pulmonary lesions that are produced by 3MI and D3-MI are the same, but a larger dose of D3-MI is required to cause the same extent of damage. While bronchial damage is observed in mice that received 25 mg/kg of 3MI, no damage is apparent when the same dose of D3-MI is administered. In addition, the LD50 for D3-MI is greater than the LD50 for 3MI; 735 mg/kg and 578 mg/kg, respectively. Furthermore, there is a significant increase in wet lung weights of mice that received 100 mg/kg or 300 mg/kg 3MI, but no increase in animals that received 100 or 300 mg/kg D3-MI. These results suggest that methyl C-H bond breakage is required for in vivo pneumotoxicity of 3MI in mice. In addition, these deuterium isotope effects support a mechanism of pneumotoxicity which includes the recently proposed imine methide electrophilic intermediate. (Supported by USPHS Grant #HL13645.)

NEW INVESTIGATOR PRESENTATION THE IMMUNOTOXICITY OF CATIONIC AMPHIPHILIC DRUGS. L.J. Sapers, B. Wierda and M.J. Reesor. West Virginia Univ. Medical Center, Morgantown, WV.

We have shown that cationic amphiphilic drugs (CADs) have an immunotoxic potential. We have also determined that the immunotoxicity associated with chlorpheniramine (CP), a widely studied CAD, is based on a drug-induced inhibition of an event which occurs very early during lymphocyte activation. Such an event involves the hydrolysis of phosphatidylinositol by phospholipase C to yield inositol phosphates (IP) and diacylglycerol (DAG) as products. IP and DAG then function as mediators of a transmembrane signal for the continuation of the immune response. It was the purpose of these studies to determine the effects of CP on this phosphatidylinositol pathway. We demonstrated that formation of IP in lymphocytes increases progressively over a 2 hour period following Con A stimulation (153%, 316% and 565% of control at 30, 60 and 120 min, respectively). In contrast, lymphocytes pre-incubated with 10-5 M CP for 60 min, then stimulated with Con A for 2 hours in the presence of 10-5 M CP, exhibit a significantly depressed IP formation (75% of control). In addition, CP also inhibited the activity of phospholipase C (IC50 = 0.58 mM), the enzyme responsible for the formation of IP during lymphocyte activation. These results suggest that the mechanism associated with CP-induced immunotoxicity may be mediated by suppression of IP formation during activation. Supported by a fellowship from the Procter & Gamble Company.

Industrial exposure to isocyanates has been known to cause respiratory and/or dermal hypersensitivity in some individuals. Early detection of sensitization is important to prevent occurrence of severe allergic reactions. Serologic screening for IgE antibodies requires isocyanate-conjugate antigens be prepared, characterized and evaluated for ability to detect specific antibodies. This study was undertaken to define physical-chemical characteristics of MDI-protein antigens which are effective in detecting antibody populations in animals experimentally sensitized to MDI via the inhalation and dermal routes, and in patients with MDI asthma. Antigens were prepared by reaction of MDI with serum albumin at pH 7.4. Ultraviolet absorbance spectra indicated an average of 17 haptens per molecule protein. Analysis by isoelectric focusing indicated a minimum of four fractions with isolectric points of 4.21, 4.40, 4.61 and 4.75. Using Western blotting, two bands were observed to react strongly with antibodies from a patient with MDI asthma. Further purification of the antigen conjugates will enable production of antigens appropriate for early detection of MDI sensitivity. Supported by NIHES ES01532.


The prediction of respiratory sensitisation potential has been hampered by the lack of a well-validated animal model. Karol (1983) was able to induce sensitisation to TDI in guinea pigs by administering the material as a vapour for 5h day⁻¹ for 5 days; pulmonary responses were seen two weeks later following challenge with a TDI-serum albumin conjugate; sensitisation was confirmed by the presence of homocytotropic (IgGₐ, and IgE) antibodies. We have investigated the Karol model using both TDI and TMA. TDI was administered at concentrations ranging from 0.7 to 4.2ppm. An increased respiratory rate was seen at challenge in only 1/3 experiments; TDI-specific antibodies were detected by PCA and ELISA in 2 experiments, however the titres were dependent upon the hapten density of the conjugate used for their detection. TMA was administered at concentrations ranging from 1 to 100ug ⁻¹. In 2 experiments no changes in respiratory rate were detected at challenge, but high titres of TMA-specific antibodies were found in animals sensitised with ≥15ug ⁻¹. Further work is essential before the guinea pig model of Karol could be considered for wider application in toxicology testing. Ref Karol, MH (1983). Toxicol Appl Pharmacol, 68, 229-241.

GUINEA PIGS IMMUNIZED BY INJECTION WITH HDI OR DES-N ACTIVATOR EXHIBIT PULMONARY SENSITIVITY RESPONSES. J.C. Stadler and N.D. Krivanek. E.I. duPont de Nemours and Co., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

Guinea pigs have been used to demonstrate pulmonary sensitization to various materials. The best immunization/challenge protocol for testing sensitizing capacity of a material has not been defined. This study was aimed at examining several isocyanates and optimizing the test protocol. Toluene diisocyanate (TDI), hexamethylene diisocyanate (HDI), and a paint activator that contains the bisure of HDI (Des-N) were investigated. Groups of guinea pigs were exposed by inhalation to TDI, HDI, or activator for 3hrs/day for 5 days. At 21 days, 3 of 5 animals responded to pulmonary challenge with TDI conjugated to guinea pig serum albumin (GSA), but none exposed to HDI or Des-N responded similarly to HDI-GSA. Subsequently, guinea pigs were immunized by either dermal application or injection of HDI or activator. Dermal sensitivity responses to injection of HDI conjugates were achieved in all groups. Cytolytic antibodies to HDI were present in both groups sensitized by injection and in the group sensitized by dermal application of HDI. Pulmonary responses to HDI-GSA occurred in guinea pigs injected with HDI or activator. Results indicate some capacity of HDI or activator to initiate pulmonary sensitivity; however, this would not have been apparent if only the immunization by inhalation protocol had been employed.

IMMUNOLOGICAL RESPONSE FOLLOWING EXPERIMENTAL SENSITIZATION TO SUBTILISIN THROUGH INHALATION EXPOSURE. J.A. Hillbrand, P.S. Thorne, and M.H. Karol. Dept. Industrial Environmental Health Sciences, Univ. of Pittsburgh, Pittsburgh, PA.

Groups of guinea pigs were exposed to known concentrations of subtilisin by inhalation, acutely or subchronically. The antibody (Ab) response of individual animals was compared with the occurrence of respiratory sensitization. Both an ELISA and a latex agglutination assay were used to detect specific Ab. Acute studies included exposure to 6 doses from 0.0083 to 15 mg/m³ for 1 or 5 days, 15 min/day. Dose dependence for Ab formation was observed below 1.9 mg/m³ and a threshold was seen above this level, regardless of respiratory response or days of initial exposure. Pulmonary sensitivity was observed in animals exposed to 1.9 and 15 mg/m³ but not 0.0083 or 0.041 mg/m³. Responses to inhalation challenge ranged from moderate (with immediate and delayed onset) to anaphylactic. In the subchronic study, exposure for 11 weeks to 0.00068 plus 6 weeks to 0.0051 mg/m³ produced significant levels of IgG or IgM Ab in 3 of 25 animals by week 11 and 7 of 25 animals at the end of the study. This exposure failed to produce pulmonary sensitivity upon challenge, even though acute exposure to this cumulative dose produced pulmonary reactions in 63% of the animals. Hence, Ab production can be used as an indicator of exposure but not as an indicator of pulmonary sensitivity. Supported by NIHES ES01532.
The fate of 2,4-dinitrochlorobenzene (DNCB) a potent skin sensitizer, and 2,4-dichloronitrobenzene (DCNB) a non-sensitizer, was compared following their application to the skin of BALB/c mice. The dose applied was 50 μL of a 1% solution in acetone. The chemicals were detected in frozen sections of skin and draining lymph nodes by immunofluorescence using a monoclonal anti-DNCB antibody which recognized both DNCB and DCNB. Cutaneous APCs were localized in similar assays using monoclonal anti-Ia antibodies. The study showed that only the sensitizer (DCNB) was able to bind to cells in the epidermis, including Ia+APCs (presumably Langerhans cells), and to induce the movement of some of these cells out of the epidermis into the dermis and eventually into the draining lymph nodes. This could not be attributed to differences in the protein reactivity or skin permeability of DNCB and DCNB as experiments with radiolabeled chemicals showed that they were equally reactive in vitro with serum albumin and that they could be absorbed to the same extent across mouse skin in vivo. The sensitization potential of a chemical may therefore be dependent on its ability to associate with and stimulate the efflux of cutaneous APC to the draining lymph nodes.

Human peripheral blood lymphocytes (PBL) were exposed to various concentrations of T-2 toxin in vitro during co-culture with optimal mitogenic concentrations of phytohemagglutinin (PHA), concanavalin A (Con A) or pokeweed mitogen (PWM). PBL cultured for 7 days with PWM mitogen in the presence of T-2 toxin were utilized to assess the antibody (Ab) producing ability using an indirect hemolytic plaque assay. Similar cultures were utilized to assess the effects of T-2 toxin on Con A-generated suppressor cells. T-2 toxin inhibited the proliferative response to Con A at a lower concentration (1.6 ng/ml) as compared to PHA (2.4 ng/ml) and PWM (2.4 ng/ml). The antibody producing ability of lymphocytes was inhibited by T-2 toxin at concentrations exceeding 3.2 ng/ml. Time course studies indicated that maximum suppression of the proliferative response to mitogen stimulation was obtained when the toxin was added at the initiation of cultures. However, a reduced proliferative response was still evident when the toxin was added 6 hours before harvesting the 72 hour cultures. T-2 toxin did not induce or interfere with the generation of suppressor cells by Con A.

(Taken from NSERC)
The hypothesis that DMN-induced humoral immune suppression is mediated by reactive intermediates was tested by determining the distribution of reactive species to known target organs (liver and kidney) and to immune organs (peripheral blood, bone marrow, thymus, and spleen) after exposure to 6 mg/kg and 2 uCi of $^{14}$C-(methyl)-DMN. Most of the radioactivity resided in acid insoluble material which increased with the number of exposures and correlated with suppression of the In Vitro antibody response to sheep erythrocytes (SRBC). The magnitude of TCA insoluble radioactivity in spleen homogenates was similar to both liver and kidney. Fractionation of spleens from exposed animals into various cell types indicated greater association of acid insoluble radioactivity with T- and B-lymphocytes as compared to macrophages. Fractionation of spleen cell lysates into protein, DNA and RNA indicated that most radioactivity was associated with the nucleic acid component. Pretreatment of mice with aminooacetanilide (AAN), a DMN demethylase inhibitor, reduced acid insoluble dpm in the spleen. These results suggest that the immunotoxicity of DMN is mediated by reactive metabolites capable of altering cell function by methylation of key macromolecules. (Supported by NIH ES03564 and ES07087).

Bone marrow stroma consists primarily of two cell types - macrophage-like stromal cells (MSC) and fibroblastoid stromal cells (FSC). Proliferation of colony stimulating activity (CSA) is postulated to be influenced by MSC secreted regulatory factors. The goal of this study was to determine if hydroquinone treatment of enriched MSC cultures altered the ability of FSC to support CSA dependent granulocyte/macrophage colony formation (G/M-CFU-C). MSC cultures of either FSC or MSC were derived from B6C3F1 mouse bone marrow cells. Day 6 MSC cultures were treated with hydroquinone (10$^{-6}$ M) for 2 days and then were added to day 13 enriched MSC cultures at different cell concentrations. Two days later these FSC cultures with treated MSC were overlaid with hemopoietic cells in soft agar. After 7 days of incubation, FSC and MSC colonies were monitored by enumerating the number of G/M-CFU-C that developed in agar. FSC cultures which contained hydroquinone treated MSC generated 50% fewer G/M-CFU-C than controls. These results demonstrated that MSC exposed to hydroquinone can inhibit FSC dependent colony formation. The mechanism responsible for this effect may involve the Inhibition of CSA released from co-cultured MSC. Supported by NIOSH grant OH01542.

Previous work in our lab has shown DMN exposure affects CMI through changes in myelopoiesis. DMN exposure does not affect the ability to generate pleuripotent stem cells or form IL-3 responsive colonies. Only the generation of CSF-1 colonies was affected, resulting in an increase in the number of colonies with fewer cells per colony. DMN exposure resulted in an increased proliferative response to GM-CSF between 36-60h of culture with a decrease in proliferation towards CSF-1 at 60-72h. Examination of the sera for alterations in the signals controlling myelopoiesis demonstrated a net decrease in the content of CSF-1 but no changes in the levels of two inhibitory factors, transferrin and lactoferrin. These results indicate DMN exposure is affecting myelopoiesis at the level of the GM/CSF responsive cells by altering their responsiveness to this growth factor. Supported by PHS grants ES03468 and CA08210.

Parallel human and rat studies are in progress which compare physiological and biochemical changes following exposure to O$_3$ (0.4 ppm, 2 hr). Nasal and pulmonary lavage fluids are collected 16 hours postexposure. Only control subjects have been examined to date. For measurement of toxicity and sensitivity, we have improved an HPLC assay with UV detection for nucleotides (ATP, ADP, AMP, cyclic AMP, NADP, and NAD), and HPLC-electrochemical detection assays for ascorbic acid (AAH), uric acid (UA), a-tocopherol (AT), and glutathione (GSH). Studies to date show similar biochemistry of human and rat lavage fluids. Alveolar macrophages from 12 human subjects of both sexes had mean AH$_2$, UA and GSH levels of 146, 11, and 497 ug/10$^5$ cells, respectively. The same cells had AT concentrations of 18 ug/mg phospholipid. AT in the surfactant phase of human lavage fluid was 9.3 ug/mg phospholipid. AH$_2$ was present in lung and nasal lavage fluids of rats at levels as high as 25 ug/mg protein (4-10x higher than in the whole lung). Adenine nucleotides were also found in both nasal and lung lavage fluid supernatants in high quantities (20 to 50 ug/mg protein). This abstract does not necessarily reflect EPA policy.
ORNITHINE DECARBOXYLASE ACTIVITY: A SENSITIVE MARKER OF LUNG RESPONSE TO O₃ AND NO₂. Nabil M. Elsayed and M. Mohammad G. Mustafa. Divisions of Pulmonary disease, and Environmental and Occupational Health, University of California, Los Angeles, CA.

Inhalation of O₃, NO₂, or a mixture of both, result in lung biochemical alterations. We undertook this work in search of sensitive markers that may have pathophysiologic relevance. We exposed rats, 90 days old, to a mixture of O₃ and NO₂ (0.45 +1.8 ppm) or to filtered air, for 3 days. After exposure we sacrificed the rats and analyzed their lungs for biochemical changes. We found that Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities, reflecting one of the lung's antioxidant defense systems, increased significantly (80% and 65%, p<0.05). But, ornithine decarboxylase (ODC) activity increased 500%, p<0.001. Increased ODC activity may reflect greater response of polyamines, which were shown to be associated with DNA synthesis, to oxidant exposure. The data also suggest that ODC activity can serve as a sensitive marker of the lung response to environmental levels of oxidants. Supported by grants NIH, HL-27309 and EPA, R812869-01.


To investigate the role of the daily time period of O₃ exposure on the toxicological response, rats were exposed to 0.8 mg O₃/m³ for 12 h diurnal (lights on), 12 h nocturnal (lights off) or 24 h continuous periods. After 3 days exposure, the nocturnal and continuous exposures resulted in significant, comparable increases (15-30%) in pulmonary antioxidant enzyme activities, whereas the diurnal exposures caused no effect. Single nocturnal exposure resulted in significant increased in total protein, albumin and relative influx of neutrophilic granulocytes in lung lavage fluid (500, 960 and 1400% respectively), which were about twice those after similar diurnal exposure, while the total nucleated cell numbers were only marginally affected. These data suggest that pulmonary effects mediated by nocturnal O₃ exposure are larger than the diurnal exposure response and may thereby govern continuous exposure-mediated changes. Animal toxicity studies on air pollutants may therefore include exposure regimens which synchronize with the actual human exposure and activity pattern. These results may have repercussions for the evaluation of existing O₃ exposure experiments.

PULMONARY FUNCTION IN THE OUTBRED MALE BLOTCHY MOUSE AFTER 8 WEEKS OF 5.0 PPM NO₂. M.A. Stevens,1 D.L. Costa,2 R. Farb,3 G. Mechanic,4 Northrop Services, Inc, RTP, NC; 2US Environmental Protection Agency, RTP, NC and 3Dept of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC. Sponsor: J.S. Tepper.

Nitrogen dioxide (NO₂) is an oxidant pollutant which leads to mild emphysema in rodents after subchronic exposure to concentrations of 30 ppm. However, the outbred blotchy mouse, a spontaneously emphysemic animal will develop severe emphysema after only 4 weeks inhalation exposure to 20 ppm NO₂. To assess the sensitivity of this susceptible subpopulation to high ambient levels of NO₂, we measured pulmonary function in normal male and outbred blotchy male mice for up to 8 weeks of 5.0 ppm NO₂. Lung volumes, diffusing capacity, total respiratory system compliance and the distribution of ventilated air were unaffected by NO₂ in the normal mice. While unexposed blotchy mice exhibited lower diffusing capacities, higher compliance values and higher wet lung weights than the normal mice, the NO₂ exposure did not lead to a significant acceleration of development of functional emphysema in these animals. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
In a variety of settings, individuals may be acutely exposed to nitrogen dioxide at concentrations well beyond those associated with urban air pollution. Accordingly, this study was undertaken to assess lung alterations brought about by acute, short duration exposures to high concentrations of NO₂. Fischer 344-rats were exposed to NO₂ at mass concentrations of 90, 135, 180, and 270 mg/m³ for durations of 2, 5, 15, and 30 min. Control groups consisted of colony animals and rats exposed to clean air. Animals were sacrificed at 4, 8 or 18, 24, and 48 hrs after the exposures for the following lung measurements: lung wet weight (LWW), right cranial lobe wet weight (RCLWW), right cranial lobe dry weight (RCLDW), and total lung capacity (TLC). Following fixation, the lungs were subjected to histopathologic analyses. No major changes were found in LWW, RCLWW, RCLDW, or RCLDW/RCLWW after the animals were exposed to the NO₂ at the above mass concentrations for durations of ≤5 min. Based on increases in LWW, RCLWW, and RCLDW, the threshold effect level following NO₂ inhalation for 15 min was found to be >75 ppm but <100 ppm. Histologic evidence of lung damage paralleled the severity of lung injury indexed by the lung measurement data.

Exercise during the inhalation of toxic agents can increase their toxic effects in the lung. Little is known, however, as to how exercise following a toxic insult may impact on the severity of the lung’s response. We exposed Fischer-344 rats to a sublethal concentration of NO₂ (150 ppm) for 30 min. One group of NO₂ exposed rats was exercised on a treadmill for 5 min 1 hr after exposure. Another exposed group was exposed to 5% CO₂ 1 hr after exposure as a control for the increased VE component of exercise. All NO₂ exposed-exercised rats died within 6 hr thereafter. No animals died after NO₂ exposure followed by CO₂ inhalation. The following lung measurements were made at the time of death or 6 hrs after the NO₂ exposures: wet lung weight (WLV), right cranial lobe wet weight (RCWW), and right cranial lobe dry weight (RCWDW). Significant increases in WLV, RCWW, and RCWDW were found in rats exposed to NO₂ followed by exercise compared to NO₂ exposure and rest only. These results indicate exercise following NO₂ inhalation can potentiate the severity of the toxic response. Increasing ventilation alone after exposure to NO₂ does not appear to be the mechanism involved. [This work was supported by the DoD and conducted under the auspices of the DoE].

Blood from mice was analyzed for carbon monoxide (CO) and cyanide (CN). Using an amount of Douglas fir wood or Nylon 6 which when thermally decomposed produced sufficient smoke to kill 50% of the animals (LC₅₀) the mean % carboxyhemoglobin (COHB) found with wood was 70% and the mean blood CN with Nylon 6 was 0.063 mg% while COHB was only 5%. Mice were exposed to CO or HCN singly or together to determine their LC₅₀ within 30 min of exposure. These were 3,000 ppm for CO with 65% COHB level and 177 ppm for HCN with 0.094 mg% CN in blood. Low oxygen was also used, 7.7% O₂ yielded 50% lethality. A combination of HCN and low O₂ revealed the greatest additivity in a composite LC₅₀ of 89 ppm HCN and 13.8% O₂ with a mean blood CN level of 0.059 mg%. Lethal blood levels and air levels of HCN can thus be reduced by approximately 50% when low oxygen is present. Supported under NBS Grant NBS79NA00009.

To determine a correlation between phosgene exposure concentration and persistence of lesions after exposure, Fisher 344, male rats were exposed to 0.0, 0.1, 0.25, 0.5, and 1.0 ppm phosgene for 4 hr. Rats were killed immediately (0 hr), 24, 48, 72, and 168 hr postexposure. Histopathological and biochemical changes were both concentration and time dependent. The pathological lesion occurring 24-48 hr after exposure, was multifocal in nature, and primarily affected the alveolar ducts and associated alveoli. It was characterized by the presence of fibrin, Type II cell hyperplasia, alveolar macrophage infiltration and interstitial fibrosis. The biochemical changes occurred 48-72 hr post-exposure and included significant increases in glutathione (GSH) selenium-dependent and selenium-independent peroxidases, GSH-r-SH transferase and sulfhydryls. Non-protein sulfhydryls were significantly decreased at 0.5 and 1.0 ppm phosgene. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

The pulmonary response to the inhalation of various levels of 2-MF (50, 100, 160, 200, 240, 360 and 480 ppm) was investigated in Sprague-Dawley rats. Groups of randomly selected female and male rats (weight range: 180-204 g for females and 264-290 g for males; 10 rats/sex/dose level) were exposed to 2-MF (1x6 hours) and kept in observation for 14 days. Prior to necropsy, the lungs were infused in situ with buffered glutaraldehyde and selected tissues from female and male rats were sampled for examination.

Slight perivascular and/or peribronchial edema were detected in rats exposed to 100, 160 and 200 ppm whereas moderate edema and perivasculan and/or peribronchial fibroplasia were observed only in rats exposed to 240 and higher levels of 2-MF. In addition, treatment-related findings included mixed and fine vacuolation and necrosis of the liver and atrophy of the spleen white pulp (240-480 ppm). (Work supported in part by the Canadian Panel on Energy Research and Development).

CARCINOGENICITY AND GENOTOXICITY OF 1,3-BUTADIENE (BD). R. Melnick, J. Reycroft, M. Shelby, B. Chou*, R. Miller*, R. Tice#. NTP, NIH, Bethesda, MD; Battelle Northwest, Richland, WA; Brookhaven National Laboratories, Upton, NY.

A previous inhalation study revealed that exposure of male or female B6C3F1 mice to 625 or 1250 ppm BD (6 hr/day, 5 day/wk for 60 wk) resulted in increased incidences of neoplasms at multiple organ sites (Muff et al., Science 227:540, 1985). A subsequent 2-year study is in progress to better characterize the carcinogenicity of BD. Male and female B6C3F1 mice have been exposed 6 hr/day, 5 day/wk to BD vapors at concentrations ranging from 6.25 to 625 ppm. After 37 weeks, 32% of the males and 12% of the females exposed to 625 ppm BD have died with lymphomas. Lymphomas were also observed in males and females exposed to 200 ppm BD, and in males exposed to 12.5 ppm BD. In addition, 14% of a male group exposed to 625 ppm BD for only 13 weeks (stop-study group) have died with lymphomas. The diagnosis of lymphoma was based on necropsy observations. Bone marrow cytogenetic studies of male mice exposed to 6.25, 62.5, and 625 ppm BD for 2 wk showed dose-related statistically significant increases in SCEs at 6.25 ppm, micronucleated polychromatic erythrocytes at 62.5 ppm and chromosomal aberrations at 625 ppm. Thus, the carcinogenicity of BD has been demonstrated at lower concentrations and with shorter exposure periods than in previous studies, and genotoxicity observed at 6.25 ppm.


We assessed how several lavageable lung constituents are affected in the rat following thoracic x-irradiation. Fischer-344 rats were irradiated with 7.5 Gy (Low Dose) and 15 Gy (High Dose) of 250 Kv-x-rays; control groups consisted of sham-irradiated animals. After 1, 5, 9, and 13 weeks, the animals were sacrificed and their lungs were lavaged. Cell numbers and types, lavaged were determined, and the lavage fluids were analyzed for phospholipid (PL), protein (P), and histamine (H) contents. >95% of the lung free cells harvested from all groups were alveolar macrophages (AM). No changes in AM numbers following the Low Dose were found. The High Dose brought about an = 3 fold increase in AM numbers by week 5, and AM numbers continued to be elevated over the course of the study. PL in lung lavage fluids from the x-irradiated groups increased = 1.5 fold by week 1. PL continued to be elevated for the Low-Dose group until week 9. PL for the High Dose group were not significantly different from control values after week 1. P increased only in the High Dose group at weeks 5 and 9, and latter in the Low Dose group at week 13. H in the lavage fluids scaled with the appearance of interstitial mast cells in lung sections (High Dose). Our results indicate the PL and P responses to x-irradiation are not concurrent, and the appearance of interstitial mast cells and lavageable H does not correlate with increases in lavageable PL.

INHIBITION OF FC-MEDIATED PHAGOCYTOSIS IN PULMONARY ALVEOLAR Macrophages (PAM) FROM MICE EXPOSED TO MAINSTREAM CIGARETTE SMOKE. E. Celik and E. Celik, Tobacco and Health Research Institute and the Graduate Center for Toxicology, University of Kentucky, Lexington, KY.

Male C57Bl mice were exposed twice daily to mainstream cigarette smoke from University of Kentucky reference cigarettes (2R1) for 25 to 32 weeks. Elevated levels of blood COB and pulmonary AHH activity indicated effective inhalation of smoke by animals. PAM monolayers were established by culturing cells obtained by bronchoalveolar lavage and used for phagocytosis assays. Antibody (Ab) dependent and independent phagocytes were assayed with rabbit anti sheep erythrocyte IgG (EA)-and tannic acid-treated sheep erythrocytes as target particles, respectively. Macrophages and particles were incubated for 1 hr., and free erythrocytes were lysed by brief hypotonic shock. The macrophage monolayers were fixed with glutaraldehyde for examination by oil emersion phase contrast microscopy. In smoke exposed (SM) groups, the percentage of PAMs phagocytizing EA was only 50% of that observed in room (RC) and sham (SH) control groups. Phagocytic capacity, defined as the number of erythrocytes per cell, was the same for all three groups. Ab-independent phago- cytosis assays showed no appreciable differences among RC, SH and SM groups. The data suggest an inhibition of Fc-mediated phagocytosis in PAMs from smoke exposed mice. (Supported by NIH 5-41033).
Previously we compared lung responses of rats and mice to inhaled dusts. In the present studies, we have expanded our scope to examine the in vivo and in vitro lung responses of several species following inhalation of carbonyl iron (CI) particles. Two strains of rats, 3 strains of mice and 1 strain of hamsters and guinea pigs were exposed to an aerosol of CI for brief periods at 100 mg/m³. Electron microscopy of dissected lung tissue revealed that particle deposition patterns in the distal lung were identical for all species although increased numbers of particles were deposited in rats and mice compared to guinea pigs. Time course studies showed that increased numbers of rat pulmonary macrophages (PM) migrated to sites of CI deposition and phagocytized particles (PM) in vivo and in vitro compared to the other 3 species. Chemospecific studies showed that rat PM migrate better to dust-activated chemotactic factors (C5a) while other 3 species respond to n-formyl peptides. In addition, a PM inflammatory response was observed only in the mouse. Our data indicate that the rat is the most efficient species in clearing inhaled particles while hamsters and guinea pigs may respond best to bacteria.

In contrast to the more stringent legislation adopted in many countries for the registration of the active ingredients of pesticides now in use, there is a lack of critical data and registration guidelines concomitant with the use of many of the approximately 2,000 inert ingredients in tens of thousands of pesticide formulations that have been generally recognized as safe and have been on the market for decades. These inert pesticidal ingredients span both a broad spectrum of structure or class as well as use category, e.g., solvents, carriers, diluents, suspending or dispersing agents, etc. The populations at potential risk to these agents, which in many applications are unlabeled and unspecified and categorized as inert substances with only a percentage range given, cover the occupational formulation and application sectors as well as the general public. The objective of this review is to specifically highlight a representative cross-section of inert ingredients from both use and structural considerations illustrating potential toxicities including carcinogenicity, genetic effects, neurotoxicity, and immunotoxicity.

Pesticides which pose chronic health hazards, such as tumor production in experimental animals, should undergo risk assessment for proper risk management. The health hazard must be satisfactorily mitigated before a pesticide can be safely used in workplace. In order to extrapolate animal testing results to human use situations it is essential to properly estimate human dosimetry. This paper dealt with cancer risk assessment using conventionally available mathematical models. Human dosimetry estimates were derived from field worker exposure studies and dermal absorption studies in rats. Cancer risk assessment was performed by K. S. Crump Company. Taken into account were the varying levels and extents of exposure of workers performing different types of jobs. The estimated risk by way of three mathematical models shows that the extra cancer risk due to oxadiazon exposure is very low even in a worst case scenario. No additional risk management is needed at this time other than adhering to the prescribed label. This forms the basis of our regulatory decision.

Species differences in male reproductive toxicity of a pyrrolidine herbicide. G. L. Sprague, and D. W. Frank, Stauffer Chemical Company, Toxicology Department, Farmington, CT.

Comparative long-term dietary studies in mice, rats and dogs were conducted with 1-(m-trifluoromethylphenyl)-3-chloro-4-chloromethyl-2-pyrrolidine (R-40244), a herbicide. Rats were fed constant dietary levels of 0, 40, 100 or 400 ppm R-40244 for 2 years. Average doses over the 2-year study were 0, 2, 4 and 16 mg/kg/day, respectively. Testes weights were mildly reduced after 12 and 24 months at 16 mg/kg but not at 2 or 4 mg/kg R-40244. Necropsy and microscopic findings of toxicity were noted at all study intervals (only at 16 mg/kg). Testicular changes included atrophy of seminiferous tubules, interstitial cell hyperplasia and edema and medial hypertrophy and degeneration of small muscular arteries. Epididymal changes included degeneration of sperm within the epididymides and microtubular hyperplasia. No reproductive organ changes were noted in males at 2 or 4 mg/kg or in females at any level. No male reproductive organ changes occurred in a 6-month dog or 24-month mouse study using maximum doses of 30 and 100 mg/kg/day, respectively. In conclusion, repeated exposure to high dietary levels of R-40244 produced a rat specific male reproductive tract toxicity.
EVALUATION OF HOST RESISTANCE AND IMMUNITY IN MICE EXPOSED TO ALDICARB. P.T. Thomas, H.V. Ratajczak, W.C. Elsenberg, M. Furedi-Machacek, K.V. Ketela, and P.W. Barbera, IIT Research Institute, Chicago, IL.

Reports have suggested that aldicarb, a widely used carbamate insecticide, suppresses the immune response when administered at low levels to mice in drinking water. To develop an immune profile of this compound, adult female Swiss Webster and Balb/cJ mice received distilled water only or water containing 0.1, 1.0, 10, 100 or 1000 ppb of aldicarb ad libitum for 34 days. Functional parameters measured after exposure included resistance to infectious viral challenge, quantitation of splenic antibody-forming cells and circulating antibody levels to sheep erythrocytes and splenic T- and B-lymphocyte blastogenesis to mitogens and alloantigens. To supplement the functional assays, complete blood counts, differential leukocyte counts and body and relative organ weights were measured. Gross and histopathologic examinations of tissues relevant to the immune system also were performed. Aldicarb exposure did not alter any immunologic or toxicologic parameter measured, when compared to vehicle controls in either strain of mice. The results suggest that aldicarb is environmentally relevant exposure concentrations are not immunotoxic in rodents.

COMPARATIVE DIETARY TOXICITY OF 2 PYRIDYLPHENYL-ETHERS IN RATS AND MICE. A. C. Katz, G. M. Zwicker and G. L. Sprague, Stauffer Chemical Company, Toxicology Department, Farmington, CT.

Methyl-3-hydroxy-4-[4-(trifluoromethyl)-2-pyridyloxy]phenoxy pentanoate (SC-1084) or 3-hydroxy-4-[4-[3-chloro-5-(trifluoromethyl)-2-pyridyl]-oxy] phenoxy ethyl pentanoate (SC-1075) were administered to Sprague-Dawley rats or CD-1 mice at dietary levels of 0, 40, 100, 250, 700 or 2000 ppm for 4 weeks. In mice, 2000 ppm SC-1075 or SC-1084 produced 100% or 0% mortality, respectively. SC-1075 reduced feed consumption and body weights at 250-2000 ppm and SC-1084 had no effect. Evidence for anemia resulted at 700 ppm SC-1075 and 2000 ppm SC-1084. Increased liver weights and increased serum SGPT and SGOT activities were noted with both compounds but the effect was seen at lower doses of SC-1075 than SC-1084. In rats, SC-1075 produced anemia and an effect on the liver at the same dietary levels as those seen with mice. The only difference was that no deaths occurred in rats. SC-1084 also produced equivalent effects at comparable dietary levels in mice and rats. This study demonstrated that SC-1075 and SC-1084 produced a target organ effect in the liver and SC-1075 was more potent and also produced anemia. Findings in rats and mice were similar and effects in the liver for both species were comparable on a mg/kg rather than a mg/m2 basis.

EFFECT OF CYCLODIENE COMPOUNDS ON CALMODULIN IN RAT TISSUES. B.D. Mehrotra, K.S. Moorthy and D. Dessiah, Department of Chemistry, Tougaloo College, Tougaloo, MS, and Department of Neurology, University of Mississippi Medical Center, Jackson, MS.

Our previous studies have shown that cyclodiene compounds are potent inhibitors of Ca" pump activity in CNS and heart. Since calmodulin (CaM) is known to regulate calcium pump activity, we have determined the CaM levels in rats treated with cyclodiene compounds. Rats were treated P.O. with nldrin (A), dieldrin (D), and endrin (E) at different doses for 10 days. Ca" ATPase and Ca uptake were measured in synaptosomes (S.E.) and heart endoplasmic reticulum (E.R.) in CaM depleted and non-depleted fractions. CaM levels were determined in both tissues by RIA method. A, D and E showed a dose dependent decrease of Ca" ATPase and Ca uptake in both tissues. The decrease was observed with fractions having endogenous CaM, but no effect was seen in CaM depleted fractions. Addition of exogenous CaM to the fractions showing decreased calcium pump activity restored the original level of activity. Rats treated with A, D and E showed a dose dependent decrease of CaM levels. These in vivo results suggest that cyclodiene compounds inhibit CaM regulated pump in CNS and heart.

(Supported by NIH/MBRS Grant No. PR 08110.)

TISSUE GLUTATHIONE AND MERCAPTURIC ACID EXCRETION FOLLOWING ACUTE INHALATION EXPOSURE TO 1,3-DICHLOROPROPENE. G.D. Fisher and W.W. Kilgoare. Department of Environmental Toxicology. University of California, Davis, CA.

1,3-Dichloropropene (DCP) is extensively used in agriculture as a fumigant to control parasitic nematodes. Two hundred to 250 g rats were subjected to inhalation of DCP by nose-only exposure for one hour. Urinary excretion of the mercapturic acid of cis-DCP (3C-NAC) and tissue glutathione (GSH) levels were determined. 3C-NAC was measured in rats exposed to concentrations of up to 800 ppm DCP. The quantity of 3C-NAC contained in 24 hr urine collections correlated with the log of the exposure concentration. GSH content was measured in heart, kidney, liver, lung and testes of rats exposed to concentrations of up to 2000 ppm DCP, and nasal tissue of rats exposed to concentrations of up to 225 ppm DCP. Heart, kidney, liver and testes GSH content was not affected at concentrations of 800 ppm DCP or less. Lung GSH content was decreased, but remained relatively constant, approximately 70% of control, at exposure concentrations of 2 to 1000 ppm DCP. However, nasal tissue GSH displayed an exposure concentration-dependent decrease that coincided with 3C-NAC excretion. These results indicate that nasal tissue GSH serves as the principal source for conjugation to DCP. (Research supported by NIEHS Training Grant ES07859-08)
CHARACTERIZATION OF MULTIPLE FORMS OF CYTOCHROME P-450 FROM AN INSECTICIDE RESISTANT STRAIN OF HOUSE FLY (MUSCA DOMESTICA) M.J.J. Ronis, W.C. Dauterman, and E. Hodgson, Toxicology Program, N.C. State Univ., Raleigh, NC

There is much evidence to implicate metabolism by the microsomal monoxygenase system in insect resistance to pesticides. Both qualitative and quantitative changes in cytochrome P-450 isozymes appear to be associated with resistance in a number of house fly strains, including the Rutgers strain, which has multiple cross resistance including the organophosphates, diazinon and malathion.

Three forms of cytochrome P-450 have been characterized from microsomes prepared from abdomens of Rutgers flies using chromatography on phenyl sepharose, DEAE-cellulose, CM-sepharose, hydroxyapatite and on an affinity column of house fly cytochrome b5 bound to sepharose 4B. The forms show differences in M.W. on SDS-PAGE gels and their substrate specificity towards the insecticides aldrin and phorate is currently being investigated using reconstituted systems containing cytochrome P-450, cytochrome P-450 reductase, cytochrome b5 and diacylphosphatidylcholine.

DT-DIAPHRAGMASE AND QUINONE DETOXIFICATION. A.S. Atallah, J.R. Landolph and P. Hochstein, Institute for Toxicology and The Cancer Center, University of Southern California, Los Angeles, CA.

DT-diaphorase (E.C. 1.6.99.2) catalyzes the two-electron reduction of quinones in the presence of NADH or NADPH. A variety of substances are known to induce its activity in animals. It is also characterized by high sensitivity to inhibition by the anticoagulant dicoumarol. A permanent fibroblast cell line (10%) derived from C3H mouse embryo has been used to study the toxicity of some model quinones under conditions in which DT-diaphorase activity was altered, either through treatment with inducing agents, e.g., Sudan III, 3-methylcholanthrene or with the inhibitor dicoumarol. The quinones utilized included menadione, 1,2 naphthoquinone, 1,4 naphthoquinone, duroquinone and daunomycin. In all instances, induction of DT-diaphorase in this cell line was associated with increased protection against quinone toxicity as measured by a colony survival technique. In contrast, in all instances in which dicoumarol was utilized to inhibit DT-diaphorase the sensitivity of cultured cells to quinones was enhanced. The results obtained are consistent with a view that DT-diaphorase competes with one-electron quinone-reducing enzymes, which generate autoxidizable semiquinones, and forms more stable hydroquinones as an initial step in the detoxification of such compounds.

CONTRACTILE RESPONSES OF THE FROG SLOW SKELETAL MUSCLE TO U-40481: ROLE OF EXTRA-CELLULAR CALCIUM Y.C. Revikumar and J.A. Rieger*, School of Pharmacy, Northeast Louisiana University, Monroe, LA and *College of Pharmacy, University of Oklahoma, Oklahoma City, OK

\[ \text{N'-}(2,4\text{-xylyl})\text{-N-methylformamidine (U-40481) is an active metabolite of the pesticide amidraz. We have previously shown U-40481 to noncompetitively inhibit ACh, KCI, and caffeine induced contractions in the frog rectus abdominis muscle (Toxicologist 5: 1985). However, U-40481 also caused slow, sustained contractions in the rectus muscle at high bath concentrations. The present studies were performed to determine the mechanism of the contractions produced by U-40481 in rectus muscles from Rana pipiens. U-40481A (0.25-5 mM) caused dose-dependent contractions over 30 min. While cumulatively added ACh produced a maximal tension of 12.3 g Tension/g muscle (gT/g), 2.5 and 5 mM U-40481A produced about 90 gT/g. Neither a subthreshold concentration of KCI (10 mM) nor procaine HCl (1.5 mM) affected the tension elicited by 1 mM U-40481A. However, the tension produced by 5 mM U-40481A in Frog-Ringer's solution (FR) without added calcium was only 22% of that in FR with normal calcium content. Also, verapamil (10 mM) significantly decreased U-40481A-induced tension. It appears that U-40481A produces contractions of the frog rectus muscle primarily by facilitating influx of calcium ions from the medium.}


Cyanide (CN) interaction with isolated hepatocytes was studied further in hepatocyte suspensions and cultures to examine the relation between cytotoxicity indicators and their mechanistic implications (The Toxicologist 4:127). Hepatocytes were isolated by collagenase perfusion from Sprague-Dawley rats (270-370 g; male) and incubated in hormone-supplemented culture medium at 37°C and 70 oes/min in closed, 25-ml flasks under 95% air:5% CO2. ATP depression and LDH release from the cells showed quantitatively similar responses in suspensions and cultures to CN concentration and incubation time. Inhibition of O2 consumption was the earliest and most sensitive change detected. Half-maximal changes in different parameters were estimated from the data to be: O2 consumption inhibition, 78 μM; ATP/AODP depression, 0.14 mM; ATP depression, 0.20-0.24 mM; urea synthesis inhibition, 0.11 mM; LDH release, 1.5 mM. The CN concentration at which 50% inhibition of O2 consumption occurred is in the range of plasmatic levels in rodents at lethality and at which liver cytochrome oxidase inhibition has been reported. The results are consistent with the hypothesis that complexation of cyanide by cytochrome oxidase initiates toxicity and that target organ toxicity depends on differences in the organ's capability to compensate for the oxidative stress produced. (Supported by DAMD17-82-C-2211).
USE OF AN IN-VITRO SYSTEM TO STUDY THE EFFECTS OF LOW LEVELS OF LEAD ON THE BLOOD-BRAIN BARRIER. A.M. Gehbalt and G.W. Goldstein, Departments of Pediatrics and Neurology, University of Michigan, Ann Arbor, MI. Sponsor: M.J. Erbehe.

Brain capillaries are a major target tissue for the toxic action of lead. Pathologic studies in children poisoned with lead and in several animal models reveal a breakdown in the normally tight blood-brain barrier (BBB). Under normal conditions, the brain capillary endothelial cells are sealed together by continuous tight junctions. Glial cell interaction with endothelial cells plays an important role in the regulation of the BBB. We are investigating the cellular and biochemical effects of low-levels of lead on an in vitro model of the BBB by co-cultures of endothelial and glial cells. Each cell type was identified morphologically and histochemically. Lead was cytotoxic to glial cells (LD50 ~300µM), a concentration ten times less than that at which visible cytotoxicity was first detected in endothelial cultures. Co-cultures exhibited a lead sensitivity intermediate to that of the two cell types. After 6 days in co-culture grossly visible nodules of cells were formed. 10 µM lead increased the number of nodules while higher concentrations were inhibitory. In summary, lead alters the interaction of endothelial and glial cells in vitro, indicating a potential mechanism for its toxicity in vivo. (NIH ES02380)

AFFINITY PURIFICATION OF CLOFIBRIC ACID BINDING PROTEIN(S) FROM RAT LIVER. N.D. Lalwani, K. Alvaes, M.K. Reddy and J.K. Reddy, Department of Pathology, Northwestern University Medical School, Chicago, IL 60611

Previous studies have shown that 3H-nafenopin, a structural analog of the hypolipidemic peroxisome proliferator, clofibrate, binds to a saturable protein pool in the rat liver cytosol. In order to identify this protein pool, we have utilized affinity chromatography using clofibr symbolic acid as a ligand, immobilized on AH-Sepharose 4B. Cytosol was prepared in 10mM Hepes (pH 7.5), 1mM EDTA, 1mM dithiothreitol, 1mM benzamidine, 0.3mM PMSF, 10% glycerol and 0.4M KCl from the livers of F344 male rats. The affinity bound proteins were eluted in the presence of free 1mM clofibrate acid. A major binding protein with 70KD molecular weight and few minor proteins were co-purified as ascertained by SDS-polyacrylamide gel electrophoresis. The electrophoretic profile of clofibrate acid binding proteins was similar to that isolated by ciproflaxin and nafenopin affinity columns. Immunoblotting using anti-serum against nafenopin binding 70KD protein showed immunological similarity with the 70KD protein obtained from clofibrate acid and ciproflaxin affinity columns. The similarities between these binding proteins suggest a common mechanism, possibly receptor mediated, for the induction of peroxisome proliferator pleiotropic response. Supported by NIH grants CA 30816 and GM 23750.

DETECTION OF DNA CROSS-LINKING BY GRAVITY-FLOW ALCALINE ELUTION. J.R. Hincks and R.A. Coulombe, Jr, Center for Environmental Toxicology, Utah State University, Logan, UT.

The gravity-flow alkaline elution method was modified to detect DNA cross-links. Cultured bovine kidney epithelial cells (MDBK) labeled with [3H] thymidine were exposed to known cross-linking agents, followed by exposure to 600 R X-ray, loaded onto PVC filters, lysed and then eluted by gravity with 42 mL solution (pH 12.2). The extent of DNA cross-linking was determined by the decrease in the amount of DNA eluted from cells exposed to the test compound + X-ray compared to cells exposed to X-ray alone. UV light (254 nm) at 10 J/m2 + X-ray produced no change in DNA eluted over X-ray alone, but 20 and 40 J/m2 + X-ray produced a significant (p<0.01) reduction in DNA eluted (18% and 35% respectively) over X-ray alone. Cells treated with 0.25, 1.0, or 4.0 µM nitrogen mustard for 1 hr and exposed to X-ray produced a significant (p<0.05) dose-dependent reduction (10% to 57%) of DNA eluted compared to X-ray alone. Cells incubated with 20, 40 and 60 µM mitomycin C for 1 hr then exposed to X-ray produced significant (p<0.05) reduction in DNA eluted of 23%, 42% and 45% respectively compared to X-ray alone. These results indicate gravity-flow alkaline elution can also be used for the detection of DNA cross-links. (Supported in part by USPHS Grant ES03591).


We have shown earlier that in vitro influence of MX and phorbol esters (PE) on cAMP-PK were different from those observed for PE in other cell and intact animal systems. At present, the effects of MX on Type I and II cAMP-PK IEZ activity and [3H]-cAMP binding were examined in vitro. MX (2.2 nM-10 µM) produced a dose-dependent inhibition (2-14%) of the cytosolic-cAMP/cAMP-PK activity ratios as compared to control. Type I IEZ showed a similar inhibition (8-21%), while MX caused marked variations in Type II IEZ activity (98-111% of the control). At the same dose range, MX also caused a dose-dependent inhibition (27-55%) of cAMP binding in Type I IEZ and cytosol, whereas cAMP binding to Type II IEZ was independent of mirex concentration. Most importantly, the diminution of PK activity ratios and cAMP binding showed a significant correlation both for crude cytosol (r=0.98) and Type I IEZ (r=0.99); a less correlation (r=0.61) existed between these two activities in the case of Type II IEZ. The results suggest that the effects of MX on cAMP-PK may be due to its interference on the cAMP binding to the holoenzyme complex thus releasing less catalytic subunits, and may be an important mechanism to account for the observed MX-induced inhibition of cAMP-PK in vivo. (Funded by BRSG/NIH S07RR05399-24)
Gossypol, a polyphenolic binaphthalene-dialdehyde, produces both general toxic and antifeedant effects in mammals and insects. The cellular mechanisms by which gossypol exerts these effects are not clear. In this study, the interaction of gossypol with the mitochondria isolated from house fly thorax and beef heart was characterized. Gossypol at 1 µM inhibited 32.4% and 64.2% of Mg2+-dependent ATPase from house fly and beef heart mitochondria, respectively. This action appears to be both species and dose dependent. A concentration of 2.5 µM gossypol was high enough for almost a complete inactivation of the soluble mitochondrial ATPase from insect thorax (FI) and beef heart (F1).

The data suggested that the mechanism of gossypol action is completely different from that of oligomycin, dicyclohexyl carbodiimide (DCCD) and p,p'-DDT. Kinetic studies showed that gossypol inhibits FI and F1 competitively. In comparison with the well-known ATPase inhibitor p,p'-DDT which can not inhibit the soluble ATPase, gossypol inhibited FI and F1 with Ki values of 0.125 and 0.136 µM, respectively. So, the specific action of gossypol with the soluble mitochondrial ATPase may cause a conformational change of the enzyme that distort the substrate site thereby prevents the substrate from binding to its site(s).

The relative competitive binding affinities of 12 polynuclear aromatic hydrocarbons (PAHs) for the rat hepatic cytosolic 4S binding protein were determined using [3H]-benzo[a]pyrene as the radioligand. With the exception of triphenylethylene, triptycene and 4H-cyclopental[de]benzanthracene, the EC50 values for the remainder of these compounds were between 1.25 x 10−1 to 2.5 x 10−5 M with 1,2,5,6-dibenz[a]anthracene being the most active ligand. A comparison of the relative cytochrome P450 (2C) receptor and 4S binding affinities and aryl hydrocarbon hydroxylase (AHH) induction potencies of these hydrocarbons demonstrated the following: (a) 5 PAHs, exhibited high to moderate binding affinities for the 45 and 95 cytosolic proteins (EC50 values < 10−6 M) and induced AHH in rat hepatoma cells; (b) 3 compounds, namely, triphenylethylene, triptycene and benzo[a]pyrene exhibited high affinities for the 4S binding protein (< 1.25 x 10−7 M), low affinities (EC50 values > 10−5 M) for the Ah receptor protein and did not induce AHH in vitro. The results suggest that the 45 protein may not be involved in AHH induction; however, preliminary data indicate that PAH ligands for the NS binding protein modulate this induction process. (Supported by the Center for Energy and Mineral Resources.)

The aim of the present study was to elucidate molecular mechanisms of cephalosporin nephrotoxicity. Effect of cephaloridine and therapeutically used cephalosporins on renal microsomal membrane proteins were investigated. Male Wistar rats (200 g) were treated i.v. with 1200 mg/kg of cephaloridine, cefazidime, cefoxitin and cefotaxime for 5 days. Subsequently, rats were killed and the effects on renal tubular transport, gluconeogenesis and protein composition of various renal subcellular fractions were quantified. Renal accumulation of p-aminobenzoate and tetraethylammonium as well as renal gluconeogenesis were decreased by cephaloridine, cefazidime and cefoxitin whereas cefotaxime showed no effect. Effects on protein composition were analysed by one- and two-dimensional SDS gel electrophoresis. Cephaloridine, cefazidime and cefoxitin caused significant changes in the polypeptide pattern only in the renal microsomal fraction whereas cefotaxime had no effect. Cephaloridine and cefazidime induced a polypeptide of molecular weight 44000 whereas cefotaxime showed no effect on the polypeptide pattern. The effects of cephaloridine on renal microsomal proteins and nephrotoxicity could be ranked as follows: cephaloridine > cefazidime > cefoxitin > cefotaxime.

Mechanisms for the release of alkaline phosphatase (AP) from cultured hepatocytes exposed to TAMOXIFEN, DIPHENYLYLDACTOIN, OR PREDNISOLONE 21-ACETATE. D. E. Amacher and C. V. Higgins. Drug Safety Dept., Pfizer Inc., Groton, CT.

Tamoxifen (Tam), diphenylylhydantoin (Dph) and prednisolone (Pred) are unrelated drugs that elevate canine serum AP of hepatic origin. In this study, primary rat or dog hepatocytes were dosed with these 3 drugs for either 2 or 24 hrs. Intracellular AP, and the extracellular activities of the cytosolic enzymes lactate dehydrogenase (LDH), aspartate aminotransferase (AST), or alanine aminotransferase (ALT) were then monitored 24 hrs after the establishment of monolayer cultures. In dog hepatocytes, Tam (250-1000 µg/ml) caused a 10-fold increase in extracellular AP and, at all but the lowest dose, 2-fold release of ALT, LDH, and AST. In rat hepatocytes, 2 hr exposure to Tam caused >10-fold elevations of extracellular AP, ALT, and LDH activities. Two hr exposure to 250-1000 µg/ml Dph or Pred clearly elevated extracellular AP in both rat and dog cell cultures, but had no effect on LDH, AST, or ALT. Intracellular AP activity also increased after 2 or 24 hr exposure of dog hepatocytes to Dph, were generally unchanged for Pred, but decreased with similar exposure to Tam. Mechanistically, the dose-related hepatic release of AP by Tam suggests general cytotoxicity, but the release of AP following Dph exposure was apparently due to AP enzyme induction.
The effects of nucleoside toxins and the drug dilazez on cell growth, cell viability, and the recovery of cell growth were determined. L5178Y mouse lymphoma cells were treated with a formycin derivative during an incubation at 37°C. Concentrations of 5.0 M formycin A, 120 µM formycin B, or 170 µM 5'-deoxoformycin A produced an arrest of cell growth for one day and small increases in the nonviable cell count as determined by the trypan blue exclusion method. Although 5'-deoxoformycin A (gift of Dr. L.B. Townsend) is not converted to a 5'-nucleotide by enzymic phosphorylation, the nucleoside is a cytotoxic agent. The cell counts increased during an additional two-day "recovery" incubation in growth medium. Addition of 50 µM dilazez two hr after toxin treatment produced an accelerated two-day recovery of cell growth in comparison to controls. (Dilazez is a vasodilator and an inhibitor of nucleoside transport.) There is little information available on the mechanism of cell recovery from the action of different toxicants. These findings indicate an intracellular-extracellular turnover of nucleoside toxins. The cell recovery technique could be useful in drug selectivity studies of transformed cells. (Supported by grant 82-0261 from the Air Force Office of Scientific Research.)

Small cell cancer of the lung is one of the leading types of cancer in smokers. However, no single constituent of smoke nor any combination of the many hazardous agents contained in smoke has ever succeeded in inducing pulmonary small cell cancer in animals. The pulmonary endocrine cells believed to be the origin of this cancer type are rare in healthy adult mammals although abundant in the perinatal age period. They also have been shown to increase in number in rabbits exposed to hyper or hypoxia as well as in hamsters treated with nitrosoamines. We postulated that it requires two joint factors to make pulmonary endocrine cells capable to proliferate and progress into small cell cancers: significant imbalance in pulmonary endocrine cells and coincident exposure to nitrosoamines. Both of these conditions are uniquely present in a smoker's lung. To test this hypothesis, we exposed Syrian golden hamsters to a significant oxygen imbalance and treated them at the same time subcutaneously with diethylthiobarbituric (18 mg/kg). 90% of the animals developed multiple lung and lung-carcinogenic small cell cancers of the lung within 8 weeks of treatment. (Operated by Martin Marietta Energy Systems, Inc. for the U.S. Dept. of Energy.)

In vivo and in vitro effects of the organophosphorus defoliant DEF on catfish acetylcholinesterase. C. Kabi, Ecotoxicology Laboratory, Duke University, Durham, NC. Sponsor: M.B. Abou-Donia

The effects of the organophosphorus defoliant S,S,S-trimethyl phosphorothioate (DEF) on channel catfish brain acetylcholinesterase (ACHE) were investigated using in vivo and in vitro techniques. Catfish (Ictalurus punctatus), 25-40 g wet weight were exposed in 50 liter aerated, static aquaria to initial doses ranging between 0.3-8.0 µg/l for 30 days. Following exposure, brains from survivors were homogenized in phosphate buffer, with ACHE activity being determined using Elsässer's spectrophotometric method. Further experiments using sublethal doses focused on the time course of ACHE inhibition and recovery. In vivo approaches, brain homogenate was fractionated by differential centrifugation, with 10,000g and 100,000g fractions being utilized. These fractions were incubated with DEF, with aliquots being tested for ACHE activity at times ranging from 2 to 30 min. Incubations were performed with and without cholinesterase inhibitors. In vivo results indicated a dose-related decrease in brain ACHE activity, with the LC50 corresponding to approximately 70% reduction in ACHE activity. Sublethal exposures indicated a time-dependent decrease in activity during the exposure period, with activity reduction occurring 2 days after transfer to DEF-free aquaria. Recovery was slow, requiring nearly one month to regain 50% of control activity. In vivo evidence using substrates and inhibitors were specific for true or pseudo ACHE indicated that fish brain ACHE is a true ACHE. Fish brain ACHE is not affected by DEF alone, but in the presence of microsomes and RAPDH, DEF is activated to potent anticholinesterase agent. The necessity for activation most likely is related to the leg noticed in in vitro exposures.
While there is concern over the chronic contamination and uptake of Pentachlorophenate and its salts by temperate species of fishes, there is paucity of information on their effects on tropical species. Presently, effects of Sodium pentachlorophenate on food intake, conversion and energetics of the freshwater catfish Heteropneustes fossilis have been studied. Juvenile fish were maintained in toxicant-free water and in concentrations of 33 and 66 ug/l of NaPCP for 30 days and fed daily on different rations of the food, Rasbora daniconius muscle. The daily food consumption of R. fossilis in toxicant free water was higher than that of fish maintained in 33 or 66 ug/l NaPCP. As compared to the controls, the maintenance level of fish in the two levels of the toxicant increased nearly by 55.6 and 80%. A reduction in growth rate of fish exposed to the bicarbonate was apparent through a reduction in conversion efficiency. Changes in specific dynamic action levels clearly indicate that NaPCP acts as a metabolic stressor in the fish impairing food conversion efficiency and production.

Paper mill waste discharges contain a variety of toxic chemicals including hydrogen sulfide, mercaptans, and fungicides. The present investigation assessed the impact of a paper mill waste discharge on a river in North Central Louisiana. A variety of biological and chemical parameters were examined at two sites above (1 and 2) as well as two sites below (3 and 4) the mill discharge. The effluent was highly colored permitting significantly less light penetration at site 3 (9.3 cm) than at sites 1 (26.1 cm) and 2 (29.6 cm). However, the color appeared to have no influence on the species diversity index, considered the best single means of assessing biological integrity in freshwater streams and rivers. Sammen-Wiener Diversity Index values of 2.20, 2.61, 2.95, and 3.60 were determined for sites 1, 2, 3, and 4, respectively, indicating a normal progression of diversity. However, an unusually high number (7) of hybrid Centrarchid species were collected at the site of discharge, indicating a disruption of the natural environment. A variety of toxicity bioassays also were conducted. Despite the objectionable color, the paper mill waste discharge appeared to have no significant impact on the endemic fish population.

There is a need for toxicological information on 1,4-dithiane, an environmental contaminant found in and around locations where mustard gas (bis-[chloroethyl]sulfide) has been disposed. In a 90-day rat subchronic study, in which CD strain rats were dosed by gavage at 0, 105, 210, and 420 mg/kg/day (30 rats/sex/dose group), no overt toxicity, treatment-related mortality or treatment-related clinical chemical, hematologic, or ophthalmologic changes were found. The female livers and the male kidneys were significantly heavier (p <0.05) in the treated animals. Antispastic crystals of undetermined chemical composition were deposited in the olfactory mucosa of both sexes. These crystals were present in similar amounts in both sexes of the high and intermediate dose groups. In the low dose group, the crystals were present in greater amounts in the females. The crystals were not observed in the control animals. Other treatment-related manifestations were eosinophilic cytoplasmic granulation of the convoluted renal tubule cells in the high dose males and minimal hypertrophy of the centriflobular region of the liver in both sexes. (Supported by Army Medical Research & Development Command Project Order 85PP5870).

The antibiotic sulfadimethoxine (SDM) has been shown to be efficacious in the treatment of various bacterial diseases in fish. Currently, little information is available regarding the pharmacokinetics of SDM in the rainbow trout. Aortic calcium-infusion catheter tubes were utilized in pharmacokinetic studies to allow single dose administration by i.v. (42 mg/kg) or gavage (42 mg/kg, or 126 mg/kg in food) and blood sample collection (i.v.) in free swimming fish. After i.v. administration (Na+ SDM) α and β elimination phases were demonstrated with respective plasma half-lives of 0.68 and 16.9 hrs. The systemic clearance was 24.4 ml/min. The apparent oral bioavailability varied with dose and chemical form. Na+ SDM administered at 42 and 126 mg/kg and the free base at 42 mg/kg exhibited bioavailability values of 65, 50 and 34%, respectively. The peak plasma concentrations ranged between 10 and 20 hours. SDM was well distributed throughout the body tissues and fluids with the greatest concentration in the bile. The volume of distribution (Vd) in the trout was 597 ml/kg. SDM was bound 17.3% to fish plasma proteins. These results demonstrate pharmacokinetic parameters useful for therapeutic considerations in the trout. (Supported by FDA-CVM-85-1 and ES-01985).
TRICHLOROACETATE EFFECTS ON LACTATE AND GLUCOSE METABOLISM. M.F. Davis, West Virginia Univ. Med. Ctr., Morgantown, WV.

Trichloroacetate (TCA) and dichloroacetate (DCA) are produced during chlorination disinfection of drinking water. DCA has been shown to decrease plasma and liver lactate concentrations, and decrease gluconeogenesis from lactate. TCA has not been studied. The goal of the present studies was to determine if TCA has similar effects. Male and female SD rats were given 3 doses of either TCA or DCA by gavage (2 ml/kg). Equimolar doses were used (0.92 or 2.15 mmol/kg), as neutral solution; controls received the equivalent Na load. In agreement with previous studies, DCA decreased plasma lactate in males (0.99 ± 0.059 to 0.423 ± 0.038 mmol/ml) and females (0.937 ± 0.040 to 0.490 ± 0.053 mmol/ml) and elevated 3-hydroxybutyrate in males (0.100 ± 0.017 to 0.160 ± 0.017 mmol/ml). TCA decreased plasma lactate in males (0.559 ± 0.065 to 0.296 ± 0.022 mmol/ml) and females (1.100 ± 0.171 to 0.653 ± 0.064 mmol/ml). In females decreases of liver lactate (2.310 ± 0.23 to 1.30 ± 0.18 mmol/g wt) and plasma glucose (180 ± 6 to 135 ± 5 mg/dl) were found. These results suggest that TCA has effects on intermediary metabolism similar to those of DCA and that females are more susceptible to these effects. (Supported by EPA Coop. Agree. CR811906; and does not represent EPA policy.)


The major phenobarbital-inducible form of rat liver microsomal cytochrome P-450, designated P-450b, is extremely sensitive to the inhibitory effects of Emulgen 911; the non-ionic detergent used to purify this and other forms of cytochrome P-450. By substituting the zwitterionic detergent, CHAPS, for Emulgen 911, we have purified cytochrome P-450b without the use of non-ionic detergent. The protein is designated P-450*, to distinguish it from cytochrome P-450b purified with the use of Emulgen 911. NADPH-cytochrome P-450 reductase was also purified with and without the use of non-ionic detergent. The absolute spectra of cytochrome P-450b and P-450* were indistinguishable, as were the CO- and metmyoglobin-difference spectra of the dithionite-reduced hemoproteins. When reconstituted with NADPH-cytochrome P-450 reductase and lipid, cytochrome P-450b and P-450* oxidized testosterone in the 16a-, 16b- and 17-position at nearly identical rates. The N-demethylation of benzphetamine and aminopyrazine, and the 3-hydroxylation of benzo[a]pyrene were also catalyzed by cytochrome P-450* at the same rate catalyzed by P-450b. The source of reductase had no effect on the catalytic activity of P-450b or P-450*. These results indicate that the catalytic activity of cytochrome P-450b is not compromised by residual contamination with the non-ionic detergent, Emulgen 911. (Supported by NIH grants ES-03765, ES-00166 and ES-07079.)

REGULATION OF RAT LIVER MICROSOMAL CYTOCHROME P-450 AND UDP-GLUCURONOSYLTRANSFERASE. A.J. Sonderman, M.P. Arlotto and A. Parkinson. Kansas University Medical Center, Kansas City, Kansas.

Treatment of rats with pregnenolone-16α-carbonitrile (PCN) markedly induces liver microsomal cytochrome P-450p and UDP-GT-d1, a glucuronosyl transferase active toward the digitoxin metabolite, digitoxigenin monodiglotoxioside. In the present study, we have characterized the regulation of these two enzymes in rats treated with different xenobiotics. Like PCN, treatment of rats with dexamethasone (DEX), spironolactone (SPN), troglitazone (TAO), or estradiol (EE) induced both cytochrome P-450p and UDP-GT-d1. However, compared to PCN and DEX, both TAO and EE preferentially induced cytochrome P-450p, whereas SPN preferentially induced UDP-GT-d1. Treatment of rats with phenobarbital, chlordane or Aroclor 1254, modestly induced both cytochrome P-450p and UDP-GT-d1. Neither enzyme was inducible by treatment of rats with 3-methylcholanthrene or digitoxin. The induction of cytochrome P-450p and UDP-GT-d1 by PCN followed similar dose-response curves. Although cytochrome P-450p and UDP-GT-d1 are differentially affected by the age and sex of the rats, the two enzymes responded similarly, but not identically, to xenobiotic treatment. This suggests that cytochrome P-450p and UDP-GT-d1 are co-ordinable but not coordinately regulated. (Supported by NIH grants ES-03765, ES-00166 and ES-07079.)


A cytochrome P-450 isozyme, which we call PB-2, has been purified from phenobarbital (PB)-induced dog liver microsomes. The microsomal concentration of PB-2 increases 6-fold with PB treatment, which correlates well with the 5-fold increase in the microsomal metabolism of 2,2',4,4',6,6'-hexachlorobiphenyl (2,2'-HCB) upon PB induction. Anti-PB-2 antibodies inhibit >90% of the microsomal activity towards 2,2'-HCB, and in a reconstituted system, PB-2 metabolizes this substrate at a 3-fold greater rate than that seen in PB-treated liver microsomes. In addition, PB-2 metabolizes 2,2'-HCB to at least three putative hydroxylated metabolites which co-elute with those formed by control and PB-induced dog microsomes. A protein with similar chromatographic behavior to that of PB-2 has been isolated from control dog liver microsomes. This protein is identical to PB-2 in regard to mobility on SDS-PAGE, activity towards 2,2'-HCB, reactivity with anti-PB-2 IgG, and amino terminal acid sequence. These results strongly suggest that PB-2 is the major hepatic P-450 isozyme responsible for metabolizing 2,2'-HCB in uninduced dogs, and likely explain the unique ability of dogs to metabolize and eliminate this compound in vivo. (Supported by NIH Grants T32ES 07091 and K06ES00151, and BRSG Grant S07RR05605.)
MECHANISM-BASED INACTIVATION OF CYTOCHROME P-450 ISOZYMES BY ANALOGS OF CHLORAMPHENICOL. N. Miller, C. Balfour, and J. Halbert, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ

Isozyme-specific inhibitors of cytochromes P-450 may be useful in elucidating the role of particular isozymes in xenobiotic metabolism or in suppressing the bioactivation of xenobiotics and enhancing detoxification. Chloramphenicol (CAP) is a selective inactivator of rat liver cytochromes P-450, inactivating 6 of the 12 isozymes monitored. Over 20 analogs of CAP have been tested to determine the importance of various functional groups in regulating the ability of CAP to inactivate the major phenobarbital-inducible isozyme (PB-B). The dihalomethyl moiety and the propanediol side chain are important in determining both the efficacy and isozyme selectivity of inactivation. Unlike CAP, N-(2-p-nitrophenoxy) dichloroacetamide (PNO2DCA) is an effective inactivator of the major β-naphthoflavone-inducible isozyme (BNF-B), as well as of PB-B, in vivo and in vitro. Alkaline hydrolysis and enzymic digestion of the covalently modified isozymes has shown that PNO2 DCA is metabolized by both cytochromes P-450 to oxamyl chlorides which bind to lysine and other amino acid residues of the enzymes. The mechanism by which PNO2DCA inactivates BNF-B differs significantly from that by which CAP inactivates PB-B, although both involve an impairment of the transfer of electrons from NADPH-cytochrome P-450 reductase. Supported by NIH Grants T32 ES 07091, ES 03619 and ES 00151.

INVESTIGATIONS INTO THE ROLE OF CYTOCHROME P-450 INDUCTION AND GLUTATHIONE DEPLETION IN THE DIFFERENTIAL HEPATOTOXICITY OF THE ISOMERS OF DICHLOROBENZENE. E.R. Stine and L.G. Sipes, Dept. of Pharmacology and Toxicology, University of Arizona, Tucson, AZ

The isomers of dichlorobenzene (DCB) exhibit dramatic differences in hepatotoxicity. In male P-384 rats, 24 hr SGPT activities following 1.8 mmol DCB/kg (i.p.) were 4080, 360 and 24 units/ml for o-, m- and p-DCB, respectively. The role of bioactivation in the toxicity of o-DCB is indicated by the marked elevation in SGPT activity in phenobarbital (PB) pretreated animals (1670 units/ml), following an otherwise nontoxic dose 0.9 mmol/kg. Interestingly, PB pretreatment produces similar potentiation of m-DCB toxicity at 0.9 mmol/kg (21340 units/ml), while p-DCB shows no such induction of toxicity. Hepatic GSH concentrations are reduced at 90, 180 and 300 min after a 1.8 mmol/kg dose of either o- or m-DCB, a dose which produces toxicity only for o-DCB. Phenobarbital (250 mg/kg) depletes hepatic GSH to 15% of control levels within 2 hr, reportedly without effect on drug metabolism activity. Such GSH depletion produces an approximately equivalent elevation in SGPT activity following a 1.8 mmol/kg dose of o- and m-DCB (5749 ± 648 and 4732 ± 857 units/ml, respectively). Investigations are ongoing into the interrelationship between P-450 mediated bioactivation and the conjugation of these reactive intermediates to GSH, relative to the differential hepatotoxicity of the isomers of DCB. (NTP-N01-ES-3-5031 and NIH T32 ES 07091.)

INDUCTION OF MICROSOMAL CHOLESTEROL EPOXIDE HYDROLASE BY CLOFIBRATE. E.L. Finley and E.B. Hammock, Dept. of Entomology, University of California, Davis, CA.

Cholesterol epoxide hydrolase (mChol) is a microsomal enzyme that converts cholesterol-5,6-epoxide to cholesterol tetrol (CT). CT is a powerful hypcholesterolemic that retards intestinal cholesterol absorption and inhibits hepatic cholesterol synthesis. Rat and mouse hepatic mChol activities are unresponsive to administration of virtually all of the classical enzyme inducers (phenobarbital, 3-methylcholanthrene, Aroclor, et al.). However, we have found that inclusion of the potent hypolipidemic, clofibrate (ethyl chlorophenoxyisobutyrate) in the diet (0.5%, w/v, 14 days) induced hepatic mChol activities in the mouse (1.8-fold) and rabbit (2.9-fold). Renal mChol activities were also induced in the rat (1.5-fold) and rabbit (2.3-fold). Ciprofibrate, another phenoxyacetate hypolipidemic, also induced mouse hepatic mChol activity (1.8-fold). Clofibrate is commonly prescribed to normalize serum cholesterol in patients with genetic hypercholesterolemas. At present, the precise mechanism(s) of clofibrate action is unresolved, though, like CT, clofibrate is known to depress cholesterol biosynthesis and increase fecal excretion of neutral sterols. The specific response of mChol activities to hypolipidemic agents suggests a possible role in the mechanism of clofibrate-induced hypocholesterolemia, perhaps through CT.

TEMPERATURE ACCLIMATION (TA) OF RAINBOW TROUT MARKEDLY ALTERED ARYL HYDROCARBON HYDROXYLASE (AHH) KINETICS BUT SLIGHTLY ALTERED CYTOCHROMES P-450. L.R. Curtis, H.M. Carpenter, L.K. Siddens, D.E. Williams and D.R. Buhler. Oak Creek Lab of Biology and the Marine and Freshwater Biomedical Ctr., Oregon State Univ., Corvallis, OR.

The kinetic constants for AHH of hepatic microsomes from fish TA at 10 or 18°C were determined at physiologic (14°C) and nonphysiologic (24°C) temperature. When assayed at 24°C Vmax for 10°C TA AHH was twice that of 18°C TA but when assayed at 14°C the difference increased to 4-fold. The Km differed less than 2-fold between 10 and 18°C-TA at 14 and 24°C but Km increased 10 and 5-fold, respectively with decreased incubation temperature. Total P-450 was 0.46 and 0.35 nmol/mg-protein for 10 and 18°C TA acclimated fish, respectively. Western blots demonstrated less than 2-fold reduction in the specific AHH isozyme in the 18°C-TA. These data indicated within a physiological range, higher activity of AHH when temperature is raised above that of TA than when it is lowered. Assays conducted near standard temperature (24°C) for monoxygenase determinations in fish masked TA-dependent differences in AHH. Since TA markedly altered liver lipids of rainbow trout (Am. J. Physiol. 235:F191) and TA dependent differences in P-450 content and isozyme patterns persisted, the study appeared insufficient to explain kinetics differences, we propose a lipid-mediated mechanism for TA modulated AHH kinetics. Supported by DS03850.

Methacrylonitrile (MeAN) is a monomer in the production of plastic elastomers and coatings. It is highly toxic by dermal, respiratory and oral routes. We are studying its biochemical and toxic effects in rats and mice. MeAN (0.5 L/D) was given orally to male Sprague Dawley rats (0.1 mmol/Kg) and Albino mice (0.13mmol/Kg). Animals were sacrificed at designated times and glutathione (GSH) concentrations determined in various organs. Within 6 h, in the rats, we observed a significant decrease in GSH levels (70% of control) in the liver, kidney, and brain. Other organs including heart, lung and spleen also showed significant decrease in GSH levels. In the mice however, the significant decrease in GSH concentrations occurred within 3 h. These results suggest that there may be a species difference in MeAN toxicity. Incubation of MeAN with GSH and cystein and the TLC analysis showed the appearance of new distinct spots (RF, 0.88). This indicates that MeAN may form adducts with GSH and cystein in vivo producing S-cyanoacetyl glutathione and S-cyanoacetyl cystein. These findings suggest that MeAN toxicity in rats and mice may be mediated through depletion of GSH.

(Supported in part by S06RR08038-16 and PAU 5303)
THE EFFECTS OF SUBCHRONIC PULMONARY EXPOSURE TO BENZO(A)PYRENE ON IGA IMMUNITY IN MICE. C.T. Schmitzlein and R.A. Rhoades. Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN.

A model was developed to study the effects of pulmonary exposure to benzo(a)pyrene (BaP) on locally produced IgA antibody to sheep red blood cells (SRBC). Mice were primed intratracheally (IT) or intraperitoneally (IP) with SRBC and challenged IT 14 days later. The number of IgA antibody-forming cells (AFC) peaked 6 days after challenge in the lung-associated lymph nodes (LALN), spleen, and lung. To study the effects of BaP on this IgA response, mice received an IT instillation of BaP (40mg/kg) or phosphatidyl choline vehicle for 5 consecutive days prior to antigen priming. The BaP-treated mice primed IT had a significant decrease in the number of IgA AFC/10^6 cells in the LALN compared to vehicle-treated mice. However, the LALN had greater cell numbers. Therefore, the total IgA AFC in the LALN from BaP-treated mice was not significantly different from vehicle-treated mice. In contrast to the IgA response in the LALN, the IgA response in the lungs from BaP-treated mice primed IT was significantly decreased compared to vehicle-treated mice. In the second series of experiments, mice were primed IP rather than IT after BaP exposure. In these mice, the IgA response in the LALN, spleen, or lung did not differ significantly compared to vehicle-treated mice. These data suggest that, after pulmonary exposure to BaP, the route of antigen priming plays an important role in the production of IgA memory cells. This work was supported by NHL grant ES-05319.

ACQUIRED IMMUNOLGIC TOLERANCE TO TRIMELLITIC ANHYDRIDE DURING A 13-WEEK INHALATION EXPOSURE. C.L. Leach, N.S. Hatoum, C.R. Zeiss, D.M. Talsma, and F.J. Garvin, IT Research Institute, Veterans Admin., and AMOCO Corp., Chicago, IL.

Trimellitic anhydride (TMA) is a chemical intermediate which causes immunologic lung injury. An animal model was developed in which rats were exposed to TMA during a 2-week inhalation study. Rats exhibited TMA-specific serum antibody (Ab) and lung lesions similar to humans. To assess the subchronic effects of low TMA concentrations and the long-term recovery potential, rats were exposed to 0, 2, 15 or 53 ug/m^3 for 13 weeks with recovery periods. Rats serially sacrificed showed typical TMA lung lesions through the first 6 weeks, after which the lesions began to diminish. Similarly, rats bled regularly for Ab showed high titers through the first 6 weeks, dropping sharply thereafter. An interim 6 5-week sacrifice showed a dose-dependent increase in Ab and lung lesions. At the end of the 13-week exposure Ab and lesions were reduced. After a 3-week recovery Ab rose sharply but rats challenged with TMA showed few lesions. After a 38-week recovery neither the challenged nor non-challenged rats exhibited lesions. Results indicated that immunologic tolerance was induced by long-term, low-dose exposure to TMA. This tolerance is consistent with human findings in that Ab is a necessary but not a sufficient prerequisite for inducing immunologic lung disease.

EFFECT OF PHOSGENE ON LUNG NATURAL KILLER CELL ACTIVITY. G.R. Burleson, L.B. Fuller, and L.L. Keyses. Northrop Services, Inc., Environmental Sciences, RTP, NC. Sponsor: J.A. Graham

Phosgene is a colorless, toxic gas used in many chemical and pharmaceutical manufacturing processes. This study was designed to investigate the effect of acute phosgene exposure on pulmonary natural killer (NK) cell activity in Fischer 344 male rats. NK cell activity was measured using effector cell from whole-lung homogenate to evaluate immunocompetence. The whole-lung homogenate was prepared as a single-cell suspension by finely mincing lung tissue followed by collagenase digestion. Effector:target cell ratios of 100:1, 50:1, and 25:1 were used in a standard 4 hr 51-Chromium release assay with YAC-1 target cells. Inhalation exposure to phosgene at ppp for 4 hr resulted in a suppression of whole-lung NK cell activity. The suppression of NK activity was evident 20 hr after exposure, but not when assayed immediately after or 44 hr after exposure. Exposure to 0.5 ppm phosgene suppressed lung NK cell activity, however 0.25 ppm phosgene had no effect. Exposure to 1.0 ppm phosgene had no effect on spleen or peripheral blood NK cell activity. Thus, the suppressed pulmonary NK cell activity after phosgene exposure was not manifested systemically. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

THE ROLE OF COMPLEMENT IN MEDIATING CLEARANCE OF INHALED PARTICLES. DB Warheit, Du Pont - Haskell Laboratory, Newark, DE.

Inhaled asbestos fibers activate in vivo a complement-derived chemotaxant for pulmonary macrophages. This mechanism is not unique to asbestos. Accordingly, I postulated that complement-mediated mechanisms facilitate lung particle clearance by stimulating macrophage recruitment and phagocytosis of inhaled particles.

Normal and cobra venom factor (CVF) treated rats as well as C5-normal (C5+) and C5-deficient (C5−) mice were exposed to aerosolized carbonyl iron particles for 1, 3, or 6 hrs at a concentration of 100 mg/m^3. Quantitative in situ measurements by scanning electron microscopy included particle deposition, macrophage migration and particle endocytosis. Time course studies showed that increased numbers of macrophages in normal animals migrated to deposition sites and phagocytozed carbonyl iron particles (p<0.01) compared to similarly exposed, complement-depleted rats and mice. These data correlated with in vitro chemotaxis and phagocytosis results using normal or CVF-treated and CI-activated sera to opsonize CI particles (p<0.05). Using an analytical chemistry method (ICP) on digested lung tissue, we showed that normal animals cleared CI at a faster rate than decomplemented (CVF) rats. Evidence supports the concept that complement activation facilitates lung particle clearance.

It was demonstrated that systemic complement activation following intravenous injection of cobra venom factor causes acute lung microvascular injury, which is associated with the release of oxygen radicals from activated neutrophils. Evaluations of the leukocyte responses and products of lipid peroxidation in both tissues and plasma were performed. The current study demonstrates that acute lung injury, measured by LDH isoenzyme release and lung vascular permeability levels in complement-activated rats, is paralleled by the appearance of products of lipid peroxidation (conjugated dienes, lipid hydroperoxides, fluorochrome substances) in both plasma and tissue of lungs but not liver, kidney or spleen. Companion experiments in which animals were pretreated with interventions found to protect against acute lung injury such as hydroxy radical scavengers (dimethyl sulfoxide and dimethyl thioether), or catalase, and the iron chelators (apomeltoferin and deferoxamine), also dramatically attenuated plasma and tissue levels of products of lipid peroxidation. These results suggest an important role of neutrophil-derived oxygen radicals in acute lung microvascular injury following systemic activation of the complement system.

Delta-9-tetrahydrocannabinol (Delta-9-THC), the major psychoactive component of marihuana induces immunosuppression in animals. We have shown that Delta-9-THC decreases host resistance to HSV2 vaginal infection in both guinea pigs and mice. In order to clarify the level at which the drug decreases resistance, its effect on lymphocyte blastogenesis was determined. Female B6C3F1 mice were inoculated intraperitoneally for 4 days with Delta-9-THC at doses ranging from 15mg/kg to 100mg/kg. On day 2 of drug treatment animals were inoculated intravaneously with HSV2 (10^8 PFU). Splenocytes were assayed for proliferative responsiveness using HSV2-infected (MOI = 5), methanol-fixed mouse embryo fibroblasts as targets. A drug-induced dose-dependent suppression of the blastogenic response was recorded for splenocytes obtained from animals treated with 50 mg/kg (P < 0.05) and 100 mg/kg (P < 0.01). Delta-9-THC, cannabidiol treatment did not affect the blastogenic response to HSV2. These results suggest that Delta-9-THC inhibits proliferative lymphocyte responsiveness in virus infection.


The effects of O_2 on susceptibility to influenza virus were assessed using 2 different exposure regimens and a variety of indicators of susceptibility. Mice treated with 1 ppm O_2 for 5 consecutive days (3 hrs/day) showed significantly enhanced mortality provided infection occurred on the 2nd day of exposure. This exposure regimen also caused notable differences in histopathology of the lung and increases in lung lavage fluid protein content and wet and dry lung weights of some of the days assayed post infection. No differences in pulmonary function or virus titers in the lung were noted. Because virus titers in the lung were not affected by this exposure regimen, the effects of O_2 on influenza infected mice did not appear to be due to immunosuppression but may have been due to additive effects of the virus and O_2. When the experiment was altered such that virus infection occurred on the last day of exposure there were no effects on mortality but there were significant increases in virus titers in the lung, suggesting that in this case effects may have been due to immunosuppression. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

INHIBITION OF THE BLASTOGENIC RESPONSE TO HERPES SIMPLEX VIRUS 2 (HSV2) BY DELTA-9-TETRAHYDROCANNABINOL. P.J. McMeneney, E.M. Mishkin, and G.A. Cabral. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA. Sponsor: A.E. Munson.

Recombinant human interleukin-2 (rIL-2) has pharmacologic activity in mice and rats, but little has been reported on its toxicologic profile in rodents. A fluid retentive syndrome has been reported in mice and humans and this provides the basis to use the rodent as a model to explore the observed toxicity in humans. We have studied the effects of various dosing regimen on the fluid retention syndrome that occurs in mice and rats treated with high doses of rIL-2 and which bears resemblance to the major toxic effects of IL-2 in humans. Daily intraperitoneal (IP) dosing of mice at doses of 40,000 units b.i.d. produced pleural effusion and/or ascites and mortality in some mice between 3 to 7 days of administration. Much higher doses and longer duration of administration were required to produce such effects by once a day administration. As IP dose of 4 million units, once a day for 14 days, resulted in mortality and fluid accumulation of similar degree to an IP dose of 100,000 units b.i.d. for 7 days. Prominent hematologic effects in mice included increased percent neutrophils and thrombocytopenia. Serum transaminases (ALT, AST) were markedly elevated. Icterus occurred at high doses. Intraperitoneal administration was more effective in producing fluid accumulation than intravenous administration of rIL-2. Doses per gram body weight that were toxic in mice produced ascites, icterus, and mortality in rats. Marked peripheral eosinophilia was not noted but perivascular eosinophilic cuffing was present in lungs. The pleural and ascitic fluids resembled plasma and were highly cellular. The cells were greater than 95% Thy 1 positive and were highly cytotytic for P815 and YAC tumor cells. These studies extend toxicity findings in rodents and demonstrate that toxicity is markedly affected by dosing regimes.


103


104

A NEW MODEL TO STUDY THE INFLAMMATORY POTENTIAL OF SURGICAL IMPLANTS. R. M. ROBERTSON, R. G. R. A. BEAULIEU, AND D. L. LANKIN, UNIVERSITY OF MARYLAND, BALTIMORE, MD

105


106

HIGHLY PURIFIED CD3-SELECTED HUMAN CM-2 (CD2) CYTOTOXIC T-LYMPHOCYTES FROM BLOOD DONORS. R. A. EVANS, J. R. HELD, AND D. KOCH. AMERICAN RED CROSS, LOS ANGELES, CA

107


108

HIGHLY PURIFIED CD3-SELECTED HUMAN CM-2 (CD2) CYTOTOXIC T-LYMPHOCYTES FROM BLOOD DONORS. R. A. EVANS, J. R. HELD, AND D. KOCH. AMERICAN RED CROSS, LOS ANGELES, CA

109

Unleaded gasoline and numerous other petroleum-derived hydrocarbons induce renal toxicity by causing accumulation of hyalin droplets in male rats. Studies were performed to quantify the conjoint effect of gasoline and estradiol (an inhibitor of hepatic α2u-globulin synthesis) in the kidney of male rats. Rats were treated with gasoline (2.0 mL/kg, p.o., 9 days) and estradiol (1 mg/kg, s.c., 9 days, starting on the 8th day of gasoline treatment). Animals were serially sacrificed at 3, 6, and 9 days after the last dose of gasoline. Gasoline alone caused a 5.2-fold increase in α2u-globulin with concurrent proliferation of hyalin droplets. However, within 3 days of terminating gasoline administration, renal α2u-globulin content returned to normal values. Estradiol decreased renal α2u-globulin content by 60%, 80% and 95% on post-exposure days 3, 6, and 9, respectively. By post-exposure days 6 and 9, estradiol decreased renal α2u-globulin content to 60% and 50%, respectively, of that in rats allowed to recover from gasoline without hormone treatment. These findings suggest that renal hyalin droplet accumulation is directly related to the availability of α2u-globulin.

THE REVERSIBLE BINDING OF 2,2,4-TRIMETHYLPENTANE (TMP) TO RENAL α2u-GLOBULIN (α2u) IN MALE FISCHER 344 RATS. E.A. Lock, M. Charbonneau, J. Strasser, and J.S. Bus. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

TMP produces nephrotoxicity in male but not in female rats which is characterized by an increase in protein droplets in proximal convoluted tubular cells and an increase in the male-specific protein α2u in the kidney. We have examined the relationship between radioactivity derived from [3H]-TMP and renal proteins in male and female rats. F-344 rats were dosed with [3H]-TMP (500 mg/kg, 230 μCi/kg) and killed 24 hr later. The kidneys were removed, homogenized, a 100,000×g fraction was prepared and applied to a Sephadex G75 column. Radioactivity from TMP in male cytosol resolved in two peaks, one of which contained about 25% of the radioactivity co-eluted with α2u (19000 d). Cross-reaction with a monoclonal antibody to α2u confirmed that these fractions contained α2u. The remainder of radioactivity eluted in the low molecular weight (MW) range (<1000 d). Radioactivity from TMP in female kidney cytosol resolved into only one peak in the low MW range. Dialysis of male cytosol led to a loss of the low MW components, but radioactivity (25%) still remained associated with the nondialyzable fraction. Dialysis against SDS led to loss of this binding. These studies provide the first evidence for a reversible binding between a probable metabolite of TMP and the male rat specific protein in the kidney. (Supported in part by IRSST Quebec)
LACK OF COVALENT BINDING OF 2,2,4-TRIMETHYLENETANE (TMP) TO G2u-GLOBULIN (g2u). D.J. Loury, T. Smith-Oliver, and R.E. Butterworth. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

TMP, a component of unleaded gasoline, produces nephrotoxicity in male but not female rats. It has been suggested that aldehyde metabolites of TMP form Schiff-base adducts with lysine groups of the male rat specific protein g2u, thus impairing its catabolism by renal lysosomes, and resulting in formation of protein droplets and kidney toxicity. We have examined the ability of C-TMP to bind to g2u in metabolically competent primary hepatocyte cultures (PHC) from male F344 rats or in the intact animal. SDS-PAGE analysis showed that g2u was synthesized in PHC from male but not female rats. PHC were incubated with 0.1 and 0.5 μg C-TMP for 15 hr. Samples were treated with cyanoacrylate (CB) to stabilize any putative Schiff-base adduct, and subjected to SDE-dialysis and SDS-PAGE. No binding of radioactivity to g2u or effect of CB was observed. Rats were treated with 300 mg/kg C-TMP 10, 16 and 22 hr before sacrifice. 16% of the dose was excreted in the urine. Analysis as above revealed no binding to g2u in liver, blood, kidney cortex or urine. As a positive control, C-formaldehyde was added to male rat liver. g2u Schiff-base adducts formed extensively and were stabilized by (CB). These studies indicate that covalent binding of TMP to g2u is not the mechanism by which TMP produces nephrotoxicity.


The unique susceptibility of the kidney to cephaloridine (CPH) toxicity has been attributed to the ability of proximal tubular cells to actively transport and accumulate CPH. However, the effects of high intracellular CPH concentrations on cell viability in non-target tissues are not known. These experiments were designed to evaluate CPH toxicity in target (kidney) and non-target (liver) cells exposed to equally high intracellular concentrations of CPH. Renal cortical slices or suspensions of isolated hepatocytes were prepared from naive male F-344 rats and incubated at 37°C in a bicarbonate buffer supplemented with 1.5 mM (kidney) or 1.6 mM (liver) CPH. Intracellular CPH concentrations in hepatocytes exposed to 60 mM CPH were 3.5-5 fold greater than renal slices incubated with 1.5 mM CPH. Malondialdehyde (MDA) production and enzyme leakage increased in a concentration and time dependent fashion in CPH treated renal slices. However, CPH did not affect viability of hepatocytes as reflected by trypan blue exclusion and enzyme leakage. Thus, high intracellular CPH concentrations in hepatocytes do not result in toxicity. Factors other than intracellular accumulation of CPH must contribute to the unique susceptibility of the kidney to CPH toxicity.


DCM is a byproduct of chlorination and contaminates many drinking water supplies. Little has been reported concerning its toxic effects, although maleic acid in a known nephrotoxin. Experiments were performed on male, Sprague-Dawley rats housed in metabolism cages. Twenty-four hr urine samples were monitored for volume, osmolality, glucose, protein, etc. Blood samples were assayed for renal and hepatic function parameters, and renal slice experiments were conducted. DCM was given i.p. Approximately 20% mortality was observed after 100 mg/kg administered daily for 3 days. Tetroethylammonium accumulation by renal cortical slices was altered 24 hr after DCM, while p-aminophenylurate uptake was unaffected. GPT & GOT showed modest increases in plasma. At 150 mg/kg DCM caused a moderate increase in urinary glucose concentration (2-3 x control). Daily administration (3 days) of DCM (100 mg/kg) had no effect on GSH and variable effects on liver GSH. DCM (3 days, 100 mg/kg) after mercuric chloride (0.5 mg/kg, 1 dose) resulted in additive effects on renal function and an enhanced mortality. Twenty-four hr after the last DCM dose, renal GSH was elevated. Urinary glucose excretion and blood urea nitrogen were not different from that caused by mercury alone. (Supported by AF Con. #F49620-86-C-0096).
Evidence for Role of Phospholipase C in Gentamicin-Induced Renal Phospholipidosis in Rat. S. Kacew, Dept. of Pharmacology, Univ. of Ottawa, Ottawa, Ontario.

A prominent feature associated with gentamicin (GEN)-induced nephrotoxicity is the development of phospholipidosis in renal cortex of rat. Previous in vitro studies demonstrated that GEN inhibited a phosphoinositol (PI)-specific phospholipase C prepared from rat renal cortex. The objective of this study was to determine the effects of GEN in vivo on kidney phospholipid metabolism. GEN (105 mg/kg/day) was administered (s.c.) daily for 4 days to male Sprague Dawley rats. GEN significantly increased the renal concentration of total phospholipid (TPL), PI, phosphatidylerine (PS), sphingomyelin (S) and phosphatidylycholine (PC). The levels of phosphatidyethanolamine (PE) and phosphatidylglycerol (PG) remained significantly different from control. As expected, GEN significantly decreased alkaline phosphatase, an index of brush border membrane function, and Na⁺-K⁺ ATPase, a marker of basolateral membrane activity. GEN produced a significant reduction in renal phospholipase C activity. In contrast, GEN did not markedly alter pulmonary phospholipid levels or the activity of phospholipase C. Our data show that GEN in vivo induces renal phospholipidosis via inhibition of phospholipase C and this observed effect is tissue specific. (Supported by the Medical Research Council of Canada).

Development of a Comprehensive Bioassay for Assessing Lung Toxicity to Inhaled Dusts. D.B. Warheit, Du Pont-Haskell Lab., Newark, DE.

Using a multidisciplinary approach, we have developed a comprehensive method for evaluating the lung toxicity of inhaled materials. To validate the method, rats were exposed to aerosols of either silica (Si) or carbonyl iron (CI) (0.025 mg/m³). Fluids and cells from sham and exposed animals were recovered by lavage (BAL) and measured for differentials and cytchemistry. Pulmonary macrophages (PM) were cultured and studied for morphology, chemotaxis, in vitro and in vivo phagocytosis by SEM. Additional animals were fixed either for lung digestion-clearance studies or for histopathology (LM), SEM and TEM, where particle deposition and translocation were assessed. Our results showed that deposition patterns were similar, but brief doses of Si elicited a permanent inflammatory response (PMN) concomitant with increases in LDH and proteins in BAL (p<0.01). PM functional capacities were depressed (p<0.05). Histopathologic changes were observed within 1 month after exposure. In contrast, 6 hr or 3 day exposure to CI produced no cellular, cytotoxic or permeability changes at any time postexposure. PM function was either enhanced or unchanged from controls. These data demonstrate that Si is substantially more toxic to the lungs than CI and validates this method as a reliable bioassay for evaluating the lung toxicity of inhaled materials.


Carbon Black (CB) was used as a probe of the pulmonary retention and clearance of submicron particles. F344/COBS CD/Crl rats were exposed 20 h/day, 7 days/week for 1, 3 or 6 weeks to either 7±2 mg/m³ CB or filtered air. The CB aerosols of 0.28 μm MMAD (±1.15) were generated with a Wright Dust Feed-Cyclone generating system. Lung and hilar lymph node CB burdens were determined following the exposure and during one year post-exposure. The lung burdens were 1.1±0.1 mg, 3.5±0.2 mg and 5.9±0.1 mg, respectively, after 1, 3, and 6 weeks of exposure. Clearance of the CB from the respiratory system was incomplete at the end of one year. Of the initial lung burden, 8%, 46% and 61% remained in the lungs of 1, 3 or 6 week exposed animals. After exposure, the hilar lymph nodes contained 0.2%, 0.9% and 2.0% of the lung burden. At one year post exposure, CB transport from the lungs accounted for a rise in lymph node burdens to 1%, 21% and 27% of the initial lung burden for the 1, 3 and 6 week exposures. The retention of CB in the lung and lymph nodes combined was 9%, 67% and 89% of the initial lung burden respectively for the 1, 3 and 6 week exposed animals. These results show that lung burdens consisting of milligram quantities of an "inert" dust severely decrease lung clearance and increase lymphatic transport of inhaled particles.

Retrosternal, Caudal Mediastinal Tissue as a Translocation Site for Particles Clearing From the Pleural Space Compartment (PSC). B.E. Lehnert, Los Alamos National Laboratory, Los Alamos, NM

Some particle types appear in the pleural space compartment (PSC) following lung deposition. We previously reported particles deposited in the PSC are cleared from this compartment with an overall t 1/2 ≈ 5-7 days. Further studies have revealed that the clearance process is biphagic with the earliest and most rapid phase having a t 1/2 ≈ 0.3 days and a slower, macrophage associated phase having a t 1/2 ≈ 6 days. Approximately 80% of the particles cleared from the PSC during the early phase of clearance. This observation has been complemented by the finding that ≈75% of the rapidly cleared particles become associated with the retrosternal, caudal mediastinal tissue. Such rapid particle translocation to this site is independent of carrier vehicle volume used to instilled particles over a range of 0.3-1.4 mL. This finding suggests free particles are not translocated to the mediastinal tissue site merely as a function of consecutive pleural fluid flow. However, when carrier vehicle only is instilled into the PSC at a time when pre-instilled particles are contained in pleural macrophages, the lavageable burden of particles in the PSC 24 hrs later decreases with increasing carrier vehicle volume loads.
PROPYLENE CHLORHYDRINS, a mixture of 1-chloro-2-propanol (1-CP) and 2-chloro-1-propanol, are used as chemical intermediates for the manufacture of propylene oxide and have been identified as potential air pollutants. The purpose of these studies was to determine the biological fate of 1-CP in rats after inhalation. F344/N male rats were exposed nose-only for 6 hr to 245 µg/L air (77 ppm) and 25 µg/Na sample (8 ppm) of 14C-1-CP. Steady-state levels of 14C (pmol/ml) in blood were reached during the first 2-4 hr of exposure. Elimination of 14C via urine and 14CO2, the two major pathways for excretion, was independent of exposure concentration. Two metabolites were detected in urine, one of which was tentatively identified as S-(2-hydroxypropyl)-cysteine. The liver, kidneys, large and small intestines, lungs, spleen, trachea, and nasal turbinates contained the highest initial concentrations of 14C within 1 hr after exposure. The data from these studies indicate that after inhalation, 1-CP is widely distributed and rapidly metabolized and eliminated.

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DEPOSITION AND METABOLISM OF PROPA NO L AND ACETONE VAPORS IN THE NASAL CAVITY. J.B. Morris and D.G. Cavanagh, School of Pharmacy Univ. of Connecticut, Storrs, CT.

To explore the effects of local metabolism on nasal deposition of inspired gases, the deposition of propanol (a metabolized vapor), and acetone (a non-metabolized vapor) was measured in vivo in the surgically isolated upper respiratory tract of anesthetized Syrian hamsters. Acetone deposition could be described by a ventilation-perfusion (V-P) model with a perfusion rate of 46 µl/min. At an inspiratory flow of 200 ml/min, propanol deposition efficiency was greater than that predicted by the model and was dependent upon the inspired concentration; local metabolism rates of 3.8 µg/min were estimated from this dependency. Nasal propanol metabolism rates in vivo averaged 2.2 µg/min. This value was not different from the 3.8 µg/min obtained in vitro (p>0.05). A concentration-dependency on propanol deposition was not observed at 38 or 71 ml/min, suggesting minimal metabolism at these flows. After correcting for metabolism, propanol deposition could be described by the V-P model with a perfusion rate of 50 µl/min. Thus, while metabolism enhanced propanol deposition, the effect was only observed at the highest flow rate, suggesting ventilation rate may influence the metabolic fate of inspired propanol in nasal tissues.

A new model has been developed to assess the physiologic and behavioral performance of exercising guinea pigs while subject to intoxicating atmospheres. The apparatus consists of a rubberized wheel driven at 0-1 m/hr by a variable speed motor. The guinea pig runs on the surface of the wheel while enclosed in a 3 liter ventilated exposure chamber. This chamber is a whole body plethysmograph by design, so that A0 and f along with the measurements of V02 and VCO2 can be made. "Performance" during toxic conditions and variable running speeds is not only evaluated by pulmonary function and metabolic integrity but also by a clear behavioral incapacitation endpoint, "collapse". Running guinea pigs were exposed to CO (4250-7100 ppm) for 30 min at V02 3x's above baseline. All animals were then placed back into the apparatus without the occurrence of death. These concentrations were at least 2x's lower than that which produces similar times to incapacitation in sedentary guinea pigs. This guinea pig model is sensitive to CO and potentially other gases or mixtures and could prove to be useful in evaluating the potential to escape during toxic inhalation exposures. NBS Grant GONAB44001.


Toxicology and carcinogenesis studies of chronic inhalation exposure to methyl methacrylate (MMA) were conducted. MMA, a liquid monomer, is used as a chemical intermediate in the manufacture of plexiglass and other acrylic products and as a "bone cement" in orthopedic and dental surgery. Groups of 50 male F344 rats were exposed to MMA at 0, 500, or 1,000 ppm and female F344 rats at 0, 150, or 500 ppm and groups of 50 male and 50 female B6CF1 mice at 0, 500, or 1,000 ppm, 6 hrs a day, 5 days a week for 102 weeks. The doses were selected based on rats of 30-day studies. Body weights and survival rates of the groups of male and female rats and mice exposed to MMA were similar to those of their respective controls except that survival rate of the high dose group of male mice was higher than that of controls. Inhalation exposure of methyl methacrylate for 102 weeks did not induce increased incidences of neoplasms in male and female rats and in female mice. However, incidences of inflammation and degeneration of the olfactory epithelium in the nasal cavity of male and female rats and inflammation, hyperplasia, cytoplasmic inclusions in the epithelial cells, and degeneration of the olfactory epithelium in the nasal cavity of male and female mice were significantly (p<0.05) increased.


Both hexamethylene disiocyanate trimer (HDIt) and methyl isocyanate (MIC) are known to produce pulmonary irritation in man and animals; however, the responses observed following similar exposures were very different. Two groups of male guinea pigs were exposed via inhalation to 121 mg/m3 HDIt and 10 ppm MIC over 3 hours. Using a previously described method to obtain flow-volume (F-Vt) loops, measurements were made during air or 10% CO2 challenge prior to and at intervals following exposure. A maximum response to HDIt occurred 24 hours post-exposure which included an increase in respiratory frequency (f) and a decrease in tidal volume (VT) during both air and CO2 breathing. F-Vt loops were rectangular in shape and showed evidence of airflow limitation near the end of expiration. Guinea pigs exposed to HDIt recovered to normal within 5-6 days. Exposure to MIC produced severe airflow limitation during air breathing and CO2 challenge. Decreases in both f and VT were recorded and these changes persisted beyond 5 days post-exposure. According to criteria published by Schaper et al. (TAP, 1985), the response observed following exposure to HDIt is characteristic of lung restriction, while the effects of MIC inhalation can be classified as obstruction. Supported by NIHS Grant ROI ES02747.

The purpose of this study was to develop a model to describe the uptake of VDF, an important plastics monomer. Male Fischer 344/N rats were exposed nose only to concentrations of VDF ranging from 30 to 5,000 ppm. Tidal volume (mean, 1.1 ml/breath) and respiratory frequency (mean, 148 breaths/min) were not influenced by exposure concentration. Blood levels of VDF, obtained by GC analysis of samples from rats with indwelling jugular cannulas, increased with increasing exposure concentration, from 15 ng VDF/ml blood at 30 ppm to 700 ng VDF/ml blood at 5000 ppm. VDF tissue/air partition coefficients, were determined experimentally to be 0.07, 0.27, 0.8, 1.0, and 0.29 for water, blood, liver, fat, and muscle, respectively. These values were incorporated into the physiological model. Model predictions agreed with the experimentally determined data in that time to reach steady-state blood levels of VDF was less than 15 min. After cessation of exposure, blood levels of VDF decreased to below 4 ng/ml by 2 hr. Results suggest that uptake of VDF is governed by physical properties such as solubility in biological fluids. (Research supported by NIEHS through IAA 222-T01-ES-20092 under U.S. DOE Contract No. DE-AC04-76EV01013.)


Concern over potential hazards associated with sudden chemical releases into the environment from existing and proposed chemical facilities has prompted evaluation of the possible adverse effects resulting from a brief inhalation exposure. When acute toxicity data are not available for short exposure times, estimates are often made from extrapolated TLVs. The literature was evaluated to obtain TLV and acute toxicity information on ten acutely toxic compounds. Estimated safe concentrations, extrapolated linearly from TLVs, were compared to reported LC₅₀s. The extrapolated values for hydrogen cyanide, nitrogen dioxide, carbon monoxide, furan, hydrogen disulfide and acrolein provided little margin of safety since they ranged by factors of 0.6 to 9 compared to the LC₅₀s. Extrapolation of a 4- or 1-hour LC₅₀ to 30, 10, 5 or 1 minute produced values which exceeded the reported LC₅₀ for the shorter exposure time by up to 5-fold. These results indicate that the practice of using values which are linearly extrapolated from TLVs (or even 4-hour LC₅₀s) to shorter exposure times can underestimate the risk.


A small, and easy to manufacture, low flow aerosol generator was designed and characterized. An air flow as low as 1.5 L/min can be used to aerosolize liquids. Two liters per minute were used to generate stable aerosol concentrations with polyethylene glycol. Liquid flow into the generator was maintained with a syringe pump. Flow rates of 0.06 mL/hr to 1.6 mL/hr produced aerosol concentrations of 270 to 1400 mg/m³ in a 200 mL exposure chamber. The aerodynamic mass median diameter, as determined with an impactor, was about 2.4 μm with a geometric standard deviation of about 3.7. Aqueous solutions of sodium chloride were easily aerosolized and a stable output (less than 10% variation) was obtained as long as the feed rate from the syringe pump was constant. This generator should find application where expensive or highly toxic solutions must be generated.

NEW INVESTIGATOR PRESENTATION


Platinum antitumor drugs, the prototype of which is cis-DDP, exert their biological activity by binding to DNA. In order to study replication blockage and mutagenesis by this compound, a duplex bacteriophage M13 genome has been constructed containing the major cis-DDP adduct, a single cis-[Pt(NH₃)₂Cl(Cp)]⁺ intrastand crosslink (G-G). Initially, a 12-base pair insertion mutant of M13mp18 was constructed in which a duplex deoxyoxygenucleotide, d(TCTAGGCTCTG)-d(GAGGGGGTTAGA) was inserted into the unique Hind II restriction site: the underlined bases constitute a new Stu I site. An M13 genome containing a 12-base gap in the minus strand was created by hybridizing the circular, plus-strand genome of the insertion mutant against Hind II-cleaved M13mp18 duplex DNA. The platinated dodecamer, d(TCTACGCGCTCTG) was ligated into the gap with high efficiency, to create the site-specifically adducted genome, M13-12A. Plasmid DNA from 30 μg of the (G-G) crosslink conferred Stu I resistance to the genome, while restriction at other nearby sites was unaffected. Removal of the platinum by treatment with cyanide restores the Stu I sensitivity.

32
INDUCERS OF CYTOCHROME P450D.


Inducers of rat liver microsomal cytochrome P450D are noncovalently but tightly bound by the cytochrome. We investigated the ability of two inducers of cytochrome P450D, Aroclor 1254 and isosafrole (ISF), to affect the microsomal activation of aminoanthracene (AA) to mutagen(s) for S. typhimurium strain TA98. Cytochrome P450D from rat liver microsomes from ISF treated animals has a bound inhibitory metabolite of ISF which was displaced by incubating microsomes in 250 mM butanol at 37°C for 30 min. followed by Sepharose CL-6B chromatography. Butanol treatment increased ISF induced microsomal mutagenesis by 41%, whereas butanol had no effect on non-induced microsomal activation of AA. Butanol treatment of Aroclor 1254 induced microsomes failed to remove any of the Aroclor 1254, or affect AF mutagenesis. However, AF mutagenesis was inhibited 99% by addition of Aroclor 1254 (75 nmol/plate) to butanol treated ISF induced microsomes. Therefore it is likely that inducers of P450D can inhibit its ability to metabolize AF to mutagen(s). These results indicate that the results of the Ames assay using either purified P450s or S-9 from induced animals can be negatively affected by the inducers of cytochrome P450D. Supported by NIH grant ES03505.

SULFITE-DEPENDENT MUTAGENICITY OF 7,8-DIHYDROXY-7,8-DIHYDROBENZ(a)PYRENE. G. A. Reed and A. D. Bye. University of Kansas Medical Center, Kansas City, KS

Benzo(a)pyrene (BP) and sulfur dioxide (SO2) are ubiquitous air pollutants and are both found in tobacco smoke as well. Although SO2 itself is not carcinogenic, concurrent administration with BP enhances the respiratory tract carcinogenicity of BP. SO2 exists in biological systems as its hydrated form, sulfite (SO32-). We have previously detailed the conversion of the proximate carcinogenic BP metabolite 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene (BP-7,8-diol) to the ultimate carcinogen BP diol epoxides during SO2 autoxidation. We report here that SO32- converts BP-7,8-diol to forms highly mutagenic to S. typhimurium strain TA98, supporting the formation of diol epoxides by their interaction. The SO32- dependent mutagenicity occurs at SO2 concentrations from 0.5 to 10 mM, and is blocked by inhibitors of SO2 autoxidation. SO2 is neither toxic nor mutagenic in these assays. This SO32- dependent conversion of the proximate mutagen and carcinogen BP-7,8-diol to its active forms provides a plausible mechanism for the observed enhancement of BP carcinogenicity. Supported by a grant from the Speas Foundation.

ENHANCED IN VITRO MUTAGENICITY OF DIMETHYLNITROSAMINE FOLLOWING ETHANOL PRETREATMENT IN RATS. E. Monosson and J. G. Babish. Department of Pharmacology, New State College of Veterinary Medicine, Cornell University, Ithaca, NY

The interaction between alcohol consumption and cytochrome P-450 levels may be important in the metabolic activation of various chemical carcinogens present in foods such as polymeric aromatics or nitrosamines. These studies were designed to determine the influence of ethanol pretreatment to rats on the capacity of hepatic S9 to metabolically activate various promutagens in the Salmonella typhimurium assay.

Rats were treated with four doses of a 50% aqueous ethanol solution (5 ml/kg body weight) at 48, 42, 24 and 18 hours before killing. control rats were treated with saline following the same regimen. Hepatic S9 from control and treated rats was used at concentrations of 10, 50 and 150 µl per plate with tester strains His G46, and TA 100, for DMN and benzo(a)pyrene (BP) and 2-aminoanthracene (2AA), respectively. All promutagens were tested at 7 dose levels which had previous demonstrated a linear dose response with the test organism.

Ethanol pretreatment did not affect the dose response curves for BP or 2AA. However, the S9 from the 48-hour ethanol treatment was a more efficient activating system for DMN than S9 from the saline treated rats.

GENOTOXICITY OF PYRROLIZIDINE AND LARKSPUR ALKALOIDS DETECTED BY ALKALINE ELUTION. J. R. Hincks, R. A. Coulombe, Jr., F. R. Steritz and R. Holymyx. Center for Environmental Toxicology, Utah State University, Logan, UT, Dept. of Chemistry, Colorado State University, Fort Collins, CO, and Western Region Research Laboratory, USDA, Berkeley, CA.

Pyrrolizidine alkaloids (PA) have previously been studied for possible mutagenic and carcinogenic activities. Several PA's have been shown to be positive in various unscheduled DNA synthesis assays. In this study, we investigated the interaction of these compounds with DNA in cultured bovine kidney epithelial cells (MDBK) using gravity-flow alkaline elution. Six PA's and nine larkspur alkaloids isolated from Delphinium sp. were tested. Compounds were incubated for 4 hrs at doses ranging from 1x10^{-4} M to 1x10^{-7} M. Six PA's (sanchonelline, senecliphylline, riddelline, monocrotaline, sarracine, and halosupine) induced low levels of DNA single-strand breaks. Detection of DNA cross-links by a modification of this procedure indicated that many of these compounds interact with DNA by producing both single-strand breaks and DNA cross-links. (Supported in part by USPHS Grant ES03591).
INDUCTION OF HYDROGEN PEROXIDE-MEDIATED CHROMOSOME DAMAGE IN VITRO BY PHENOLIC ANTIOXIDANTS. B.J. Phillips, D. Anderson and S.D. Gangolli. BIBRA, Carshalton, UK.

Butylated hydroxyanisole (BHA) stimulates \( \text{H}_2\text{O}_2 \) production by Aroclor induced rat liver microsomes in the presence of a NADPH-generating system. This may be due either to interference with microsomal oxidase function or to the formation of metabolites such as 2,4-di-tert-butylhydroquinone (DBHQ) which readily autoxidises to form superoxide and \( \text{H}_2\text{O}_2 \).

Using cultured Chinese hamster ovary (CHO) cells, which have previously been shown to be sensitive to the genotoxicity of enzymatically-generated \( \text{H}_2\text{O}_2 \), BHA caused chromosome damage only in the presence of a metabolic activation system. The effect was observed with a standard S9-mix preparation but was more marked with washed microsomes, free of catalase. Exogenous catalase substantially reduced microsomal-mediated damage. DBHQ, in the absence of microsomes, produced a marked catalase-sensitive clastogenic effect.

It is concluded that redox-active metabolites of BHA can cause indirect genetic damage in vitro, but that enzymes such as catalase which are likely to be present in high concentration in vivo offer a high degree of protection from this effect.

(Supported by the UK Ministry of Agriculture, Fisheries and Food.)

DIFFERENTIAL ACTIVATION OF BENZIDINE CONGENERS BY RAT HEPATIC ENZYMES TO MUTAGENIC PRODUCTS IN THE SALMONELLA TEST. Michael H. Iba, Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.

The activation of 3,3'-dichlorobenzidine (DCB), o-tolidine (TLN), o-dianisidine (DIN) and benzidine (BZ) to mutagens was compared in the Ames test, using Salmonella typhimurium TA98 and three liver enzyme systems: (i) S9, (ii) S9+acetol coA (S9-Ac), and (iii) microsomes (M). Each from control, DCB-, 3-methylcholanthrene- (MC-) and phenobarbital-(PB-) pretreated rats. Only DCB was activated in all three systems in the order: S9 > S9-Ac > P. BZ was activated only in S9 and S9-Ac but the latter was more effective than the former. TLN and DIN were not substantially activated by any of the three systems. Activation of DCB but not its congeners was enhanced in systems from rats pretreated with DCB, MC or PB. When estimated on the basis of cytochrome P-450 content, DCB activation was enhanced only by DCB-pretreatment. DCB activation was not inhibited by carbon monoxide; however, it was inhibited totally by either dichlorophenylphenoxyethylamine (DPEA) or antibody to NADPH cytochrome P-450 reductase but partially by a-naphthoflavone. The data suggest that DCB but not its congeners may be activated extensively by hepatic cytochrome P-450 and that the most effective cytochrome P-450 species may be the ones induced by DCB itself.
The genetic toxicity of DCE is thought to result from direct conjugation of the parent compound with GSH. We have tested the hypothesis that fed rats will suffer more hepatic DNA damage than fasted rats since fasting depletes hepatic GSH. DNA damage in male Fisher-344 rats exposed by inhalation to DCE for 4 hr was measured with the alkaline elution technique. Fed rats exposed to 50, 100, and 500 ppm DCE suffered significant, dose-responsive DNA damage and fasted, DCE-exposed rats had significantly more DNA damage than the fed rats. Possible explanations include: 1. The DCE-GSH conjugate does not damage DNA, 2. The amount of DCE-GSH adduct is not greater in fed rats than in fasted rats, and 3. Fasting-related changes in hepatic nuclei may alter the elution behavior of DNA and thereby confound detection of fed/fasted differences in the actual amount of DNA damage. (Supported by USEPA CR812556. Does not necessarily reflect EPA policy.)

Chlordecone (CD) and CCl_4 combination has been shown to cause greatly potentiated hepatotoxicity in rats. Since inhibited hepatocyte regeneration was implicated as the underlying mechanism, possible involvement of genotoxic events was investigated. Rats (250-300 g) were given an oral dose of 10 mg CD/kg followed by a single i.p. injection of 100 μl CCl_4/kg on the third day. Hydroxyurea (500mg/kg) was given immediately after the CCl_4 treatment and 1 hr later hepatocytes were prepared from the livers. We monitored the unscheduled DNA synthesis (UDS) by the 'nuclei procedure' and by an in vitro DNA assay. DNA damage was assessed both by the DNA unwinding technique and by ADP-ribosyltransferase (ADPRT) activity in digitonin-permeabilized hepatocytes. No UDS was detectable in the hepatocytes of CD + CCl_4 treated rats in contrast to CD and CCl_4 (p < 0.05) treatment groups. DNA strand breaks occurred only in CD pretreated rats. ADPRT activity was inhibited significantly in animals treated with CD + CCl_4 or with CD alone but was stimulated in CCl_4 treatment group. Genotoxic mechanisms may be involved in the CD + CCl_4-induced suppression and hepatocellular regeneration and repair in these livers. (Supported by USEPA R-811072 and Fogarty Int. Ctr. TW0336.)

OVINE URINARY METABOLITES OF ETHOXYQUIN: H.L. Kim, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX. Sponsor: S. Safe.

Ethoxyquin (EQ) is an antioxidant which induces hepatic glutathione, glutathione S-transferases, and numerous drug-metabolizing enzymes in rodents. Pretreatment of mice and sheep with EQ reduces the toxicity of pyrrolizidine alkaloids and butterweed, respectively. The ovine urinary metabolites of EQ were examined and compared with that of the rat. Sheep were given feed containing EQ (140 mg/kg/day) for 12 days and the 24 hr urine samples were collected on days 2, 3, 13, and 16. Rats were given EQ (80 mg/kg/day) orally for 7 days and the urine samples were collected daily. EQ and its metabolites were extracted from urine samples and analyzed by GC-MS. EQ and hydroxy-EQ were readily detected in urine samples of both rat and sheep, but dihydroxy-EQ and other metabolites were found in rat urine only. Neither EQ nor its metabolite were detected in sheep urine collected on day 16, four days after the EQ feeding ended. EQ was also analyzed by reversed phase HPLC and the detection limit was about 1 ng. This rapid HPLC method was applied to quantitate the EQ content in the feed samples. (Supported by the Texas Agricultural Experiment Station.)
2,2,2-Trifluoroethanol (TFE), the toxic metabolite of the anesthetic agent fluroxene, is further metabolized to trifluoroacetic acid, which accumulated to maximum serum concentrations 16 to 24 hr after TFE administration to rats. To determine the metabolic pathways of TFE male Wistar rats were pretreated with various metabolic inhibitors and inducers of P-450 metabolism, and TFE toxicity and metabolism were assessed. Pyrazole, disulfiram, isonicotinamide, diethylthiocarbamate, and 2-allyl-1-isopropylacetamide pretreatment significantly decreased the in vivo metabolism of TFE by 53-100% and the toxicity (as assessed by mortality and leukocyte count alterations). Ethanol-inducible rat increased metabolism of TFE by 65% 24 hr after TFE administration but not toxicity. We conclude that hepatic ethanol-inducible P-450 catalyzes the metabolism of most of the TFE but this metabolism is not associated with TFE toxicity, which probably arises by extrahepatic metabolism of TFE by another P-450 form. An "activated" intermediate is not TFE and is responsible for the toxicity associated with TFE since studies with 2,2,2-trifluoroacetaldehyde excluded it or its metabolite from being the toxic entities.

(Funded by NIH Grant GM 23029)

MODIFICATION OF XENOBIOTIC METABOLISM BY DIETARY INDOLES: A NOVEL MECHANISM. C.A. Bradley and L.F. Bjeldanes. Univ. of California, Berkeley.

In an effort to understand the mechanism by which dietary indoles modify carcinogen metabolism, we have investigated the potency of 3-, and 1,3-substituted indoles on the induction of hepatic monooxygenases in the rat. Oral intubation of indole-3-carbinol (IC3), 3,3'-dinitrophenol (3,3-DNP), 1-methoxyindole-3-carbinol (MCIC), or 1-methoxyindole-3-carboxaldehyde (OMBA) at 31 μmol/animal led to increases in hepatic ethoxyresorufin O-deethylase activity (EROD, 15, 5, 6, and 7-fold over control), while intubation of indole (IND), 3-methylindole (3MI), indole-3-carboxaldehyde (IC3CHO), or indole-3-acetonitrile (IAN) was without effect. For the eight indoles tested, their was a strong relationship between instability in acidic solution, and capacity to induce hepatic EROD. Further experiments indicated that IC3 did not induce hepatic EROD when dosed i.p. (thus bypassing the acidity of the stomach). Acid treatment of IC3 generated a reaction mix (RXM) that induced EROD after i.p. or p.o. dosing. Chromatography on silica indicated that at least 4 different condensation products, with the ability to induce EROD, are present in RXM. These results strongly support the hypothesis that dietary indoles influence monooxygenase levels via a series of condensation products generated upon introduction of "acid sensitive" indoles into the low pH environment of the stomach.

Intermediate Metabolism of Harmol (HA): DOSE-DEPENDENT GLUCURONIDATION AND SULFATION. D. Goon and C.D. Klassen. Univ. of Kansas Medical Center, Kansas City, KS.

Previous studies have shown that HA metabolism is primarily extrahepatic and that the major metabolite formed is harmol-sulfate (HA-SO₄). The aim of this study was to characterize the intestinal metabolism of HA in the rat with an in situ isolated jejunal loop preparation. HA (2-200 μmoles) was injected directly into the isolated loop and blood samples were collected for 1 hr. HA and its metabolites were separated by thin-layer chromatography and quantitated fluorometrically. At the 2 μmole dose, HA was extensively glucuronidated while only trace amounts of HA and HA-SO₄ were detected. Glucuronidation was saturated at 2 μmoles as higher doses of HA failed to further increase plasma concentrations of harmol-glucuronide (29 nmoles/ml). Comparison of jejunal UDP-glucose levels following 20 μmoles HA versus saline-exposed controls revealed no difference while UDP-glucuronic acid (UDP-GA) was decreased 85% (28 and 188 μmoles/g tissue, respectively). In contrast to glucuronidation, increasing doses of HA resulted in increased sulfation of HA. At the highest dose of HA (200 μmoles), similar amounts of HA were glucuronidated and sulfated (21 and 24 nmoles/ml, respectively). These data indicate dose-dependent intestinal metabolism of HA with glucuronidation predominating at low doses and sulfation increasing with dose. Intestinal glucuronidation of HA appears to be limited by depletion of the co-substrate, UDP-GA. (Supported by USPHS Grants ES-03192 and ES-07079)

EFFECT OF 17α-ETHYNYLESTRADIOL ON THE INDUCTION OF P450 BY 3-METHYLCOLANTHRENE IN CULTURED HEPATOCYTES. S.A. Sundstrom, J.F. Sinclair, P.R. Sinclair, & L.E. Smith. Dept. of Pharmacology & Toxicology and Biochemistry, Dartmouth Medical School, Hanover, NH, and Veterans Administration Medical Center, White River Jct., VT Sponsor: R. Borison

17α-Ethynylestradiol enhances the 3-methylcholanthrene mediated induction of cytochrome P450 by cultured hepatocytes. 17α-Ethynylestradiol alone did not increase P450 nor did it potentiate the induction of P450 by phenobarbital-like inducers. Microsomes from cells treated with either 3-methylcholanthrene or the combination of 3-methylcholanthrene and 17α-ethynylestradiol had identical catalytic activities per P450 content for 7-ethoxyresorufin-O-deethylase or for aryl hydrocarbon hydroxylation. Immunoactivity studies with antisera to the 60K P450 induced by 3-methylcholanthrene indicated that the 17α-ethynylestradiol-mediated increase in enzyme activity is associated with increased accumulation of this protein. In addition the antisera completely inhibited 7-ethoxyresorufin-O-deethylase activity induced by the combination of 3-methylcholanthrene and 17α-ethynylestradiol. We suggest that the additional P450 induced by the combination of 17α-ethynylestradiol and 3-methylcholanthrene was the same isozyme induced by 3-methylcholanthrene alone.
INHIBITION OF RAT LIVER MICROSOMAL CYTOCHROME P-450 IN VIVO. M.P. Arlott, A.J. Sonderfan and A. Parkison, Kansas University Medical Center, Kansas City, Kansas.

Troleandomycin (TAO) has been shown to bind selectively to cytochrome P-450, forming a stable complex. The present study indicates that a single ip injection of TAO can completely and selectively inhibit the catalytic activity of cytochrome P-450 in vivo. Zoxazolamine paralysis time and heparinobital sleeping time were used to measure the induction of cytochrome P-450 by phenobarbital (PB), 3-methylcholanthrene (MC) and pregnenolone-16α-carbonitrile (PCN) in Long Evans rats. A single injection of TAO completely reversed the inductive effect of PCN, a potent inducer of cytochrome P-450. The inductive effect of PB, a weak inducer of cytochrome P-450, was partially reversed by TAO. TAO had no effect on the inductive effect of MC, which does not induce cytochrome P-450. In untreated mature rats, zoxazolamine paralysis and heparinobarbital sleeping time were shorter in male rats than in female rats, and were prolonged in male but not female rats by TAO. This result is consistent with the higher constitutive levels of cytochrome P-450 in mature male rats. Metabolism of zoxazolamine and heparinobital measured in vivo showed an excellent inverse correlation with in vivo zoxazolamine paralysis and heparinobarbital sleeping time. A single dose of TAO reversed the ability of PCN to protect rats against digoxin toxicity, suggesting that induction of cytochrome P-450 is an important factor in the toxic effect of PCN. (Supported by NIH grants ES-03765, ES-00166 and ES-07079.)

EFFECTS OF ANESTHETICS ON THE BILARY EXCRETION OF SULFHYDRLS (SHs) AND Cd. C.A. White and C.D. Klaassen, Univ. of Kansas Medical Center, Kansas City, KS.

Biliary excretion of some metals, such as Cd, appears to be related to the excretion of SHs. Anesthetics influence the pharmacokinetics of many compounds and previous data suggest they might alter SH excretion. The purpose of this study was to examine the effects of anesthetics on the biliary excretion of SHs and then determine their subsequent effects on the biliary elimination of Cd in rats. SHs were quantitated by HPLC/EC. $^{106}$CdCl$_2$ (10 μmol/kg) was administered iv and measured radioiodometrically. Ketamine (100 mg/kg)-xylazine (10 mg/kg) mixture (KX), when compared to either, pentobarbital, or urethane, significantly increased bile flow (2-fold), biliary SH concentration (2.2-fold), and SH excretion rate (3.8-fold), while hepatic concentration of SHs was unchanged. KX increased the cumulative amount of Cd excreted over a 2-hr period by 46%, whereas the biliary concentration of Cd was significantly higher (30%) in the urethane-anesthetized rats. The biliary excretion rate of Cd was 50% higher during the first hr after KX, but the differences between the KX- and urethane-anesthetized rats diminished to only a 17% difference at 2 hr. This decrease in Cd excretion may result from a decrease in the concentration of unbound Cd in liver. In conclusion, KX increases the biliary excretion of SHs and Cd. However, the increase in the biliary excretion of SHs and Cd is not directly proportional. (Supported by USPHS Grants ES-01142, ES-03192, and ES-07079)

COMPARATIVE EFFECTS OF BUTYLATED HYDROXYANISOLE (BHA) ON IN VIVO AND IN VITRO METABOLISM OF AFLATOXIN B$_1$ (AFB) IN RAT AND MOUSE LIVER. D.H. Monroe and D.L. Eaton, Department of Environmental Health, University of Washington, Seattle, WA.

Mice are resistant to the carcinogenic effects of AFB, and BHA pretreatment has been shown to increase the resistance of rats to the carcinogenicity of AFB. To further elucidate the biochemical mechanisms underlying the species and chemical-induced resistance to AFB, in vivo and in vitro studies were performed to assess the relationship between activation/inactivation bioconversion pathways and covalent binding of AFB to hepatic DNA in both mice and rats given BHA. Male SD rats or female CD-I mice were fed 0.75% BHA for 10 days, then given $^3$H-ABF. Bacterial AFB-DNA adduct formation in vivo in mice was only 1.4% of that measured in rats, and BHA treatment had no effect on AFB-DNA binding. However, BHA treatment in rats decreased AFB-DNA adduct formation 5.4-fold. The rate of activation of AFB to the epoxide was 3.4-fold greater in control mice relative to control rats. BHA pretreatment increased the activation of AFB in mice 3.3-fold, but had no effect on oxidative metabolism in rats. Control mice had 52 times more OSH-S-transferase (GST) activity towards the AFB epoxide than control rats, but epoxide hydrolase (EH) activity in mice was 52% of the activity in rats. BHA increased GST activity only marginally in mice but 3.2-fold in rats. BHA increased EH activity in both species. In vitro the presence of cysteic GST at endogenous concentrations totally prevented the formation of AFB-TRIS-diol, suggesting that EH activity is not an important detoxification pathway for AFB in vivo. These results further demonstrate that elevation of GST activity, and thus inactivation of AFB-epoxide, is the critical component in species and BHA-induced resistance to AFB carcinogenicity, and that the rate of formation of the epoxide is a relatively unimportant determinant under conditions of high GST activity. (Supported by NIH grants ES-05415 and T32 ES-07032).

EFFECT OF VOLATILE ANESTHETICS ON THE BILARY CLEARANCE OF CHOLEPHILIC CHEMICALS. J.B. Watkins III, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN.

Exposure to volatile anesthetics depresses hepatic UDP-glucuronic acid levels, which can effect the excretion of glucuronidated chemicals into bile. The present study has examined the effect of halothane (HAL), isoflurane (ISO), enflurane (ENF) and sevoflurane (SEV) on the plasma disappearance and biliary excretion of acetaminophen (APAP–330 μmol/kg iv) and digoxin (0.10 μmol/kg iv) in Sprague-Dawley rats. Control rats were anesthetized with urethane. All volatile anesthetics reduced UDP-glucuronic acid levels by 50 to 75%. Total biliary excretion of APAP was depressed by all anesthetics, but there were no differences in pharmacokinetic parameters. Excretion of APAP–glucuronide and APAP–sulfate was depressed, although that of APAP–glutathione was increased. Total clearance and steady-state volume of distribution of digoxin were decreased by only HAL, and there were no changes in biliary excretion. Urinary clearance of digoxin was increased by all volatile anesthetics, whereas biliary clearance was decreased by HAL and ENF. These data indicate that biliary clearance of these chemicals and their metabolites can be affected by exposure to these volatile anesthetics. (Supported by AMA Education and Research Foundation and Pharmaceutical Manufacturers Association Foundation).
149 COMPARATIVE TOXICOLOGY STUDIES OF THE MONOMER AND POLYMER OF 1,2-DIHYDRO-2,2,4-TRIMETHYLMETHYLMERCURY (SCHERMANN, T. 1974) IN F344 RATS. M. E. French, W. Eastin, and A. G. Mans, NTP/NIAMS/RTP, NC and *Southern Research Institute, Birmingham, AL. Sponsor: R. S. H. Yang

1,2-Dihydro-2,2,4-trimethylquinoline (DHTMQ) (CAS No. 147-47-7) 14-day studies were conducted by administering monomer or polymer in acetone to rats (5, 20, 200, 1000, or 2000 mg/kg) or mice (0, 15, 150, 1500, 7500 mg/kg) via skin-paint application for two weeks (1x/da, 5 da/wk, 2 consecutive days before sacrifice). Studies with 14C-DHTMQ indicated that accumulation of radiolabel occurred in urine, feces, skin (dose site), skin (ear), liver, fat, and kidney. Of the 14C-DHTMQ monomer dose applied by skin paint, 80% was absorbed and 20% was volatilized off the skin surface. DHTMQ monomer treatment caused mortality where the polymer treatment did not. Significant body weight depression was dose related and occurred in both monomer and polymer treatments to rats of both sexes but not mice. DHTMQ monomer, but not polymer treatment resulted in dose-related clinical sign differences in rats and mice. Dose-related changes were observed in gross and clinical pathology, organ/body weight ratios, and pathology that paralleled histopathological changes and earlier chemical disposition data in both rats and mice treated with either DHTMQ monomer or polymer. Target organs and no-effect levels were determined.

150 New Investigator Presentation

ALTERED GANGLIOSIDE EXPRESSION WITHIN NEONATAL MOUSE CEREBELLAR CELLS FOLLOWING IN VIVO EXPOSURE TO METHYLMERCURY. A. Jacobs, W. M. Maniscalco, and J. N. Finkelstein. Toxicology Training Program and Division of Neonatology, University of Rochester School of Medicine, Rochester, NY.

An enhancement in the adhesiveness of dissociated neonatal mouse cerebellar cells, as indexed by an acceleration in reaggregation rate following in vivo methylmercury (MeHg) treatment, has recently been demonstrated in our laboratory. To determine whether altered ganglioside expression could account for changes in cell recognition/adhesion associated with MeHg exposure, gangliosides were extracted from 3 days postnatal mouse cerebellum following treatment 24 hours prior to cell isolation with 0 or 4 mg/kg MeHg. By using both approaches, we have followed the distribution of MeHg and the inorganic Hg resulting from its demethylation. Twenty-four hours after a single exposure to 8 mg Hg/kg as MeHg, autoradiographs show that Hg is concentrated in the cortex of the kidney, with significantly more label associated with tubules than with glomeruli. Little demethylation has occurred at this stage, so no inorganic Hg is detected. After one week, the distribution of MeHg has not changed, but inorganic Hg appears in a highly localized pattern restricted to the apical regions of cells of proximal tubules. At two weeks, both forms are distributed as at one week, with increasing deposits of the inorganic form in the proximal tubules. The pattern of distribution supports the hypothesis of other investigators (e.g. Klein et al., 1973) that kidney damage after MeHg exposure may result from exposure of the kidney to inorganic Hg, rather than from exposure to MeHg per se.

151 AGE DEPENDENT DIFFERENCE IN CHEMICAL FORM OF BILIRARY METHYLMERCURY IN RATS. T. Ueno, A. Naganuma and N. Imura. Dept. of Public Health, Sch. of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo, Japan.

The major chemical form of methylmercury (MeHg) in rat bile is believed to be MeHg-glutathione (MeHg-GSH), but some inconsistent results have also been reported. We have observed age dependent changes in chemical form of biliary MeHg of rat. Sephadex G-15 chromatography of fresh bile, obtained from 4-week-old male Wistar rats (A rat) 2 hr after injection of MeHgCl (5 μmol/kg, i.v.), revealed that the most (94%) of MeHg was associated with cysteinyl-glycine (CysGly) and only 5% of MeHg was associated with MeHgGSH in the bile from 16-week-old rats (B rat), however, 40% of MeHg was observed as MeHg-GSH and 60% was MeHg-CysGly. These results may reflect the alteration in the content of non-protein thiols (NPT) or GSH in rat bile by aging. Actually the amount of CysGly in the fresh bile samples from A and B rats accounted for 70% and 30% of the total NPT, respectively. It was confirmed that the GSH in rat bile increased from 23% to 68% with increase in age (from 4 weeks to 16 weeks), but total NPT did not change. Since Cys was hardly observed, these bile samples did not contain pancreatic juice which can also form CysGly from GSH. Ratio of CysGly to GSH seems to determine the form of biliary MeHg.

152 MICROSCOPIC DISTRIBUTION OF ORGANIC VS INORGANIC HG IN MEH - EXPOSED MOUSE KIDNEY. P. M. Roeder and B. Kates, Department of Obstetrics and Gynecology, University of Rochester, Rochester, NY. Sponsor: T. W. Clarkson.

Hg localization techniques based on the affinity of silver for reduced Hg allow visualization of inorganic Hg in tissue (Magos et al., 1985; Ogg and Moller-Madsen, 1985), while use of MeHg allows localization of all forms of Hg (Roeder and Kates, 1986). By using both approaches, we have followed the distribution of MeHg and the inorganic Hg resulting from its demethylation. Twenty-four hours after a single exposure to 8 mg Hg/kg as MeHg, autoradiographs show that Hg is concentrated in the cortex of the kidney, with significantly more label associated with tubules than with glomeruli. Little demethylation has occurred at this stage, so no inorganic Hg is detected. After one week, the distribution of MeHg has not changed, but inorganic Hg appears in a highly localized pattern restricted to the apical regions of cells of proximal tubules. At two weeks, both forms are distributed as at one week, with increasing deposits of the inorganic form in the proximal tubules. The pattern of distribution supports the hypothesis of other investigators (e.g. Klein et al., 1973) that kidney damage after MeHg exposure may result from exposure of the kidney to inorganic Hg, rather than from exposure to MeHg per se.

Supported by NIH ES01247 and 01248.
CHRONIC EXPOSURE OF HUMANS TO CADMIUM STIMULATES PULMONARY INFLAMMATION MEASURED BY BRONCHOALVEOLAR LAVAGE. C.S. Rose, B.J. Fisher, A.A. Fowler. Sponsor: R.A. Carchman. Medical College of Virginia, Richmond, VA

Humans chronically exposed to cadmium are at increased risk for clinically important loss of pulmonary function. To test the possibility that cadmium may alter immune effector systems in humans, as has been found in animals, we performed bronchoalveolar lavage (BAL) in 27 workers exposed to cadmium for an average of 14 ± 3 years. The urinary concentration of cadmium in each patient was greater than 10 µg/liter. The total cell count and total protein content (µg/ml) of BAL fluid from both 17 cadmium-exposed smokers (456 ± 10^2 ± 138; 150 ± 12 µg/ml) and 10 nonsmokers (214 ± 10^2 ± 44; 174 ± 14 µg/ml) were significantly higher compared to the results of BAL fluid obtained from 12 nonsmoking control subjects (67 ± 10 ± 11; 109 ± 10 µg/ml). The percentages of cells in the BAL classified as macrophages (88%), polymorphonuclear leukocytes (5%), and lymphocytes (7%) were similar among cadmium-exposed smokers and nonsmokers, and nonsmoking controls. Our results provide the first direct evidence that chronic cadmium exposure in humans is associated with alveolar epithelial injury independent of cigarette consumption. We speculate that pulmonary macrophages may play a role in modulating lung injury in chronic cadmium exposure.

KINETICS OF ELEMENTAL MERCURY OXIDATION BY A SUSPENSION OF WASHED ERYTHROCYTES. S.P. Sichak, J.B. Hursh, and T.W. Clarkson. Division of Toxicology, University of Rochester School of Medicine, Rochester, NY

Elemental mercury is oxidized in the erythrocyte to the mercuric ion. This oxidation is thought to be catalyzed by catalase compound I which is formed by reaction of catalase with hydrogen peroxide. By using a "degassing" procedure which enabled us to prepurify the mercury (elemental versus oxidized) in a suspension of washed red blood cells, we were able to measure the rate of oxidation as a function of substrate concentration and hematocrit. We were also able to observe the effects of added hydrogen peroxide on the oxidation rate. The rate of oxidation was directly proportional to hematocrit and appeared to follow Michaelis-Menten kinetics over the range of substrate concentrations (10-20 ng/ml) we used. The rate of oxidation was stimulated by the addition of hydrogen peroxide and the amount of stimulation was proportional to the amount of hydrogen peroxide added. We conclude that the enzymatic reaction responsible for mercury oxidation in the red blood cell is saturable and that the addition of hydrogen peroxide during the zero-order-oxidation phase increased the V_max of the oxidation reaction.


Female C57 mice were fed diets containing 0.25, 5, or 50 ppm cadmium (Cd) for 18 mo starting at 70 d of age. After 12 mo of Cd exposure, mice were ovarietomized (OV); controls were sham-operated (SH). Mice were sacrificed 6 mo after surgery. At 50 ppm Cd, the calcium (Ca) content of femurs and lumbar vertebrae of OV mice were 32% and 17% lower, respectively, than those of SH mice (p<0.05); at 0.25 ppm Cd, OV-induced decreases were much less: 14% for femurs (p<0.05) and 0% for lumbar vertebrae. Similarly, at 50 ppm Cd, Ca to dry weight ratios of femurs and lumbar vertebrae of OV mice were 27% and 9% lower, respectively, than those of SH mice (p<0.05); at 0.25 ppm Cd, OV-induced decreases were much less: 10% for femurs (p<0.01) and 0% for lumbar vertebrae. Microradiographs showed that Ca contents of bones from 50 ppm Cd/OV mice were clearly lower than those of other groups. Enhanced bone loss in OV mice in response to Cd exposure may provide insight into the etiology of Itai-Itai disease and may explain the increased risk of postmenopausal osteoporosis in women who smoke. Work supported by the U.S. Department of Energy, Office of Health & Environmental Research, under contract No. W-31-109-ENG-38.
APPLICATION OF SUBCELLULAR FRACTIONATION IN STUDYING THE NEPHROTOXICITY OF METALS. M. Dobretz and X.J. Andersen, Robens Institute, University of Surrey, Guildford, UK and Dept. of Biochemistry, Medical University of South Carolina, Charleston, SC. Sponsor: B.A. Fowler.

The association of cadmium and other metals with macromolecules suggests that subcellular techniques are particularly useful in studying these uptake, intracellular transport and the toxic mechanism of metals in the kidney. Subcellular techniques, based on differential pelleting and sedimentation in density gradients as developed for studying the heterogeneity of kidney lysosomal populations and other organelles, have been used to examine the renal handling of IV-administered dual labelled 109Cd-35S thionine (a more potent nephrotoxin than Cd2+). At time intervals of 10, 30, 90 min and 24 h after administration, the subcellular redistribution of the isotopes indicates that cadmium is rapidly transferred from exogenous to endogenous metallothionein and is efficiently retained in the cytosol compartment while the exogenous metallothionein is rapidly degraded in the endosome and lysosome compartments. The process of uptake of Cd-thionine thus appears to be instrumental in the retention of cadmium in the kidney. Preliminary experiments also illustrate the usefulness of subcellular approaches in studying the nephrotoxic mechanisms of other metals, e.g. gold, nickel and platinum.

CELLULAR UPTAKE AND METABOLIC REDUCTION OF PERTINENT TO TRIVALENT ARSENIC AS DETERMINANTS OF CYTOTOXICITY AND TRANSFORMATION. P. Bertolero, G. Pozzi, E. Sabbioni and U. Saffiotti. National Cancer Institute, Frederick, MD, Mario Negri Institute, Milan, Italy and European Community Joint Research Center, Ispra (Varese), Italy.

Cellular uptakes of 73 As-labelled sodium arsenite (AsO33-) and arsenate (AsO43-) in BALB/C3T3 Cl A31-1 cells were dose-dependent, highest in the first h and decreasing thereafter. At 3x10^-6 M, uptake was 4-fold higher for AsO33- than AsO43-. Cytotoxicity (24 and 72 h exposures) was higher for AsO33- than AsO43-, but when correlated to As cell burden it showed no significant difference for the 2 forms. Both AsO33- and AsO43- induced neoplastic transformation, showing relative frequencies of 31%. Either form of absorbed As showed >90% recovery from cytosol after 1-24 h exposures. Exposure to AsO33- yielded 100% as AsO33- in cytosol, but AsO43- yielded >70% as AsO33-, showing a high rate of intracellular metabolic reduction. No methylated metabolites were detected by ion exchange chromatography. AsO33- to AsO43- oxidation occurred in cell-free medium (30% after 24 h incubation), but less so (4%) in the presence of cells. AsO43- was recovered unchanged from cell-free medium (24 h incubation), but yielded up to 5% AsO33- when incubated with cells. Release of AsO33- by cells exposed to AsO43- was dose-dependent. Glutathione depletion by diethyldithiobenzoate inhibited reduction of AsO43- to AsO33- by these cells up to 25% of controls.


The purpose of this study was to determine the role of transcription, translation and protein degradation in the induction of MT-I and MT-II in rat liver following Zn treatment. Concentrations of MT-I and MT-II, quantitated by HPLC, were similar at 6 h after administration of 1 mmol Zn/kg, but thereafter the concentration of MT-II was always higher than MT-I. MT-I and MT-II mRNAs, increased coordinately following Zn administration, reaching maximum levels 6-9 h after Zn treatment. At this time, MT-II mRNA was about 2 times more abundant than MT-I mRNA. In parallel with the increase in mRNA levels, the maximum relative rates of synthesis for both proteins was also observed 6-9 h after Zn administration. At this time, there was approximately 2 times more 35S-cysteine into MT-II than into MT-I. However, at no other times were differences in the relative rates of synthesis of MT-I and MT-II observed. Half-lives of the isometallothioneins, determined by pulse-labeling experiments, were calculated to be 12.2 ± 0.8 hr and 21.9 ± 3.0 hr for MT-I and MT-II, respectively. These results suggest that differences observed in the synthesis and degradation of MT-I and MT-II lead to a greater and more prolonged induction of MT-II following administration of Zn. (Supported by USPHS Grants ES-01142, ES-07079, BRSG07 RR0357 and a Procter and Gamble Fellowship).


Dose effects of magnesium carbonate (MgCarb) on carcinogenesis and natural killer cell (NK) modulation by nickel sulfide (Ni3S2) were studied. Male F344/NCrCr rats, 50-90 g, received single injections of 2.5 mg Ni3S2 alone or combined with MgCarb and were observed for 15 y. The NK activity was determined with the use of the 51Cr/YAC-1 test for blood and spleen, and the Ox-8 histochemical method for the muscle over the first 3 wk after injection. Local administration of MgCarb up to the Mg/Ni molar ratio of 1 inhibited the carcinogenicity of Ni3S2 in a dose-related manner; final incidence of sarcomas decreased from 100% to 55% and the latency increased from 25 to 39 wk. Higher doses of MgCarb did not exert further effect. Distant injection of MgCarb did not change the potency of Ni3S2. Ni3S2 had no influence on the activity of NK cells in blood and spleen. MgCarb alone did not affect the NK activity in blood but doubled it transiently in the spleen 24 h after injection. In the muscle, Ox-8 positive cells became abundant around MgCarb but were not found close to Ni3S2. This inhibitory effect of Ni3S2 was partially reversed by MgCarb. The results indicate a dose-dependent and strictly local character of the inhibition by MgCarb of Ni3S2 carcinogenesis, as well as a possible role of NK cells in the mechanisms of this inhibition.
COMPARATIVE CYTOTOXICITY OF NICKEL OXIDES AND NICKEL-COPPER OXIDES TO RAT, MOUSE AND DOG PULMONARY ALVEOLAR MACROPHAGES IN VITRO. J.H. Benson, R.F. Henderson, J.A. Pickrell, and R.F. Singh, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Nickel oxides (NiO) and Ni/Cu oxides (Ni/CuO) are encountered during the refining of nickel ore. It is important to determine the effect of temperature of formation and relative Ni and Cu content on their toxicity. Six NiO's formed at temperatures ranging from 400 to 1045°C and 4 mixed Ni/CuO's containing 47.6 to 73.6% Ni were screened for their cytotoxicity to P388/N rat alveolar macrophages (AM) in vitro. The most toxic NiO's and Ni/CuO's were evaluated for cytotoxicity to Beagle dog and B6C3F1 mouse AM. Median lethal concentrations (LC50's) for all NiO compounds to rat AM were >1000 µg/ml, with the NiO calcined at 650°C, being more toxic than NiO's calcined at >650°C. LC50's for Ni/CuO oxides to rat AM ranged from 400 to >1000 µg/ml. Ni/CuO's toxicity was directly related to % Cu in the compound. Ranking of species sensitivity was dog > rat = mouse. Results indicate: 1) calcination at elevated temperatures reduces the toxicity of NiO's, 2) toxicity of the Ni/CuO's is probably due to their Cu, rather than their Ni content, and 3) macrophages from dogs may be more sensitive to the effects of Ni compound exposure than macrophages from rodents. (Research funded under U.S. DOE Contract No. DE-AC04-76EV01013.)


Physiological pharmacokinetic models are gaining acceptance for making predictions of a chemicals effects in humans based upon experimental results obtained in animals. In developing a physiological pharmacokinetic model for nickel, the partitioning of this metal between plasma and various organs had to be determined. Rats were given a bolus injection of nickel as 63NiCl2 followed immediately by a constant infusion of 65NiCl2 solution. Steady state concentrations of 1.9, 9.1, and 33.1 ng Ni/ml of plasma were produced at infusion rates of 46, 172, and 586 ng Ni/hr. Tissue concentrations of Ni increased as a linear function of infusion rate, however the tissue/plasma ratios were not altered by varying the infusion rates. Mean tissue/plasma ratios were obtained for the following tissues: liver (0.17), kidney (9.90), spleen (0.20), lung (0.51), heart (0.19), intestine (0.21), thymus (0.21), testes (0.18), and muscle (0.09). These data have been incorporated into a multicompartmental model for the estimation of tissue burdens of nickel in humans following various routes of administration. (Supported by NIH Grant ES07031, RR01693, and CA14236).


This program presents in videotape format the fundamental concepts of toxicology and relates to employees that "an ounce of protective prevention is worth a pound of cure". The program defines basic toxicological terms and identifies routes of entry, acute and chronic effects. Carcinogenicity, and mutagenicity are explained. The importance of personal protective measures is stressed. Additional videotape programs in hazard communications, industrial hygiene, and risk assessment topics are also presented. These programs provide an effective means of communicating basic concepts in toxicology and related topics to non-scientists. CCRIS: CHEMICAL CARCINOGENESIS RESEARCH INFORMATION SYSTEM. T. Cameron, M. Stump (NCI); D. Tidwell (NLM); S. Olin, T.Junghans (Tracor Jitco). Sponsor: G. Guzto

Information will be presented on the CCRIS data bank indicating its usefulness to scientists and non-scientists, such as media personnel, students and teachers. The scientifically evaluated and fully referenced data bank has been developed and maintained by the National Cancer Institute (NCI), the National Library of Medicine (NLM) and Tracor Jitco, Inc. It contains carcinogenicity, tumor promotion, and mutagenicity test results on a broad spectrum of chemicals. Data are derived from the scanning of primary journals, current awareness tools, and a special core set of sources, including a wide range of NCI reports. Studies and results have been reviewed and evaluated by experts in carcinogenesis and mutagenesis and only adequate findings are included. CCRIS is organized by unique chemicals and now contains information on over 1200 chemicals. CCRIS is resident and searchable in the NLM's TOXNET system. Utilizing a free text search capability, a powerful and flexible command language, and a variety of online user assistance features, TOXNET offers state-of-the-art user-friendly searching. A poster/paper format will be used for the presentation. The ease of searchability of CCRIS will be demonstrated on a terminal and participation encouraged.

In response to the needs of professionals such as extension agents, water-resources experts, community decision makers, engineers, and others who must consider the implications of toxic chemicals in their work, we have created an easy-to-use instructional program that provides the user with the background needed to understand specific chemical information found elsewhere. Using plentiful original computer art, animation, user selection of examples, interactive exercises, simulations, and gentle, ungraded tests, this PILOT program presents concepts of toxicology to the interested adult who is assumed to have had some college science. The concepts, which include terminology, routes of entry, dose-response, individual susceptibility, defenses against poisoning, health effects, risk assessment and many others, are presented in the context of societal problems caused by the use of chemicals, and the sources of scientific uncertainty about the hazards of chemicals. The program runs on an IBM PC/XT or compatible with a color monitor or an enhanced graphics adapter.


Understanding CHEMICALS and how they interact with PEOPLE is essential to informed citizenship in our society. The goal of the Chemical Education for Public Understanding Program (CEFP) is to use concepts and processes drawn from biology, chemistry, physics, environmental science, toxicology, and related fields, to enhance middle/junior high school students and the general public to chemicals and their importance in their lives.

Risk comparison and risk management is essential if citizens are to effectively participate in decision making regarding utilization of potentially toxic substances. The CEFP Risk Comparison Module communicates these ideas to the public through the use of a unique "insurance scheme." Materials developed, outcomes from their use, and a direct experience using them will be provided as an example of the CEFP approach. Evidence from use with school and community groups that clearly indicate the value of the approach will be presented.

TOOLS FOR COMMUNICATING TOXICOLOGY TO THE LAYMAN. S.B. McCollister. The Dow Chemical Company, Health and Environmental Sciences, Midland, MI

The Dow Chemical Company has prepared a variety of communications materials aimed at increasing the understanding by lay persons of issues related to the effects of chemicals on human health and the environment. A 15-minute videotape, "How Much Is Too Much?", describes in general the toxicologic evaluation process and focuses on dose response and benefit vs. risk. Written materials include the following: 1) "Who Protects our Health and Environment?", a 21-page booklet that traces the development of industrial toxicology and describes in some detail, but in lay language, the basic principles of toxicology and the various types of toxicity studies; 2) "Life is in the Balance" (20 pages), a booklet addressing risk vs. benefit, including different types of risk and assessment of risk; 3) "Pathway to a Healthier Tomorrow", a brochure which describes epidemiology studies in 16 pages; text covers various methods used, data collection and evaluation, and the potential pitfalls of epidemiology studies; 4) "A Challenge to Fear", a 24-page booklet which addresses cancer in the U.S., including cancer rates and factors; 5) "Parts per billion ... Parts per trillion", a small leaflet which puts trace levels of contaminants in understandable terms. All materials will be on display. Complimentary copies of the booklets will be available.


Regulatory decisions depend on scientific data but are not necessarily made by scientists, therefore it becomes part of the role of the toxicologist to translate complex data into a form that can be readily understood by non-scientists. Two media, motion picture and slide-tape programming, can be particularly effective in this endeavor and have been shown to numerous visitors to CIIIT. The motion picture The Mucociliary Apparatus presents the concept of the airway clearance system and its role in the protection of the nasal epithelium from inhaled toxic compounds. This is achieved through the use of 16 mm photomicrography to show the system working, diagrammatic representation of nasal structure and an animated sequence to show how cilia propel mucus. Simple language is used and inter-species comparisons are made to help simplify complex concepts. A slide tape program entitled Behind the Barrier provides an effective way to tour a barrier type animal facility where visitors are not permitted. It also shows various animal husbandry procedures. While movie presentations may be expensive and difficult to undertake, slide tape presentations are a simple inexpensive way to present toxicological concepts in a pre-recorded program format and aid toxicologists in reaching a wider audience.
One of the most fundamental concepts in toxicology is the relationship between the degree of exposure (dose) and the severity of the toxic effect (response). This relationship can be depicted using a dose-response curve, and quantitative measures of toxicity can be derived from such curves. The most common quantitative measure of acute toxicity, the LD₅₀, can be derived from a dose-response curve which plots the percent mortality at each dose against the log dose. This computer simulation allows the participant to generate dose-response curves of this type and to read the LD₅₀ from the curves. The simulation is designed so that the participant can examine dose-response curves for a number of different chemicals administered to the same species and also the curves for the same chemical administered to a number of different species. The simulation can thus be used to: (1) illustrate the basic dose-response concept; (2) the use of the dose-response curve for determining a quantitative measure of toxicity; (3) the variation in dose-response for different chemicals; and (4) the species dependence of the dose-response. The simulation is flexible so that some or all of the principles can be illustrated in a particular application.

Responsibility for both implementation and enforcement of environmental laws has been increasingly delegated to state agencies. As a result, there is a growing need for scientific and legal expertise among state and local-level environmental advocacy organizations. To address that need, the Environmental Defense Fund (EDF) created the Environmental Information Exchange. The program is intended to provide state groups with critical information to support their own arguments and strategies for environmental and health protection. Based on a unique combination of EDF staff resources, computer links and outside database resources, the network seeks to encourage generation of an ever-growing database consisting of questions, issues and answers on various issues of concern.

As a response to the need for public information about environmental and occupational health risks, UMDNJ-Robert Wood Johnson Medical School has developed a model program to provide information and services to the general public, lay and professional employees, small industry (fewer than 100 employees), schools, and physicians. The foundation for this program is its Resource Center, which produces educational materials on specific risk issues. The program is guided by an Advisory Committee representing 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. EOHIP is designed to be a model program based in New Jersey that can be replicated in other states.
Two slide/tape programs were developed to present basic concepts of toxicology to non-scientists. One slide/tape program titled "Toxicology: The Science of Poisons" presents an overview of the science of toxicology, and introductory information about general manifestations of toxicity. Acute and chronic toxicity are explained and the organ systems affected by toxicants are reviewed. Dose-response relationships, toxicokinetic processes, and the concept of half-life are explained and demonstrated visually. The second in the series, titled "Toxicity Testing", explains the process of toxicity testing of chemicals used as food additives or pesticides. Information about how acute, subchronic and chronic studies are performed is presented and reproductive and oncogenicity testing are explored in detail. The target audience for the program is grade 12. The program has been widely shown in California and well received.

The subchronic dermal toxicity of Metsulfuron methyl (Methyl 2-[[[4-(methoxy-6-methyl-1,3,5-triazin-2-yl)-amino]-carbonyl]-amino[sulfonyl]-benzoate - CAS No. 74223-64-6) was investigated by applying either 0, 125, 500 or 2,000 mg/kg to the shaved back of rabbits (10 per test group, 5 of each sex) on 21 consecutive days. The dermal treatments were 6 hours a day. Rabbits were observed daily. Hematologic and clinical pathologic studies were conducted on all rabbits prior to testing, 1 day following the 21st dose, and after a 14-day recovery. Pathologic examination was conducted on 6 rabbits per group the day following the 21st dose; the rest after a 14-day recovery. Sporadic weight loss, slight diarrhea, and small pustules at the treatment site were seen with similar frequency and severity among all groups including the controls. Clinical pathological examinations revealed no compound-related effects. Mild testicular degeneration was seen in a few test rabbits but the focal nature, mild degree of involvement, distribution through the dose groups, and lack of dose response indicated that the effect is not related to the test chemical. Metsulfuron methyl at doses up to 2,000 mg/kg failed to exhibit any signs of toxicity following dermal exposure.

Carbofuran, a broad spectrum carbamate insecticide/nematicide, was tested in a repeated dose dermal toxicity study to assess possible health hazards associated with subchronic dermal exposure. Carbofuran technical was applied to the shaved skin of New Zealand White rabbits (6/sex/group) for approximately 6 hours/day at dosages of 0, 10, 100, or 1000 mg/kg for a total of 21 consecutive days. There were no statistically significant differences in body weight, clinical chemistry, hematology, cholinesterase activities (plasma, erythrocyte, or brain), or organ weights between control and treated animals. Brain cholinesterase activities were analyzed using brain slice and brain hemisphere homogenates; activities were statistically different. No treatment-related lesions were observed following gross necropsy or histopathologic examination of tissues. Minor changes (increased skin thickness, increased lymphocyte infiltration) were observed in treated skin (including saline controls) compared to untreated skin. Under the conditions of this study, Carbofuran was practically non-toxic, and the no-observed-effect level (NOEL) for male and female rabbits was 1000 mg/kg.

The subchronic dermal toxicity of Ethion technical was evaluated in two 7-day range-finding & two 21-day dermal toxicity studies in New Zealand White rabbits. Studies were conducted at doses of 0.05, 0.10, 0.20, 0.50, 1.0, 5.0, 10, 50, 100, 250, 350 & 500 mg/kg/day, applied to the shaved skin of 2 rabbits/sex/group for 6 hrs/day/7 days. Toxic effects included plasma & brain cholinesterase inhibition & skin irritation at doses >10.0 mg/kg and erythrocyte cholinesterase inhibition at >50 mg/kg. A 21-day study was conducted with 6 rabbits/sex/group at doses of 0, 1.0, 3.0, 25 & 250 mg/kg/day. A NOEL for toxicity of 3.0 mg/kg in females was based on plasma & brain cholinesterase inhibition. A NOEL for skin irritation of 1.0 mg/kg in females was based on histological skin changes. Brain cholinesterase was depressed in males at all doses. Two procedures for determining brain cholinesterase levels were evaluated. Homogenization of brain halves gave more consistent results than brain slices. Since brain slices were used in the previous studies, a second 21-day study was conducted using brains at doses of 0, 0.10, 0.25, 0.50, 0.80, 1.0, 3.0, & 25 mg/kg. A NOEL for toxicity & skin irritation of 0.80 mg/kg in males was based on brain cholinesterase data and histological skin changes.
Chlorpyrifos, an organophosphate, is the active ingredient in DURASAN® and LORSBAN® brand insecticides. Five rats were given 0.5 or 25 mg 14C-chlorpyrifos/kg p.o. or 15 daily 0.5 mg/kg oral doses of unlabeled chlorpyrifos followed by 0.5 mg 14C-chlorpyrifos/kg on the 16th day. Males and females were sacrificed 72 and 144 hr post-dosing, respectively. Most (84.0%) of each dose was excreted in the urine. Urinary metabolites were 3,5,6-trichloro-2-pyridinol (TCP; approx. 15%) and the glucuronide (approx. 80%) and sulfate (approx. 5%) conjugates of TCP. Another 5.6 to 11.4% of the dose was found in the feces. No 14C was found in the expired air. Except as noted below, 14C concentrations in the blood, bone, brain, carcass, fat, gonads, heart, lungs, kidneys, skin, skeletal muscle, and spleen were below the limit of quantitation (<0.01% dose/g). Small amounts of 14C, <0.14% dose/g, were found in the fat of males at all dose levels, and in the liver of males and the fat of females given the 25 mg/kg dose. These data confirm previous reports that the rat rapidly metabolizes and eliminates chlorpyrifos in the urine as TCP and conjugates.

*Trademark of The Dow Chemical Company.

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**HEPATIC AND EXTRAPARENCHYMAL MICROSOMAL MONOOXYGENATION OF PHTHALEIN: S. Kinsier and E. Hodgson, Toxicology Program, North Carolina State University, Raleigh, NC**

Both the cytochrome P-450-dependent monoxygenase system (P-450) and the FAD-containing monoxygenase (FMO) are involved in the metabolic oxidation of organophosphate insecticides, with many of these compounds being substrates for both systems. Selective inhibition was utilized to determine the relative contributions of P-450 and FMO in the microsomal sulfidation of phoratoxin in liver, lung and kidney in male and female mice. Inhibition of one enzyme system while retaining full activity of the other was achieved by heating microsomes at 50°C for 90 seconds to inactivate the FMO of liver microsomes, while an antibody to cytochrome P-450 reductase eliminated cytochrome P-450 activity in liver, lung and kidney microsomes. Reaction rates (nmol of phorate sulfide formed/min/mg microsomal protein) were highest in liver microsomes, with relative contributions being 77% by P-450 and 23% by FMO. In lung microsomes, overall reaction rates were lower, and P-450 and FMO contributed 45% and 55%, respectively. Kidney microsomes have greater FMO activity, relative to P-450, than either liver or lung microsomes.

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Mouse livers perfused in situ with the phosphorothioate pesticide methyl parathion resulted in the presence of the toxic metabolite methyl paraaxon (MPO) in the effluent perfusate. Mouse whole blood detoxified MPO, but not rapidly enough to prevent transport of at least some MPO to other tissues. Hepatic disposition and biotransformation of MPO was altered by changes in protein binding to perfusate, but not by changes in the perfusate flow rates.

Pretreatment of mice with phenobarbital (PB) daily for four days (80 mg/kg, ip) induced hepatic microsomal activation of MPO to MPO in vitro. However, effluent perfusate of livers from PB pretreated mice did not contain MPO. Yet MPO was produced intrahepatocically since hepatic cholinesterase levels were depressed compared to livers from PB pretreated mice perfused without MPO. Furthermore, PB pretreatment antagonized the acute toxicity of MPO in vivo.

These data indicate the net result of MPO metabolism by normal livers is metabolic activation, whereas the net effect in PB pretreated mice is detoxification. This enhanced detoxification could account for, at least in part, the antagonism of MPO toxicity observed following PB pretreatment. MPS (Supported by Grant ES04353 from NIEHS).
Carbosulfan was administered to rats at dietary concentrations of 0, 10, 20, and 250 ppm for three successive generations. Consistently lower body weights were observed in F1 and F2 adult males receiving the 250 ppm diets while a smaller effect was reported for females in this group during portions of gestation and lactation but not during the growth phases. Fup body weights and/or borers were significantly lower at most age intervals between birth and weaning for all litter sets in the 250 ppm group. Neonatal survival was significantly decreased for the F1-a, F1-b litters in the 250 ppm group. There were no effects on reproductive functions and no treatment-related histopathologic findings were observed at levels up to 250 ppm.

Treatment of rats (Charles River, male and female 6-8 wks old) with dietary mirex at 40 ppm for 1 week caused hyperglycemia, hypercholesterolemia, and inhibition of adrenocorticoid synthesizing enzymes. Hypoglycemia (59 mg/kg in treated vs 89 mg/kg in controls) was associated with hyperinsulinemia (228 mcu/ml in treated vs 128 mcu/ml in controls) 4 days following mirex treatment at 400 mg/kg body wt., without any effect on serum glucagon (81 ng/l in treated and 77 ng/l in controls).

The animals lost BW (164 g to 134 g in mirex treated vs 165 g to 162 g in controls), because of hypophagia and lipid mobilization. Further treatment at 400 mg/kg BW caused 75% mortality (LT-50 = 10.3 days) and the survivors showed drastic BW reduction, severe hyperglycemia (15 mg/kg mirex vs 118 mg/kg controls), hyperlipidemia, fat depletion, and severe hypophagia. Fatty livers (lipogenesis and glycogen accumulation) can be explained by hyperinsulinemia and lack of glucagon stimulation. Hypoglycemia and concomitant insulinopenia, lack of stimulation of glucagon secretion, as well as lack of lipogenesis in adipose tissue and hyperphagia by hyperinsulinemia indicate that adrenal glucocorticoid insufficiency may be causing an increase in ACTH which may increase insulin secretion and lipolysis.

Pregnancy has been suggested to modify both the hepatic and renal elimination of chemicals and thus may influence their toxicities. 14C-Endrin was administered i.v. to nonpregnant mice (NP) (25 g) or day 18 pregnant mice (P) (50 g). In NP receiving 5 mg/kg, seizures occurred after 244 ± 13 sec at brain [14C] of 5.9 ± 0.2 µg/g. These parameters were significantly reduced in P at 5 mg/kg (188 ± 11 sec; 4.5 ± 0.1 µg/g). When P received 2.5 mg/kg, an equal mass of endrin relative to NP receiving 5 mg/kg, brain [14C] at seizure was lower than NP (4.8 ± 0.1 µg/g) but time to seizure was significantly extended (319 ± 34 sec). Pregnancy may increase sensitivity to the acute toxicity of endrin or result in a different metabolic profile.

14C-Paraquat was administered i.v. to NP (15 mg/kg). P received paraquat so as to achieve equivalent initial blood levels. BUN was significantly increased in NP compared to P at all sacrifice times from 4 to 48 h. BUN increased to 170% of control levels in NP. A maximum 54% increase was observed in P. Similar but not significant results were seen with urine glucose. Pregnancy reduced the acute renal toxicity of paraquat. This may be the result of more rapid elimination of the compound (t 1/2 from plasma: P = 3.7 ± 0.1 h; NP = 11.9 ± 1.5 h).

To assess the mutagenic potential of the herbicide glyphosate (N-phosphonomethylglycine), in vitro and in vivo assays were conducted to evaluate endpoints of gene mutation, chromosomal effects, and DNA damage/repair. Microbial assays were conducted with S. typhimurium, E. coli, and B. subtilis. Glyphosate was not mutagenic in reverse mutation assays with Salmonella strains TA1535, TA1537, TA1538, TA100, or TA98 or in E. coli WP2 hfr either in the presence or absence of metabolic activation systems. No DNA damage was observed in the B. subtilis rec-assay. Employing mammalian in vitro systems, no mutagenic activity was detected in either the CHO/HGPRT point mutation assay or in the rat hepatocyte primary culture/DNA repair (UDS) assay. No clastogenic activity was detected in an in vivo cytogenetics assay with rats, and no dominant lethal mutations were observed in mice exposed to glyphosate. The results of these studies in mammalian and bacterial cell culture systems and in whole animal systems consistently indicate that glyphosate is not mutagenic.
ABSORPTION OF GLYPHOSATE IN THE MALE FISCHER RAT. C.R. Ducson and I. Glenn Sipes. Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.

Glyphosate, the major component of the non-selective, broad spectrum herbicide Roundup®, has an oral LD₅₀ of 5600 mg/kg in rats. Absorption across the gastrointestinal membranes is thought to be minimal, since a 60-90% of a single oral dose of glyphosate is rapidly eliminated unchanged in the feces. Thus, distribution and retention of glyphosate by tissues should be minimal. The purpose of our study is to further investigate the absorption of glyphosate in the rat. Male Fischer F344 rats were given glyphosate at 5.6 or 56 mg/kg, p.o., and then feces, urine, and tissues were collected, oxidized to ¹⁴CO₂ (except urine), and analyzed for ¹⁴C using liquid scintillation counting. The elimination of glyphosate given at 5.6 and 56 mg/kg resulted in total recoveries of ¹⁴C of 98.6 ± 7.9% at 96 hr and 93.1 ± 3.5% at 77 hr, respectively. Treating rats with 5.6 mg/kg glyphosate before or after treatment with 0.5 ppm Roundup® in the drinking water for 16 days resulted in total recoveries of 102.5 ± 3.4% and 103.8 ± 3.4%, respectively. Less than 2% of the dose remains in the tissue after 24 hr. Glyphosate appears to be rapidly eliminated from the body and is not retained in the tissues. Also, there is no apparent change in the elimination pattern when rats are treated with 0.5 ppm of the commercial formulation, Roundup®, for 16 days. Supported by ES-3-5031.


Clopyramid (3,5-dichloropicolinic acid) was fed in the diet to B6C3F1 mice and Fischer-344 rats at dose levels of 0 (control), 100, 500, or 2000 (mice) and 0, 15, 150, or 1500 (rats) mg/kg body weight/day for up to 2 yrs. In the mice, the only evidence of toxicity was body weight depression in males of the highest dose group. In top dose rats, decreased food consumption & body weights, increased liver & kidney weights, and pathologic, but non-tumorigenic, macroscopic and microscopic stomach changes occurred. The microscopic changes also occurred in the stomachs of a few middle dose rats. The gross change consisted of increased prominence of the gastric limiting ridge. The histopathologic change consisted of thickening of the epithelium of the anterior surface of the limiting ridge, more readily apparent in males. No evidence that clopyramid causes malignant or nonmalignant tumors was found in either rats or mice. The stomach changes in the rats did not occur in the mice. The no-observed-effect-level (NOEL) was 500 and 2000 mg/kg/day in male & female mice, respectively, and 15 mg/kg/day in both male and female rats.


Since the herbicide propanil is an aromatic amide and many other aromatic amides are genotoxic via N-hydroxy (N-OH) metabolites, N-OH derivatives of propanil and 3,4-dichloroaniline (3,4-DCA) were synthesized and tested for genotoxicity. Propanil, N-OH-propanil, 3,4-DCA, and N-OH-3,4-DCA were not mutagenic in S. typhimurium TA97, TA98, or TA100 (+/- S9) using concentrations up to 200 μg/ml. The compounds also produced negative responses in the CHO/HGPRT mutation assay (+/- S9) as well as in the rat hepatocyte unscheduled DNA synthesis (UDS) assay using concentrations up to 1000 μg/ml. Although the compounds were not genotoxic in these assays, genotoxicity was observed. The cytotoxic response in hepatocytes was quantified by lactate dehydrogenase (LDH) release using chemical concentrations up to 50 μg/ml. All the compounds increased LDH release in a dose-dependent fashion at 24-hours. Although propanil is cytotoxic, these data suggest that it may not pose a significant genotoxic hazard.


This study was conducted to characterize the nature and reversibility of histologic changes in the small intestine of mice induced by dietary administration of 6000-ppm captan. Groups of 35 male CD-1 mice received captan for 3 to 20 months (mo). Treatment periods (T) were followed by up to 12 mo recovery (R) where captan was removed from the diet. Captan produced diffuse hyperplasia, focal hyperplasia (FH) and neoplasia (N) in the epithelium of the proximal small intestine. Incidence of FH increased with age and duration of treatment. At 3 mo, diffuse hyperplasia was lower in incidence than FH and was observed with less severity in older animals. Adenomas were first seen at 9 mo and adenocarcinomas at 18 mo. Incidences of FH but not N were reduced by recovery. At 18 mo, 67/12R and 12T/6R mice had incidences of FH similar to controls. Incidences of N at 12 and 18 mo for 67/6R and 12T/6R mice were greater than controls and similar to the incidences in 12T and 18T mice. At least 6 mo treatment was required for tumor development.

A subchronic nose-only inhalation toxicity study of chlorpyrifos, an organophosphate insecticide, was conducted in male and female Fischer 344 rats at 0, 5, 10, or 20 ppb chlorpyrifos for 6 hrs/day, 5 days/week for 13 weeks in ARO-designed nose-only inhalation chambers. Urinalysis, hematologic, clinical chemistry, and pathologic evaluations were performed at the end of the study with no effects detected. No treatment-related signs of toxicity or changes in body weight gains were noted during the course of the study. In addition, no changes in either plasma, red blood cell, or brain cholinesterase activities were detected at the end of the study. These data suggest that chlorpyrifos is not toxic to rats by the nose-only inhalation route following 13 weeks of exposure at concentrations approaching the theoretical maximum vapor concentration (25 ppb at 25°C).


To investigate a possible central role in chlordecone (CGL)-induced hypothermia, colonic temperature (CT) was measured following infusions of CGL into the lateral ventricle, third ventricle or the cisterna magna in male Fischer-344 rats. Compared to vehicle-control rats, infusions via chronic indwelling cannulae of 40, 320 or 800 μg CGL into the lateral ventricle or of 320 or 800 μg CGL into the third ventricle did not significantly change CT. However, intracisternal (IC) infusions of 80, 160, 320 or 800 μg CGL produced significant hypothermia (maximally 2.2°C) from 0.5 to 4 or 6 hr. IC infusion of 320 μg CGL significantly decreased CT and increased tail skin temperature (ST). CGL is known to induce nor-epinephrine (NE) release in the brainstem, and NE is involved in tonic vasomotor control within the medulla. IC infusions of NE (16 μg) significantly decreased CT and increased ST. CGL-induced hypothermia appears to be associated with vasoconstrictory effects that are mediated by a brainstem adrenergic mechanism.

PERIPHERAL AND CENTRAL ENZYME INHIBITION BY THREE OP'S. M. Fairgrieve-LaVave and B. Magnus Francis. Dept. of Veterinary Biosciences and Inst. for Environmental Studies, University of Illinois, Urbana, IL.

Fenthion is suspected of causing severe and long-lasting nervous system damage in both humans and animals. It does not inhibit brain neurotoxic esterase (NTE) in chicks. However, it caused abnormal gait and a long delayed ataxia in chicks. To test the hypothesis that fenthion affects peripheral more than central nervous system enzymes, we compared 3 OP's using 4 assays: inhibition of NTE and AChE in brain, inhibition of NTE in lymphocytes, and of AChE in red blood cells (RBC). Deoxynromolytophospho (DBL) induces OPIDN, and was the positive control; fenthion (FTR), which does not induce OPIDN, was the negative control. Chicks were treated po or dermally with 1 or 2 doses of 5 mg/Kg FEN; 75 mg/Kg FTR or 50 mg/Kg DBL. Enzyme inhibitions were measured at different intervals after dosing. As expected, DBL caused both peripheral and central NTE and AChE inhibition. FTR and FEN caused AChE inhibition both in the RBC and brain, but neither peripheral nor central NTE inhibition. Thus, the severe neurological effects caused by FEN can not be explained by selective inhibition of peripheral NTE or AChE in the absence of central inhibition of these enzymes.

KINETICS OF INHIBITION AND REACTIVATION OF MAMMALIAN BRAIN ACETYLCHOLINESTERASE (AChE) BY ORGANOPHOSPHINATE (OPIN) ESTERS. J.K. Marquis and R.D. MacCallum, Boston Univ. School of Medicine, Boston, MA.

The kinetics of AChE interaction were studied with the following 4-nitrophenyl-OPIN compounds: (I) chloromethyl(phenyl)-, (II) trifluoromethyl phenyl (methyl)-, (III) 4-methoxyphenyl(methyl)-, (IV) isopropyl(phenyl)-, and (V) bis-(2-thienyl)-. Inhibition, spontaneous reactivation, and oxime-induced reactivation were measured in purified enzyme and in enzyme chemically modified with water-soluble carbamimidates. K1 for control enzyme: I ≥ II ≥ III ≥ IV > V. A major shift in potency (decrease) was observed in modified enzyme only for compound III. Spontaneous reactivation was measured at 26 and 24 hrs. Control values at 6 hrs: I = 45%, II = 24%, III = 41%, IV = 31%, V = 33%. Spontaneous reactivation was significantly enhanced in modified enzyme and the time to 100% reactivation was significantly reduced. Reactivation induced by pralidoxime and chloidoxime was also studied. Neither the level of reactivation nor the time to maximum reactivation was significantly altered by these conditions of chemical modification. It is suggested that pharmacological agents designed to enhance spontaneous reactivation may serve as useful chemopreventive agents. (Supported by USARO DAAE 29-85-K-007.) Compounds generoslly supplied by Dr. C. Lieske, ICD, APG.)
INFLUENCE OF DIETARY PROTEIN LEVELS ON ESTERASE ACTIVITY IN RATS. L.E. Butler and W.C. Dayteman. Toxicology Program, N.C. State Univ., Raleigh, NC.

An increase in toxicity of pesticides has been shown to occur in rats fed protein deficient diets. The NRC has set nutritional requirements for lab animals with a recommended dietary protein level of 12%. Standard lab chow average 20-25% protein and are designed for optimal growth rate. In this study, male weanling rats, divided into three groups, were fed for 30 days on three purified isocaloric diets containing 5, 12, or 22% casein ad lib. A fourth group was fed a standard lab chow ad lib. Esterase activity was determined using various substrates. These findings will be discussed.


The measurement of blood cholinesterase activity has been used as an indication of exposure to cholinesterase inhibitors especially organophosphate compounds. The variability of "normal values" not only from person-to-person, but for the same person at different times, has made interpretation of the results difficult (Duncan, R.C., et al., 1986). The importance of adequate individual baseline data has recently been recognized by the U.S. EPA in the draft Farm Worker Protection Regulation (40 CFR-170.35). This paper presents baseline cholinesterase data, both red blood cell (RChE) and plasma (PChE), obtained from sixty to ninety persons employed in a specialties chemical manufacturing facility. Cholinesterase baselines were determined in the Fall of 1981 and 1982 and in the Spring of 1983. The same collection procedure and analytical method were utilized at each interval. Whole blood samples were obtained by finger puncture and were collected in heparinized Natelson tubes. After centrifugation, PChE and RChE were determined with an automatic clinical chemistry analyzer (Baker Centrifichem 400), using a modified Ellman method. The baseline cholinesterase values were analyzed to obtain estimates of the variability of the "normal values" from person-to-person and for the same person over time.

RESULTS OF A ONE-YEAR DIETARY TOXICITY STUDY IN BEAGLE DOGS ADMINISTERED CYHEXATIN. D.M. Bond, R.J. Kociba, D.G. Keyes, J.M. Wall and S.K. Weiss. The Dow Chemical Company, Midland, MI.

Cyhexatin, an active acaricide, is registered for use on a variety of fruits, nuts and ornamentals. In an effort to supplement toxicological data, a one-year dietary toxicity study in male and female Beagle dogs has been conducted. Targeted dietary levels of 0.05, 0.50 or 0.75 mg cyhexatin/kg body weight/day were provided to 6 dogs/sex/dose level. In-life parameters monitored included health status, body weights, food consumption, clinical chemistry, hematologic, urinalysis, gross and histopathology. Possible treatment-related effects consisted of a trend toward lower body weights for the high-dose female dogs during the last 4 months of the study. In addition, relative heart weights of female dogs given 0.75 mg/kg/day were statistically increased when normalized to body weight. This increase, however, was not apparent when body weight differences were controlled by normalizing heart weight to brain weight and was not associated with any clinical or pathologic effects in the heart. Evaluation of all parameters indicated that under the conditions of this study, the no-observed-effect level of the body weight difference level was determined to be between 0.5 and 0.75 mg/kg/day, with 0.75 mg/kg/day considered to be the no-observed-adverse-effect level for both sexes of Beagle dogs.
Rats were treated in vivo by oral intubation with different doses of toxaphene for three days and the levels of whole brain biogenic amines isolated through alumina absorption were analysed by high performance liquid chromatography. The depletion in brain biogenic amines was significant and dose dependent with a maximum depletion amounting to 87% for dopamine and 74% for norepinephrine in 75 mg/kg toxaphene dosed rats. No significant changes in brain epinephrine levels were observed in any of the toxaphene treated groups. Toxaphene treatment had no significant effect on the total brain weight of the animals. The greater incidence of mortality in the higher dose rats parallel remarkable depletion in dopamine and norepinephrine levels and such alterations may account for the toxic effects of toxaphene. The decrease in the levels of dopamine (37%) and norepinephrine (43%) in the lowest toxaphene dosed groups (10 mg/kg) was appreciable and may represent the early signs of the adverse effects on CNS function since they occur before any overt symptoms of neurotoxicity of toxaphene become apparent. (Supported by NIH/MBRS Grant #080407).

Enzyme linked immunosorbent assays (ELISA) are slowly being accepted as a method for pesticide residue analysis. A specific ELISA for the rice field herbicide molinate (5-ethyl hexahydroazepine-1-carbothioate) has been previously reported with sensitivity of about 10 ng/ml. This report focuses on assay improvements utilizing newly synthesized hapten and new antibodies. These assays have been screened for improved sensitivity and specificity. The previously reported assay was validated against the classical gas chromatographic method by analyzing field and spiked water, air and soil samples. Samples were extracted in either toluene or pentane/dichloromethane following accepted residue techniques. Extracts were split and organic extracts analyzed by gas chromatography. The organic extracts saved for ELISA, were solvent exchanged into 1,2-propanediol/acetone and analyzed. The correlation between methods was good except that ELISA results were generally about 10% higher than gc results. Verification of the ELISA method was also conducted using a laboratory field test a radiolabeled molinate. Using these data, a guideline for validation of ELISA as an analytical method has been suggested. Supported in part by California Department of Food and Agriculture.

Endothal is an important herbicide that is similar in structure to the natural vesicant cantharidin. To characterize the effects on hepatic tissue, endothal and cantharidic acid (the dicarbosyl form of the anhydride) were administered ip to mice at 5-times their LD50 values, i.e., at 75 and 10 mg/kg, respectively. Within 45 min both compounds caused dramatic liver enlargement and congestion. Liver weights and hepatic Hb content increased to 136% and 200%, of control values, respectively. Hepatic glycogen metabolism also increased as evidenced by reductions in glycogen content and glycogen synthase activity, and by increases in glycogen phosphorylase and blood glucose levels. Microsomal Mg2+-ATPase was significantly reduced by both treatments indicating functional damage to the membranes. However, none of these effects were detected in vitro with toxicant concentrations up to 100 μM. Neither compound affected the following parameters: mitochondrial Mg2+-ATPase, microsomal lipid peroxidation, hepatic triglyceride or GSH content, or serum GPT levels. The results show that endothal and cantharidic acid alter hepatic biochemical processes, but that the observed lesions represent secondary effects in vivo and not the primary mechanism of action. (Supported in part by NIH/MBRS Grant 080407).

Decreased penetration of pesticides in insects is one of the mechanisms responsible for pesticide resistance. An in vitro method was developed in order to study penetration through the cuticle. In vivo and in vitro penetration of fenvalerate was measured at 0, 1, 3, 6, 12 and 24 hr time intervals. In the in vitro studies, the medium was collected and the cuticle was washed with acetone. The medium, cuticular wash and the cuticle were assayed for radioactivity. The cuticular wash contained the majority of the fenvalerate at all time intervals. At 24 hrs, approximately 5% of the dose was located in the cuticle while only 2% actually penetrated. In the in vivo studies, insects were treated topically with the same dose at the same time intervals. They were washed with acetone and penetration was determined. Since penetration in insects is determined by assaying the whole carcass after a cuticular wash, the fenvalerate found in the medium and the cuticle from the in vivo studies were combined. The rate of penetration of fenvalerate was similar in both in vivo and in vitro studies.
STABILIZATION OF BACILLUS THURINGIENSIS STRAIN HD1-1 AND HD2-1 AGAINST UV-LIGHT.
Samia Y. El-Touny and Chester M. Rimel.
Toxicology Program, N.C. State University, Raleigh, NC. Sponsor: Walter Dauterman.

The toxicity and the UV bioinactivation of 4 preparations of Bacillus thuringiensis (BT) Berliner were studied. Stabilized and non-stabilized preparations of BT strain HD1-1 were assayed against the corn ear worm (CEW), and stabilized and non-stabilized preparations of BT strain HD2-1 were assayed against the cabbage looper (CL). Thuricide (48B), a commercial formulation of BT, was used as a standardized preparation. HD1-1 was toxic to CEW with an LD₅₀ of 2.6X10⁶ IU and HD2-1 was toxic to CL with an LD₅₀ of 800 IU. Thuricide was toxic to CEW and CL with an LD₅₀ of 2.9X10⁶, 2X10⁷ IU respectively. The UV-studies revealed that purified crystals of HD1-1 and HD2-1 were unstable when exposed to soft UV-light source and 50% of the toxic activity was lost after 15-20 hours. Stabilized preparations were significantly persistent and photolytically stable and 90% of the toxicity remained for 30-40 days. It was found that the activity of BTI and BT strain HD2-1 was directly related to fluorescence of tryptophan.

METHYL MERCURY CYTOTOXICITY: THE PROTECTIVE EFFECTS OF GLUTATHIONE ON MICROTUBULAR ARCHITECTURE. L. Kromdah, L. Jamali and L.O. Trombetta
St. John's University, Jamaica, NY

Mouse neuroblastoma Neuro-2A (C1300) cells differentiated with 2.0% Fetal Bovine Serum (FBS) in Dulbecco's modified Eagle's medium were exposed to 25 μM methyl mercury (MeHg) for 1 hour. Treated cells appeared rounded with the loss of processes. These cells also contained numerous cytoplasmic vacuoles. Immunohistochemical staining directed against α-tubulin revealed severe alterations in microtubular architecture. These changes included condensation of reaction product with concomitant loss of fibrillar structure. Administration of 10 μM glutathione (GSH) 1 hour before and 12 hours after exposure to MeHg had no saving effect on microtubular structure. However, simultaneous administration of GSH with MeHg dramatically prevented cytotoxicity. This observation suggests a saving effect on GSH on microtubular organization and/or the prevention of the injurious effects of MeHg on microtubular polymerization.


Microtubules (MTs) are thought to be a principal cellular target of methylmercury (MeHg), a potent neurotoxicant. We examined the effects of MeHg on MTs of neurons and glia induced to differentiate from pluripotent murine embryonal carcinoma stem cells by retinoic acid. MTs were stained with an anti-tubulin antibody and examined by indirect immunofluorescence microscopy. MeHg-induced injury was time-dependent, but not uniform in all cells. After 30 min exposure to MeHg (6 μM) MTs were largely absent from fibroblast-like cells and glial cells but were preserved in most neurons. MT preservation in neuronal perikarya was variable. MTs in neurons were largely preserved following this treatment. After 1 hr exposure MTs were absent in neuronal perikarya. MTs were present in most neurons. However, the intensity of the labeling in neurites was reduced relative to control neurites, suggesting a reduced number of MTs. Contrary to existing vivo data, neuronal MTs were not selectively vulnerable to MeHg-inducing injury. The known heterogeneity of MTs within neurons may underlie the cells variable response to MeHg. (Supported by NSERC).

SUBCHRONIC TOXICITY OF ALKYL LEAD. PART 2. MICROSCOPIC AND ULTRASTRUCTURAL NEUROPATHOLOGICAL EFFECTS. P.B. Little, C.A. Franklin, A. Gilman, V.E. Valli, A. Yagminas and D.E. Villeneuve
Health Protection Branch, Ottawa, Canada, and Ontario Veterinary College, Guelph, Ontario.

Groups of male rats (5 per group) were dosed orally with inorganic lead (200 mg/kg bw), triethyl lead (3-EL) (0.2 or 1.0 mg/kg bw) or tetraethyl lead (0.2 mg/kg bw) 5 days per week for 13 weeks. Following glutaraldehyde perfusion nervous system tissues were harvested and examined by light microscopy using H&E and Bielschowsky staining. The lumosacral nerve from each rat was stained with Toluidine Blue and selected areas examined ultrastructurally. In rats dosed with 1.0 mg/kg 3-EL significant lesions (Wallerian degeneration) were apparent in ventral and particularly lateral tracts. Less but qualitatively similar changes were seen in the group administered inorganic lead. Ultrastructurally, electron dense bodies were apparent in lumosacral nerve Schwann cell cytoplasm and in unmyelinated axoplasm; additionally a reduction of neurofilaments and neurotubules was apparent in some of these affected axons from animals dosed with 1.0 mg/kg 3-EL.
The time course of light and ultrastructural changes in spinal cord and peripheral nerves were studied in adult male Sprague-Dawley rats 2-21 days after a single oral dose of 8.25, 17.5 or 35 mg/kg TMPB. At 21 days, the 8.25mg/kg dose of TMPB produced chromatolysis in motor neurons and axonal atrophy in both dorsal and ventral roots. Higher doses induced pronounced chromatolysis and more extensive atrophy with marked proximal axonal degeneration at earlier times. Occasional axonal swellings in the white matter contained neurofilamentous accumulations. Following 35mg/kg, reactive astrogliosis were seen in areas of spinal cord demonstrating axonal degeneration and macrophages were present beneath the myelin sheath of sensory fibers in the dorsal root ganglia, indicative of early secondary demyelination. In addition, neuropathological changes were evident at spindles and motor endplates. The extent of axonal alterations in relation to chromatolysis appeared dose-dependent and suggests that at least some of the axonal pathology arises secondary to altered perikarya functions. Supported by NS-23325 and ES-04078.

Chlorine dioxide (ClO₂), considered as an alternative to chlorine for drinking water disinfection, has been shown to retard cell proliferation in the CNS of developing rats, possibly as a result of hypothyroidism (Taylor and Pfohl (1985), Orme et al (1985)). Following direct exposure of rat pups to 14 mg ClO₂/kg/day from postnatal (pn) days 1-10, we detected a decrease in forebrain weight at pn days 21 and 35. No change in thyroid status (T₃, T₄ levels; liver α-GPD activity) was evident. We have examined the morphology of the forebrain at pn day 35 in rats exposed to ClO₂ as above. No stained sections of cortex from Krieg’s areas 2, 4 and 18 show no gross pathological lesions. Cortical thickness in these areas was unchanged and cortical layers contained the expected cell types. Laxol fast blue staining showed no evidence of myelin loss. In toluidine blue stained sections the intensity of staining and number of cells staining positive for Nissl substance were unchanged relative to sections from control animals. These results indicate a normal migration and differentiation of cortical neurons in developing rat brain following direct ClO₂ exposure. This abstract does not necessarily reflect EPA policy. (Supported by a NRC Research Associateship).

The purpose of this experiment was to study the effect of lead on the development of the explanted otocyst in culture. Gravid female mice were killed on gestation day 12.5 or 16. Otocysts were microdissected from each embryo or fetus. Otocysts were cultured, Pb(NO₃)₂ was added to the experimental medium at concentrations up to 5 mM. On days 0, 2, 4, 6 and 8 in culture, otocysts were processed for histological evaluation. Otocysts were examined for structural development and differentiation of sensory tissues. In cultures without lead the sensory epithelium patches in the cochlea and vestibular system increased in size. In the 12.5 gestation-day otocysts, development was characterized by the coiling of the cochlea. The development of the 16 gestation-day otocyst in culture was characterized by differentiation of the sensory epithelium. In lead-treated cultures, there were changes in the structural development in 12.5 gestation-day cultures but not in sensory epithelium differentiation in 16 gestation-day cultures. The embryonic and fetal susceptibility of the developing inner ear to lead is compared.

Chronic prenatal exposure to ethanol results in a broad spectrum of effects termed the fetal alcohol syndrome. The role of acute ethanol ingestion, "binge drinking," in the production of the fetal alcohol syndrome has not been established. The present study investigated the morphologic effect of maternal acute ethanol ingestion on the developing cerebral cortex of the fetus. Pregnant Charles River (CD) rats were intubated with 2.5 g/kg (three times daily) or 4.5 g/kg (twice daily) of ethanol and blood alcohol levels were measured on gestational days 14 and 15, a critical period for the development of the cerebral cortex. On gestational day 21 no resorptions were found at either dose. Transverse 5 μm serial sections of gestational day 21 brains were examined for the presence of cortical malformations. Total dose 18 g/kg: severe distortion of cortical layers in half of the fetuses; heterotopias accounted for most other abnormalities. Total dose 15 g/kg: minor distortion of cortical layers in half of the fetuses; heterotopias and cysts common. It is proposed that a prenatal "binge" of ethanol during a critical period results in alterations in neuronal proliferation and migration in the developing rat cerebral cortex. Supported in part by NS16694 and ES07079.
VINCA ALKALOIDS INHIBIT THE NEURON PERIKARYAL RESPONSE TO AXOTOMY. A.I. Soifer, A. Moretto, M.I. Sabri, and P.S. Spencer. Institute of Neurotoxicology, Albert Einstein College of Medicine, Bronx, N.Y.

We have used vinca alkaloids (known inhibitors of axon transport) to explore the role of retrograde axon transport (RT) in activating neuron perikaryal repair responses to axon transection. Mouse lumbar dorsal root ganglia (DRG) (L4-L6) were excised 48 hours following unilateral transection of the sciatic nerve. Ornithine decarboxylase activity (ODC), the rate limiting enzyme in polyamine synthesis, was assayed in pooled DRG samples. ODC activity in DRGs ipsilateral to transection was increased 10-20 fold over contralateral values. Typical ODC activities in ipsilateral and contralateral pooled DRG samples were respectively, 5.78 ± 1.0 and 0.34 ± 0.04 pmol 14CO2 released/h/3 DRG- (x ± SEM, n=4). Systemic administration of single doses of either vincristine (1 mg/kg) or vinblastine (5 mg/kg) immediately prior to axotomy attenuated ODC induction in ipsilateral DRG by 30% and 47%, respectively. Maximum inhibition of ODC induction occurred at the 5 mg/kg dose level. Vinblastine (0.1-1 mM) did not inhibit ODC activity in vitro, suggesting that direct inhibition of ODC activity in the DRG is unlikely. In summary, agents that disrupt RT attenuate DRG regenerative activity. This suggests that neuronal response to axonal injury is mediated by RT. Interruption of this mechanism may be etiologically linked to the distal axon degeneration which follows repetitive exposure to vinca alkaloids. Supported by DH 02085, NS 19611.


Axonal injury elicits a perikaryal response (the retrograde reaction) characterized by dramatic hierarchical shifts in de novo synthesis, particularly involving enzymes supporting synaptic transmission. A sensitive neurohistochemical method which focused on decreasing levels of de novo acetylcholinesterase (AChE) synthesis following axotomy was developed as a model for investigating chemically-induced neuropathies. Hypoglossal nerves were sectioned unilaterally and rats were given DFP, 2.5 mg/kg i.m., 48 hr post-axotomy to completely and irreversibly inhibit pre-existing AChE. Eight hr post-DFP, the level of de novo AChE synthesis in individual hypoglossal cell bodies was determined histochemically and quantitated by microdensitometry. A decrease in de novo AChE synthesis occurred between 24 and 48 hr postoperatively. In contrast, total AChE levels (pre-existing plus newly synthesized AChE) in non-DFP treated rats could not be demonstrated until 72-96 hr. The de novo AChE method provides a sensitive index of neuronal remodelling following traumatic insult. Supported by NS-23325.

ELEMENTAL DISTRIBUTION IN RAT DORSAL ROOT GANGLION CELLS AND IN INJURED AXONS. R. M. Lofachtin, J. Lowery, J. Elchberg and A. J. Saubermann. University of Houston, College of Pharmacy, Houston, TX.

Based on previous studies of cell injury (Trump et al., SEM III:1-14, 1979) it is possible that neurotoxins such as acrylamide and 2,4-hexadiene cause axonopathy by perturbing ion homeostasis. Since nerve damage induced by these agents is associated with perikaryal chromatolytic-type changes, the subcellular distributions of Na, F, S, Cl, K and Ca were determined in control rat (n=7) dorsal root ganglion (DRG) cells by x-ray microprobe analysis. Four cellular compartments (cytoplasm, mitochondria, nucleus and nucleolus) were examined using ultrathin, frozen, unfixed DRG sections. In all intracellular compartments the concentrations (mmol element/kg dry wt) of Na, S and Cl were relatively low whereas Ca was not detectable. The levels of P were highest in the cytoplasm and nucleolus with lower levels of this element found in mitochondria and nucleus. The nucleus exhibited highest concentration of K whereas nucleolus, cytoplasm and mitochondria had, in descending order, lower levels of K. To study the effects of injury on elemental distribution, sciatic nerves (n=4) were subjected in situ to axonotmesis. Axoplasmic concentrations of Cl and Na increased dramatically (3 and 6 fold respectively) while K levels decreased (55%). Mitochondria exhibited similar changes in these elements but also Ca and P levels were markedly elevated. In sciatic nerves from streptozotocin-treated rats (20 wks diabetic) the only observed elemental change was a sharp decrease (50%) in axoplasmic and mitochondrial K. These data demonstrate the elemental compartmentalization of DRG cell bodies and the characteristic changes in subcellular elemental distribution which occur in response to injury. Supported by NIH Grant ES0 3830-01.


In experimental animals, systemic administration of DOX produces neuronal cell death in the dorsal root ganglia (DRG). However, DOX is also accessible to CNS neurons by retrograde axonal transport from peripheral target organs. DOX (10 mg/kg) was injected into the medial gastrocnemius muscle of rats, and neurotoxicity examined 2-6 weeks later. Retrograde delivery of DOX to the DRG and anterior horn cells (AHC) by 2 days was confirmed by fluorescence microscopy. In contrast to systemic administration, no degeneration of neuronal perikarya was observed. However, axonal degeneration occurred, often at very proximal locations. Neurofilamentous axonal swellings were observed proximally at 4 weeks and more distally at 6 weeks; occasional swellings were found to contain membranous material. Morphometric analysis at 6 weeks revealed the presence of some atrophic sensory fibers in the DRG. Segmental demyelination was also noted in the sciatic nerve and roots. The results suggest that sublethal neuronal injury by DOX can result in axonal alterations without degeneration of the neuronal perikaryon. Supported by NS-23325 and ES-04078.
Organophosphate (OP) neuropathy is associated with degeneration of peripheral nerves and spinal tracts of hens given a single dose of OP which increases ages >70% of Neuropathy Target Esterase (NTE). A dose of DBDCVP (1 mg/kg sc) exceeded this threshold in brain (B), spinal cord (SC) and sciatic nerve (N), induced a typical OP neuropathy with distal axon degeneration both in N and in ventral and dorsal-lateral columns of lumbarosacral and cervical SC, respectively. Hens treated with 0.75 mg/kg sc DBDCVP, in which NTE inhibition was <70% in N and >80% in SC, developed a spastic/paralytic gait without clinical evidence of peripheral neuropathy. The characteristic pattern of axonal degeneration was present in SC, while N was essentially unaffected. A dose of DBDCVP (0.188 mg/kg sc) which caused >60% NTE inhibition in N and SC and >75% in B, produced no clinical abnormalities. This discrepancy in the pattern of NTE inhibition is unlikely to be related to regional differences in NTE sensitivity since in-vitro legs of DBDCVP for B, SC and N NTE were comparable (4.0, 20 min, 37°C). Other possible explanations include differential distribution or destruction of DBDCVP. These data support the idea that the OP target NTE is located in the axon. It is apparent that B NTE may be an imperfect monitor for enzyme activity in parts of the nervous system where OP-induced axonal degeneration actually develops. Supported by NS 19611 & Italian Ministry of Education.


Chlorpyrifos (0.0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate) was evaluated for potential to cause subchronic organophosphate-induced delayed neurotoxicity (OPIDN). An earlier study showed a single oral dose LD50 in mature unanesthetized chicken hens of 50 mg chlorpyrifos/kgBW (95% confidence limits 30 and 83.4 mg/kgBW). To evaluate for OPIDN, laying chicken hens (10/group) were gavaged with chlorpyrifos in corn oil at dose levels of 1, 5, or 10 mg/kg body weight/day, 7 days/wk, for 13 wks. Tri-ortho-cresyl-phosphate (TOCP), 10 mg/kgBW/day, was the positive control; corn oil was the negative control. Central and peripheral nervous system tissues from all positive and negative control birds and the chlorpyrifos high dose (10 mg/kgBW/day) group was examined microscopically. Birds at 10 mg chlorpyrifos/kgBW/day lost weight, and some developed transient acute organophosphate toxicity. Birds given chlorpyrifos showed no clinical signs or histopathologic lesions characteristic of OPIDN. Birds given TOCP developed both the clinical signs and histopathologic lesions of OPIDN. It was concluded that under the conditions of the study chlorpyrifos did not induce OPIDN.
199 EFFECT OF DELTA-9-TETRAHYDROCANNABINOL ON RAT NEUROBLASTOMA CELLS. E.M. Mishkin, P.J. McHermey and G.A. Cabrall*. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA. Sponsor: A.E. Munson

Delta-9-tetrahydrocannabinol (Delta-9-THC) is the major psychoactive component of marijuana. Thus, the objective of this study was to determine the effect of the drug on the growth kinetics and morphology of nerve cells using rat B103 neuroblastoma cells as a model. Delta-9-THC in doses ranging from $10^{-7}$M to $10^{-4}$M inhibited cellular growth in a dose-dependent fashion. This inhibition was correlated with alterations in cell morphology which included rounding of cells, retraction of neurites, blebbing of the cell surface, and exfoliation of the plasma membrane. Cytoplasmic alterations included distension of the endoplasmic reticulum and Golgi apparatus, and macrovacuolization. These changes were accompanied by cytoskeletal reorganization in the absence of alteration in total cytoskeletal protein. Autoradiography using $^3H$-Delta-9-THC demonstrated that the drug was confined to the cytoplasm and often associated with macr vacuoles. These results suggest that Delta-9-THC targets cellular membranes thereby altering neuroblastoma cell growth and behavior.

220 EFFECT OF PHENOBARBITAL PRETREATMENT ON THE GUINEA PIG MODEL OF HALOTHANE-ASSOCIATED HEPATIC NECROSIS. R.C. Lind, A.J. Gandolfi, and B.R. Brown, Department of Anesthesiology, University of Arizona, Tucson, AZ

A guinea pig model of halothane (C₆H₉BrCl; H) hepatotoxicity was evaluated for the influence of enhanced metabolism via phenobarbital (PB) pretreatment on the degree of resulting hepatic injury. Outbred male Hartley guinea pigs were pretreated with 1 mg/ml PB in their drinking water for 6 days. Control and PB-treated animals were exposed to 1% v/v halothane for 4 hr in either 21 or 80% O₂. PB treatment increased hepatic microsomal CYP P-450 levels by 50% and enhanced H metabolism as indicated by increased plasma trifluoroacetic acid (2.7x), bromide ion (2.4x) and fluoride ion (1.5x) levels following H exposure. PB-treated animals developed significantly less hepatic injury following H exposure as indicated by plasma ALT at 48 hr post (H only = 360 ± 415, PB + H = 47 ± 29, x ± SD, p<0.05, N=19 in each group) and by histopathologic evaluation of hepatic tissue (H only; 9/19 with extensive centrilobular necrosis, PB + H; 1/19). Future studies with other PB-like inducers and other hepatotoxins will be required to determine if the PB-related hepatoprotective effect is unique in this animal model.

(NIH AM 5715)

221 SEX AND STRAIN SPECIFICITY IN HEPATOTOXICITY OF PYRAZOLE IN MICE. Lucy M. Anderson, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick, MD

Pyrazole is a potent inhibitor of alcohol dehydrogenase but also an inducer of cytochrome P450 enzymes acting on N-nitrosodimethylamine (NDMA) demethylation activity was measured in 9000g supernatants of liver homogenates from control and pyrazole-treated mice, with 2 mM NDMA as substrate and formaldehyde as the measured endproduct. Basal levels of this enzyme activity were slightly higher in the P and CBA males (16.1 ± 1.1 and 13.8 ± 1.3 nmoles CHD/mg protein/20 min, respectively) compared with females (11.0 ± 4.3 and 5.4 ± 0.8 for P and DBA females) and males of other strains (10.2 ± 1.9 for DBA/2, 9.9 ± 1.3 for C3H, and 11.8 ± 2.4 for SJL). Induction of NDMA demethylase by pyrazole at 200 mg/kg was comparable in all strains and in both sexes and did not correlate well with toxicity, e.g., induced levels of 26.4 ± 2.2 in CBA males, 18.4 ± 1.8 in SJL males, and 21.8 ± 6.6 in P females. Pyrazole hepatotoxicity and induction of cytochrome P450 may involve different mechanistic pathways.
Periportal hepatic necrosis caused by allyl alcohol (AA) is a consequence of metabolism to the reactive aldehyde acrolein by alcohol dehydrogenase. Subsequent metabolism of acrolein to acrylic acid by aldehyde dehydrogenase (ALDH) is a potentially important detoxification reaction. In the present study in male Wistar rats, calcium cyanamide (CC, 14 mg/kg, p.o.) a relatively specific inhibitor of ALDH, potentiated the acute in vivo hepatotoxicity induced by AA (25 or 37.5 ul/kg, i.p.), determined by histopathology evaluation, plasma markers of hepatic damage and biochemical changes in the liver at 4 and 24 hours post-AA. In suspensions of isolated rat hepatocytes incubated up to 2 hours, cyanamide at concentrations of 5 to 100 μM caused a dose dependent increase in the toxicity of 125 μM AA determined microscopically and by the lactate dehydrogenase latency test. Calcium cyanamide in vivo and cyanamide in isolated hepatocytes were not toxic in the absence of AA. In conclusion, these results are consistent with the hypothesis that AA is metabolized to a reactive metabolite (acrolein) which is subsequently detoxified, at least in part, by ALDH.

Stimulation of H₂O₂ generation by fatty acids: effect of chain length. Jeffrey A. Handler and Ronald G. Thurman, Dept. Pharmacology, University of North Carolina at Chapel Hill, NC

Peroxisomal β-oxidation of fatty acids generates H₂O₂ and increases ethanol (E) metabolism significantly via catalase in the presence of 4-methylpyrazole (4MP), an inhibitor of alcohol dehydrogenase. Rates of fatty acid-stimulated E uptake, however, are much higher than reported rates of H₂O₂ generation. The purpose of this study, therefore, was to measure H₂O₂ generation and E uptake in perfused livers in the presence of fatty acids of varying chain length. H₂O₂ generation was determined from the time necessary for catalase-H₂O₂ measurement spectrophotometrically through a lobe of the liver to return to basal values after the addition of a known quantity of methanol or E in the presence of 4MP. Rates of H₂O₂ generation and 4MP-insensitive E uptake were similar with all fatty acids studied. Basal rates of H₂O₂ generation and 4MP-insensitive E uptake were 9 to 17 μmol/g/hr. The medium chain, 2-methyl palmitate, which stimulated H₂O₂ generation maximally to 81 μmol/g/hr (100%). Stimulation of H₂O₂ generation diminished as the chain length was decreased (8:0 = 60%; 6:0 = 20%) or increased (14:0 = 90%; 16:0 = 90%; 18:0 = 30%; 18:1 = 60%). It is concluded that H₂O₂ generation from fatty acids occurs at high rates with a chain-length specificity similar to that reported for isolated peroxisomes. Thus, 4MP-insensitive E uptake may be a new way to study H₂O₂ generation from toxic chemicals under nearly physiological conditions in the intact liver.

Effect of Diquat on the distribution of iron in rat liver. D.W. Reif, L.P. Seales, C.E. Thomas and S.D. Aust. Dept. of Biochemistry, Michigan State University, East Lansing, MI

The generation of organic and oxygen free-radical species in vitro has been shown to cause release of iron from ferritin resulting in the promotion of lipid peroxidation. It is suggested that a similar mechanism occurring in vivo may result in cellular damage. We report that thirty-six hours following an intraperitoneal injection of a non-lethal dose of diquat, male Sprague-Dawley rats (n = 10) showed no significant difference in total hepatic ferritin levels versus controls (n = 12). However, total ferritin-bound iron decreased by 30 nmol Fe/g of liver. Additionally, hepatic levels of low molecular weight chelatable iron (LMWC-Fe) species increased by 30 nmol Fe/g of liver (p <.01) in treated animals, a four-fold increase in LMWC-Fe levels above control. These results suggest that diquat toxicity may be associated with the lipid peroxidation resulting from the release of iron from ferritin, and that iron release/lipid peroxidation may be a process common to other free radical mediated toxicities. Supported by NIH grant GM33443.


Approximately ten chemicals are known to increase hepatic peroxisomes and to increase the incidence of liver cancer in Fisher 344 rats. To assess this relationship, F-344 rats (both sexes) were fed DINP (0.1 - 2.0%) for 13 weeks or fed DINP (0.03 - 0.6%) for 2 years. Electron microscopy was used to semi-quantitate the induction of hepatic peroxisomes in 2 rats per sex per dose. In the 13 week study, peroxisome proliferation was shown to be dose dependent. A no observed effect level was observed at 0.5%, a weak effect observed at 0.5% and a clear induction effect was observed at ≥ 1.0% of DINP. In the 2 year study, no treatment related induction of peroxisomes was observed in either male or female rats at any dose. Chronic treatment of DINP at the estimated maximum tolerated dose did not increase the incidence of liver tumors as compared to control rats. In conclusion, this study provides clear evidence that DINP, a weak peroxisomal proliferating agent, produces no significant increase in liver cancer at chronic doses producing minimal or no effects on hepatic peroxisomes in rats.
TRIDIPHANE (2-(3,5-DICHLORO)-2(2,2,2-TRICHLORO-ETHYL)OXIRANE), A HERBICIDE PEROXISOME PROLIFERATOR
D.F. Moody and B.D. Hammock, Univ. of California, Davis, CA

Tridiphane is a newly registered epoxide herbicide which is
synergistic for atrazine via inhibition of plant glutathione-S
transferase (GST). Tests in mice treated with tridiphane to
assess its action on mammalian enzymes involved in epoxide
metabolism demonstrated an induction of liver cystolic epoxide
hydroxylase (EH). Increases in this enzymatic activity have
previously been limited to peroxisome proliferators. In mice
injected with 250 mg/kg tridiphane for 3 days (ip in corn
oil) increases of pepsinogen were seen in hepatocytes
examined by electron microscopy. The following changes
(relative to controls) were noted in liver: relative liver weight:
1.65; mitotic index, 30.08; cystolic acetyl transferase activity:
3.16; glutathione resistant oxidation of palmitoyl CoA: 2.28;
microsomal EH: 1.40; cystolic EH: 1.47; serum cholesterol:
0.96; and serum triglycerides: 0.30. These changes were
similar to those seen in mice receiving 500 mg/kg clofibrate,
the prototypical peroxisome proliferator. Dissimilar responses
to the 2 compounds were found for liver cystolic GST's.

Tridiphane increased the conjugation of 3 and decreased the
conjugation of 2 substrates, while clofibrate decreased or had no
effect on the conjugation of the 5 compounds tested. These
findings extend the association between increases in cystolic EH
and peroxisome proliferation. The association of the latter with
carcinogenesis warrants close attention to the chronic effects of
tridiphane. (Supported in part by USPHS Grant R01-ES02710-
06. Dr. Moody's current address is: Center for Human
Toxicology, Univ. of Utah, Salt Lake City, UT 84112.)

A COMPARISON OF PROTECTION AGAINST ANIT
INDUCED HEPATOTOXICITY OFFERED BY AN E1
AND AN E2 PROSTAGLANDIN. C.P.
Chengells, D.C. Dodd, T.M. Dennis.
Product Safety Assessment, G.D. Searle
Research and Development, Skokie, IL

The purpose of these experiments was to
determine if 16,16-dimethyl PGF2α and
misoprostol, a PGF1 derivative, share in
common the ability to afford protection against a-naphthylisothio-
cyanate (ANIT) induced hepatotoxicity. Each prostaglandin was given at dosages of
20, 60 and 200 mg/kg sc 24, 18 and
0.5 hrs before and 6 hr after ANIT 30
mg/kg po. There were 10 male
rats/dosage group and appropriate
control groups were included. All
animals were sacrificed 24 hrs after
ANIT. ANIT caused the expected
increases in serum alanine and
aspartate aminotransferases, alkaline
phosphatase and total bilirubin. This
was accompanied by severe necrotizing
cholangitis and increases in liver
weights. These effects were attenuated at all 3 dosages of the E2 but not the
E1 prostaglandin. Hence, the property
of offering protection against ANIT
induced hepatotoxicity may have very
specific structural requirements.

TOXICITY OF MICROCYSTIN FROM MICROCYSTIS AER-
UGINOSA IN RATS: MORPHOLOGIC AND SERUM CHEMISTRY
ALTERATIONS. S.B. Hooser, V.R. Beasley, W.
Carmichael, W.M. Haschek, College of Veterinary
Medicine, University of Illinois, Urbana, IL,
and Wright State University, Dayton, OH.

A cyclic heptapeptide hepatotoxin produced by
strain 7820 of Microcystis aeruginosa was given in
to 50 rats. A very sharp dose-response was
seen. Clinically non-affected animals had no
lesions, while all clinically affected animals
died with severe hepatic necrosis. In both
males and females the approximate LD50 was 120
μg/kg and survival time was 18 to 32 hours.
Male rats given 150 μg/kg were killed at intervals
from 10 min to 24 hr. In the liver, lesions at 30 min consisted of mild centrilobular
hepatocyte disassociation and degeneration.
Lesions progressed until at 60 min massive
centrilobular and midzonal hepatocyte necrosis
with severe hemorrhage were seen. In pulmonary
vessels, eosinophilic globular debris and infarc-
tions, hepatocytes were present from 1 to 9 hours.
In the kidney, at 18 to 24 hrs many cortical tubules were dilated and contained small
amounts of eosinophilic material while
glomerular capillaries were often filled with
cosinophilic, granular material. ALT was mark-
edly elevated beginning at 40 min. BUN and
creatinine were elevated by 9 to 12 hrs. Serum
glucose decreased markedly over 24 hrs. The
mechanism of toxicity remains to be elucidated.

INHIBITION OF TAUCHELATE (TC) EFFLUX FROM
RAT HEPATIC CANALICULAR MEMBRANE VESICLES
(CMV) BY GLUTATHIONE DISULFIDE (GSSG).
J.C. Griffiths, T.F.M. Akerboom, and H. Bies. Institut
für Physiologische Chemie I, Universität Düsseldorf, Düsseldorf, FRG. Sponsored by J.F. Kehr.

The liver transports anions across both the
sinusoidal and canalicular membranes. In clearing bile acids such as TC, the rate-limiting step is
the excretion across the canalicular membrane.
Perfused liver studies showed that GSSG is specif-
ically transported into bile and that these
oxidants which raise the cystolic GSSG concentration inhibit the
hepatic release of TC (Akerboom et al., J. Biol.
Chem. 259, 5538, 1984). The efflux of preloaded raclalabeled TC from right-side out CMV can be used as an in vitro model (Meier et al., J. Biol. Chem.
259, 10614, 1984).

TC efflux from CMV was inhibited at 2 mM
half-maximal concentration of GSSG. The inhibition of TC transport was transient, and the maximum effect of 50% inhibition (with 5 mM GSSG)
was observed at 15 and 30 sec; by 60 sec, there no longer
was inhibition of TC efflux. Preincubating the CMV with GSSG prior to the initiation of transport for 0,
5 or 30 min at 37°C showed no difference in the
dose-response curve of TC efflux indicating that
covalent binding to membrane proteins may not play a significant role. The inhibitory effect of GSSG
was abolished with the addition of N-ethylmaleimide. Reduced glutathione (5 mM) had no effect on
transport. (Supported by Deutsche Forschungs-
gemeinschaft.)
HEPATOBILIARY FUNCTION IN RATS FOLLOWING SUBCHRONIC EXPOSURE TO AMIODARONE. R.A. Young and H.M. Mehendale. Dept. Pharmacol. and Toxicol., Univ. MS Medical Center, Jackson, MS

Amiodarone (Am), an antiarrhythmic agent, has been implicated in hepatotoxicity. Our objective was to evaluate Am-induced hepatotoxicity in rats. Am in methylcellulose was administered by gavage for 10 days to male Sprague-Dawley rats (225-250 g) at doses of 5, 50, 150, and 500 mg Am/kg (4 rats/group). Controls received methylcellulose only. Additional rats (4/group) were maintained on a 4 week dietary protocol using Am (50 ppm and 1500 ppm) in powdered rat chow. Controls received diet lacking Am. Following treatment, rats were anesthetized and the femoral vein and common bile duct cannulated. This allowed for administration of readily excretable phenolphthalein glucuronide (PG) (3 mg/rat) and collection of bile. Rats administered Am by gavage or in the diet exhibited an impairment of biliary PG excretion without a commensurate cholestatic effect. Serum enzyme levels (SGOT, SGPT, ICD) were not affected by either treatment protocol. Am and the major metabolite, desethylamiodarone, were predominant in bile and liver, but were also detected in other tissues and bile of treated rats. The data suggest Am-induced alteration of hepatobiliary secretory function, which may manifest itself prior to detectable biochemical indices of toxic ity (Supported by ES 07045).


Synthetic mixtures of lead acetate (PbAc), CC14 and monochlorobenzene (MCB) were given orally at three dose levels, once daily for seven days, to male rats. Animals were killed on the eighth day and the effects of treatment on liver, kidney, and hematopoietic system were evaluated. CC14, either alone or with MCB and PbAc, caused decreased weight gain and significantly decreased adrenal, testes/ lung, spleen and kidney/brain weight ratios. No significant hematological changes were found in any group. All groups treated with CC14 exhibited elevated SGPT and alkaline phosphatase activities; these effects were neither enhanced nor antagonized by other compounds. BUN was elevated in all CC14-treated groups. MCB enhanced this action but PbAc appeared to antagonize the enhancing effect of MCB. Both PbAc and CC14 decreased red cell ALAD activity. The action of the two chemicals was generally additive. Light microscopic examination showed mild hepatic injury in all CC14-treated rats. These studies suggest that these common environmental chemicals, when given at low doses, do not show marked synergistic or antagonistic effects. (Supported by the NSF Industry/University Cooperative Center for Research in Hazardous and Toxic Substances).

CYCLOHEXIMIDE IN METHYL ISOBUTYL KETONE POTENTIATION OF TAUROLTICHOCOLIC ACID-INDUCED CHOLESTASIS. L. Dahlström-King and G.L. Plaa, Dép. de pharmacologie, Université de Montréal, Montréal, Québec, Canada.

Methyl isobutyl ketone (MIBK) potentiates the hepatocarcinogenic properties of haloalkanes and the cholestatic response to tauroliothicolic acid (TLC) or a combination of Mm-bilirubin. Induction of microsomal enzymes by ketones plays a major role in the potentiation of the hepatocarcinogenic properties of haloalkanes, but the role of protein synthesis in augmenting the effect of cholestatic agents has not been investigated. Groups of six male Sprague-Dawley rats were treated by gavage with corn oil or MIBK (7.5 mmol/kg) for 3 days; TLC (10 and 15 mg/kg) was injected iv 18 hr after the last gavage; bile flow was measured over 15- or 30-min periods. Cycloheximide (C6) was injected (0.5 and 1.0 mg/kg) ip along with the last gavage prior to TLC. MIBK increased the cholestasis by ~25% with TLC (10 mg/kg). C6 injected ip in conjunction with the last gavage diminished the cholestatic response. However, the reduction of the cholestatic response in MIBK-pretreated animals was less than that observed in corn oil-pretreated animals. This suggests that MIBK-induced increases in hepatic protein synthesis may be involved in MIBK potentiation of TLC-induced cholestasis. (Supported by MRC, Canada and FCAR, Québec, Canada)


Complex waste mixtures were lethal and hepatotoxic in male F344 rats exposed by gavage and evaluated 24 hours later. Seven of the 10 treated samples produced death at dosages ranging from 1 to 5 ml/kg body weight, with 4 of the 7 causing 100% mortality at 5 ml/kg. These wastes have been partially chemically characterized and the lethality could not be explained on the basis of the concentrations of the identified organic compounds. Eight of the 10 samples were hepatotoxic based on histopathological examination; 6 of the 8 were centrifugal toxicants and two were pericellular toxicants, indicating different mechanisms of injury. The waste varied greatly in the severity of hepatotoxicity, with one sample producing minimal centrifugal vascular degeneration at 5 ml/kg and another causing marked centrifugal necrosis at 1 ml/kg. Increased liver weights and increased values of serum alanine transaminase, aspartate transaminase, lactic dehydrogenase, bilirubin, and alkaline phosphatase were also observed. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
SK&F 101772 (bis-[1,2-bis(diphenylphosphine)ethane] gold(I) chloride is an antineoplastic agent with activity in a variety of in vivo and in vitro tumor models. Administration of a single i.v. dose of SK&F 101772 to male beagle dogs (272 mg/m^2) markedly elevated serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities within 48 hrs. Histologic analysis revealed multiple areas of necrosis distributed throughout all zones of the liver. Suspensions of hepatocytes isolated from naive male beagle dogs rapidly lost viability (measured as lactate dehydrogenase leakage), reduced glutathione (GSH) and ATP when exposed to SK&F 101772 concentrations greater than 20 uM. The loss of ATP preceded cell death whereas GSH depletion paralleled cell death. SK&F 101772 (20 uM) markedly stimulated hepatocyte oxygen consumption while 100 and 200 uM markedly inhibited cellular respiration. Both the stimulation and inhibition of hepatocyte oxygen consumption preceded cell death. These data demonstrate that SK&F 101772 is hepatotoxic to dogs. SK&F 101772-induced liver injury may be the result of altered mitochondrial function.

In order to establish a tissue bank for human in vitro liver xenobiotic biotransformation and toxicological studies, a system was developed for freezing liver slices. Liver tissue was procured from the Arizona Organ Bank or from resections and placed in cold Sack's solution. The tissue was cooled and mechanically precision-cut to 250 um slices. Slices were cryoprotected in 10% dimethyl sulfoxide/fetal calf serum (FCS) solution then frozen by either submerging in liquid N2, or slow freezing (1°C/min), stored, and later thawed in 37°C FCS. Control and cryopreserved slices were maintained for up to 6 hr in dynamic organ culture. Viability of cryopreserved tissue was assessed by intracellular K+ retention, protein synthesis and histological evaluation. Biotransformation activities were measured by parent disappearance and metabolite appearance for standard substrates. These experiments showed that the tissue retained 60-80% of its control viability and was actively biotransforming xenobiotics. The intrinsic variation between tissue specimens and the effect of cryopreservation on the multitude of drug metabolic enzymes involved will require extensive comparative studies. (Supported in part by NIEHS NO1-ES-55112)

A new method for obtaining metabolically active tissue slices for species comparisons has been developed. Fresh liver tissue blocks were embedded in soft agar and mechanically sliced at a thickness of 0.375 mm using a McIlwain tissue chopper. Slices obtained in this manner consumed oxygen and expired C-14 carbon dioxide from C-14 butyric acid linearly for up to four hours. The rate of oxygen consumed and C-14 carbon dioxide evolved was proportional to protein and tissue wet weight. The effect of media composition on oxygen consumption and non-protein sulfhydrl levels will be reported. The respiration, oxidation of butyric acid and xenobiotic metabolism have been compared using liver slices from rats and dogs. These results indicate that embedding of the tissue prior to slicing yields uniform liver slices that are metabolically active and suitable for species comparisons. Furthermore, the method can be readily adapted to non-hepatic tissues.

We have developed methodology to cut tissue slices of defined size and thickness and under quasi-physiological conditions. These slices, when incubated under conditions which assure optimal exposure to oxygen and nutrients, stay viable for many hours and can be used as tools in biochemical toxicology. Both biochemical conditioning of the slices as well as measurement of various biochemical parameters is possible. On the other hand, histology, immuno- and enzyme histochemistry and autoradiographic studies allow for localization of damage within the tissue. These possibilities are illustrated for halogenated benzene toxicity in the liver which can be demonstrated to be more pronounced in the centrilobular region by a combination of vital staining procedures binding studies followed by autoradiography histology and histochemical analysis. This demonstrates the potential suitability of slices for complex studies of cell specific toxicity as an alternative to the isolated perfused organ and in vivo studies.
THE EFFECT OF DIFFERENT CULTURE MEDIA ON THE TOXI-
CITY OF MONOCHLOROBENZENE, AND THE DICHLOROBE-
ZENES. R.L. Fisher, S.M. Wishnie, L.G. Sipes, A.J. Gandolfi, C.L. Krundieck and K. Brendel. Departments of Pharmacology and Toxicology, Arizona Health Sciences Center, Tucson, AZ.

Rat liver slices were incubated in dynamic organ culture using different types of incubation media and the hepatotoxicants, monochlorobenzene (MCB), 1,2 dichlorobenzene (1,2 DCB), 1,3 dichlorobenzene (1,3 DCB) or 1,4 dichlorobenzene (1,4 DCB). 1.0 M of these hepatotoxicants produced no toxicity when incubated with Waymouth's medium with 10% fetal calf serum (FCS) and glucose but were substantially toxic when incubated in Krebs-Henseleit buffer supplemented with glucose. In order to identify what components in Waymouth's medium reduced the toxicity of these compounds, the Krebs-Henseleit buffer was supplemented with FCS, bovine serum albumin (BSA), vitamins, or amino acids. FCS completely inhibited the toxic response produced by all of the compounds. 1.0% BSA significantly reduced the toxicity responses produced by MCB and 1,4 DCB but had no effect on 1,2 DCB or 1,3 DCB. Amino acids significantly decreased the toxicity induced by 1,3 DCB and, to a lesser extent, 1,2 DCB. Vitamins had no effect on the hepatotoxicity of MCB or any of the DCBs. These results imply that the mechanisms of toxicity are different for these chlorobenzenes. Determining the factors which modulate the toxicity of chlorobenzenes should help elucidate their mechanisms of hepatic injury. (Supported in part by NIEHS 1ES-55112)

COVALENT BINDING OF 14C-BROMOBENZENE METABOLITES TO LIVER SLICES IN VITRO. G.M. Becker, R.L. Fisher, L.G. Sipes and K. Brendel. Departments of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

Bromobenzene (BB) is known to induce centrilobular hepatic necrosis. It was uncertain whether the susceptibility of the centrilobular zones of the liver to BB toxicity was due to increased formation of reactive metabolites in this zone or to differences in blood flow or other factors. BB has previously been shown to induce cellular necrosis, Kt and lactate dehydrogenase release from precision-cut, 300 μm thick hepatic slices in vitro. To determine if reactive intermediates were formed in vitro, male Sprague Dawley rats were induced with phenobarbital and later liver slices cut. Slices were incubated in stainless steel mesh rollers in vials at 37°C in Waymouth's medium, and 14C-BB added at 0.25 to 1.0 mM. Slices were removed, Soxhlet-extracted with methanol and acetone for 3 days to remove non-covalently bound radioactivity and counted. Samples for autoradiography were fixed, extracted for one week with ethanol, embedded, sectioned and autoradiographed. 14C-BB metabolites bound to slices in a dose- and time-dependent manner. Moreover, the binding was 2.7 times greater in centrilobular zones than peripheral zones. This suggests that centrilobular susceptibility to BB toxicity may be due to the increased formation of BB-reactive intermediates in this zone. (Supported in part by NIEHS 1ES-55112)
Methodology has been developed to cryopreserve precision-cut porcine liver slices. Livers were harvested from weanling domestic pigs (9-12 kg), placed in cold Sack's solution, cored and cut mechanically into slices of 250 μm thickness. The slices were then exposed in batches to the cryoprotectant, frozen in individual stainless steel wire mesh baskets, placed in stainless steel storage vessels under liquid nitrogen, and thawed in 37°C culture medium. Our experiments have shown several conditions to be equally effective. Fast freezing and thawing (in excess of 1000 °C/min) and slow freezing (0.5-5°C/min) combined with fast thawing have resulted in significant retention of viability as measured by intercellular potassium content, protein synthesis and leakage of cytosolic enzyme markers. The effects of dimethyl sulfoxide (DMSO), proline, trehalose, glycerol and polyvinylpyrrolidone as cryoprotectants have been compared at various concentrations and 10% DMSO was found to offer effective cryopreservation. From several media and sera examined, fetal calf serum provided optimal protection of the slices. Compared to other laboratory animal tissues, pig liver slices seem to offer the first viable model for liver slice cryopreservation. (Supported in part by NIEHS N01-ES-55112).

PROTEIN AND DNA SYNTHESIS IN PRIMARY CULTURES OF POSTNATAL RAT HEPATOCYES


We have extended the validated assay (Norbury et al., The Toxicologist 6:115, 1986) for measuring cytotoxicity in vitro to include tests for protein and DNA synthesis. Primary cultures of postnatal rat hepatocytes were exposed to various concentrations of chlorpromazine (CPZ), erythromycin estolate (EE), and erythromycin stearate (ES). Protein and DNA synthesis were measured by incorporation of 3H-leucine and 3H-thymidine, respectively. The release of lactate dehydrogenase and aspartate aminotransferase was also determined. CPZ and EE significantly inhibited both protein and DNA synthesis at concentrations of 20 to 40 μM, which were below those at which enzyme release occurred. ES inhibited DNA synthesis without significant enzyme release at the highest concentration tested, 160 μM. Protein synthesis was only marginally affected. These data suggest that protein and DNA synthesis are sensitive and reliable indicators of toxicity in vitro.

MIREX DECREASES THE UPTAKE OF 3β-ESTRADIOL-17β-D-GLUCURONIDE (E217G) AND 3β-TAUROCHOLATE (TC) INTO ISOLATED FEMALE RAT HEPATOCYES (1RH).

Steve Teo and Mary Yore, Dept. Pharmacol. and Grad. Ctr. for Toxicol. Univ. KY, Lexington, KY.

The insecticide mirex decreases biliary excretion of a variety of drug metabolites. In order to determine if the uptake of organic anions across the basolateral membrane is affected, the uptake of E217G, a cholesstatic steroid, and TC, a bile acid, was studied in mirex treated (50, 25 & 12.5 mg/kg/day p.o. for 3 days) and untreated (corn oil) Sprague-Dawley rats. 1RH were prepared 2 days after the last dose and viability (88-95%) determined by trypan-blue exclusion. Two (0.5 & 50 μM) concentrations of E217G were used since high and low affinity carriers have been identified in its carrier-mediated uptake. Mirex (50 mg/kg) significantly decreased (p<0.01) the uptake (pmoles/min/mg protein; Mean ± SEM; n=4-6) of E217G (0.5 μM) from 43.8±5.00 to 13.2±1.31, of E217G (50 μM) from 563±65.4 to 327±32.7, and of TC (10 μM) from 171±21.8 to 85.6±16.2. Uptake of 0.5 & 50 μM E217G and 10 μM TC were also significantly reduced by 35, 27 & 42% respectively at 12.5 mg/kg mirex. The reduction of E217G uptake was more profound at the low (0.5 μM) concentration at all 3 mirex doses. These data indicate that mirex inhibits uptake of E217G primarily at the high-affinity carrier and is also a potent inhibitor of TC uptake.

The NRDA is based on the lysosomal incorporation of the dye by viable cells in culture. Toxicity due to chemical exposure is correlated to the amount of dye extracted from the remaining viable cells as measured by visible spectrophotometry. This study compared the applicability and sensitivity of the NRDA against conventional in vitro toxicity measures such as trypan blue dye exclusion and alanine and aspartate transaminase (ALT, AST). Cells from untreated rats were exposed to carbon tetrachloride (CT), chloroform (CF), chlorobenzene (CB) and selenite (Se) at 2.5 μM to 10 mM for 24 hr. All measures were in agreement in ranking chemical toxicity: Se > CT > CB > CF at 24 hr. However, the NRDA was a more sensitive indicator of toxicity at lower levels of chemical than other measures tested. CT cytotoxicity was also monitored over 72 hr at 1.0, 2.5 and 5.0 mM. ALT and AST activities in medium did not accurately reflect CT cell damage at > 24 hr, probably due to enzyme degradation, while NRDA values remained a consistent indicator of toxicity throughout the 72 hr period. Thus, the NRDA is a rapid, inexpensive and sensitive measure of in vitro chemical hepatotoxicity. (This study does not necessarily reflect EPA policy).


SKF 101772 (bis-[1,2-bis(diphenylphosphine)-ethene]gold(I)chloride), an experimental antineoplastic agent, is hepatotoxic in vivo as well as cytotoxic to hepatocyte suspensions. The earliest manifestations of SKF 101772 toxicity to isolated hepatocytes include ATP depletion, stimulation of respiration and extensive blebbing of the plasma membrane. These changes are eventually followed by LDH leakage and cell death. ATP was decreased to 60% of control after 30 min exposure to 10 μM SKF 101772 whereas LDH leakage was not significantly different from control until 120 min. Fructose (50 mM) resulted in a pattern of ATP depletion similar to SKF 101772, however, the cells did not leak LDH and appeared morphologically normal. In addition, both compounds (15 μM 101772 or 50 mM fructose) produced a rapid and extensive activation (2-4 fold) of phosphorylase A. These data suggest that ATP depletion is not the single, primary biochemical event responsible for SKF 101772 toxicity. Furthermore, an increase in cytosolic calcium, as estimated by phosphorylase A activation, also may not play a major role in organo-gold-induced cell lethality.

BOVINE HEPATOCYTE CULTURES - A TOOL FOR THE MEASURE OF XENOBIOTIC CYTOTOXICITY AND METABOLISM IN LARGE ANIMALS. J.A. Wnieweski and L.R. Shull. Dept. Environ. Tox., Univ. of Calif., Davis, CA

Isolated hepatocytes from laboratory animals are routinely used to study xenobiotic cytotoxicity and metabolism. More recently, we have harvested hepatocytes from larger, non-laboratory animals, e.g. cattle, to assess their potential as a tool for such research in large animals. In the present study, hepatocyte cultures from calves were exposed to 0, 10 and 100 mM carbon tetrachloride (CCL4), over a 16-hr time period. Lactate dehydrogenase (LDH) leakage, a common indicator of cytotoxicity in rat hepatocytes, was too low to be a useful indicator in bovine cells. Likewise, no evidence of lipid peroxidation, as measured by the thiorbarbituric acid reaction with malondialdehyde, was observed. However, a dose-related decrease was evident in ATP content, ochreoscyamin 0-deethylase and 7-hydroxyochrousamin glucuronidation, even though the control levels of cytochrome P-450-linked activities decreased over time. Although common indices of CCL4 cytotoxicity in rat hepatocytes, e.g. LDH leakage and lipid peroxidation, were not evident in bovine cells, CCL4-induced effects on metabolic activities indicate that bovine cells are capable of bioactivating xenobiotics. However, different methods may have to be used to assess the extent of any toxic effects. Based on the present study, we feel bovine hepatocytes have great potential as a tool to measure xenobiotic metabolism and cytotoxicity.


SKF 101772 (bis-[1,2-bis(diphenylphosphine)-ethane]gold(I)chloride) is an antineoplastic agent that is hepatotoxic to dogs in vivo and is highly cytotoxic to dog hepatocytes in vitro. Preliminary data have indicated that mitochondria may be targets of SKF 101772 injury. In order to define the mechanism of action of SKF 101772-induced cytotoxicity in more depth, studies were carried out with isolated rat hepatocytes. Suspensions of isolated rat hepatocytes were approximately 4-fold more susceptible than dog hepatocytes to SKF 101772-induced injury. Rat hepatocytes rapidly lost ATP in a concentrated-related fashion when exposed to SKF 101772 concentrations greater than 12 μM. SKF 101772 also caused a marked stimulation of cellular respiration at 12 and 25 μM. In isolated rat liver mitochondria, SKF 101772 stimulated state 4 respiration and decreased the ADP/O ratio. Gold chloride had similar effects on rat liver mitochondria. These data indicate that SKF 101772 behaves as an uncoupler of oxidative phosphorylation in rat liver mitochondria. Therefore, SKF 101772-induced cell injury, and possibly hepatotoxicity, may be the result of selective uncoupling of mitochondria.
ANTIPROTEASE ACTIVITY ASSOCIATED WITH PROTECTION FROM HEPATOCELLULAR TOXICITY BY GALACTOSAMINE.
J.R. MacDonald and C. White. Dept. of Pathology, School of Medicine, San Francisco, CA

Increased total cellular LDH activity following cytoprotective treatments of galactosamine (GAL)-challenged hepatocytes suggested a stimulated recovery of protein synthetic rates or inhibited degradation. Both possibilities were evaluated in cytoprotection models in rat hepatocyte monolayers. Hepatocyte cultures were established from untreated male, Sprague-Dawley rats and cells challenged with 4 mM GAL in vitro, or from GAL-challenged rats (400 mg/kg IP in prior to cell isolation). Cytoprotective cysteine treatments (10 uM, 3h post GAL) did not stimulate recovery of protein synthetic capacity measured by pulse labeling cellular protein with 35S-methionine. Exposure of control cells to the antiproteases methylamine (5mM) or leupeptin (300uM), or cysteine (30uM) increased cellular LDH >10% within 4h. Leupeptin (a thio) protease inhibitor, but not methylamine (lysosomal protein degradation inhibitor) inhibited GAL-induced loss of cell viability in culture when added 12h post GAL(4mM) These results suggest that protease activity may be an important late component of toxic cell injury subject to modulation by post-toxicant cytoprotective agents. Further studies are necessary to discern relative roles of lysosomal vs. nonlysosomal proteolytic activity in cell injury and potential mechanisms of inhibition by cytsine. (Supported by NIH grant AM 82943).

INHIBITION OF FATTY ACID OXIDATION IN ISOLATED RAT HEPATOCYTES BY A TETRAZOLE-SUBSTITUTED ALKOXYACYTETONE. P. I. Eshoo and P. S. Foxworthy. Lilly Research Labs., Toxicology Division, Greenfield, IN.

It was recently demonstrated that certain tetrazole-substituted alkoxyacetophenones produced peroxisome proliferation in rodents. Since it has been suggested that induction of peroxisomal β-oxidation may result from alterations in normal fatty acid metabolism, these studies were performed to determine the effect of 4,6-[1-tetrazolo-5-yl]hexylxoyacetophenone (4-THA) on fatty acid oxidation in rat hepatocytes. 4-THA caused a concentration-dependent inhibition of ketogenesis from 0.5mM palmitate and oleate, but had little effect on ketogenesis from oleate alone. Inhibition of ketogenesis was characterized by a decrease in the 8-hydroxybutyrate:acetoacetate ratio. 13CO2 production from 0.5 mM 13C-oleate was increased by 4-THA, but total oxidation (13CO2 + acid-soluble 14C) was decreased. The addition of carnitine up to 4mM did not affect inhibition. Decreasing the concentration of oleate from 0.5mM to 0.2mM increased inhibition. In cells isolated from fasted rats, ketogenesis from 0.5mM palmitate was not inhibited by 4-THA. The data demonstrate that the tetrazole compound is capable of altering fatty acid metabolism in the liver of normal fed rats. Whether this effect is causally related to peroxisome proliferation will require further investigation.

INHIBITION OF CARNITINE PALMITOYL TRANSFERASE I IN RAT LIVER MITOCHONDRIA BY A TETRAZOLE-SUBSTITUTED ALKOXYACYTETONE. P. S. Foxworthy and P. I. Eshoo. Lilly Research Labs., Toxicology Division, Greenfield, IN.

We have recently demonstrated that 4,6-[1-tetrazolo-5-yl]hexylxoyacetophenone (4-THA), a peroxisome proliferator in rodents, inhibits ketogenesis from oleate, but not oleoate, in hepatocytes from fed rats. Accordingly, experiments were done to determine if 4-THA was inhibiting the outer carnitine palmitoyl transferase (CPT-I) in rat liver mitochondria. CPT-I was assayed essentially as described by Gamble and Cook (JBC 250:9516, 1985) using 200 uM 14C-carnitine, and 0.1 mg mitochondrial protein in 0.5 ml. With 50 and 100 uM palmitoyl CoA (Pal CoA) as substrate, 100 uM 4-THA had little effect on CPT-I. At 25 uM Pal CoA and below, inhibition of CPT-I was inversely proportional to the concentration of Pal CoA. 100 uM 4-THA increased the Km for Pal CoA from 16.7 to 73.7 uM. Inhibition of CPT-I by 4-THA was dose-dependent at Pal CoA concentrations of 5-25 uM. The inhibitory activity of 4-THA was independent of carnitine at concentrations of 20 to 500 uM. CPT-I activity was higher in mitochondria isolated from fasted rats than in fed, but the inhibition of CPT-I by 4-THA, studied as a function of Pal CoA concentration, was similar to that of fed rats. The data indicate that 4-THA is a competitive inhibitor of CPT-I with respect to Pal CoA.


Primary rat hepatocytes (PHH) have been used to measure peroxisome proliferation induced by a wide variety of chemicals such as diphenyl ether and hypolipemic drugs. We report here on the response of rodent and primate hepatocytes to several known inducers using cyanide-insensitive palmitoyl CoA oxidation (PCO) as an indicator for peroxisomal enzyme induction. Hepatocytes were isolated by collagenase perfusion from male Sprague-Dawley rats, a cynomolgus monkey, and a male human liver (organ transplant donor) and chemically treated in attached cultures for 24 to 96 hours. Treatment of PHH with 125 uM ciproflurate (CIP) or 750 uM clofibrate (CLO) gave maximal increases in specific activity (nmol/min/mg protein) over DMSO controls (2.5 ml/ml) of 6-fold and 3-fold, respectively, under the assay conditions. Primate hepatocytes exhibited 2.5 fold less activity than PHH 4 hr after isolation. Neither CIP, CLO nor 4,4-((1,3-benzodioxol-5-yl)methyl)amino-benzene acid (DL-940) caused an increase in PCO activity over DMSO controls in primate hepatocytes. These studies indicate that in PHH CIP is a much more potent inducer of peroxisomes in vitro than CLO and that PHH are more sensitive to peroxisome proliferation than primate hepatocytes.
Hepatocyte suspensions were used to investigate the relationship of molecular structure to cytotoxicity in 6 isomeric dinitrotoluene (DNT). Hepatocytes were isolated from male, Sprague-Dawley rats (250-350 g) and incubated and 1.5 x 10^7 cells (viable)/ml for 4 hr at 37°C under 95% air:5% CO_2_. Ortho-para-substituted isomers (2,3-3,4-DNT) inhibited protein synthesis (PS) with low IC50 values (0.16 mM, 0.25 mM, and 0.24 mM, respectively) and over a narrower response range (0.08 to 0.4 mM). Meta-substituted isomers (2,4-, 2,5-, and 3,5-DNT) were less potent (IC50 = 0.52 mM, 0.52 mM, 0.53 mM, respectively) and exhibited a broader concentration range (up to 12 mM and above). Lipid peroxidation, assessed by TBA reactants and ethane evolution, was either not detected or, with 2,5-DNT, marginal and not well reproduced. Lactate dehydrogenase (LDH) release occurred at higher concentrations than PS inhibition, but exhibited similar relative potency. Atomic charges for all atoms in the DNT molecules were calculated using the semi-empirical MINDO molecular orbital method. A linear correlation was developed between log EC50 concentration for LDH release and C-atomic charge for carbon atoms bearing reactive nitro groups. This correlation was used to predict EC50 values for untested nitroaromatic compounds. (Supported by DAMD17-85-C-5016).

The effect of digitonin in the periferal (pp) and perivenous (pv) perfused rat liver in situ was investigated by analysis of changes in the release of cytoplasmic and mitochondrial marker enzymes. After 10 min of preperfusion digitonin was added in a conc. of 5 ng/ml, flow rate 30 ml/min. Eluates were taken every 10 sec. and analyzed for the activities of alanine aminotransferase (ALT), glutathione (GSH), glutathione-S-transferase (GST) and succinic dehydrogenase (SDH). Comparing the data from the pp and pv perfused liver the activity of ALT shows and earlier release by the pp-perfusion. The preferential pp-zonation of ALT is known from microdissection studies (Z. Physiol. Chem. 363, 375-380, 1982). For GST no predominant zonation was found, whereas for GSH a slight preferential location in the pp-region was observed. The SDH fraction eluted at 250 sec. In conclusion the periferal and perivenous zonation of enzymes may be important for their role in the detoxification of drugs and chemicals.
CHARACTERIZATION OF GLUTATHIONE DEPENDENT INHIBITION OF NADPH INDUCED PEROXIDATION OF ISOLATED RAT LIVER NUCLEI. M.A. Timmenstein and D.J. Reed. Oregon State University, Corvallis, OR

Glutathione (GSH) is known to play an important role in protecting cells against oxidative stress. The present study indicates that GSH protects isolated rat liver nuclei against NADPH induced peroxidation. Nuclei were isolated from homogenized rat liver by discontinuous sucrose gradient centrifugation. Peroxidation was induced by 1.7 mM ADP, 0.11 mM EDTA, 0.1 mM FeCl₃ and 1 mM NADPH. Peroxidation levels were assayed by measuring the formation of thiobarbituric acid reactive products and the disappearance of phospholipid unsaturated fatty acid moieties. Increasing concentrations (0.1 to 1.0 mM) of GSH produced longer lag periods (15 to 30 min) prior to the onset of lipid peroxidation. This GSH induced lag period was abolished by prior treatment of nuclei with trypsin, thiol modifying reagents, disulfides, or heating nuclei at 50°C for 15 min. Nuclei which were incubated with GSH catalyzed the conversion of cumene hydroperoxide to cumyl alcohol. This activity was also inhibited by thiol modifying reagents, disulfides, and heating nuclei at 60°C for 15 min. The data suggest that a GSH dependent peroxidase is associated with rat liver nuclear membranes which is capable of inhibiting lipid peroxidation. Supported in part by NIH grant ES01978.

HEPATIC TOXIC EFFECTS OF ACROLEIN IN MALE SPRAGUE-DAWLEY RATS. J. Horvath, G. Witz, and C. Witmer. JGPT-UNH-Robert Wood Johnson Medical School/Rutgers University, Piscataway, NJ.

Alpha,beta-unsaturated aldehydes are cytotoxic compounds which are known to express part of their toxicity through alkylation of cellular thiol groups of cellular constituents. The present studies examined the effects of the alpha,beta-unsaturated aldehyde acrolein (ACR) on hepatic thiol groups in relation to liver toxicity. Incubation of hepatocytes from male rats with ACR (1mM) for 20 min. at 37°C reduced the number of total-SH groups by 34%, while incubation with 0.1 and 1mM ACR reduced GSH by 37 and 68%, respectively. Injection of lmmole/kg ACR into the portal vein of male rats revealed changes characterized by focal areas of pale coloration upon gross examination 24 hours later. Microscopically, these areas contained regions of coalescing coagulative necrosis. At 15 min. post-injection, rats given lmmole/kg ACR showed decreases in total-SH and GSH of 29 and 67%, respectively. At 24 hr. post-injection, these sulfhydryl pools were comparable to controls. Total-SH and GSH were comparable to controls at 15 min. and 24 hr. post-injection in rats given 0.1mmole/kg ACR. These results suggest that the depletion of -SH may be a contributing factor in the hepatic lesion caused by ACR.


Ethyl Acrylate, dissolved in corn oil, was administered orally by gavage once daily (5xweek) to Fischer 344/N rats for two weeks at dosages of 0 (corn oil control), 2, 10, 20, 50, 100 and 200 mg/kg. Stomach weights and histopathologic observations of the stomach were recorded at one and two weeks. Ethyl acrylate produced increases in stomach weights (8-8%) at 20, 50, 100, and 200 mg/kg at one week, and (19-8%) at 50, 100, and 200 mg/kg at two weeks. Primary histopathologic changes were limited to the non-glandular (forestomach) portion of the stomach. Manifestations of irritation occurred in a dose-related manner at 50, 100, and 200 mg/kg at one week and at 100 and 200 mg/kg at two weeks with the incidence and severity of these changes generally lower at two weeks. In addition, dose-related epithelial hyperplasia and hyperkeratosis occurred at 20, 50, 100, and 200 mg/kg at both time intervals. At two weeks, these changes generally were more severe than at one week at 200 mg/kg, were comparable at 100 mg/kg, and were less severe at 20 and 50 mg/kg. Ethyl acrylate by gavage in corn oil produced no changes in the forestomach of rats at 10 mg/kg or less.


Ethyl Acrylate was administered to Fischer 344/N rats in the drinking water at concentrations of 0 (control), 200, 1000, 2000 and 4000 ppm for 13 weeks. Stomach weights were recorded and the non-glandular (forestomach) and glandular portions of the stomach were evaluated histopathologically after 1, 2, 4 and 13 weeks of treatment. Ethyl acrylate produced increases in stomach weights (7-37%) and changes in histopathology of the forestomach at 1000, 2000 and 4000 ppm at all time intervals. No lesions were observed in the glandular stomach in any group. The predominating microscopic change noted in the forestomach was diffuse hyperplasia of the squamous epithelium. This change was generally minimal (basal cell hyperplasia only) at 1000 Ppm, minimal to mild at 2000 ppm and minimal to moderate at 4000 ppm at all time intervals with no apparent change with time. Hyperkeratosis generally occurred in rats at 2000 and 4000 ppm in conjunction with hyperplasia. Other microscopic changes of the forestomach indicative of irritation were noted in rats at the two highest concentrations at 1 and 2 weeks only. Ethyl acrylate in the drinking water produced minimal changes at 1000 ppm (70 mg/kg/day) and no effects at 2000 ppm (17 mg/kg/day).

Ethyl acrylate was dissolved in corn oil and administered orally by gavage to Fischer 344/N rats once daily (5 x week) for 13 weeks at doses of 0 (corn oil control), 20, 100 and 200 mg/kg. Additional rats were dosed at 200 mg/kg for 4 weeks and then placed on recovery for 9 weeks. Stomach weights were recorded and the non-glandular (forestomach) and glandular portions of the stomach were evaluated histopathologically at 4 and 13 weeks. Stomach weights were increased (7-63%) at 100 and 200 mg/kg after 4 weeks and at all doses after 13 weeks. No lesions of the glandular stomach were observed in any group. Histopathologic changes of the forestomach were observed in all treatment groups at 4 and 13 weeks. The histopathologic changes in the forestomach included dose-related diffuse hyperplasia and/or hyperkeratosis of the squamous epithelium of the forestomach. Changes indicative of ongoing irritation occurred in rats at 100 mg/kg (minimal) and 200 mg/kg at 4 weeks and 200 mg/kg at 13 weeks. Focal papillomatous hyperplasia was observed overlying these foci of ongoing irritation in the 200 mg/kg group only at 4 and 13 weeks. Forestomach lesions, present after 4 weeks of dosing (200 mg/kg), were completely reversible after 9 weeks of recovery.

POTENTIATION OF RETINYL ACETATE (RA) HEPATOTOXICITY BY BUTYLATED HYDROXYTOLUENE (BHT). D.L. McCormick, T.A. Bultin, and C.J. Detrisac. IIT Research Institute, Chicago, IL

The natural retinoid, RA, and the phenolic antioxidant, BHT, both inhibit mammary carcinogenesis in rats; co-administration of RA and BHT results in increased carcinogenic activity. However, chronic exposure to RA + BHT also induces biliary hyperplasia and hepatic fibrosis. This study was conducted to characterize the temporal, pharmacologic, and histopathologic parameters of the RA - BHT hepatotoxic interaction. Using a 3x3 matrix design, female Sprague-Dawley rats were fed chow diet supplemented with (per kg): 0, 125, or 250 mg RA and/or 0, 250, or 5000 mg BHT; 3 rats per group were killed after 30, 60, 90, 120, or 180 days on diet. BHT induced hepatomegaly but no gross pathology at any time point. The 125 dose of RA alone induced no gross hepatotoxicity, but fibrosis and biliary hyperplasia were seen at 120 and 180 days in rats fed 250 mg BHT. Hepatic fibrosis was observed at 120 and 180 days in 1/3 rats fed 250 mg BHT; this incidence was increased to 2/3 at both time points in groups fed 250 mg RA + 5000 mg BHT. These data suggest a threshold hepatotoxic activity for these dose levels of RA; BHT potentiated RA hepatotoxicity. BHT reduced liver vitamin A concentration at all RA dose levels; thus, mechanisms other than increases in hepatic vitamin A levels must underly this toxicologic potentiation. (Supported by contract N01-CP-41063 from the NCI, DHEW).

STIMULATED AND NON-STIMULATED ORNITHINE DECARBOXYLASE ACTIVITY (ODC) IN GASTRIC MUCOSA OF MINATURE SWINE. R. Raybourne, J.W. Bier, D.W. Gaines, and J. Frieden, CPSAN, FDA, Washington, DC and Laurel, MD

Increased levels of ODC are associated with cell proliferation and regeneration following inflammation and tissue damage. Increases in this enzyme have been observed in rodent gastric mucosa following treatment with sodium chloride (Blochem. Biophys. Res. Commun. 121:1027, 1984). The effect of oral administration of NaCl and the model tumor promoter TPA on ODC in gastric mucosa of 6-8 mo. old male miniature swine (Normal:Hanford) was investigated. The pyloric region of the stomach had a lower level of the enzyme than the fundic or cardiac regions in untreated animals. Treatment with 15 mg/kg NaCl produced large increases in all 3 regions, with the greatest relative increase in the pylorus. Treatment with 250 mg/kg NaCl or 2 mg of TPA produced significant, but less dramatic increases. Most of the increased enzyme activity was in the villus tip region rather than in the gastric crypts. No increase in the amount of the inflammatory mediator leukotriene B4 was observed in the mucosal extracts. ODC appears to be a useful enzymatic marker for the regenerative events which occur subsequent to tissue damage in gastric mucosa.

ENDOSCOPIC ASSESSMENT OF GASTRIC MUCOSAL DAMAGE BY ASPIRIN IN DOGS. PROTECTION BY Ro 22-1327


Ro 22-1327, a synthetic PGE2 prostaglandin, was used to protect the gastric mucosa from aspirin damage.

Doses of 0, 0.1, 1 and 10 mcg Ro 22-1327 were given by gavage to fasting dogs (2-3 dogs/treatment) in PEG-400 and water, followed one hour later by 0 and 650 mg aspirin in 1% CMC by gavage. Endoscopy was done two hr later and gastric mucosal lesions were graded. Serial blood samples were obtained from fasting dogs (6 dogs/treatment) treated with 0 and 1 mcg/kg Ro 22-1327 followed one hr later by 650 mg aspirin for determination of total plasma salicylate. Acid secretion was studied in other dogs prepared with gastric pouches, after treatment with Ro 22-1327 and stimulation by food.

Pretreatment with 1 and 10 mcg/kg of Ro 22-1327 protected the gastric mucosa from aspirin damage. Ro 22-1327 did not affect food-stimulated gastric acid secretion (P > 0.05) nor total plasma salicylate levels (P > 0.05).
The NPSH levels and organ weights of the forestomach (FS) and glandular stomach (GS) of male P344N rats were determined to investigate the induction of inflammation and hyperplasia by the irritant, EA. Following 2 weeks of daily gavage dosing of EA in corn oil at 0, 2, 10, 20, 50, 100, and 200 mg/kg; rats were killed at various times after the final dose. FS weights at EA doses greater than 20 mg/kg were increased (214% of control at 200 mg/kg) 24 hr after the last dose. This increase in the ambient weight of the organ was due to inflammation and hyperplasia. The FS weight increased further in the first 6 hr after the last dose in an acute inflammatory response. The ambient NPSH level of the FS was elevated after repeated EA dosing (reaching 198% of control at 200 mg/kg), but the NPSH concentration was depleted immediately following gavage dosing (reaching 12% of control 6 hr after a 200 mg/kg dose). The GS did not have similar changes in organ weight or NPSH level in response to EA. EA dosed in the drinking water at the same total body burden did not result in similar increases in organ weight or NPSH content. The results indicate that repeated gavage dosing of EA results in an increase in the detoxification capacity of the FS that remains insufficient to detoxify bolus EA doses greater than 20 mg/kg.

It has been reported that exposure of cells in vitro to various metals results in the cross-linking of proteins to form a polymer. Using two-parameter flow cytometric analysis, it has been observed that the addition of zinc sulfate, lead acetate, aluminum chloride, aluminum lactate, or cadmium acetate to logarithmically growing murine erythroleukemic cells (MELC) results in nuclear perturbation at specific phases of the cell cycle. After washing, 1x10⁶ cells were lysed with nonionic detergent (NP40) and incubated with RNase for 15 minutes. The nuclei were stained with propidium iodide (PI, 50μg/ml) for DNA and fluorescein isothiocyanate (FITC, 1.5 μg/ml) for protein. The nuclei were analyzed with an Ortho 50H flow cytometer for green fluorescence (FITC), red fluorescence (PI), and 90° light scatter. Treatment with lead, zinc, or cadmium resulted in an increase in 90° light scatter and protein fluorescence. Exposure to aluminum did not increase 90° light scatter but decreased protein stainability. These results indicate that the metals alter the internal structure of the nucleus and affect protein stainability. Flow cytometric analysis appears to be a highly sensitive method for studying the interaction of metals with the nucleus.

A method has been developed to investigate the transport of metals across hepatic plasma membranes. Sinusoidal and canalicular membranes were isolated from rat liver homogenates using the method of Aronson and Touster (J. Cell Biol. 47, 604-618, 1970). Compared to homogenates, canalicular membranes were enriched 53 fold in Mg ATPase and 17 fold in 5'-nucleotidase, marker enzymes for canalicular plasma membranes. Canalicular membranes were not enriched in Na/K ATPase, a marker for sinusoidal plasma membranes. Sinusoidal membranes were enriched 21 to 25 fold in Na/K ATPase and Mg ATPase activities and were only enriched 5 fold in 5'-nucleotidase. These membrane preparations showed low enrichment in markers of other organelles. The enrichment of glucose-6-phosphatase, a marker for endoplasmic reticulum, was 1.3 fold for these membranes. Differences between the two membrane preparations were also demonstrated using SDS-PAGE. A rapid filtration technique was used to determine the characteristics of metal binding to these membranes. Binding of 109 Cd to these membranes was found to be time dependent and maximal binding was reached after 20 min. Furthermore, this data indicates significant differences in 109 Cd binding between the two membrane preparations.
EFFECTS OF AGE AND SEX ON HEPATIC AND RENAL METAL AND METALLOTHIONEIN LEVELS IN CONTROL AND CALCIUM EXPOSED RATS
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Age and sex differences in zinc (Zn), copper (Cu), and metallothionein (MT) levels were investigated in 2 to 84 day old control and Cd-treated (10 umol CdCl₂/Kg) rats. While hepatic Zn content increased with age, the Zn concentration was the highest between days 5 and 8. Both hepatic MT content and concentration were the highest between days 5 and 8, and then declined to adult levels by day 31.Renal Zn, Cu, and MT content and Cu and MT concentrations increased with age in both males and females. The adult males accumulated significantly greater Zn and Cu than the females. In animals injected with Cd, the hepatic Cd content in females was higher than the males. Also, the 31 and 84 day old animals accumulated more Cd and had higher Zn and MT concentrations than the younger animals. The 84 day female MT levels were significantly greater than those of the males. The adult males accumulated less Cd in their kidneys than the young males. In weaning animals, the males accumulated a greater percentage of injected Cd in their kidneys whereas in the adults the females accumulated more Cd. These results point to hormonal modulation of metal and MT metabolism in liver and kidney. (Supported by Grant #ES03187).

EFFECT OF SULFHYDRL-DEFICIENT DIETS ON HEPATIC METALLOTHIONEIN (MT) LEVELS IN RATS.
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Sulfhydryls perform important roles in the detoxication of chemicals. For example, glutathione (GSH) detoxifies numerous electrophiles, and MT, a low molecular weight protein rich in cysteine, detoxifies metals such as Cd. Low concentrations of sulfhydryls in the diet are known to decrease the hepatic GSH content and, thus, increase the toxicity of a number of chemicals. However, it is not known whether dietary restriction of sulfhydryls lowers hepatic MT content. Therefore, rats were fed diets without cysteine but with varying concentrations of methionine (0.15, 0.3 or 0.6%) for 8 days. Control diet contained 0.3% each of cysteine and methionine. GSH concentrations in liver were decreased to about one-third of control values in rats fed the diet containing 0.15 and 0.3% methionine. In contrast, hepatic MT concentration was unaffected by these sulfhydryl-deficient diets. The rats on the various diets were also challenged by the administration of Zn (3 mmol/kg, sc), which increased hepatic concentration of MT 45-fold in control rats. In rats maintained on the sulfhydryl deficient diets, Zn elevated hepatic MT to the same level as in controls. In conclusion, restriction of dietary sulfhydryls markedly decreases the hepatic concentration of GSH but does not decrease the hepatic concentration of MT or its induction by Zn. (Supported by USPHS Grants ES-01142, ES-03192 and ES-07079).

METAL COMPOSITION OF METALLOTHIONEIN (MT) INFLUENCES INHIBITION OF 8-AMINOLEVULINIC ACID DEHYDRATASE (ALAD) IN LEAD (Pb).
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The effect of MT metal composition on inhibition of ALAD by Pb in vitro was evaluated. Pretreatment of rats with Zn resulted in activation of hepatic and renal ALAD and prevented the inhibition of this enzyme by Pb. Pretreatment with Cd attenuated Pb inhibition of hepatic ALAD, decreased the activity of renal ALAD, but did not affect Pb inhibition of renal ALAD. Cysteolic metal-binding patterns disclosed that Pb co-eluted with Zn and Cd bound to liver and kidney ZnMT, and liver Cd,ZnMT while minimal binding of Pb to kidney Cd,ZnMT was observed. Cd,ZnMTs from liver and kidney had Cd/Zn ratios of 2 and 5, respectively. Inhibition of purified ALAD by Pb was also attenuated by purified MTs in the following order: liver ZnMT = kidney ZnMT > liver Cd,ZnMT > kidney Cd,ZnMT. Thus, since Pb has been shown to displace Zn but not Cd from MT, liver and kidney ZnMTs and liver Cd,ZnMT with a low Cd/Zn ratio readily decrease the free pool of Pb available to interact with ALAD. The data demonstrate that the capacity of MT to alter the intracellular distribution of Pb and mediate the inhibition of ALAD by Pb is dependent upon the relative metal composition of the MT. (Supported in part by NRS Grant ES-03070).

KIDNEY SYNTHESIZES LESS METALLOTHIONEIN (MT) THAN LIVER IN RESPONSE TO CdCl₂ AND Cd-METALLOTHIONEIN (Cd-MT).
L. E. Sendelbach and C. D. Klassen.
Univ. of Kansas Medical Center, Kansas City, KS

Acute exposure to Cd produces liver injury, whereas chronic exposure results in kidney injury. Tolerance to the hepatotoxicity is observed during chronic exposure to Cd due to the induction of MT. The nephrotoxicity elicited by chronic Cd exposure purportedly results from renal uptake of Cd-MT synthesized in liver. The change in target organ from liver to kidney might be due to a lower amount of MT synthesized in the kidney in response to Cd-MT. Therefore, the purpose of the present study was to quantitate hepatic and renal MT induced by CdCl₂ and Cd-MT. MT levels in mice were assessed using the Cd-heme method 24 hr after administration of CdCl₂ (0.5-3.0 mg Cd/kg) and Cd-MT (0.1-0.5 mg Cd/kg). In both liver and kidney, MT reached higher levels following CdCl₂ (220 and 60 μg/g, respectively) than Cd-MT (20 and 35 μg/g, respectively), probably because higher dosages of CdCl₂ than Cd-MT are tolerated. Cd-MT produced 19 and 3 μg MT/µg Cd in liver and kidney, respectively, while CdCl₂ produced 11 and 6 μg MT/µg Cd, respectively. In conclusion, induction of MT occurs in the liver and kidney after the administration of CdCl₂ and Cd-MT. However the kidney is less responsive than liver to both forms of Cd which may contribute to making it the target organ of toxicity after chronic Cd exposure. (Supported by USPHS Grants ES-01142 and ES-07079).

68

C3H/HeJ (C3H) mice are more sensitive to Cd-induced hepatotoxicity than DBA/2J (DBA) mice. MT, which exists in two forms, has been implicated in the detoxication of Cd. This study was initiated to evaluate the relationship between the hepatic concentration of MT isoforms and hepatotoxicity in these two strains after Cd administration. CdCl₂ was administered (2.5-80 μmol Cd/kg, sc) to male mice of both strains. Livers and blood were removed from 6 hr to 5 days thereafter. Hepatic MT-I and MT-II were separated and quantified by HPLC/AAS. Serum ALT activity was measured to assess Cd-induced hepatotoxicity. In both strains, Cd caused a dosage- and time-dependent increase in hepatic MT-I and MT-II content. MT-I concentration was at all times and dosages approximately 2.2 times greater than that of MT-II in both strains. One day after 20 μmol Cd/kg, hepatic MT-I and MT-II in C3H (Cd sensitive) mice were 50% greater than in DBA mice. In addition, serum ALT activity in C3H mice was 58% higher than in DBA mice. CdCl₂ at a dosage of 80 μmol Cd/kg, produced no deaths within 72 hr after administration to the DBA strain, whereas 65% of the C3H mice died within 24 hr after administration of 40 μmol Cd/kg. In conclusion, these data suggest that the strain difference in Cd-induced hepatotoxicity cannot be attributed to differential induction of MT isoforms or total MT content. (Supported by USPHS Grants ES-01142 and ES-07079)

Induction of metallothionein (MT) isoforms by zinc, cadmium and dexamethasone (DEX) in mice. W.C. Kershaw, L.D. Lehman-McKeeman and C.D. Klaassen. Univ. of Kansas Med. Ctr., Kansas City, KS

We have previously demonstrated that in rats, Zn or DEX treatment increase hepatic MT-II more than MT-I, whereas Cd produces a similar increase in MT-I and MT-II. However, it has been shown in mice that these chemicals induce approximately twice as much MT-I mRNA as MT-II mRNA. Therefore, the purpose of the present study was to determine the effect of these compounds on hepatic concentrations of MT isoforms in mice. Male C57BL/6 mice were injected sc with various dosages of Zn (375-6000 μmol/kg), Cd (5-80 μmol/kg) or DEX (10-1000 μmol/kg) and livers were removed 24 hr later. MT-I and MT-II were separated and quantified by HPLC/AAS analysis. Approximately 25% of the mice died after the highest dosage of each inducer. Maximal induction of total MTs following Zn, Cd or DEX administration was 46-, 27- and 10-1 times control values, respectively. Following all dosages of metals, the ratio of the hepatic concentration of MT-I/MT-II was approximately 2.4. However, after DEX the ratio of MT-I/MT-II was approximately 1 at all dosages except the highest at which the ratio increased to 1.7. In summary, Zn, Cd and DEX induced MT-I to a greater extent than MT-II in mice, an observation which is consistent with previously reported induction of MT mRNAs. (Supported by USPHS Grants ES-01142 and ES-07079 and a Procter and Gamble Fellowship)

Comparison of the toxicity of CdCl₂ and Cd-metallothionein (Cd-MT) in isolated rat hepatocytes. L.E. Sendelbach, W.M. Bracken and C.D. Klaassen. Univ. of Kansas Medical Center, Kansas City, KS

Inorganic Cd distributes mainly to the liver and produces hepatotoxicity, while Cd-MT distributes primarily to the kidney and produces nephrotoxicity. Cd-MT has also been demonstrated to be more toxic than Cd in cultured kidney cells, but it is not known if Cd-MT is more toxic to all cultured cells or if there is a good correlation between in vitro and in vivo toxicity. Therefore, hepatocytes, which were isolated and grown in monolayer culture for 24 hr, were incubated with CdCl₂ (1-100 μM) or Cd-MT (3-100 μM Cd). The intracellular K⁺ content was quantitated 24 hr later as an index of toxicity. K⁺ concentration of the hepatocytes was decreased 50% by 4 μM CdCl₂, while 20 μM Cd-MT produced similar injury. In the intact animal, zinc induces the synthesis of MT and decreases the hepatotoxicity of Cd. ZnCl₂ added to the media (100 μM) for 24 hr before exposure to Cd or Cd-MT increased the intracellular MT concentration 700%. This elevation in MT reduced the toxicity of CdCl₂ about 5 times but did not alter the toxicity of Cd-MT. In summary, CdCl₂ is more toxic to cultured hepatocytes than Cd-MT, and MT decreases the toxicity of CdCl₂ in the hepatocytes, similar to that observed in the intact animal. This indicates that cultured hepatocytes appear as an excellent model for examining the hepatotoxicity of Cd. (Supported by USPHS Grants ES-01142 and ES-07079)


The ontogeny of rat hepatic MT-I and MT-II was determined. MT-I and MT-II, first detected on gestation day 18, increased coordinately during late gestation reaching maximum levels of approximately 490 and 500 μg/g, respectively, which were maintained from postpartum days 1 through 7. Thereafter, MTs decreased, reaching adult levels (approximately 10 μg/g liver) between 28 and 35 days of age. MT-II was significantly higher than MT-I in 14- and 21-day-old rats. In contrast to protein concentrations, MT mRNAs reached maximal levels during late gestation which were maintained throughout the first three weeks postpartum. Additional experiments indicated that Cd (1-30 μmol/kg), Zn (100-3000 μmol/kg) and dexamethasone (0.3-10 μmol/kg) increased hepatic concentrations of MT-I and MT-II and their respective mRNAs in 14-day-old rats, despite the pre-existing high levels of protein and mRNA at this time. These results indicate that hepatic MT-I and MT-II and their mRNAs increase coordinately during fetal development. Differences between the decline of the isoproteins and their mRNAs suggest that there may be translational control of hepatic MTs during neonatal development. However, induction of MTs by Cd, Zn and dexamethasone suggests that translational control of MTs in developing rats is overcome by these agents. (Supported by USPHS Grants ES-01142, ES-07079, BRSGS07 RR0537 and a Procter and Gamble Fellowship)

69
Most mammals, including primates, show a high sensitivity to Cd-induced testicular lesions. A deficiency of metallothionein (MT), a low-M₆ metal-binding protein clearly linked with Cd tolerance, has been observed in rat testes and may explain the sensitivity of this species. Little is known about the low-M₆ metal-binding proteins in primate testes and thus, this study examined the nature of these proteins in the Patas (Erythrocebus patas) monkey. Control testis was compared to Zn-treated (300 µM/kg, sc. 24, 48, and 72 h prior to isolation) liver which is known to be rich in MT. A major low-M₆ Zn- or Cd-binding protein was seen in both tissues after gel-filtration of cytosol. This protein was extractable by heat-treatment and acetone precipitation from both sources. Such extracts were further separated by reverse phase HPLC. Four distinct hepatic forms were isolated by HPLC, all of which proved to be MT by amino acid analysis (>75% Cys, etc.). In contrast, two testicular forms were seen both of which were very different from MT in amino acid content (2% Cys). These results suggest that the low-M₆ Zn-, Cd-binding protein in the Patas testes is not MT and further support the assertion that MT deficiency is a key factor in the marked testicular sensitivity to Cd toxicity.

The initial cytosolic ligands for lead in rat kidney have approximate molecular weights of 10,000 (10K) and 63,000 (63K) and have been shown to facilitate the transport of lead into rat kidney nuclei in vitro (Mistry et al., J. Pharm. Exp. Therap. 232:462-469, 1985). Purification studies of the 10K PbBP using Sephadex G-75 and DEAE ion exchange chromatography showed that Pb binding to this protein was stabilized at pH 8.6 and that PbBP eluted after liver metallothionein (MT) I and II standards from the DEAE column. PbBP also eluted after the endogenous Zn or Cd/Zn-induced renal MT's by this procedure. SDS polyacrylamide gel electrophoresis of the purified PbBP showed it to be an apparently homogeneous peptide with a calculated mass of 8300 Daltons. Amino acid analysis of the purified PbBP demonstrated a high glutamic and aspartic content with significant levels of phenylalanine and leucine but little cysteine. These data indicate that the 10K PbBP is not an MT but rather a novel carboxyl-rich, metal-binding protein with an amino acid composition similar to that previously reported for the lead intranuclear inclusion body protein (Moore et al., Lab. Invest. 29:488-494, 1973) suggesting that it may play a role in the formation of these structures. (Supported in part by NRSA Training Grant 5T32 ES 07126).
INHIBITION OF MICROTUBE REASSEMBLY BY CADMIUM, ARSENITE, AND METHYLMERCURY IN CULTURED FIBROBLASTS. B.A. Perrino and I.K. Chou. Dept. of Microbiology, Boston Univ. School of Medicine, Boston, MA.

Sponsor: A.E. Rogers

Cell proliferation requires coordinated cycles of disassembly/reassembly of cytoplasmic microtubules (MT) and the mitotic spindle. Cd, CH, Hg, and As have been shown to cause MT disassembly in cultured cells. To investigate the effects of these metal compounds on MT reassembly, MT in 3T3 cells are disassembled with colcemid, and then allowed to reassemble following colcemid removal. MT elongate outward from the centrosome region in a radial pattern, toward the plasma membrane. MT regrowth was monitored by fluorescence microscopy and the kinetics of MT reassembly in control and metal treated cells examined by measuring MT lengths at specific times after colcemid removal. We demonstrate that exposure of 3T3 cells to micromolar concentrations of Cd, CH, Hg, or As inhibits MT reassembly in a dose dependent manner. Furthermore, the kinetics of MT reassembly in metal treated cells differ from each other. These results indicate that MT reassembly in 3T3 cells is impaired by Cd, CH, Hg, and As, and suggest that inhibition of MT assembly by these metals may be involved in their cytotoxicity. The reassembly of MT following recovery from colcemid pretreatment may be a useful system for studying the mechanisms of metal-induced inhibition of MT assembly in living cells.

DISTRIBUTION-INDUCING AGENTS PROTECT 3T3 CELLS FROM CADMIUM TOXICITY. C. Shopsis & B. Eng. LARC, Rockefeller Univ., NY, NY. Sponsor: D.M. Stark

A group of diverse chemicals can induce differentiation in erythroleukemia cell culture systems. Tests of 8 of these agents, using a cell protein accumulation cytotoxicity assay (Shopsis & Eng., Toxicol. Lett. 26:1 1985), indicated that they also protect 3T3 cells from Cd++ toxicity. The Cd concentrations required to reduce cell protein accumulation by 50% (IC50) were 5 and 7 µM for 24 and 48 hr exposures, respectively. Simultaneous exposure of cells to Cd and 1X dimethyl sulfoxide (DMSO) raised these P50 to 3 to 40 µM (24 hr) and 24 µM (48 hr). Addition of DMSO 6 hr after the initiation of a 24 hr Cd exposure still reduced Cd toxicity. Other differentiation-inducing agents found to antagonize Cd toxicity were ouabain, hexamethylene bisacetamide (HMA), dimethylformamide, heparin, hypoxanthine (Hx), N-butylate and dimethyl acetamide. DMSO and HMA also protected LMTk cells cultures in a low serum medium from Cd toxicity. Non-differentiation inducing solvents (methanol, ethanol, methyl ethyl ketone and butanol) did not antagonize Cd toxicity. DMSO, HMA, Hx and butylate did not protect cells from Zn or Hg induced toxicity, suggesting that direct induction of heavy metal-binding agents may not be the mechanism of protection from Cd. In an acute cytotoxicity assay with a 4 hr Cd treatment period, metal toxicity was not antagonized by these differentiation-inducing agents. Supported by Revlon Inc.

ANTAGONISM OF CADMIUM CARCINOGENESIS IN THE WISTAR RAT BY ZINC TREATMENT: DOSE AND ROUTE DEPENDENCE. M.P. Walske and S. Rehm. Laboratory of Comparative Carcinogenesis, National Cancer Institute-FCFR, Frederick, MD

Soluble cadmium (Cd) salts induce both injection site (IST) and testicular tumors (TT) in rats. Others have previously shown that zinc (Zn) salts given at a dose equal to Cd antagonize both Cd-induced IST and TT. This study was designed to extend these findings by using several levels and routes of Zn. Adult male Wistar (Crl:WI) BR rats received single Cd doses (30 µM/kg, sc). Zn was given sc (three doses of 1, 0.3, or 0.1 µM/kg, at -6, 0, and +18 h relative to Cd) or po (100 ppm in drinking water, -2 to +104 weeks). The study lasted 104 weeks. Zn given sc caused a dose-related reduction in TT in rats given Cd from >70% (Cd alone) to 10% (Cd + high Zn sc). Unlike previous studies, Zn sc did not affect IST formation (Cd alone, 13 tumors/30 animals vs. Cd + high Zn, 9/29). In rats in which Cd-induced TT were prevented by sc Zn, an elevation of prostatic tumors occurred (Cd + high Zn, 30%; Control, 11%). Oral Zn almost eliminated tissue reaction at the injection site, reducing the number of Cd-induced IST to 1/30. Zn po, however, did not reduce Cd-induced TT (Cd alone, 22/30; Cd + Zn po 24/30). These results show that Zn inhibition of Cd carcinogenesis is dose and route dependent and also depends on the target site in question.

CADMIUM CARCINOGENICITY: INFLUENCE OF DIETARY COPPER AND SELENIUM ON ANTIOXIDANT ENZYMES AND TRACE METAL LEVELS. J.J. Sprosw, L. Trombetta and J.S. Jamall. St. John's University, N.Y.

Weanling Sprague-Dawley rats were fed a diet containing 0.5 ppm selenium (Se) with 0, 10 ppm copper (Cu). Rats were treated with either 5 mg Cd (as CdCl2) via osmotic minipumps (Alzet 2002) (Cd-Cu) or fed 50 ppm Cd in their feed (Cd-D) for 7 weeks. Cd-D resulted in a 50% reduction in cytosolic superoxide dismutase (SOD). Cd-Cu rats also exhibited 28% reductions (P<0.05) in SOD in rats fed 10 and 50 ppm Cu. Cd-D rats also exhibited 1B% (P<0.05) reductions in glutathione peroxidase (GSH-Px) activity in rats fed either 10 or 50 ppm Cu. Catalase activity was unaffected by either Cd treatment. No increase in lipid peroxidation was observed. Cd-Cu rats exhibited 40% increase (P<0.01) in heart Se levels at all levels of dietary Cu. Heart Cu levels were significantly lower (30%) in rats fed 50 ppm Cd in the diet. The significance of these alterations will be discussed in the context of morphological lesions observed. (Supported by NIH Grant ES03370)
Weanling Sprague-Dawley rats were fed a low-Se diet containing 50 ppm copper (Cu) supplemented with either 0, 0.1 ppm or 0.5 ppm selenium (Se) for 7 weeks. There were two sets of Cd-treated rats: One set (Cd-OP) received 5 mg Cd (as CdCl₂) via osmotic minipumps (Alzet 2002). The second set (Cd-D) received 50 ppm Cd admixed with their feed. Rats fed the low-Se diet and treated with Cd-D exhibited a 30% decrease in GSH-Px activity. Rats fed the basal diet and treated with Cd-OP exhibited a 32% increase in Se-independent GSH-Px. Supplementation of the feed with 0.1 or 0.5 ppm Se prevented the effects of Cd on both enzymes. Treatments of rats with Cd-OP resulted in a 19% reduction in GSH-Px activity only in the low-Se group. No significant alterations were observed in cytosolic SOD activity in either of the sets treated with Cd. No increase in lipid peroxidation was observed in any of the Cd-treated groups. Alterations in renal trace metal levels will be presented in the context of the biochemical lesions observed. (Supported by NIH Grant ES03370)

Weanling Sprague-Dawley rats were fed a diet containing 50 ppm copper (Cu) with either 0, 0.1 ppm or 0.5 ppm selenium (Se) for 7 weeks. There were two sets of Cd-treated rats: One set (Cd-OP) received 5 mg Cd (as CdCl₂) via osmotic minipumps (Alzet 2002). The second set (Cd-D) received 50 ppm Cd admixed with their feed. Superoxide dismutase (SOD) activity was reduced by 29-44% in rats fed 50 ppm Cd (Cd-D) irrespective of dietary Se. Cd-OP treated rats showed a 34% reduction in SOD activity only in rats fed the basal, low-Se diet. Se deficiency, in the absence of Cd treatment, resulted in a 41% increase in SOD activity. Glutathione peroxidase (GSH-Px) activity was not affected by either Cd treatment. The significance of these biochemical changes will be discussed in the context of alterations in cadmium, selenium, copper and zinc levels in the lung of these rats. (Supported by NIH Grant ES03370)

Previous studies from this laboratory have suggested that methyl mercury chloride (CH₃HgCl) and cadmium chloride (CdCl₂) alter Na⁺,K⁺ ATPase possibly by binding at two different sites. In the present study binding of (²²⁴) ouabain was completely blocked by 5 x 10⁻⁷ M CH₃HgCl whether preincubated in the presence or absence of 5 x 10⁻⁷ M ATP. On the other hand CdCl₂ abolished this process only when the preincubation was carried out in the absence of ATP. Preincubation in the absence of 5 x 10⁻⁷ M MgCl₂ (E₀ conformation) followed by incubation in its presence, although resulted in total inhibition of (²²⁴) ouabain binding by CH₃HgCl, the inhibition by CdCl₂ was not significant. CdCl₂ had no effect on Mg²⁺-independent ouabain binding. When both ATP and MgCl₂ were omitted during preincubation followed by their inclusion during incubation, the nature and degree of effects of CH₃HgCl and CdCl₂ remained essentially the same as those observed in the presence and absence of the nucleotide alone. All effects of CH₃HgCl and CdCl₂ in the presence and absence of ATP and Mg²⁺ suggest that while CdCl₂ affects the high affinity nucleotide-binding site, CH₃HgCl affects the ATPase system possibly by binding to some nonspecific allosteric sites.

UPTAKE OF INORGANIC MERCURY (Hg) BY HUMAN ERYTHROCYTES (RBC) IN VITRO. J.L. Cox and E.J. Kasraei. Grad. Ctr. for Toxicology and Dept. of Neurology, Univ. of Kentucky, Lexington, KY.

Hg binds to RBCs and increases RBC fragility, presumably by interacting with membrane sulphydryl groups. Much work has centered on the binding of organic mercurials by cells. In the current study, uptake of inorganic Hg by RBCs is examined.

RBCs from healthy volunteers were washed and incubated for 10-120 min in 10 mM HEPPES buffer containing varying concentrations of Hg-203 at 0 and 37°C. The % Hg uptake by intact RBCs vs. Hg was biphasic at all time points and at both temperatures. For example, Hg uptake declined from 56±7% (37°C) to 36±6% between 0.001-1.0 μM Hg (37°C, 10 min). However, uptake increased from 36±6% to 91.9±0.9 as the (Hg) varied from 1.0-100 μM. Hemolysis did not exceed 15% despite nearly quantitative Hg uptake from the incubation medium at 100 μM Hg.

These results suggest Hg uptake by RBCs may be mediated by two distinct, concentration-dependent mechanisms. The first appears to involve saturable binding of Hg by membrane ligands. The second may represent an alteration of the membrane, opening the cell to massive Hg influx particularly at higher (Hg). A detailed investigation of membrane Hg ligands will be necessary to establish the precise mechanisms involved. (Supported by grants from the NDA, NIH (AA05931, NS00768), and the VA Research Service.)


Aminociduria (Auria) is a well-known effect of heavy metal poisoning. Fractional reabsorption (FR) of filtered AA is depressed by inhibition of unidirectional efflux from the lumen across the brush border (BB) (PSEB 139:1032, 1972). AA transport across basolateral membranes (BL) appears even more sensitive to metals than that at BB: the cellular steady state accumulation (S) of reabsorbed AA in cortex rises following metal intoxication, in spite of the fall in reabsorbed load (RL); TET, the transit time of AA across cells, is lengthened. For instance, 2 days after SC HgCl₂ injection (10 μmol/kg), RL of cycloleucine (in μmol/min) fell to 22% of control, while S rose 3-fold; TET increased from 40 to 120 sec. Qualitatively similar results were obtained with Cd and Ni. Inhibition of BL transport, however, does not alter overall reabsorption: Thus, p-CI-mercuribenzoate (50 μmol/kg IV 20 min earlier) depressed BL transport of aspartate by 64% without altering its FR. These findings extend earlier conclusions that a) BL transport is sensitive to metals; b) BL carriers play a major role in AA reabsorption; and c) metals can act at multiple sites in a single cell type. (Supported by NIH grants ES-00159 and ES-02453)

TIME COURSE OF MERCURY EXPOSURE IN CULTURED HUMAN ERYTHROCYTE CELLS. M.D. Alco, M.L. Taub, and P.J. Kostyniak. SUNY at Buffalo, Toxicology Research Center, Buffalo, NY.

Previous studies with cultured proximal tubule cells have shown that intracellular glutathione (GSH) levels increased after exposure to mercuric chloride (MC) suggesting a dynamic protective role for GSH during mercury exposure. The purpose of this study was to document the time course of mercury uptake and disposition by intracellular protein and GSH. Cultured cells were used at confluence and exposed to MC (0.04-25 μM) for 1 to 72 hrs. Uptake of MC into cultured cells increased within 24 hrs at medium concentrations (<5 μM). At 24 hrs 1, 84, 77, 66, and 36% of the MC originally present within the medium accumulated within the cells at concentrations of 0.04, 0.2, 1, 5, and 25 μM respectively. However, the subcellular distribution of mercury between protein and non-protein (GS) binding sites appeared to be in dynamic flux for all MC concentrations and time points studied. Non-protein bound mercury increased with time in a biphasic manner, with an initial lag phase lasting approximately 6 hrs. This lag phase accounted for a larger proportion of the total non-protein component with increasing MC concentrations. It was not clear whether equilibration between protein and non-protein binding sites had occurred within 72 hrs. A relative shift in binding occurred from non-protein to protein sites as the intracellular level of mercury within the non-protein fraction approached the absolute cellular GSH concentration. These effects occurred without evidence of cytotoxicity and support a protective role for GSH in cells exposed to MC. This research was supported by grants GM07229, GM07142, the Johns Hopkins OAR, and a Sigma Xi Grant-in-Aid of Research Award.

The effects of arsenic gas on the hematopoietic system were studied following prolonged exposure. Male and female rats and female mice were exposed to arsenic via inhalation over a concentration range of 0-50 ppm in a series of 14-28 day pilot experiments. Fanned cell volumes (PCV's) were in the normal range at 0.50-5.0 ppm arsenic but a marked, dose-related splenomegally was observed. Similar results were obtained in mice exposed to arsenic concentrations of 0.5-5.0 ppm. Hematology studies in rats exposed to arsenic concentrations of 0.5, 2.5, or 5.0 ppm for 14 or 28 days showed release of immature red cells. Examination of peripheral blood smears demonstrated moderate polychromatosis, moderate anisocytosis, low numbers of nucleated erythrocytes and an increased occurrence of Howell-Jolly bodies. The presence of these DNA fragments indicates either an increase in the rate of red blood cell division and/or decreased aplastic function. These observations are consistent with a regenerative response following a hemolytic episode. The data from these studies also indicate a similar hematological response of rats and mice to arsenic and suggest that arsenic, at well-tolerated concentrations, produces a dose-related stress on the hematopoietic system characterized by a regenerative anemia.


Arsine gas is a potent hemolytic agent but the effects of prolonged exposure to tolerated concentrations of this gas are relatively unstudied. The present multidisciplinary study was undertaken to evaluate the low level effects and mechanisms of arsenic toxicity. Using both sexes of Fischer 344 rats and B6C3F1 mice, acute and subchronic exposures were conducted for 6 hr/da for 14 continuous days or 5 da/wk for 13 weeks to 0-50 ppm arsenic (current TLV is 0.05 ppm). Chamber concentrations were maintained within 10% of nominal levels using a computerized control system interfaced with a photolization detector/gas chromatograph. Exhaust effluents were scrubbed of arsenic with whetted, activated carbon. Experimental data, defined and collected according to research protocol specifications, were validated and audited according to FDA-GLP regulations. Data were also managed via a computerized, interactive information management and retrieval system.


A series of short-term inhalation exposures has been conducted to study arsenic toxicity in rats and mice. Male and female rats and female mice were exposed to 0.01 to 50 ppm arsenic 6 hours a day for 14 consecutive days. Male and female rats were exposed 5 days a week for 4 weeks to concentrations of 0.3 to 5.0 ppm. All groups exposed to 10 ppm or higher experienced 100% mortality within 4 days or less while those exposed to less than 5.0 ppm showed no overt signs of toxicity. There were no dose dependent differences in body weights with the exception of male rats exposed to 5.0 ppm for 4 weeks. Packed cell volumes were depressed in rats at 2.5 ppm and in mice at 5.0 ppm. Both species showed a linear increase in relative spleen weight with increasing exposure concentration ranging from 110% of control at 0.5 ppm to 260% of control at 5.0 ppm. Microscopic examination of the spleen from exposed male and female rats showed sequestration of erythrocytes within the red pulp, hemosiderin accumulation within macrophages, and increased erythropoiesis. The increased spleen weights are attributed to the increased numbers of erythrocytes and erythrocyte progenitor cells relative to those in spleens in control animals.

Studies were initiated to characterize the potential of arsine and GA, used in the semiconductor industry in the manufacture of computer chips, to produce developmental toxicity. Intratracheal administration of a single 50, 100, or 200 mg/kg dose of GA on gestational day (gd) 4 to groups of 14-16 pregnant P3A4 rats produced no maternal or developmental toxicity compared to saline or placebo control groups. In contrast, a dose of the trioxodide of arsenic (48 mg/kg) or gallium (52 mg/kg) equimolar to the 100 mg GA dose caused significant fetal mortality and low fetal body weight. Arsine range-finding developmental toxicity studies conducted with inhalation concentrations of 10 ppm resulted in 100% mortality of females within 3 days of beginning 6 hr/day exposure. Arsine exposures of 0.5 to 5.0 ppm on gd 6 through 17 produced a decrease in corrected maternal body weight gain, a decrease in packed cell volume values similar to those observed in non-pregnant rats, and an increase in relative liver weight and spleen size. In the 5.0 ppm group, there were 6/14 exposed rats pregnant, compared to 12/15 in the control group. Exposure on gd 3 through 17 also resulted in fewer pregnant rats than expected. A definitive teratology study is in progress.

TISSUE DISTRIBUTION OF ARSENIC IN THE RABBIT FOLLOWING SUBCUTANEOUS ADMINISTRATION OF LEWISITE WITH OR WITHOUT BRITISH ANTI-LEWISITE THERAPY. T.H. Snyder, B.L. Joiner, P.J. Fisher, R.C. Kiser, W.B. Keys, C.L. Fisher, Battelle Columbus Division, Columbus, OH.

Studies were performed to evaluate tissue distribution of arsenic from subcutaneously administered Lewisite (L) in rabbits with and without British Anti-Lewisite (BAL or 2.2-dimercaptopropanol) treatment. Lewisite challenge doses were administered at 2.4 mg/kg (LD10) or 3.5 mg/kg (LD40). A maximally tolerated therapeutic dosage (35 mg/kg) of BAL was administered intramuscularly four times at four-hour intervals initiated one hour after L exposure. Tissue arsenic levels were determined 0, 4, 12, 24, 48 and 96 hours after L exposure with or without BAL therapy.

For both dosages (LD10 and LD40), BAL treatment increased arsenic mobilization from tissues, resulting in decreased arsenic levels in blood, brain, spinal cord, lung, liver and kidney. In contrast to similar studies using inorganic arsenic III (sodium arsenite), BAL treatment of L-exposed animals significantly reduced arsenic in target tissues, particularly in brain and spinal cord. This work was supported by U.S. Medical Research and Development Command Institute of Chemical Defense (Contract Number DAM7-83-C-3129).


In vivo 31P-NMR studies (Chen et al., BBRC, 139: 225-234, 1986) have shown that arsenite (As3 +, 10 mg/Kg, i.v.) decreases hepatic ATP with constant increases in P, and phosphomonoesters. The present studies evaluated temporal relationships between arsenite-induced mitochondrial swelling in situ, 31P-NMR of liver extracts and 31P-NMR in vivo. Correlative ultrastructural studies conducted on hepatocytes of rats sacrificed at 0.5, 1.0 and 2.0 hours showed mitochondrial swelling by 0.5 hours primarily in perportal hepatocytes. At subsequent time periods a larger area of the liver lobule was affected. In vivo NMR studies showed a two-step reduction in hepatic 31P-ATP. A moderate initial rate of reduction was observed followed by an acute drop after about 1 hour. No marked alterations in intracellular pH (7.0 for the control and 6.8 after the arsenite injection) were observed. 31P-NMR studies indicated increases in the Ala/Lac, but no obvious changes in the Glu/Lac ratio. The present studies indicate that mitochondrial swelling occurred in the perportal region of the liver lobule in concert with alterations in cellular phosphorus-containing metabolites suggesting that mitochondrial structural changes are closely related to the loss of ATP synthesizing capacity in vivo.

DIRECT DETERMINATION OF BLOOD LEAD LEVEL IN PLUMBERS USING PLASMA ATOMIC EMISSION SPECTROSCOPIC TECHNIQUES. Salah A. Soliman, Nabil S. Ahmed, Ahmed S. El-Hakary and Abd El-Khalik H. El-Sebae. Lab. of Environmental Chemistry & Toxicology, Div. of Pesticide Chemistry, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

The blood lead level in fifteen plumbers working in a phosphate fertilizer plant was determined. A direct current plasma atomic emission spectrometer was used to measure lead level in full blood samples. No extraction or previous preparations were required. The blood samples were diluted 1:1 in doubly distilled water containing triton X100 (1 ml/L), orthophosphoric acid (2 ml/L), magnesium chloride (20 mg Mg/L) and calcium chloride 95 mg Ca/L. Results were compared with that obtained for the same samples using the conventional atomic absorption spectrometric technique after treatment with ammonium pyrrolidine dithiocarbamate and extraction by methyl-2-5-butyl ketone. Results indicated that using both techniques, the lead level in blood of the tested subjects was quite high (40-86 ug/dl) compared to non-exposed workers (18-41 ug/dl). The results also indicated that the use of the direct plasma atomic emission spectrometric technique is both much easier and as accurate as the atomic absorption spectrometric method.

Knowledge of the spatial distribution of trace elements (TE) in the brain is very difficult to obtain by conventional methods due to the difficulties in dissecting the structures for analysis. Synchrotron Radiation Induced X-ray Emission (SRXE) is a very sensitive method which measures TE concentrations as low as 0.1 ppm. The advantages of synchrotron radiation (SR) are: high brightness and flux, good polarization, short time of measurement, non-destructive and multi-elemental detection. All advantages are important in toxicology. TE analyses with SR can easily be performed on sample volumes as small as 0.0001ul. The Minimum Detectable Limits (MDL) in 0.0001ul sample is 100ppb for Fe, 50ppb for Cu and Zn, and less than 1ppm for Pb and Hg. SRXE may also be used to produce 2-dimensional images using the fluorescent x-rays. The distribution of TE in freeze-dried sections of mouse cerebella were obtained by both analytical point-to-point measurements and by imaging. The average concentration of Fe, Cu, Zn, and Hg was 5.1, 0.2, 0.7, and 0.1 ppma respectively. The mercury was observed in molecular cell layer of mouse cerebella, but not in the granule cell layer or fiber tract. The distribution of these elements may be correlated with the nutritional and functional status of the animal. (Supported by NIH P41RR01838 and ES04040).

304 INTRACELLULAR LEAD-CALCIUM RELATIONSHIPS J.G. Pounds and J.P. Rosen, Brookhaven National Lab, Upton NY and Montefiore Hospital and Med Center, Bronx NY

Cellular Ca homeostasis and Ca-mediated cell functions are attractive processes to be involved in the mechanism(s) of lead toxicity. Proposed intracellular sites of Pb-Ca interactions include calmodulin, Ca-transporters, Ca-gates, etc. in a wide variety of cells and tissues. Knowledge of the Pb:Ca or Ca:Pb ratio in the different structural and functional compartments of cells is essential for identifying characterizing, and understanding the significance of these interactions. Unfortunately, it is extremely difficult to concurrently and directly measure Pb and Ca in situ due to inadequate sensitivity, selectivity, and spatial resolution of the existing techniques. We have, however, concurrently and indirectly calculated intracellular Ca-Pb and Pb:Ca ratios using kinetic analysis of dual-label Ca-45 and Pb-210 washout curves in cultured osteoclastic bone cells. The ratio of Ca:Pb half-times and rate constants is <4 supporting the concept of similar metabolism of the two elements. The kinetic distribution of Ca and Pb in the cells is not symetrical; the Ca:Pb ratio for rapidly exchanging pools is 40:1 and for the most slowly exchanging pools is 7:1. The ratio of the steady state flux across the plasma membrane is 35:1 and across the mitochondiral membrane is 30:1. These observations of Ca-Pb relationships should be useful for designing and evaluating Ca-Pb studies in subcellular systems.


Trace element status is a result of trace element nutrition and interactions. The possible interactions can be complex and are not well understood at this time. Rats were fed a trace element adequate (AIN) or a trace element replete(Purina) diet and given water containing 0 or 500ppm lead acetate for 28 days (6 rats/group) to study these interactions. Weight gain and food and water consumption were monitored. Rats were sacrificed and blood collected on day 28. Serum was pipetted (10ul) on to polyimide film and freeze dried. Trace element analysis was conducted using Synchrotron Radiation Induced X-ray Emission (SRXE). 0.01ul of serum was analyzed. Differences were observed between lead and control groups and between diets. In animals on AIN diet the serum Cu ratio of control to lead is 2.1 and 1:2 in the Purina group. There are also changes in serum trace element content between diets. The serum Rb ratio in Purina and AIN rats is 15:1 and the serum Br ratio in Purina and AIN rats is 4:1. These results show complex nutritional and toxicological trace element interactions. This is the first part of a larger study in which trace element analysis using SRXE will be conducted on several different tissues from the same rats. (Supported by NIH grants ES04040, P41RR01838)

305 LEAD INHIBITION OF Mg2+-ATP-DEPENDENT CALCIUM TRANSPORT IN ISOLATED RAT LIVER MEMBRANES. F.A.X. Scheure. Dept. of Pediatrics and Pathology, Albert Einstein College of Medicine, Montefiore Med. Ctr., Bronx, NY. Sponsor: J.P. Rosen

Low level lead toxicity (blood lead: 25-55 ug/dl) is associated with metabolic, neurobehavioral, developmental and cardiovascular disorders. Interactions between lead intoxication and calcium metabolism are well recognized. In every tissue in which lead has been examined, lead interferes with cellular calcium homeostasis resulting in an apparent increase in cellular calcium and alterations in calcium-mediated processes. The mechanism(s) by which lead increased cellular calcium are unknown. This study examines the effects of lead (10-5 to 10-4m) on Mg2+-ATP dependent calcium transport activities in plasma membrane vesicles, endoplasmic reticulum-rich microsomes and mitochondria isolated from rat liver. Lead inhibited Mg2+-ATP dependent Ca transport in all three of these membranes with half-maximal inhibition observed between 3x10-7 and 10-5 M lead. However significant inhibition of plasma membrane calcium transport is observed at lead concentration as low as 10-8M. These results reveal mechanisms by which lead may alter cellular calcium homeostasis and may represent expressions of perturbed cellular function at extremely low levels of lead. Supported by USPHS Grant R23 ES04046.
ERTHROCYTE MEMBRANE FLUIDITY AS RELATED TO BLOOD LEAD. C.R. Angla, L.R. Cook, S.J. Stehus, and R.C. Maxell. University of Nebraska Medical Center, Omaha, NE

The mechanisms of decreased red blood cell (rbc) survival, increased viscosity and decreased osmotic fragility with increasing blood lead (PbB) are still unexplained. We assessed the fluorescent polarization of diphenyl hexatriene (DPH) in rbc membranes from Pb workers (PbW) and age matched controls (C). Fluidity, or anisotropy parameter (a.p.), decreases with Pb aging. The a.p. of rbc fragments of PbB (PbB 18-40 ug/dl) was significantly lower than C with PbB 6-12 ug/dl (1.31 vs 0.86; p<0.05); the a.p. of both decreased on storage but remained significantly lower in PbW. In 53 assays of fresh, hemoglobin-free, resealed ghosts from 24 subjects (PbB 3-43 ug/dl) there was a modest but significant correlation of a.p. and PbB; a.p. = 2.97-0.31 log PbB; r=0.54; S.E.E. =0.39. The greater effect on rbc membrane fragments may relate to the dispersion of DPH into a two-sided membrane or to a selective effect of Pb on the inner membrane. We postulate that the decreased ratio of rbc membrane phosphatidylicholine:phosphatidyl ethanolamine (PC/PE) in PbW may reflect decreased transmethylation of inner membrane PE to outer layer PC with changes in methylation of the inner layer membrane protein. With no direct correlation of a.p. with total PC/PE, the bilayer kinetics of protein methyl carboxylation deserve investigation.

AGING ALTERS THE TISSUE DISTRIBUTION OF LEAD. D.A. Cory-Slechta, Division of Toxicology, University of Rochester Medical School, Rochester, NY

How the degenerative processes of aging affect toxic vulnerability has received little experimental attention. This study examined changes in the distribution of lead with age. In a cross-sectional study, male Fischer-344 rats were exposed from either 21 days of age (young = Y), 8 months (adult = A) or 16 months of age (old = O) to 50 ppm sodium or lead (Pb) acetate in drinking water for 8 months. Subsequent tissue Pb determinations revealed comparable kidney Pb levels across age groups. In Pb-exposed rats, age-related increases were found in both blood (X values, Y = 8.5, A = 15.9, O = 29.6 ug/dl) and brain Pb levels (Y = 0.13, A = 0.19, O = 0.29 ug Pb/g). Increases in liver Pb content were primarily restricted to the old rats (Y = 0.19, A = 0.26, O = 0.72). Elevation of soft tissue Pb levels were likely due to the diminished capacity of the bone to store Pb with advancing age (Y = 41.2, A = 28.4, O = 18.6 ug Pb/g). An increased incidence of morbidity and mortality was also observed in the old Pb-exposed group compared to old controls. Enhanced vulnerability to Pb emerges during the later stages of the life cycle. Supported by ES03054, ES01247, ES01246.

NON-INVASIVE DETECTION OF TIBIAL BONE LEAD IN INTACT LEGS BY L X-RAY FLUORESCENCE. L. Wielopolski, D.N. Sliatkin, J.A. Kalef-Bara, J.F. Rosen. Medical Dept., Brookhaven National Laboratory, Upton, NY; Dept. Med. Physics, Univ. Ioannina, Ioannina, Greece; Dept. of Pediatrics, Montefiore Medical Center, Bronx, NY.

An x-ray generator with a molybdenum anode was used at 40-45 kvp to produce a 0.4 cm² beam of partially polarized photons. These were directed at the antero-medial skin surface of the mid-tibia in 5 intact amputated legs and in 6 children with Pb toxicity. The x-ray spectrum, measured 90° from the incident beam, showed small but distinct peaks in the 10.9 keV region from four amputated legs and three children's legs. After the surface skin dose reached 1 rad. These peaks were attributable to Pb L₂ x-ray fluorescence. The intensities of these Pb signals are critically dependent upon the thickness of tissue overlaying the tibial bone, because L₂ x-rays are attenuated in soft tissue with a half-value layer of 0.8 mm and because most Pb in the irradiated zone is in tibial bone. Based on spectra from a leg phantom containing 30 µg Pb/gm in its pseudo-bone component, it was estimated that the limit of detection with the molybdenum anode is 55 µg Pb/gm of bone, roughly twice the median bone Pb content in normal children. The same apparatus with a silver anode has a detection limit below 5 µg Pb/g bone, and is being evaluated for screening children with suspected Pb toxicity at Montefiore Medical Center.


Lead is known to be a neurotoxin and hemotoxin in humans, and produces cancer in rats. Since the availability of lead to its sites of toxicity is determined by its unbound concentration in the plasma, a pharmacokinetic model is proposed to predict this concentration in high risk populations following intermittent exposure. The proposed model includes input functions for the oral and pulmonary routes of administration. The major tissues associated with lead distribution and toxicity are incorporated as compartments. Saturable kinetics were used to simulate the uptake of lead from the gut, and the binding of lead in the plasma and red blood cell. The input functions and parameters of the model were then adjusted to reflect the changes expected in populations known to be at high risk of lead toxicity. Computer simulation of these cases was performed using the SCop simulation procedure. The areas under the concentration versus time curves and the peak concentrations of lead in each compartment should more accurately estimate safe exposure limits and risk of toxicity.
310 INTRAVENOUS ADMINISTRATION MODE FOR LEAD INCLUSION DEVELOPMENT IN MOUSE TISSUES. E.M.R. Sorensen and M.H. Bhattacharyya, College of Pharmacy, Department of Pharmacology/Toxicology, University of Texas, Austin, TX and Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL.

In past studies, formation of lead (Pb) inclusions in the liver and other tissues of laboratory mammals necessitated prolonged exposures—up to 180 days—with drinking water exposure mode. Such an experimental design can lead to considerable variability in Pb uptake and tissue distribution because of the differences in individual exposures and their measurement (due to uniformity of Pb from solutions, differences in individual water consumption and Pb absorption, and losses of water during consumption). In this study, these limitations were overcome by inducing B6C3F1 mice by use of a single 20 mg/kg dose of intravenously administered stable Pb tagged with a radioactive tracer (Pb-210). Seven days after a single injection, the liver contained about 0.13% Pb/g or 8.8 ± 0.5% of the injected dose (ID)/g fresh weight (mean ± SE, n = 4). This level decreased to 3.9 ± 0.6% ID/g (n = 5) after 14 days. Intracellular Pb inclusions averaging 1.6 ± 0.1 μm in diameter (n = 8) were visible at both optical and ultrasonic levels within parenchymal hepatocytes. Intravenous injections of 2.6 μCl were adequate for autoradiographic localization of Pb deposits within the liver. Seven days after Pb injection, most tracts were present over endothelial lining cells of the hepatic sinusoids; however, Pb deposits within intracellular inclusions were inadequate for tract development. Autoradiographic evaluation of the spleen (averaging 1.9 mg Pb/g) showed macrophage engulfment of Pb. In summary, intravenous administration of a single 80 μg/kg dose of Pb to the mouse resulted in inclusions that were visible at the optical and ultrasonic levels within parenchymal hepatocytes, as well as in the reticuloendothelial systems of the liver and spleen. Work supported by U.S. DOE under contract No. W-31-109-ENG-38.

312 MOBILIZATION OF LEAD OVER THE COURSE OF DMSA CHELATION THERAPY. D.A. Cory-Slechta. Division of Toxicology, University of Rochester Medical School, Rochester, NY

DMSA (2,3-Diaminocaproyl-N-carboxyethyl glycine acid), an orally effective chelator, has been proposed to replace CaEDTA, the current agent of choice for treatment of elevated Pb (lead) burden. This study examined the pattern of tissue Pb mobilization over the course of a 5-day administration of DMSA. Groups of 7 rats exposed for 4 months to 50 ppm Pb acetate, in drinking water, were given 1, 2, 3, 4, or 5 daily i.p. saline (control), 25 or 50 mg/kg DMSA injections (i.n) and sacrificed 24 hours later. Blood Pb levels declined in a dose-related manner to the range of non-exposed controls over the first 4 DMSA i.n. After single DMSA i.n., kidney Pb content declined markedly to 50% of control, and then to 30% after the fifth i.n. Brain Pb values dropped gradually over the first 4 i.n. to 30% of control; a dose-related effect was apparent only in response to the first 2 i.n. Liver Pb values declined substantially after the fourth i.n. No further drop in liver lead was produced by the fifth i.n. DMSA is effective in removing Pb from critical organs without producing the redistribution we have observed with CaEDTA. Supported by ES03054, ES01247, ES01248.

311 LEAD-INDUCED ALTERATIONS OF RENAL GENE EXPRESSION WITHIN SUBCELLULAR COMPARTMENTS. P. Masry, C. Mastro, and B.A. Fowler, NIEHS, Research Triangle Park, NC.

Previous ultrastructural morphometric/biochemical studies (Fowler et al., The Toxicologist 5: 53, 1985) have demonstrated that a single intravenous injection of lead (3mg Pb/kg) produced a reversible formation of intranuclear inclusion bodies which was associated with concurrent, reversible changes in total renal gene expression in the absence of cellular toxicity. The present studies were undertaken to evaluate the subcellular distribution of these alterations in cytosolic, mitochondrial, and microsomal compartments at 4, 16, 24 and 72 hour time points by two-dimensional gel electrophoresis of in vivo labeling of proteins using [35S] cysteine/methionine. A number of changes involving both increases and decreases in the rates of synthesis of specific gene products were observed in response to lead exposure in all three fractions and at all time points examined. The number and intensity of these alterations were maximal at the 16 and 24 hour points and accounted for most of the changes observed in the whole tissue homogenates. Results of these studies indicate that Pb-induced alterations in renal gene expression are distributed among a number of subcellular compartments suggesting a stress response by these organelles at a Pb dose level which did not produce overt cell injury.


Lead persists in tissues of exposed individuals, primarily in the bone. In order to determine if previously ingested lead represents a potential risk to the developing fetus, rat dams were pretreated with inorganic lead (21 day exposure, at doses ranging from 0.2% to 2.0% lead acetate) prior to pregnancy and bred at varying intervals thereafter. Groups of females from each lead dosing level were bred at either 2, 4, 6, or 8-10 months following lead exposure. Fetal morphology and lead levels were measured at gestational day 21. The effects of lead ingestion on subsequent pregnancies were dose dependent and were persistent for at least 8-10 months. Offspring of low lead dosed dams demonstrated no observable reduction in litter size or pathology, although, lead accumulated in the offspring. At the intermediate lead dose, a 25% reduction in litter size and an increased incidence of stillbirths occurred. In the surviving offspring, widespread cerebral hemorrhages were observed. The high dose lead group was most affected with a 90% reduction in litter size, although dams appeared to be normal. The results of this study indicate that the effects of lead ingested prior to conception are particularly detrimental to subsequent pregnancies. (Supported by grants from the NIA & NIEHS.)
DOES LEAD NITRATE TREATMENT IN MOUSE LIVER INDUCE BIOCHEMICAL PATTERNS SIMILAR TO RAT LIVER?


Lead nitrate (LN) treated rat liver exhibited certain biochemical properties characteristic of hepatocytotoxic nodules in rat. It showed decreases in phase I (cytochromes P-450, b, etc.) and increases in phase II (glutathione (GSH), GSH transferase (GST), and DT-diaphorase) drug metabolizing components. This prompted us to examine the effect of LN in mouse male liver. C3H/Bl mice were injected with LN via the vein and control with saline and were killed at 0, 1, 2, 4, 7 and 15 days. Cytochrome P-450 and beta content were significantly decreased at 1 and 2 days and restored to normal level by 7 days. GSH content was significantly increased and the recovery was gradual. DT-diaphorase was increased at 4 days. In contrast GST using CDN as substrate showed maximum activity at 4 days and returned to normal by 15 days. This study showed that a decrease in phase I components is common to both mouse and rat liver after LN treatment. In contrast, only a few phase II components were increased in mouse compared to rat. This shows that there are in fact a number of biochemical responses of liver to lead nitrate shared by both mouse and rat.

EFFECTS OF EDTA CHELATION ON LEAD AND ZINC CONCENTRATIONS IN HUMANS: COMPARTMENTAL MODELS

A. Marcus, Washington State University, Pullman, WA*

Chelation of toxic metals often removes essential metals as well. A compartmental kinetic model is developed for the distribution of lead and zinc among body pools, and the inhibition of erythrocyte enzyme delta aminolevulinic acid dehydratase (ALAD) activity. The model was fitted to data on seven lead-exposed Japanese workers described in (Araki et al., Arch. Environ. Hith. 39 (1984) 363-367). The model was quite adequate for fitting plasma lead, plasma zinc, and erythrocyte ALAD response to a one-hour infusion of calcium disodium ethylenediamine tetraacetate (EDTA). Cumulative urinary elimination of lead and zinc was also generally well fitted by the model. Erythrocyte lead and zinc concentrations were less adequately fitted, suggesting that the erythrocytes consist of several kinetically distinct pools with very different degrees of trace metal removal by EDTA. Most of the lead removed by EDTA is extracted from non-blood pools. Erythrocyte ALAD responds to labile pools of lead and zinc in red blood cells.

COMPARISON OF HEPATOCELLULAR NECROSIS AND BIOCHEMICAL CHANGES IN RESPONSE TO COBALT-HEME OR LEAD NITRATE IN DIFFERENT RAT STRAINS.


Both cobalt-HEME (CH) and lead nitrate (LN) are potent inhibitors of heme biosynthesis. A comparative study was undertaken to examine necrosis and biochemical changes after CH or LN administration in different strains of rat. Male Fischer, Wistar and Sprague-Dawley (SD) rats were given CH or LN at 100 μmol/kg or saline and sacrificed at 72 hours. Both agents increased liver weights. Both also decreased phase I (cytochromes P-450, b, total microsomal heme, etc.) but only CH altered increased phase II (glutathione (GSH), GSH-transferase, GST-p and DT-diaphorase) drug metabolizing component. Using different doses of LN (25, 50 and 100 μmol/kg) only the highest dose gave moderate necrosis in Fischer and no effect in SD and Wistar rats. However, CH caused significant necrosis in all strains. Thus the hepatocellular necrosis effect of LN is clearly dissociated from its biochemical effect. Although both inhibit heme synthesis lead nitrate and cobalt-HEME appear to be acting in a very different manner at the cellular level.

EXAMINATION OF THE POTENTIAL BIOCHEMICAL MECHANISMS OF ORGANOemetAL TOXICITY.

B.L. Novicki and M.R. Kriegman. University of North Carolina, Chapel Hill, NC.

Organoemetals exert deleterious effects on a variety of cellular organelles in several mammalian tissues. The basis for toxicity has not been established. It has been proposed that dealkylation of certain organoemetals proceeds via the production of alkyl radicals. The potential for initiation of lipid peroxidation resulting from free radicals could be involved in alkylmetal toxicity. Two in vitro systems derived from livers of adult male Long Evans rats have been used to investigate the toxicity of selected organoemetals in cultured hepatocytes. Alanine aminotransferase (ALT) activity in ALT-positive (ALT-PL+) and ALT-negative (ALT-PL-) hepatocytes and the loss of viability were measured. Preincubation of the cells with 30 μmol/kg of lead and tin compounds did not result in a significant increase in ALT activity. The use of a NADPH-generating system did not result in a significant increase in ALT-PL+ but did cause a significant increase in ALT-PL-. Lead and tin compounds did produce malondialdehyde, under identical incubation conditions. These studies suggest that lipid peroxidation is not an important contributor to the cytotoxicity induced by these metalloids in these cells.
This study was designed to investigate the comparative subchronic toxicity of tri- and teta-ethyl lead in the rat. Groups of 5 male rats were administered inorganic lead (200 mg/kg bw), tri-ethyl lead (3-EL) (0.2, 1.0 or 2.0 mg/kg bw) or teta-ethyl lead (4-EL) (0.2, or 2.0 mg/kg bw) by oral gavage 5 days per week for 13 weeks. Animals dosed with 2.0 mg/kg 3-EL or 4-EL showed signs of irritability and self-mutilation after 21 and 42 days respectively and were killed at this time for humane reasons. All remaining treated animals showed reduced weight gain but no clinical symptoms. Serum lead was normal in all animals. RBC ALAD was reduced in animals receiving 1.0 mg/kg of 3-EL or 200 mg/kg inorganic lead and was accompanied by an increase in RBC protoporphyrin levels and urinary coproporphyrins. Hematologically, animals dosed with inorganic lead showed a depressed MCH and MCV. Marked histopathological changes were observed in the kidneys of animals treated with inorganic lead. Moderate cytological changes were noted in the bone marrow of animals treated with inorganic lead or the 1.0 mg/kg level of 3-EL.

Lung clearance, tissue distribution and elimination of manganese was studied in male Long-Evans rats. Animals were exposed for 1 hr at two concentration levels: 0.29 mg Mn/m³ and 2.93 µg Mn/g. Activity of Mn was measured in liver, kidney, stomach, large and small intestine, blood, urine and feces was determined on days 0, 1, 2, 7, 14, 28, 60, and 120. Inhaled ⁵⁴MnCl₂ was cleared from the lung of rats biexponentially; at the high concentration, the rapid and the slow phases had half-times, respectively, of 0.2 and 10.5 days. At the low concentration, the rapid and the slow phases had T₁/₂, respectively, of 1.8 and 12.7 days. Relative uptake into the brain was independent of inhaled concentration and did not exceed 1% of lung deposition on day 0. After the high concentration, liver and kidney Mn levels peaked immediately at the end of exposure and declined rapidly during the first two days. After the low exposure, liver and kidney accumulation was maximal on day 2 and then declined similar to the high exposure group. Relative Mn content in the GI tract was similar after high and low exposures, except for the large intestine where much higher levels were measured in the early phase after inhalation of the high concentration. These data show that concentration of inhaled Mn has a significant influence on its organ transfer and clearance rates.
ZINC AND NICKEL REDUCE CADMIUM-INDUCED MORPHOLOGICAL CHANGES IN 3T3 FIBROBLASTS. E.M.B. Sorenson, E. Borenfreund. College of Pharmacy, Department of Pharmacology, Toxicology, University of Texas, Austin, TX and Laboratory Animal Research Center, The Rockefeller University, New York, NY.

Zinc and nickel have been shown to affect the cytotoxicity of cadmium in BALB/c mouse 3T3 fibroblasts. A one to eight molar ratio of cadmium to zinc or a one to four molar ratio of cadmium to nickel reduced the toxicity following exposure to 10 μM cadmium in 3T3 cells (Borenfreund and Pueuter, 1986, Toxicon 30:121). The present study was conducted to determine whether the ultrastructural and optical alterations induced by cadmium at a slightly higher concentration (20 μM) could be reduced by simultaneous exposure of the cells to 20 μM zinc or 200 μM nickel. Following exposure, cells were preserved for optical and ultrastructural evaluation. Control cells formed continuous monolayers of platelet-like cells with superficial microvilli and occasional vacuoles, lysosomes, and mitochondria. Cells exposed to nickel had elongate nuclei and occasional superficial blebs. Zinc-exposed cells were slightly rounded compared to control cells and had electron dense cytoplasmic deposits. Cadmium-exposed cells were dispersed and swollen several times larger than control cells. Micronuclear swelling, cytoplasmic vacuolization, altered peripheral chromatin aggregation, and alterations in the endoplasmic reticulum were characteristic features of these cells. Simultaneous exposure of cells to 80 μM zinc and 20 μM cadmium resulted in the least cadmium-induced morphological alterations; however, some cells were swollen or vacuolated. In contrast, cells exposed to both cadmium and nickel were swollen and dispersed compared to controls. Micronuclear swelling, vacuolization, and electron dense lysosome-like structures were predominant in many cadmium and nickel-exposed cells. Therefore, simultaneous exposure of 3T3 cells to zinc and cadmium reduced cadmium-induced morphological alterations to a greater extent than did exposure to both nickel and cadmium. Morphological changes paralleled the viability assessments using neutral red techniques.

THE EFFECT OF NICKEL ON SPECIFIC PROTEIN BINDING TO MOUSE SATELLITE DNA. Dr. M. Latta, T. A. Kimmel, R. J. Inaba and M. Costa. Institute of Environmental Medicine, New York University Medical Center, New York, NY.

Evidence from our laboratory indicates that altered binding of proteins to heterochromatin may be involved in nickel carcinogenesis. In order to determine the importance of this effect we have begun to investigate the specific binding of protein, in the presence and absence of nickel, to mouse satellite DNA. Mouse satellite DNA comprises a significant portion of heterochromatin. The plasmid, P16, containing a 13 kb mouse satellite DNA sequence consisting of a tandemly repeated 214bp consensus satellite DNA sequence was grown and isolated using standard procedures. The 214bp consensus sequence was purified by restriction enzyme digestion of P16 with Blal, agarose fractionation and electroelution. The purified 214bp fragment was then subcloned into puc8 to facilitate end-labelling of the 214bp satellite DNA sequence. Our primary assay to determine the presence of proteins that bind specifically to mouse satellite DNA is DNAase I footprinting. Using this method the effects of nickel, or various other metal ions related to nickel carcinogenesis, on specific protein binding are being studied. We will attempt to purify any proteins whose binding to satellite DNA is significantly altered by these metal ions.


The carcinogenic effect of Ni(II) compounds may be related to their ability to displace the physiologically essential divalent cation Mg(II) from its normal binding sites. Nickel treatment of cells caused DNA damage in heterochromatic regions of DNA. Chromium, another carcinogenic metal, did not display this specificity for heterochromatin. Magnesium protected cells in culture against nickel-, but not chromium-induced transformation, chromosomal aberrations and sister chromatid exchanges. When excess MgCl2 was added to Syrian hamster embryo cell cultures during NiCl2 or K2Cr2O7 treatments, it significantly reduced the frequency of nickel-transformed colonies, but did not decrease the incidence of transformation produced by chromate. NiCl2-induced 3 times the expected amount of chromosomal aberrations in heterochromatin, while damage in euchromatin was less than expected. When excess MgCl2 was added with NiCl2, the proportion of aberrations in heterochromatin were preferentially reduced compared to euchromatin. Sister chromatid exchanges, which occurred mostly in heterochromatin following nickel treatment, were also reduced by excess magnesium. Varying the extracellular Mg(II) level had no effect on the induction of chromosomal aberrations or SCEs by K2Cr2O7.

CARCINOGENICITY OF NICKEL DITHIOCARBAMATE. M. A. Basinger, M. M. Jones. Department of Chemistry, Vanderbilt University, Nashville, TN, M. A. Holcher. Department of Pathology, Vanderbilt University, Nashville, TN, R. E. Mrak, E. M. Walker, Jr., J. L. McClellan Memorial Veterans Hospital, Little Rock, AR.

Nickel diethylthiocarbamate, a compound frequently encountered in the analytical chemistry of nickel, was found to be carcinogenic when tested by subcutaneous implantation in male ICR mice. These tumors arose after periods ranging from six months to a year and were fibrosarcomas. The particle size of the nickel dithiocarbamate used was in the range 1.5-3.5 μm, the range found by Costa and his co-workers to be optimum for phagocytosis. The tumor consisted of a solid sheet of pleomorphic tumor cells that could be seen to arise within skeletal muscle. Under electron microscopy examination the majority of the cells were fibroblastoid in appearance with the predominant cytoplasmic organelle rough endoplasmic reticulum. The results suggest that other examples of nickel compounds prepared and used for specialized purposes may also be carcinogenic.
As an environmental pollutant, Ni exists as a respirable atmospheric aerosol in urban environments. This study assesses Ni toxicity in pulmonary alveolar Type II cells. Toxicity data will then be incorporated into an extrapolation model to determine effects of inhaled Ni on man. Human (A549) and rat (L2) Type II cells were exposed to 0-50 mM NiCl₂. Toxicity was determined using four endpoints: cell proliferation, lactate dehydrogenase release, trypan blue exclusion, and electron microscopy. Cell growth in both A549 and L2 cells was inhibited in a dose dependent manner. A549 cells were inhibited to a greater extent than were L2 cells with respect to cell growth but to a lesser extent with respect to cell death with increasing Ni concentrations. By 48 hrs the ID₅₀, as measured by cell growth, was 1.3 mM for L2 cells and 0.36 mM for A549 cells. Cell death occurred only at high concentrations, greater than (13 mM). Subcellular pathology at 0.68 mM Ni included many nuclear irregularities and a markedly dilated rough endoplasmic reticulum. Thus human and rat Type II cells have different sensitivities to the same concentrations of Ni. (Supported by NTH Grant ES07031, RR01693 and CA14236.)

The relationship between metals and their role in DNA-protein interaction is being investigated in our laboratory. Chromate carried out studies to determine its ability to induce alterations in the association of proteins and DNA. CHO cells or isolated nuclei were extracted with 2% SDS followed by a second extraction in 5M urea and 2% SDS. The proteins remaining associated with the DNA following extraction were compared by one and two dimensional gel electrophoresis. The amount of radiolabeled protein per µg of extracted DNA increased with the amount of chromate treatment. Two proteins of Mr 43KDa and 53KDa having respective PI values of 5.5 and 6.5-9 constituted the most prominent proteins crosslinked to the DNA by chromate. ³²P orthophosphate labeling indicated that both of these proteins were phosphorylated. This may be due to a direct chemical effect of chromate. The characteristics of p53 suggest that this protein may be acting to promote the cell cycle of the basic p53 at this time is unknown. The biological role of alterations in DNA-protein associations may be related to the cytotoxic, mutagenic, and carcinogenic properties of chromate. (Supported by NCI Grant No. CA43070.)

CNS injury is produced by thallium (Th). Biochemical evidence shows lipid peroxidation (LP) in selected brain regions of rats. In situ localization of LP histochemically has not been demonstrated in brain. A technique was developed for localization of LP in brain regions. Results were compared to Th effects on behavior, biochemistry and morphology. Male Sprague-Dawley rats were injected i.p. 1 thr 8 days with 6 mg/kg Th acetate. Rats were sacrificed at days 2, 4, 6, 12 and 21. A modified Shiff procedure for aldehyde (SPA) on unfixed cryostat sections was performed on cerebrum (C), midbrain (M), cerebellum (Ch), brain stem (B), hippocampus (H). Fixed action patterns of behavior were measured by a computerized method. LP was measured by the thioarbituric acid procedure. At 8 days, modified SPA showed LP in Cb>Bs>H>Cx>Mb. Laminar differences in LP intensity in Cb were observed, with Myelin staining most intensely. Decreased numbers of Purkinje cell and disruption of the molecular-granular interface was observed. Phospholipid was altered chiefly in Myelin layer of Cb. Time response showed LP effects as early as 48 hours and recovery as early as day 8. Behavioral patterns suggested changes consistent with Cb injury. LP specific staining in the CNS permits identification of focal lesions by Th at early time periods which were comparable to biochemical measures of LP.

The dietary toxicities of the basic sodium aluminium phosphates KASAL and KASAL II, were examined in male rats. In addition, aluminium levels in bone were determined to estimate the possible aluminium deposition by these compounds. Groups of 25 male Sprague-Dawley rats were fed basal diet or diets containing 30000 ppm KASAL, 7000 or 30000 ppm KASAL II or 14470 ppm aluminium hydroxide for 28 days. Corresponding daily aluminium doses were 141, 67, 288, and 302 mg/kg/day. Neither form of KASAL produced detectable toxicity. Body weights and food consumption were similar in treated and control groups. No toxicologically significant changes were observed in hematology and clinical chemistry parameters or in organ weights. Gross and histopathological examination of collected tissues revealed no treatment-related changes. Femurs collected at necropsy under conditions of aluminium contamination showed no significant deposition of aluminium after dietary administration of KASAL, KASAL II, or aluminium hydroxide. All aluminium values in bone were less than 1 ppm and most values were not quantifiable. Thus, dietary administration of up to 30000 ppm of either basic sodium aluminium phosphate formulation caused neither toxicity nor significant deposition of aluminium in femurs.
VANADIUM INHIBITION OF YEAST GLUCOSE-6-PHOSPHATE DEHYDROGENASE. M.D. Cohen, A.C. Sen, and C.L. Wei Dept. of Food Science and Human Nutrition, Univ. of Florida, Gainesville, FL. Sponsor: L.O. Lim.

Vanadium is a known inhibitor of many enzymes related to phosphate use in the body. Effects on the hexose monophosphate shunt have been proposed but conclusive data were not presented. Vanadium in the +4 and +5 oxidation states inhibits the activity of yeast glucose-6-phosphate dehydrogenase. Inhibition by ammonium metavanadate (5+) is mixed-type and competitive regarding glucose-6-phosphate and NADP+ (Kd values of 2.1 and 2.7 μM, respectively). These values are on the order observed for other inorganic anions, such as sulfate and phosphate. Inhibition by vanadium oxide (4+) is mixed-type for both substrates with Kd values of 49 and 52 μM for the sugarsphosphate and NADP+, respectively. Experiments to determine if an oxidative effect contributed to the strong inhibition indicated that this was not a major factor under the conditions used. Absorbance patterns indicate no complex formation of the enzyme or NADP+ with metal. The absorbance amplitude was increased in a dose-dependent manner when the NADP+ and metal were combined. The inhibition observed appeared to be directed at the initial binding of NADP+, with subsequent disturbance of active enzyme tetramer formation.

ABNORMAL COPPER STORAGE IN THE TELEOST FISH (Morone Americana). T.E. Bunton, J. Frazier, S. Bakst, Division of Comparative Medicine and Pathology, Division of Experimental Pathology and Toxicology, Johns Hopkins University, Baltimore, MD.

The teleost fish Morone americana (white perch) was determined to accumulate massive amounts of copper in the liver (up to 1002±513 μg/g wet weight) compared to the adult white bass (3.45±0.6 μg/g) and the striped bass (Morone saxatilis; 3.49±1.17 μg/g), and the concentrations of copper increased with age. In order to document the toxic effects of copper accumulation in fish tissues, and to determine the similarities to Wilson's disease in man (hepatoliucentricular degeneration), histological, histochemical, and electron microscopic analyses of tissues were performed. There was diffuse accumulation of rubenic acid (copper) positive granules in hepatoctyes, with scattered hepatocellular degeneration and multifocal fibrosis in older fish. Ultrastructurally, there were multiple complex electron dense cytoplasmic bodies in hepatocytes and cells of melanomacrophage centers and mitochondrial alterations. Copper positive granules were also seen histologically in accumulations of macrophages in brain associated with degenerative changes, and in the spleen, kidney, gastrointestinal tract, heart, and gills. These preliminary findings of high liver copper levels, coupled with degenerative changes in liver and brain indicate that the white perch may be a useful animal model investigating copper toxicity.


Independent investigative work evaluating the Mouse Ear Swelling Test (MEST) was conducted by several cosmetic companies. The objectives of the work were to compare the accuracy and sensitivity of the MEST with various predictive procedures for allergic contact dermatitis. Pilot experiments were conducted which compared the sensitivity of the MEST to a new dermal patching procedure for mice. In both procedures, groups of either five or ten Balb/C mice were induced with either 0.05%, 0.1%, 0.25% or 0.50% 1- chloro-2, 4 Dinitrochlorobenzene (DNCB). Following the induction phases, groups of mice were challenged with 0.1% DNCB. Results showed the new dermal patching procedure to be superior to MEST in detecting low concentrations of a sensitizer. In validation studies, test materials such as Eugenol, fragrance oil mixtures, propylene glycol, ethanol and DNCB were evaluated in the MEST and compared to responses obtained from the guinea pig maximization test and when possible to human sensitization tests. Results showed the MEST to be roughly of equal sensitivity to human tests with some but not all materials and to be less sensitive than the guinea pig maximization test.
DESIGN, DEVELOPMENT AND VALIDATION OF AN ALTERNATIVE TEST SYSTEM: THE MOUSE EAR SWELLING TEST AS A CASE STUDY. S.C. Gad, Toxicology, G. D. Searle Research and Development, Skokie, IL

A multistage scheme was developed for the development, validation, and dissemination of new test systems in toxicology. Its use is illustrated by the case of the mouse ear swelling test (MEST).

The stages in the scheme are (I) defining the test objective, (II) defining a developmental test design and optimizing test design, (III) internally evaluating performance of optimized test design against a battery of known positive and negative compounds, (IV) transferring the technology to other laboratories, (V) validating the test system in multiple laboratories, and (VI) continuing to refine and evaluate test system performance and utilization. Each of these stages is demonstrated with data from the MEST development process.


Keratinocytes can be isolated with high purity from mammalian skin. Under standard conditions, cultures grow for several days before differentiating into keratin masses. Using rat epidermis, we have shown that differentiation can be inhibited for at least 2 months by reducing the calcium concentration of the medium to 0.1 mM. Under these conditions, cultures retain an epithelial morphology and synthesise DNA at a constant rate. Cells are shed into the medium and differentiate in suspension. Full differentiation can be induced by calcium addition at any time.

Keratinocyte cultures are being employed to study the genetic effects of chemicals on a system normally capable of growth arrest and differentiation. The possible indirect effects of irritants and tumour promoters can also be investigated using cultures supplemented with phagocytic cells.
(Supported by the UK Ministry of Agriculture, Fisheries and Food.)

IN VIVO AND IN VITRO MODELS FOR ASSESSING CARDIOVASCULAR TOXICITY. Z. Ruben, G.D. Searle & Co., Skokie, IL

Many xenobiotic agents induce cardiovascular toxicity. Rats, dogs and non-human primates are the most common animals from which data on toxicity of the heart and blood vasculature become available. Rabbits, mice, hamsters, chickens, turkeys, geese and other animals are less commonly used. Data from clinical signs, heart weight, necropsy, histologic, clinicochemical and hematologic findings provide a general background for determining or suspecting the presence of cardiovascular toxicity. For determining the mechanism of toxicity, electron microscopy, histochemistry, metabolism and hemodynamic studies are usually done. The effects of the genetic background and of the nervous, endocrine, urinary, respiratory, immune or other systems on the mechanism of toxicity may be important. In addition, in vitro methods using perfused hearts, isolated heart portions or blood vessels; or cultured cardiac myofibers, fibroblasts, endothelial or vascular smooth muscle cells may be highly valuable for research on mechanisms of toxicity. Correlation between morphologic changes induced by xenobiotics and functional impairment is essential for meaningful toxicity assessment because not all morphologic changes are a sign of toxicity. Examples demonstrating the above points will be presented.

A NOVEL PREDICTIVE ASSAY FOR EVALUATING THE SKIN IRRITATION POTENTIAL OF SURFACTANTS. E. Patrick and H.L. Malbach, Department of Dermatology, University of California, San Francisco, CA.

We reported that pre-treatment with croton oil increased skin reactivity of that area to irritants acting by different inflammatory mechanisms and to subsequent applications of croton oil. This observation was used to develop a predictive assay for evaluating the skin irritation potential of surfactants. One ear of 30-33 gm female ICR mice was treated with 10 μl of 0.5% croton oil in acetone. Seventy-two hours later, 10 μl of a surfactant solution was applied to the pre-treated ear of six animals. Dosing with surfactant was repeated at 24 hour intervals for a total of four applications. Four concentrations of surfactant were tested; the highest dose was also applied to a group of untreated mice. Degree of inflammation was quantitated as change in ear thickness measured with an engineer's micrometer. Thickness measurements were made before croton oil application, before each application of surfactant, 1, 2, 4, 6, and 8 hours after each application, and 24 hours after the fourth surfactant application. The irritation potentials of sodium lauryl sulfate (SLS), neodol 25-5s(AEs), Triton X-100 (LAS), and Minanol BT were compared by plotting log dose and change in pre-treated ear thickness to generate a line from which the dose required to produce a half maximal response was determined. The irritation potential of Minanol BT was > SLS > AES > LAS which is consistent with clinical experience. Open repetitive applications, non-viral measurements, use of multiple doses, and increased sensitivity of tissue pre-treated with croton oil offer many advantages over conventional animal assays for evaluating surfactants.

Efforts to eliminate or significantly reduce the use of live animals in toxicity testing have been growing. Since no validated and widely accepted alternative to in vivo dermal irritancy testing is yet available, we studied the adequacy of reducing the number of animals used per test. Data generated from six-rabbit skin irritation tests of 105 various materials were used to determine the ability of irritation scores from all of the possible combinations of 2-, 3-, 4-, or 5-rabbit subsets to predict the Draize scale score derived from 6 rabbits. There are 1575, 2100, 1575, and 630 possible combinations of 105 studies for the 2-, 3-, 4-, and 5- rabbit subsets, respectively. We classified materials using a four-level adaptability rating system based on (among other factors) the Draize score. Comparisons indicated that the 2-, 3-, 4-, and 5-rabbit scores were 88, 91, 94, and 96% agreement, respectively, with the classification assigned on the basis of the 6-rabbit score. The correlation coefficients for randomly selected subsets of 2-, 3-, 4- and 5-rabbit scores versus the Draize score were 0.977, 0.986, 0.994 and 0.996, respectively. This study indicated that 3 animals per test group allow for adequate assessment of the irritancy potential of a chemical substance. The results also conformed closely to those obtained from a previous study using eye irritancy data [The Toxicologist, 6(1):237, 1986].

USE OF EXCISED HUMAN SKIN FOR IN VITRO PERCUTANEOUS ABSORPTION STUDIES. R.L. Bronaugh, R.F. Stewart, and M. Simon. Division of Toxicology, Food and Drug Administration and Regional Skin Bank, Washington Hospital Center, Washington, DC.

The integrity of the barrier layer of cadaver skin samples was examined with regard to possible deterioration due to length of time and storage conditions. No difference was seen in values for water permeation constants (Kp) from unrefined skin or from skin frozen for a few days. Human skin could sometimes be stored at -20°C for up to a year with no change in water permeability. A rapid procedure was developed for checking barrier integrity of skin in diffusion cells before a penetration study. The percentage of the dose absorbed after 20 min contact with skin correlated with water Kp values. Changes in water permeation through human skin agreed with changes in the absorption of 7 test compounds of varying solubility properties (acetylsalicylic acid, benzo(a)pyrene, cortisone, DDT, nicotinic acid, propylene glycol, and testosterone). Water permeation is therefore considered to be a good indicator of potential changes in the barrier integrity of human skin. No correlation was observed in water Kp values and other characteristics of the donor skin samples such as age, sex, race, and length of time before skin harvest.

GENERATION OF A HUMAN CUTANEOUS EPIDERMIS, IN VITRO. L. Bernstein, and I.A. Bernstein. Toxicology Program, Dept. Environ. Ind. Health, Univ. of Michigan, Ann Arbor, MI.

An "epidermis" can be grown in vitro from human cutaneous keratinocytes using the technique for murine keratinocytes published by Vaughtman et al. (In Vitro, 22, 141-149, 1986). After trypsinization, growth medium (MEM, 10% fetal calf serum, 10 ug/ml insulin and hydrocortisone) cells from human foreskin were plated on a nylon membrane (10^5 cells/cm^2). In two weeks (when confluence was achieved), membranes were placed on plastic plates containing a layer of the medium was added so that the upper layers of cultures were exposed to the air. After two weeks of growth at the air-liquid interface up to 5 nucleated layers could be observed. Keratohyalin-like granules were seen. A week later as many as 10 layers of cornified cells could be detected. PAGE of keratins isolated from these cells was performed. The protein bands were identified by Coomassie staining and the high molecular weight keratins could be identified by immunoblot using AK2 keratin antibody. Identification of keratins of 36-67 KD was confirmed. The successful growth of human keratinocytes at the air-liquid interface offers a model which can be conveniently used in human toxicological studies without danger to the donor.

A COMPARISON OF EX-VIVO RABBIT AND RAT SKIN FOR ASSESSING SKIN IRRITATION POTENTIAL. G.J.A. Oliver and M.A. Pemberton. ICI PLC, CCL, Macclesfield, Cheshire, UK. Sponsor: Dr. E.A. Lock.

Skin irritation testing forms a part of the acute hazard assessment of most substances. The Draize rabbit skin 4-hour occluded patch test is the most commonly used experimental model. We have developed in vitro techniques for short-term organ culture of rat and rabbit skin in vitro. Organ cultures retain the permeability barrier (stratum corneum) of normal skin which regulates the kinetics of exposure of susceptible tissue and cells. Conditions for optimal viability of the skin in culture differ for the two species. Using rat epidermal slices and rabbit full thickness skin, untreated tissue remained viable for approximately 48 hours on the basis of measurement of ATP levels, leakage of intracellular enzymes (AST, GLDH, MDH) and lactate production. Following topical in vitro application, moderate and severe skin irritants caused a time dependent increase in the release of intracellular enzymes and the inhibition of lactate production in skin tissue from both species. Mild and non-irritants produced effects similar to controls. On the basis of these preliminary data, rat and rabbit tissue is of comparative sensitivity. The utility of these techniques as pre-screens to conventional in vivo studies is now being further validated using a greater number of chemicals.

Assessment of the acute toxicity of substances is a primary requirement in the overall evaluation of potential human health hazard. Consequently many acute studies have been completed but the toxicological data is often inaccessible for comparative assessment. Whereas such initiatives have been taken for the evaluation of repeat dose, i.e. mid to long term studies (Lumley & Walker, 1986), no similar analysis of acute toxicity studies has been reported. As an enhancement of the ARTWIS system for toxicological data-handling (Clapp & McNamee, 1985) a system has been developed which facilitates the comprehensive analysis of study conditions and toxicological findings for acute systemic (oral and dermal) and cutaneous (skin and eye irritation, and contact sensitisation) studies. Access to this data base allows the (1) comparison of the toxicity of chemical analogues, (2) determination of the prevalence of study outcomes, (3) optimisation of humane strategies for the assessment of acute toxicity, (4) optimisation of toxicological testing strategies. (Pemberton et al., 1987). Lumley, CE. and Walker, SR. (1986) Human Toxicol., 4,447-460. Clapp, M.J., & McNamee, JA. (1985) Med. Inform., Vol.10, No.2, 115-121. Pemberton, MA., Doe JE. & Oliver GJA. Soc. Tos. Washington, (1987).


We have previously developed an in-vitro test for assessing the skin corrosive potential of chemicals (Oliver et al., 1986). Chemicals applied in-vitro to rat epidermal slices which reduced electrical resistance below a predetermined threshold of 3.2kohms.cm² were identified as corrosive to skin. The technique was originally validated with 63 chemicals, the overall sensitivity (90%) and specificity (82%) of the test were confirmed using a further 120 compounds. The validation phase has now been extended in an intra-laboratory double blind trial in which chemical identity was undisclosed prior to chemical supply and testing. Using 34 corrosive and 35 irritant chemicals and a contact period of 24 hours, the sensitivity of the method in the double blind trial was 100% with no false negatives. The overall specificity was approximately 88%. These data provide further evidence of the performance and reproducibility of the in-vitro test method and support its potential application as a prescreen in in-vivo studies. Oliver GJA. Pemberton MA and Rhodes C. (1986) Fed. Chem. Toxic. In press.

SCHEME FOR THE RANKING AND PREDICTION OF RELATIVE POTENCIES OF DERMAL SENSITIZERS BASED ON DATA FROM SEVERAL TEST SYSTEMS. S.C. Ged, Toxicology, G. D. Searle and Co., Skokie, IL

A highly desirable use of delayed contact dermal hypersensitivity data from animal tests is an accurate prediction of the relative potency of positive agents in humans. Because of the manner in which all such animal tests are performed, wide variations of exposure conditions and concentrations (which are generally more severe than human exposure conditions) have traditionally made prediction of potency (and therefore of the extent of hazard) in humans either impossible or extremely crude. A numerical/graphical method has been developed to adjust results from suitable animal studies of all sorts for exposure conditions and allow for ranking of agents for potency and classification of relative hazards. Results from four animal test systems (MEST, EMT, GMT and Buehler) are compared with results from human studies to show that all four test systems can generate data that is usable for a relative hazard classification process though they may vary in their performance as screens.

STUDIES ON HEPATORENAL TOXICITY OF STYRENE OXIDE DUE TO REPEATED EXPOSURE. L. Lefebvre and S. Chakrabarti, Méd. du trav. et hygi. du mil., Fac. de Méd., Univ. de Montréal, Montréal, Québec, Canada

Information on hepatorenal toxic potential due to repeated low-level exposure to styrene oxide (SO) is very limited. Groups of 8 rats received ip injections of SO in corn oil at doses 0, 100 and 200 mg/kg once a day, 5 days a week for 4 consecutive weeks. Urines were collected for 24 h at the end of each week and the rats were sacrificed 24 h after the end of treatment. Hepatorenal toxicity of SO was evaluated by measuring several biochemical parameters in serum and urine. Body weights were decreased at the end of each week due to repeated exposure to SO at 200 mg/kg. Neither relative liver weights nor kidney weights were changed. Neither serum glutamic pyruvic transaminase nor serum sorbitol dehydrogenase was increased at any dose of SO during any week. Neither urinary γ-glutamyl transpeptidase nor N-acetyl-β-D-glucosaminidase nor serum urea nitrogen was increased at any dose level at any time. Urinary proteins were increased at 200 mg/kg of SO at the end of first, second and third weeks. Serum sodium was increased whereas serum potassium decreased at 200 mg/kg of SO at the end of fourth week. These results demonstrate that subchronic low-level (~16 and 33% of LD50) exposure to SO does not produce any significant hepatorenal toxicity in rats. (Supported by IRSST, Québec).
SEX-RELATED DIFFERENCES IN STYRENE-INDUCED HEPATORENAL TOXICITY IN HYPERTENSIVE RATS. S. Chakrabarti and A. Malik, Méd. trav. hyg. mil., Fac. méd., Univ. Montréal, Montréal, Québec, Canada.

The toxicity of styrene (S) is due to its reactive intermediate mediated by microsomal oxidative enzymes which are known to be modified due to hypertension. Groups of normotensive (NCX) and spontaneously hypertensive (SHR) rats (6-8 weeks old) of both sexes were treated i.p. in corn oil with 0, 0.6, and 1.0 g/kg b.w. Urines were collected for 24 h and the animals then sacrificed. An increase in urinary N-acetyl-D-glucosaminidase (NAG) at 0.6 g/kg due to hypertension and a significant interaction between S and hypertension were observed in male rats, but such effect due to hypertension only was noticed in female rats. A reduction in male SHR but an increase in female SHR rats were seen with regard to γ-GT excretion at both doses of S. An increase in urinary glucose due to hypertension and a significant interaction between S and hypertension were seen at 0.6 g/kg in male rats where such interaction was seen at both dose levels in female rats. Hypertension increased the urinary proteins at both dose levels in male rats, but decreased such excretion in female rats at higher dose of S. SGPT level was increased due to hypertension in female rats, but not so in male rats (Supported by CAPIR, Univ. Montréal).


Para-methylthiobenzamide (PMTB) exhibits nephrotoxic properties. A structural analogue of PMTB, N-methylthiobenzamide, has been shown to impair pulmonary 5-hydroxytryptamine clearance (Toxicologist 6:243). Since 5HT is nephrotoxic, we investigated the effect of PMTB on pulmonary 5HT clearance. Using the isolated perfused rat lung, we demonstrated that lung obtained from rats that received 12-hour pretreatment with a nephrotoxic dose of PMTB (3 mmol/kg, ip) exhibited reduced 5HT removal as measured by HPLC-EC. Metabolism of 5HT as measured by formation of 5-hydroxyindoleacetic acid was also depressed. This was not accompanied by changes in lung wet weight to body weight ratios. Lung angiotensin converting enzyme (ACE) activity in vitro was unchanged at both 3 and 12 hours after this dose of PMTB. Serum ACE activity was also unchanged 3 hours after PMTB administration. These findings suggest that the induction of impaired lung function after PMTB administration may contribute to its ability to cause kidney damage. (Supported by K.U. Biomedical Grant No. 4483-0712-9 and A.A.C.P./U.R.P.)

INFLUENCE OF DOSE ON THE METABOLISM AND HEPATORENAL TOXICITY OF STYRENE OXIDE IN RATS. L. LeFebvre and S. Chakrabarti. Médecine du travail et hygiène du milieu, Université de Montréal, Montréal, Québec, Canada.

Styrene oxide (SO) is a primary metabolite of styrene. In view of its large production and various uses, surprisingly few studies have been reported regarding its metabolism and hepatorenal toxicity. So present study was undertaken to examine the effect of different degrees of exposure to SO on its metabolism and hepatorenal toxicity. Pre-fasted (16 h) male Sprague-Dawley rats were treated ip in corn oil with 0, 200, 300, 375 and 425 mg SO per kg. Urines were collected for 24 h after treatment and animals were then killed. Serum sorbitol dehydrogenase and glutamic pyruvic transaminase were increased at 300 and 375 mg/kg of SO respectively. Relative kidney weight was increased with increasing dose but no further increase at 425 mg/kg. Urinary γ-glutamyl transpeptidase, N-acetyl-D-glucosaminidase and serum urea nitrogen were all increased with increasing dose of SO but reached an apparent saturation at the highest dose. Both urinary volume and sodium were initially increased at 200 or 300 mg but finally decreased at 375 or 425 mg/kg. Urinary excretions of mercapturic acids or thioethers, mandelic and phenylglyoxylic acids were all increased with increasing dose but reached an apparent saturation at 425 mg/kg. These data indicate possible dose-dependent toxicity and metabolism of SO in rats.

PHARMACOKINETICS OF ACETAMINOPHEN IN AGING SPRAGUE-DAWLEY RATS. J.B. Tarloff, R.S. Goldstein and J.B. Hook, Department of Investigative Toxicology, Smith Kline and French Laboratories, Philadelphia, PA.

Susceptibility to acetaminophen (APAP)-induced nephrotoxicity is a function of age in male Sprague-Dawley (SD) rats. The present study was designed to investigate the mechanism of the age-related increase in susceptibility of SD rats to APAP nephrotoxicity. APAP (750 mg/kg, ip) was administered to 3 and 12 mo old male SD rats. Blood was collected over 24 h and analyzed by HPLC for APAP. Renal cortical and medullary APAP accumulation (expressed as tissue/plasma ratio) was also determined. At 24 h following APAP, BUN was significantly elevated in 12 mo compared to 3 mo rats. Blood APAP concentrations were higher in 12 mo than 3 mo rats as early as 15 min following APAP administration. Blood APAP half-life was prolonged in 12 mo rats. Cortex/plasma and medulla/plasma APAP concentration ratios were not different between 3 and 12 mo rats up to 6 h following APAP. Cortical and medullary APAP concentrations (per g wet weight) were elevated in 12 compared to 3 mo rats. Thus, 12 mo rats have higher blood and renal tissue APAP concentrations. These data indicate that differences in pharmacokinetics contribute to the age-related increase in susceptibility of SD rats to APAP-induced nephrotoxicity.

The utility of renal cortical slices for assessment of chemical-induced injury is limited by loss of proximal tubular function/viability over short (3-4 hr) incubation periods. The purpose of these studies was to develop an improved incubation technique permitting use of renal cortical slices for extended (8 hr) incubation periods. Renal cortical slices were prepared from male or female Sprague-Dawley rats and were incubated individually for 1 to 8 hr in a supplemented Krebs-Ringer phosphate buffer. Proximal tubular function was estimated by slice organic ion (PAH, TEA) accumulation (S/M ratio) and gluconeogenic capacity; viability was assessed by LDH leakage. Slice accumulation of PAH (S/M ratio: 7.0 to 8.0) and TEA (S/M ratio: 11.0 to 16.0) remained constant over 8 hr; LDH leakage was 13% after 1 hr and 24% after 8 hr of incubation. Gluconeogenesis plateaued (50 umole/g slice) after 4 hr of incubation. KCl perfusion (50 and 100 umol/g) reduced slice organic ion accumulation, increased LDH leakage and inhibited slice gluconeogenesis. Gentamicin (2.5 and 5.0 mM) reduced slice organic ion accumulation but did not increase LDH leakage or inhibit gluconeogenesis.

SELECTIVE IN VITRO NECROSIS OF PROXIMAL TUBULAR SEGMENTS IN RENAL CORTICAL SLICES. CE Riegg, AJ Gandolfi, K Brendel, RB Nagle. Dept. of Pharm/Tox Univ. of AZ, Tucson, AZ

Proposed mechanisms leading to selective necrosis of convoluted (CPT) or straight proximal tubular (SPT) segments following KClO3, or HgCl2, respectively, are often attributed to blood delivery patterns, renal concentration, or feedback ischemia in the intact organ. Hence, rabbit renal cortical slices were exposed in vitro to either HgCl2, KClO3 (10 umol/mM) or ischemic conditions (0.75-5 hr) to investigate the in vivo susceptibility of proximal tubular segments. HgCl2 (100 umol) exposure demonstrated SPT damage by 12 hr leaving the CPT unaffected. Ischemia (1.5 hr) and KClO3 (100 umol; 12 hr) both caused injury to CPT without affecting SPT. Metal concentrations of 10 umol and 1 mmol had no effect or injured all cell types within the slice, respectively. Similar results were observed with 0.75 or >3 hr of ischemic exposure. Slice K'/DNA content correlated with the pathologic lesions. KClO3 (10-5 M; 8hr) inhibits anionic (PAH) but not cationic (TEA) transport by inhibiting entry mechanisms. HgCl2 (10-5 M) had no effect on transport. Higher doses of these metal inhibited all transport. Therefore, selective acute tubular necrosis relates to an innate susceptibility of tubular segments independent of physiologic feedback or blood delivery patterns proposed from in vivo studies. (Johns Hopkins CAAT)

DEVELOPMENT OF AN IMPROVED IN VITRO SYSTEM FOR PROLONGED INCUBATION OF ISOLATED RENAL TUBULES. C. E. Green, T. E. Dabbs, C. A. Tyson, and E.J. Raabekann. SHR International, Menlo Park, CA and 2National Toxicology Program, NIEHS, RTP, NC.

The application of isolated renal tubules to nephrotoxicity studies is limited by their relatively short lifespan in vitro. The aim of the present studies is to modify isolation and incubation conditions for improved tubule viability and functionality. Tubules were isolated from Fisher 344 rats by perfusion with collagenase and mild mechanical disruption. Yields were 70-90 mg protein and viability - 90-95%. Lactate dehydrogenase (LDH) release, alkaline phosphatase (ALP) stability, ATP/ADP, organic ion uptake were measured during incubations for up to 6 hr in culture medium + 2% BSA at 37°C under 95% O2:5% CO2. LDH release increased with time to 316 ± 4.0% (N=5) at 6 hr. Use of 95% air instead of 95% 95% O2 decreased LDH release by 10-25%. Adding 2% BSA to the tubule washing buffer decreased LDH release further (~10%) and appeared to stabilize ALP. Lactate (5 mmol) in the culture medium improved tubule viability slightly. Percoll gradient purification decreased viability and lowering the culture medium pH to 6.6 was without effect. In summary, the survival of isolated renal tubules was increased moderately by manipulating the isolation and incubation conditions. Additional variables are being tested. (Supported by NIEHS contract ES-65745)


The nephrotoxicity of the cysteine conjugate (DCVC) of dichloroethane is well known. In order to determine whether other solvent types like bromobenzene (BB aromatic) and acrylonitrile (ACN nitrine alkene) act on the kidney via the same mechanism, their cysteine conjugates were synthesized as cyanoethyl-cysteine (CCE from ACN) and m-bromophenyl-cysteine (BPC from BB). They were then incubated at 37°C with renal tubule suspensions isolated from Fisher-344 rats. The effects of these conjugates on organic ion uptake were compared to those of DCVC. Control uptake (T/M) of p-aminophenylate was 8.3 after 10 min incubation. T/M fell to 3.7 and 2.6 in the presence of CEC at 0.1 and 0.5 mM respectively, and to 1.9 in the presence of BB (0.5 mmoles/l) compared to 6.0 and 1.4 with DCVC (0.1 and 0.5 mmoles/l). Control T/M values for 14C-tetraethylammonium bromide was not significantly altered by the cysteine conjugates. Our data indicate that cysteine conjugates interfere specifically with the organic acid transport system but not the base uptake pathway. Further suggest that different types of organic solvents may exert their nephrotoxicity via homologous steps of the glutathione pathway occurring at the level of renal cell membrane. (MRC grant no. MA-9705).
IDENTIFICATION OF THE 2,2,4-TRIMETHYLPTENANE (TMP) METABOLITE BOUND TO MALE RAT a2u-GLOBULIN. M. Charbonneau, E.A. Lock, J. Strasser, M.J. Turner, M.G. Cox and J.A. Swenbeng. Chemical Industry Institute of Toxicology. Research Triangle Park, NC.

A reversible association between TMP-derived radioactivity and the male rat specific protein a2u-globulin (a2u) has recently been observed in kidney cytosol of TMP-treated rats. The present study was undertaken to identify the TMP metabolite associated with a2u. Kidney cytosol was isolated from male Fischer 344 rats dosed orally with [3H]-TMP (500 mg/kg, 230 μCi/kg). A Sephadex G75 column was used to separate the components of the cytosol. Radioactivity from TMP resolved in two peaks: the one which co-eluted with a2u (19000d) contained about 25% of the radioactivity. Fractions containing the a2u were pooled and concentrated using an immersible ultrafilter (10,000 m.w. cutoff). The resulting solution was acidified and a partition extraction performed using ethyl acetate. An aliquot of the organic phase was injected into a gas chromatograph equipped with a megabore DB-WAX column. The resulting chromatogram contained a peak which co-eluted with authentic 2,4,4-trimethyl-2-pentanol. Mass spectrometric analysis confirmed that the putative TMP metabolite isolated from the pooled kidney cytosol containing a2u was 2,4,4-trimethyl-2-pentanol. The relevance of this reversible association to TMP-induced nephrotoxicity remains to be evaluated. (Supported in part by IRSST Quebec)

NEPHROTOXICITY OF 2,2,4-TRIMETHYLPTENANE (TMP) METABOLITES IN MALE FISCHER 344 RATS. M. Charbonneau, E.A. Lock, J. Strasser, B.G. Short and J.S. Bus. Chemical Industry Institute of Toxicology. Research Triangle Park, NC.

TMP, a nephrotoxic component of unleaded gasoline in male rats, undergoes metabolism via pentanoic to pentanoic acids. We have examined the ability of several TMP metabolites to produce nephrotoxicity as measured by an increase in renal protein droplets, renal a2u-globulin (a2u) concentration and renal cell proliferation in male Fischer 344 rats. Rats were treated (po) with 4.4 mmol/kg of TMP: 2,2,4-trimethyl-1-pentanol; 2,2,4-trimethyl-1-pentanoic acid (2,2,4-TMP acid); 2,4,4-trimethyl-1-pentanol; 2,4,4-trimethyl-2-pentanol; 2,4,4-trimethyl-1-pentanoic acid; or 2,4,4-trimethyl-1-hydroxy-1-pentanoic acid. Protein droplet accumulation was scored in kidney slides and a2u concentration was measured in kidney homogenates 24 hr after dosing. A second series of rats were implanted with osmotic mini-pumps containing [3H]-thymidine (3H-TdR) 1 day before dosing with the above metabolites. Incorporation of 3H-TdR into renal DNA was measured 5 days later to quantitate cell proliferation. All metabolites studied led to an increase in protein droplet accumulation and renal a2u concentration. However, only TMP and 2,2,4-TMP acid increased 3H-TdR incorporation into renal DNA. These studies show that single doses of TMP-derived metabolites are equipotent inducers of protein droplet and a2u accumulation but not of cell proliferation. (Supported in part by IRSST Quebec)

THE RENAL TRANSPORT OF PENTANOIC ACIDS DERIVED FROM 2,2,4-TRIMETHYLPTENANE. E.A. Lock, M. Charbonneau, J. Strasser and J.S. Bus. Chemical Industry Institute of Toxicology. Research Triangle Park, NC.

2,2,4-Trimehtylpentane (TMP), a component of unleaded gasoline produces kidney lesions in male rats. The lesions are characterized by protein droplet accumulation and proximal tubular cell degeneration. TMP undergoes metabolism to 2,2,4-trimethyl-1-pentanoic acid (2,2,4-TMP acid) and 2,4,4-trimethyl-1-pentanoic acid (2,4,4-TMP acid). We have studied the effect of these pentanoic acids on the transport system for organic anions (PAH) and organic cations (TEA) using renal cortical slices from male rats. Thin slices of renal cortex were incubated with 75 μM [3H]-PAH and 9 μM [14C]-TEA under 100% O2 at 25°C in the presence or absence of the pentanoic acids. 2,2,4-TMP acid reduced the accumulation of PAH without affecting the accumulation of TEA in a time- and dose-dependent manner. Michaelis-Menten kinetics showed competitive inhibition of the organic anion transport system with an apparent Ki of 4 mM. 2,2,4-TMP acid reduced the accumulation of both PAH and TEA transport in a time- and dose-related manner. These studies indicate that 2,4,4-TMP acid is a substrate for the renal organic anion transport system, and suggest that the carrier-mediated transport of these metabolites may contribute to the nephrotoxicity induced by TMP. (Supported in part by IRSST Quebec)
 Gentamicin (G)-induced increases in cytosolic calcium ([Ca]i) in pig kidney cells (LLC-PK1).

LLC-PK1 cells, an established epithelial cell line derived from pig kidney, were tested as a model system for evaluating the role of calcium in gentamicin-induced nephrotoxicity. Viability was evaluated by trypan blue exclusion and [Ca]i measured using the calcium indicator, fura-2AM. Over a 2 hr time period, viability was >99% in cells exposed to 1 mM G, but was diminished to 83% and 71% in cells treated with 5 and 10 mM G, respectively. Cells (10⁶/ml) were loaded with 10μM fura-2AM and exposed to G (0.1-1kM) for 5, 15 and 30 min. [Ca]i was increased 2-fold as early as 5 min after exposure to 1 mM G (262±35 vs. 119±23 nM in controls). The effect was dose-dependent. After 1 hr exposure to 1 mM G, cells showed morphologic evidence of membrane injury by EM analysis (loss of microvilli and blebs).

In conclusion, we find that LLC-PK1 cells are sensitive to G and that exposure to G causes an early and persistent increase in [Ca]i, followed by membrane alterations. These results suggest that the primary event in the renal pathogenesis of G may involve an increase in [Ca]i.

In Vitro Toxicity of Cephaloridine and Hexachlorobutadiene in Rabbit Cortical Slices, G.H. Wolfgang, A.J. Gandolfi, K. Brendel, Dept. of Pharm/Tox, Univ. of AZ, Tucson, AZ

Cephaloridine (CPH) and hexachlorobutadiene (HCBDD) are proposed to require bioactivation prior to renal toxicity. In vitro systems were utilized to demonstrate the role of renal bioactivation in the toxicity of these compounds. Precision-cut positional renal cortical slices were obtained from male NZW rabbits and incubated in oxygenated serum-free DME/F12 media for up to 24 hr. CPH (1, 5, and 10 mM) decreased intracellular K⁺ by 38, 56, and 66%, while intracellular LDH decreased 28, 38, and 82% by 24 hr. CPH (5 and 10 mM) inhibited p-aminohippurate transport by 4 hr, while lower doses were not effective until 12 hr. CPH initially produced straight proximal tubule lesions (12 hr), expanding to the convoluted proximal tubules by 24 hr. HCBDD (1 mM) decreased intracellular K⁺ by 30, 73, and 94%, while intracellular LDH decreased 4, 47, and 90% by 12 hr. Necrosis of all cell types was evident with 1 mM HCBDD. At lower doses the lesion was limited to the proximal tubules, necrosis appearing after 4 hr (100 μM) and 8 hr (10 μM) in vitro pretreatment. Necrosis caused by CPH and HCBDD suggest that the kidney itself has the ability to activate these compounds to toxic metabolites.

90


Prolonged congestion and edema in the lung induced by Paraquat might be responsible for the subsequent pulmonary fibrosis. Although it is well known that renal dysfunction can aggravate pulmonary edema, there have been few investigations concerned with renal changes in the toxicity studies with Paraquat. Male F344 rats were intubated Paraquat dichloride at 212 mg/kg. At 3, 6, and 24 hr after the treatment, the kidneys from 4 or 5 animals at each interval were perfused with 1.25% glutaraldehyde and were examined by light and electron microscope. At 3 hr after the treatment, swelling and transformation of mitochondria, dilatation of cisterna of RER and Golgi complex, degeneration of RER, and a slight swelling of cytoplasmic matrix were observed in the proximal and distal tubules. The changes were more prominent in the distal tubules than the proximal tubules. At 8 hr, disintegration of the distal tubular cells was much advanced and accentuated by large vacuoles in the cytoplasm and widely opened space of the basal intussusception, while the architecture of the proximal tubular cells was still preserved well. At 24 hr, necrotic processes were frequently found in all course of the distal tubules but only occasional cells were necrotic in the proximal tubules. The present results indicated that Paraquat could potentiate severe renal failure substantially attributable to the distal tubular damage.


BHQ is a nephrotoxic metabolite of bromobenzene. Previous results from our laboratory have shown that RPT mitochondria (MITO) are an early target of BHQ. To determine whether MITO dysfunction is the result of protein alkylation, [14C]BHQ (0.2 or 0.5 mM) was added to a suspension of RPT (with decreased glutathione (GSH) content) at 0°C and 37°C for 15-60 min. Oxygen consumption (QO2), GSH content and covalent binding to protein were determined. Covalent binding of BHQ to protein was concentration-, time-, and temperature-dependent. At 37°C, the addition of 0.2 mM BHQ resulted in 11, 15, and 18 nmoles of BHQ bound/mg protein at 15, 30 and 60 min, respectively. Under the same conditions, QO2 was inhibited 22, 40 and 72% and GSH content decreased by 80, 92 and 97%. These data show that the majority (61% of the total) of the covalent binding occurs initially as MITO function decreases. The addition of 1 mM GSH, 5 min prior to BHQ, inhibited the covalent binding of BHQ to protein and prevented the decrease in QO2. These results suggest that BHQ-induced MITO dysfunction may result from protein alkylation. (Supported by Vet. Med. Exp. Station, Univ. of Georgia).
ROLE OF METHEMOGLOBINEMIA IN THE NEPHROTOXIC EFFECTS OF CHEMICALS. H.J. Lo and G.O. Rankin. Department of Pharmacology, Marshall Univ. School of Medicine, Huntington, WV.

Previous studies have demonstrated that anilines produce nephrotoxicity at doses which produce moderate to marked methemoglobinemia. The purpose of this study was to examine the effects of methemoglobinemia alone on renal function. In one experiment, renal cortical slices from male Fischer 344 rats were incubated (37°C, 30 min) with sodium nitrite (0-10-^-m) followed by the addition of p-aminophenol (PAMP) or betanaphthenolamine (BNA). Concentrations were continued (90 min) and the accumulations of PAMP and TEA determined. In a second experiment, methemoglobinemia was induced in male Fischer 344 rats by oral treatment with sodium nitrite (25, 50 or 100 mg/kg). Control rats received distilled water (1 ml/kg). Rats were killed at 2 or 8 h posttreatment and kidney weights, BUN concentrations, PAMP and TEA accumulation by renal cortical slices, and methemoglobin concentrations determined. Renal function was not altered by methemoglobinemia (2-95%) at 2 or 8 h. In addition, in vitro sodium nitrite had no effect on PAMP or TEA accumulation. These results suggest that methemoglobinemia alone does not alter renal function. Supported by NIH grant DK 31210.


We have previously observed a dose-related increase in blood pressure (BP), heart rate (HR), and blood volume and a decrease in plasma angiotensin II concentration in rats exposed via whole body inhalation to a high boiling (550-780°F) complex organic mixture (berry distillate, HD). The purpose of this study was to investigate cardiovascular effects of two boiling-range fractions, HD and middle distillate (MD, 350-550°F), when exposure occurred from dermal application alone. HD or MD were applied daily, 5 days/week, for six weeks at 400 mg/kg to the rat's shaved skin. After a 2-week recovery period, the femoral artery was cannulated and BP and HR were recorded in anesthetized rats. BP and HR were measured one day later in the conscious rat; then again 30 min after an injection of 5 mg/kg of captopril. BP and HR were significantly increased in the anesthetized HD-treated rat compared to the MD. Neither BP nor HR were statistically altered in the HD-treated conscious rat or the captopril-treated rat. Exposure to MD had no significant effect on BP or HR. These data suggest that HD does not act directly on the pulmonary system to produce the cardiotoxic effects and that the active components are contained in the HD rather than the MD fraction. Work supported by U.S. Department of Energy Contract DE-AC06-76RLO 1830.


The hypotensive effect of the insect repellent DEET was originally described by Ambrose (1959, Toxicol. Appt. Pharmacol. 1:97-115). These data were preliminary however, and the current studies were initiated in order to better characterize this response. Anesthetized, catherized male Sprague Dawley rats exhibited a dose related drop in mean blood pressure within 30 minutes of an i.p. injection of DEET (75% DEET, 25% ethanol). Dosages included 225, 125, 50 mg/kg. At the highest dose tested, heart rate was also significantly reduced. Adult male beagle dogs treated with 225 mg/kg exhibited decreased blood pressure, heart rate, and cardiac output. Peripheral resistance and stroke volume were not altered. ECG analysis indicated an increased R-R interval but no other changes. In a final experiment, anesthetized rats were challenged with a series of standard pharmacological agents (epinephrine, norepinephrine, acetylcholine, and histamine), then treated with 225 mg/kg DEET i.p. Blood pressure and ECG were monitored and pre and post DEET responses to the drug challenges were compared. DEET significantly reduced responsiveness to acetylcholine but not to histamine or the catecholamines, suggesting that the hypotensive effects of DEET may be due in part to an interaction with cholinergic systems.


The oral toxicity of CI-926, chemically designated as 3[4,4-(3-methylphenyl)-1-piperazinyl]-butyl]-2,4-imidazolidinedione was investigated in 13 week studies in the rat and the dog. In the rat, CI-926 was well-tolerated as a diet admixture at doses of 5, 10 and 20 mg/kg/day. Except for 20 mg/kg females, a slight suppression of body weight gain was noted during dosing and reversal phases of the study. Slight increases in mean adrenal weight were noted in females given 10 or 20 mg/kg. There were no significant clinical signs, clinical laboratory or pathologic findings. In dogs given 5, 10 or 20 mg/kg in gelatin capsules, CI-926 produced transient salivation, miosis, prolonged nicotrans and injected mucous membranes, as well as dose-related episodes of somnolence, ataxia, hypoactivity and aggression. Reduced blood pressure with reflex tachycardia occurred post-dosing. Clinical signs, which tended to accommodate by Week 8, were attributed to exaggerated pharmacologic effects of the compound. There were no consistent drug-induced clinical laboratory or pathologic findings.
ISOPROTERENOL-INDUCED VENTRICULAR FIBRILLATION IN THE RAT: INFLUENCE OF STRAIN OR DIETARY FISH OILS.
CDM, FBA, Washington, D.C.

Isoproterenol, 1 mg/kg s.c., (1) induces ventricular fibrillation (VF) in heavy (3450 g) male Sprague-Dawley (SD) rats. The present studies determined if this phenomenon was strain-specific or influenced by a diet high in fish oils (docosahexaenoic and eicosapentaenoic acid fortified cod liver oil). When male SD, 584±17 g, Wistar (W), 564±10 g, Lewis (L), 426±3 g, and F-344 (F), 344±4 g, rats (10/strain) were 201 days of age they received 1. There were no deaths by VF in either F or L rats, but in W and SD rats the incidence was 60% and 40%, respectively. All survivors had cardiac lesions; the severity of lesions was least in F rats and greatest in W rats. In the diet study, male SD rats received either regular meal, meal + 10% corn oil, or meal + 10% fortified cod liver oil. All rats were 150-172 days of age and each group weighed between 498±17 g and 535±22 g when they received the diet. The incidence of death by VF in rats receiving corn oil or fish oil for 5 weeks (5/16 & 7/16, respectively) or for 18 weeks (7/16 & 7/14, respectively) was not significantly different from that in rats fed a normal diet (7/15). These studies indicate that: 1) heavier rat strains are more susceptible to I-induced VF than are equally old rat strains, and 2) the susceptibility to I-induced VF is not altered by dietary fish oils.

THE TOXIC EFFECTS OF MAMBA SNAKE VENOMS IN PRIMARY MYOCARDIAL CELL CULTURES. P.M. Mbugua, A.A. Welder, and D. Acosta. Dept. of Pharmacology & Toxicology, College of Pharmacy, The University of Texas, Austin, Texas.

Cardiotoxins have not been identified in mamba snake venoms. For the first time, cardiotoxic effects of three mamba snake venoms was demonstrated using primary myocardial cell cultures. The cultures were obtained from hearts of 3-5 day old Sprague-Dawley rats and were exposed to various concentrations (1.10, 50, and 100 μg/ml) of three mamba snake venoms; green (Dendroaspis (D.) angusticeps), jamesson's (D. jamessoni), and black (D. polylepis) for 0.5, 1, 2, 4, and 1 hr. Cardiotoxicity was assessed and compared among the three venoms on the basis of alterations in cell morphology, beating rate activity, and cellular lactate dehydrogenase (LDH) release. No morphological alterations occurred after 24 hr exposure to 100 μg/ml of black mamba venom. However, only 0.5 hr exposure to 50 and 100 μg/ml of green mamba venom and 2 hr exposure to 100 μg/ml jamesson's mamba venom induced pseudopodia and disruption of the cell monolayer. All beating activity was lost upon immediate exposure to 50 and 100 μg/ml or all three venoms. Only cells exposed to black mamba venom were able to recover beating activity after 2 to 4 hr. No LDH release was induced in the cells after 24 hr exposure to the black mamba venom. Significant LDH leakage occurred at 1 and 4 hr with 100 μg/ml of green and jamesson's mamba venoms. These results show for the first time that the green mamba venom is potentially more cardiotoxic when compared to the black mamba (one of the deadliest snakes in the world) venom.

(Supported in part by the Fulbright Scholar Program)


Spontaneously beating MMR were prepared from 10 day old chick embryos. After 1-3 days culture, spherical MMR had formed, approximately 80-150 μm in diameter. The MMR were filmed at 37°C and a video output was used to detect edge movement via a video monitoring device. Control MMR beating rates were between 50-150 beats per minute, but individual MMR did not vary their intrinsic rates significantly. Superfusion with test agents such as 20μM propranolol or 1μM verapamil resulted in a significant negative inotropic response whilst 20μM adrenalin or 1μM ouabain caused positive inotropism. Biochemical parameters of control MMR were measured to characterise intracellular toxicological events. Intracellular ATP content was 0.34 mmol/10^6 cells; total glutathione 0.03 μmol/mg protein; carnitine accumulation 2.62 pmol/hr/10^6 cells and creatine kinase leakage 1.85/hr. These preliminary data demonstrate that MMR are biochemically functional and responsive to known cardioactive agents. Further experiments to elucidate the relationship between functional and biochemical responses of MMR to agents inducing cardiotoxicity are in progress.

THE TOXIC EFFECTS OF MAMBA SNAKE VENOMS IN PRIMARY ENDOTHELIAL CELL CULTURES. P.M. Mbugua, A.A. Welder, and D. Acosta. Dept. of Pharmacology & Toxicology, College of Pharmacy, The University of Texas, Austin, Texas.

Mamba snake venoms are among the most complex venoms with more than 30 different components. No attempt has been made to characterize the toxic potential of the mamba venoms and their constituents using in-vitro cell culture methods. Experiments were performed to evaluate the toxic effects of three mamba venoms in primary endothelial cell cultures. The endothelial cells were obtained from hearts of 3-5 day old Sprague-Dawley rats and separated from the muscle cells by a pour-off technique. After 6-7 days in culture, the cells were exposed to various concentrations (1.10, 50, and 100 μg/ml) of green (Dendroaspis (D.) angusticeps), jamesson's (D. jamessoni), and black (D. polylepis) venoms for 0.5, 1, 2, 4, and 24 hr. Leakage of cellular lactate dehydrogenase (LDH), cell viability, and morphology were used as indices of cell injury. When compared to untreated controls after 4 hr, both the green mamba venom (50 and 100 μg/ml) and the jamesson's mamba venom (100 μg/ml) caused significant LDH leakage (p<0.001) whereas the black mamba venom had no effect. The extent of reduction of cell viability by green mamba venom (50 and 100 μg/ml) and jamesson's mamba venom (100 μg/ml) after 4 hr exposure was 39% and 29%, respectively. The black mamba venom (100 μg/ml) did not reduce cell viability below that of the untreated controls. Morphologically there was complete cellular destruction with 50 and 100 μg/ml of green mamba venom after 1 hr while the result of jamesson's mamba venom caused similar damage after 4 hr exposure. No major morphological cellular alterations were observed with 100 μg/ml of black mamba venom after 24 hr. The relative toxicities of mamba snake venoms was assessed for the first time using in-vitro cell culture techniques. These results indicate that the D. angusticeps venom is the most toxic of the three snake venoms evaluated in primary endothelial cell cultures.

(Supported in part by the Fulbright Scholar Program)
Previous evidence from this laboratory suggested that allylamine (AA), an industrial aliphatic amine with known toxicity to myocardium and vascular tissue, is metabolized by vascular tissues to the reactive and noxious aldehyde, acrolein (ACR). In this study, AA (2.0 µl in 150 mg AA/kg) was given to 200-230 g rats by gavage, and total urine was collected at 24 and 48 hr. The radioactive urinary fraction was separated and purified by HPLC, and identified by comparison to an authentic synthesized metabolite. A solitary metabolite, 3-hydroxy propylacrylic acid (3-OH PPA), was identified by MS, NMR, and 2D-NMR spectroscopy. Other possible metabolites (such as 2-OH NDA) were ruled out by 2D-NMR studies. 3-OH PPA is the expected excretory product of ACR conjugation, and has been demonstrated in the in vivo metabolism of several other toxins containing allyl groups. An additional experiment with a partially purified enzyme from cultured porcine aortic vascular smooth muscle cells demonstrated ACR production (measured by HPLC) following incubation with AA. These studies suggest that AA may be initially metabolized in the media of arteries; the identified urinary excretory product indicates detoxification through conjugation with glutathione, possibly also occurring in target cardiovascular tissues. (Supported by Grant No. Hl-26189 from the National Institutes of Health.)

Methemoglobinemia resulting from exposure to heme-oxidants is a frequently cited and potentially fatal form of anemic hypoxia. Yet virtually nothing is known of the potential for concomitant oxidation of cardiac myoglobin. We employed a coronary-free fish heart model to address this question. Hemoglobin in the buffalo sculpin (Enophrys bison) was oxidized rapidly and reversibly following intraperitoneal injection with sublethal levels of sodium nitrite and hydroxylamine. Myoglobin in hearts excised at the peak effect of hemoglobin was also oxidized. For sodium nitrite, the oxidation of myoglobin exceeded that of hemoglobin. The reverse was true of hydroxylamine. In either case the effect was dose-dependent. We conclude that there is a good chance that cardiac myoglobin is oxidized in occupational or other exposures to these and related compounds. At present we are examining the physiological consequences of exposure to sodium nitrite, hydroxylamine, and aniline on the isolated, perfused sculpin heart. Supported by NEHS Grant No. ES-07060.

The sudden release of intracellular constituents upon reoxygenation of hypoxic heart tissue (oxygen paradox) appears to involve necrosis of cells damaged by hypoxia, but little is known of the mechanism. Enzyme release from rat hearts perfused by the method of Langendorff with Krebs-Henseleit medium containing 10 mM glucose was studied. Hearts were equilibrated for 30 min followed by 60 min of hypoxia and 30 min of reoxygenation. Confirming an earlier report (Ganter et al, Am. J. Pathol. B4: 327 (1976)), 5 mM cyanide 5 min before reoxygenation prevented the release of lactate dehydrogenase (LDH). However, LDH release commenced immediately upon withdrawal of cyanide suggesting an energy dependent step. Analyses of cardiac ATP and creatine phosphate revealed increases 4 min after reoxygenation or removal of cyanide, when LDH release was maximal. Removing calcium from the medium at the time of hypoxia prevented LDH release at reoxygenation. However, removing calcium after 30 min of hypoxia initiated LDH release without reoxygenation. Perfusion with 1 mM cyanide 10 min before reoxygenation decreased LDH release by about 80%. Recent work on the oxygen paradox has suggested that oxygen radical formation is not involved (Kehres et al, Free Rad. Res. Commun. [in press]). The contrasting time-dependent effects of calcium free perfusion, the need for energy, and the inhibition of LDH release by cyanide (which inhibits calcium-ATPase) suggest that, following an early calcium-dependent change, reoxygenation induces enzyme release by decreasing intracellular calcium levels. (On leave from the Div. Pharmacology, College of Pharmacy, Univ. of Texas, Austin, TX and supported by RCDA HLD1435.)

Oxygen free radicals (OFR) generated by xanthine oxidase (XO) and/or by neutrophils have been implicated in the development of ischemic reperfusion myocardial injury (Werns et al. Circulation 74:1, 1986). We examined the effects of XO inhibitors, 6MP and 6TG combined and allopurinol (AP) on ISO-MN. Male Sprague-Dawley rats (3.5 to 4.5 months old) were used. Groups of ten rats were pretreated with 6MP+6TG (10 mg/kg of each drug, sc, b.i.d) for 2 days and 2 hr before and after ISO (0.1 mg/kg, sc) on day 3 or with AP (20 mg/kg sc; b.i.d) for 4 days and 2 hr before and after ISO on day 5, with proper controls. Rats were killed 48 hr after the last treatment and MN was graded on a scale of 0 to 3. Since IRR produces leukopenia as well as increases superoxide dinitrilo activity, IRR was also tested on ISO-MN. Twelve rats were irradiated with 1000 rad. Seven days later, at the nadir of leukopenia, treated and control rats were injected with ISO and examined as above. Rats pretreated with 6MP+6TG showed normal leukocyte counts, but had significantly lower mean scores of MN than controls (0.6 vs 2.1). AP had no effect. IRR provided protection in all rats (0 vs 2.2). The protective mechanisms are being investigated.

Adult isolated myocytes allow investigation of direct cytotoxicity in the absence of effects from the vascular or neural systems present in intact animals or isolated perfused hearts. Myocytes were isolated using collagenase perfusion in Krebs-bicarbonate buffer and then placed in buffer containing 1 mM calcium with various drugs. Acute toxicity was monitored by leakage of lactate dehydrogenase (LDH) into the medium for 4 hr. The cardiotoxicity of the mixed alpha-beta agonist norepinephrine (NE) and the selective alpha-1 agonist SKF 8-89748 were examined. 100 μM NE did not increase LDH leakage over control until 4 hr. 1 mM NE had no effect at 1 hr, but caused 70% release of LDH by 2 hr when NE was well oxidized. 1 mM ascorbic acid (AA) greatly decreased the oxidation and toxicity of the 1 mM NE. SKF 8-89748 at 10 μM caused only slight leakage by 3-4 hr, but at 1 mM caused 100% leakage within 60 min. 1 mM AA did not reduce the toxicity of 1 mM SKF 8-89748. Propranolol did not inhibit the toxicity of 1 mM NE and the alpha-adrenergic antagonist prazosin did not alter the toxicity of either NE or SKF 8-89748. The toxicity of NE appears to be related to oxidation of the NE whereas the mechanism of toxicity of SKF 8-89748 remains to be determined.

SKELETAL MUSCLE SENSITIVITY TO CHOLINERGIC AGENTS DURING ORGANO Phosphoride-INDUCED DELAYED NEUROPATHY (OPIDN) IN ADULT HENS. H. El-Fawal and K. Ehrlich, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Impairment of skeletal muscle function in 7-mo-old white leghorn hens with OPIDN was investigated using a biventricular cervical nerve muscle preparation. Phenyl salicyl in phosphate (PSP) in DMSO was given im at 2 and 10 mg/kg. Hens were observed until clinical scores reached >3 (0-4 nonaffect ed to paralyzed). Hens were euthanized and nerve-muscle preparations evaluated for responses to acetylcholine (ACh) and succinylcholine (SUX). PSP-treated hens required a higher voltage for supramaximal nerve stimulation (25 ± 1 SE, n = 5 for 10 mg/kg; 23 ± 3, n = 3 for 2 mg/kg) than did vehicle-treated controls (13.5 ± 1.7, n = 6). Preparations from hens exhibiting signs of OPIDN contracted in a concentration-related manner to ACh (10^{-5} to 10^{-3} M); hens treated with PSP 2 mg/kg required higher concentrations to elicit contractions than those given 10 mg/kg. Preparations from all PSP-treated hens depolarized in response to SUX (10^{-5} to 10^{-3} M). Control hens did not respond to either ACh or SUX, which is consistent with Child and Zaimis (Br J Pharm 15:412), who found sensitivity to ACh and depolarizing agents greatly reduced in chicks >10 days old. It appears from our studies that treatment of adult hens with PSP may, therefore, reverse the age-related loss of sensitivity of skeletal muscle to cholinergic agents. Supported by NIEHS 03984.

INTERACTIONS OF INHIBITORS OF NERVE TERMINAL Ca^{2+} REGULATION WITH METHYLMERCURY (MeHg) ON SPONTANEOUS RELEASE OF ACETYLCHOLINE. P. Levesque and W. Atchison, Dept. Pharm./Tox., Mich. State Univ., E. Lansing, MI.

To gain further information on effects of MeHg on neuronal intracellular Ca^{2+} regulation, we tested the interaction of MeHg with agents known to disrupt intraterminal Ca^{2+} buffering including N,N-dimethyl-amino-8-octyl-3,4,5-trimethoxybenzoate (TMB-8, 25 μM), caffeine (CAF, 7.5 mM), (N,N-bis-(3,4-dimethoxyphenethyl)-N-methylamine) (YS035, 180 μM), and ouabain (OA, 200 μM). Miniature end-plate potentials (MEPPs) were recorded from myofibers of the rat hemidiaphragm by conventional methods during pretreatment with one of the test agents and then with the agent plus MeHg (100 μM). The typical increase in MEPP frequency (MEPP) evoked by MeHg was not prevented by OA, CAF or TMB-8 although the effect was less pronounced with TMB-8. Onset of increased MEPP was hastened by OA and CAF. MeHg application for up to 30 min after YS035 failed to increase MEPP. YS035 did not mask a potential MeHg effect due to block of postsynaptic sensitivity, since MEPPs could be elicited following YS035 treatment by use of La^{3+} (2 mM). Thus, CAF and OA which release Ca^{2+} from the SER and mitochondria, respectively, and TMB-8, which is thought to block Ca^{2+} release by SER, could not prevent the action of MeHg. YS035, which has been shown to inhibit mitochondrial uptake and release of Ca^{2+} could. (Supported by NIH grant ES03299.)

EFFECT OF DIISOPROPYL PHOSPHOROFLUORIDATE (DFP) ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF APLYSIA SILENT NEURONS. L.S. Jones, D. Lapadula, D.W. Lewis, and M. Abou-Donia. Duke University Medical Center, Durham, NC.

DFP is an organophosphate with neurotoxic effects that may be mediated through an increase in [Ca^{2+}]. As part of a study designed to test this hypothesis, we have examined the effect of DFP on the Aplysia silent neurons from the pleural ganglia. Using the single-electrode voltage-clamp technique on identified medial pleural cells, we measured the effect of DFP on the resting membrane potential (Vm), conductance, excitability, and the current-voltage relationship. Cells were dissected in a dish perfused by artificial sea water impaled with the electrode, and studied for 30 minutes prior to perfusion with 100 μM DFP, and for an hour after. Control cells were studied for the same time period (90 min.), or longer, without DFP. Control cells (5) showed little change in any of the measurements even after three hours. In the DFP cells (6), 3 became somewhat depolarized, and 4 had an increase in conductance, but in general, the DFP did not have a marked effect at this concentration. (Supported in part by NIEHS Grant No. ES02717).
IDPN selectively impairs slow axoplasmic transport, resulting in proximal neurofilamentous accumulations and distal atrophy in motor nerves. The influence of incipient atrophy of α-axons on motor nerve terminal function was investigated in cats given IDPN (50 mg/kg/week) for 5 weeks. Normal cat soleus motor nerve endings discharge repetitively to single stimuli following tetanic conditioning. Quantification of this stimulus-bound repetition (SBR), which can be recorded as antidromically conducted impulses in single motor axons of ventral root filaments, provides a sensitive index of nerve terminal status in toxic neuropathies. (Europ. J. Pharmacol. 35, 177, 1976). In normal animals, 84% of nerve endings elaborate SBR. In 3 cats given IDPN for 5 weeks, SBR was observed in only 59/133 (29%) of motor nerve endings. It is probable that axonal atrophy distorts the structural relationship between the nerve terminal and the first node, impairing the ability of the nerve terminal to generate SBR. Supported by NS-23325.

A CORRELATION OF SPINAL CORD NEUROPATHY AND SOMATOSENSORY EVOKED POTENTIALS (SSEP) IN THE RODENT MODEL OF OPIDN. R.: Veronez, M.K., Boyes, E. Padilla, USEPA/HEAL/MTD; RTP, NC.

The electrophysiological expression of the delayed neuropathy (OPIDN) associated with organophosphates (OP) has received less experimental attention than neuropathic or biochemical indices, yet might be used to detect and monitor neuropathic damage in mammals. Because of the high sensitivity of the rat's dorsal columns to OP damage, an attempt was made to correlate SSEP with progressive spinal cord degeneration over a 6 wk post-exposure period in Long-Evans 60d, male rats (n=80), doused acutely with disopropyl fluorophosphate (2.0 mg/kg, sc), at a dose that depressed neurototic esterase activity 98%. A profile of SSEP peak decrements and quantitatively determined progressive spinal cord degeneration indicated that amplitude depression of Peak P1, representing activity transmitted from the hindlimb to the somatosensory cortex, occurred coincident with minimal neuropathic cord degeneration as early as 2 wk. This suggests that SSEP may represent a useful diagnostic tool for mammalian paradigms of OPIDN.

BENZODIAZEPINES ANTAGONIZE THE FACILITATION OF THE SPINAL REFLEXES PRODUCED BY SOMAN. Barry D. Goldstein and Donnie R. Pincher, Dept. of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA.

Soman (GD) is a potent acetylcholinesterase inhibitor. It has been previously shown that GD produces a transient depression of the monosynaptic (MSR) and dorsal root (DRR) reflexes followed by a long lasting facilitation. Benzodiazepines (BD) have been used to prevent some of the pathophysiological responses produced by soman. The purpose of this study was to determine if the BD have any effect on the alterations in the spinal reflexes produced by soman.

Cats were anesthetized with ether and their spinal cords transected at Cl. A laminectomy was performed on the lumbar spinal cord. The appropriate dorsal and ventral roots were isolated and cut and the MSR and DRR were recorded by conventional electrophysiological techniques. Diazepam or midazolam was administered 15 minutes prior to the soman (10 μg/kg). It was found that the BD increased the DRR and had little effect on the MSR. However, they did block the long lasting facilitation of both reflexes produced by soman.

These data show that the BD physiologically antagonize the effects of soman on spinal reflexes. Supported by DAMD17-86-C-6007

EFFECT OF PYRIDOSTIGMINE ON TETANIC CONTRACTURE IN INNERVATED VS. DENERVATED SKELETAL MUSCLE. R.J. ANDERSON. Warner-Lambert/Parke-Davis Pharm. Research, Ann Arbor, MI

Pyridostigmine has been reported to cause histologic damage and decrements in tetanic contracture of skeletal muscle, effects which appear not to be related to cholinesterase inhibition. Decreased transmitter release and effects on postsynaptic receptors have been proposed as mechanisms for these detrimental effects. The purpose of this study was to determine whether the pyridostigmine induced decrement in tetanic contracture was due to a pre- or postsynaptic action of the drug. Rats with innervated or chronically denervated hindlimbs were given pyridostigmine (25 mg/kg) s.c. by infusion for 4 days. Tetanic contracture (20, 50, 100 Hz) was evoked by sciatic nerve or direct stimulation of the triceps surae muscles. As expected, pyridostigmine significantly decreased the contracture of innervated muscle, an effect which correlated with tetanic frequency. However, in denervated muscle pyridostigmine had no effect on tetanic contracture. These results show that innervation is required for the detrimental effect of pyridostigmine and that the drug has no direct effect on skeletal muscle. Pyridostigmine appears to interfere with transmitter release in a frequency-dependent manner, an effect which is consistent with histologic changes in the motor nerve terminal reported by others.
TIME COURSE OF ELECTROPHYSIOLOGIC DEFICITS
INDUCED BY DI-N-BUTYL-2,2-DICHLOROVINYL PHOSPHA-
TE (DCBV) IN THE ADULT HEN. D.C. Robertson*,
A.M. Mattson*, I.L. Besterveld!, G.J. Richardson!, and R.J. Anderson. Toxicology
Program, The University of Michigan and Warner-
Lambert/Parke-Davis Pharmaceutical Research, Ann
Arbor, MI.

This work investigated the time course of DBCV
induced electrophysiological deficits in hen
peripheral nerve. Groups of hens received
either corn oil alone, 4 mg/kg DBCV in corn oil,
or 30 mg/kg di-n-butyl-2,2-dichlorovinyl phos-
phinate (DCBV-P) followed by 4 mg/kg DBCV.
Brain or lymphocyte neurotropic esterase (NTE)
activities were ascertained in all groups 24
hours after dose. Hens were observed daily for
clinical signs and sacrificed 1, 7, 14 or 21
days postdose. NTE was significantly inhibited
in all treatment groups. Electrophysiological
parameters of the tibial and sciatic nerve
including conduction velocity, compound action
potential duration and amplitude, strength-dura-
tion, and relative refractory period were
obtained. Relative refractory period was
generally decreased in the tibial nerve and
increased in the sciatic nerve. Removal of the
nerve from both nerves, however, the most profound
excitation coincided with the onset of clinical signs
of toxicity. DBCV-P pretreatment prevented
these changes suggesting ageing of the OP-enzyme
complex is necessary for the production of
electrophysiological deficits.

THE INTERACTIONS OF MOWENSI AND OUABAIN ON
AN ISOLATED SENSORY NEURON P. N. Nation, S.
H. Roth, Dept. of Pharmacology and Therapeu-
tics, University of Calgary, Calgary, AB.

The ionophore antibiotic mowensen and the
cardiac ionophore ouabain were studied to
determine their effects alone and in combina-
tion on the stretch-induced discharge activity and electrical membrane properties of
the isolated crayfish stretch receptor
neuron. Extracellular and intracellular
recording techniques were used to examine changes in firing activity and membrane
electrical properties. Both drugs increased
cell discharge rate but the times of onset
and maximum effect were different for each
agent: the onset of mowensen effect occurred
rapidly and was reversible, reaching a pla-
tform within 4 minutes, in contrast to ouabain
which was slow in onset and irreversible.
Membrane electrical properties such as rate
of rise of the action potential and width of the
orthodromic and antidromic action poten-
tials were increased. These changes were
attributed to drug effects on sodium conduc-
ance. In combination, mowensen and ouabain
yielded non-additive effects, each agent
retaining its characteristic pattern of onset
and action. Supported by the Alberta Heri-
tage Foundation for Medical Research and
Alberta Agriculture.

ACTIVITY-DEPENDENT ALTERATIONS IN OPTIC TRACT
(OT) AXONS IN 2,5-HEIXANEDIONE (2,5-HD) EXPOSED
RATS. D. A. Fox and D. Impelman. College of
Optometry, University of Houston, Houston, TX.

Previously we reported that rats with 2,5-HD
distal axonopathy had supernormality in the
middle diameter, medium conduction (t2) OT group
and normal recovery in the large diameter, fast
conduction (t1) group. Recovery functions were
measured using paired-pulse stimulation to determine absolute and relative refractory periods (ARP and RRP), and amplitude and latency recovery processes. To
further investigate activity-dependent changes in
OT axons, we analyzed t1 and t2 waveforms using
single shock (SS), paired-shock (PS), and train functions (TFS). Rats were exposed to 2,5-HD in
drinking water for 52-72 days with doses from
0.44-0.70 gm/kg/day. SS data revealed similar
changes in t1 and t2 conduction velocity (~35%),
amplitude (~65%), rise time (+60%), rheobase
(~65%), and chronaxie (~100%). In contrast, dura-
tion at half-amplitude was increased 40% in t1
and 90% in t2. PS data for t2 showed amplitude
and latency supernormality (15-20%), normal ARPs,
and decreased RRRs (~60%). No changes in any t1
functions were seen. TFS showed amplitude super-
normality in t2 and no changes in t1. The selec-
tive effects on t2 following PS and TFS may be
related to an increased metabolic load on t2
(medium) compared to t1 (large) axons. This is
suggested from our EM studies showing an increase
in surface area (paramodal demyelination) to axon
diameter (swelling) ratio. Supported by ES 03183.

REVERSIBILITY OF NALIDIXIC ACID-INDUCED ACUTE
ELECTRORETINOGRAPHIC CHANGES IN CATS. F.L.
Fort, R. Yamamoto, and T. Ando. Abbott Labs, North Chicago, IL and Takeda Chemical
Ind., Ltd., Osaka, Japan

Nalidixic Acid is a quinolone-type DNA gyrase
inhibitor antibiotic which has been shown to
cause electoretinographic (ERG) changes in
cats. As an initial study of the mechanism of
these changes, their reversibility was studied.
Male cats were given a single intravenous in-
jection of 40 mg/kg (1 ml/kg) of nalidixic acid.
ERG recordings were made before and over a
period of 24 hr after injection. Maximal ERG
effects were obtained 1 hr after injection, and
almost complete recovery was observed 24 hr
after injection. ERG changes consisted mainly
of increased latency of a and b waves, decreased
amplitude of the b wave, and disappearance of
oscillatory potentials. Cats were killed at
1 hr and 24 hr after treatment for pathologic
evaluation of the retinas by gross observation
and by light and electron microscopy. There
were no anatomic alterations that were clearly
related with the ERG changes. Our results
demonstrate that acute functional retinal dis-
turbances caused by this class of drug may be
fully reversible, and that changes in retinal
function may occur in the absence of any clear
anatomic alterations.

Visual toxicity of methanol (M) is observed only in humans and monkeys. Thus, studies were initiated to develop a rodent model. Three groups of Long-Evans rats with different levels of liver folate acid were prepared by feeding either standard rat chow (G1), folate acid deficient (FAD) diet (G2), or FAD diet with 1% acetylsulfathiazole (G3). At the time of M administration (3.5 g/kg, one gavage), the liver folate concentration (G) of G1, G2 and G3 were 25, 10 and 2.4 µg/g liver, respectively. Blood M C at 24 hrs (3.1 mg/mL) and 48 hrs (2.1 mg/mL) were similar between the 3 groups. However, the blood formate C was markedly different; at both time points, it was about 3%, 25% and 300 µg/mL for G1, G2 and G3, respectively. The range of formate C (400-600 µg/mL) in G3 was similar to that found in monkeys which developed ocular toxicity after M administration by gavage. P1 and N1 peak latencies of flash evoked cortical potentials, which measure specifically the visual system function, were significantly increased in the M treated G3 rats but not G1 and G2 rats, indicating that M caused a "lesion" in the retinogeniculocortical pathway of the visual system. These data suggest that Long-Evans rats fed FAD+1% SST could be used as a rodent model for the study of M toxicity in the visual system, the target organ of M toxicity in humans.

NEUROBEHAVIORAL EFFECTS OF DIETARY RESTRICTION IN RATS. R.R. Albee and J. L. Mattsson. The Dow Chemical Company, Midland, MI.

To assess the impact of reduced body weight gain on nervous system function, 8 week old male Fischer rats were fed either 15% or 50% less food than controls. Food restriction continued for one month. Wild (15%) and severe (50%) dietary restriction resulted in a 14% and 18% reduction in body weight. Mild dietary restriction caused slight but statistically significant changes in visual evoked responses, auditory evoked responses, caudal nerve action potentials, and body temperature. Severe restriction increased the magnitude of the effects noted in the mild group, and also caused decreased grip strength. Statistical significance persisted for most changes when the data were reanalyzed by analysis of covariance, using temperature as the covariate. Decreased grip strength also remained statistically significant when body weight was used as a covariate. Somatosensory evoked responses were not affected by either mild or severe restriction. Diet restricted rats were more excitable during testing. Thus, reduced food intake can alter parameters that should be considered in the interpretation of neurotoxicological data.


Chloridine (CDM), a formamidine pesticide, increases the amplitude of selected components in rat pattern-reversal evoked potentials (PREPs) (Boyes and Dyer, Exp. Neurol. 86:434-447, 1984). Recent evidence from PREPs and pattern-onset evoked potentials (POEPs) suggests that peak P1 represents activity of a "sustained" visual subsystem (motion detection) and another peak, N2, represented responses of an "onset" visual subsystem (pattern detection) (Boyes and Hudnell, Soc. Neurosci. Abstr. 1986). The current study investigated the relative actions of CDM on sustained and transient visual functions as measured by visual evoked potentials. Rats were treated with either saline or 40 mg/kg CDM i.p., and 30 min later, PREPs and POEPs were recorded at stimulation frequencies (F) of 1, 2, or 5 Hz. At 1 and Hz 2, there was an enhancement of the negative peak thought to be N2. At 5 Hz, the waveshapes of both groups became "steady-state" in character and showed increased spectral amplitude at 1F (sustained) but not at 2F (transient). These preliminary results suggest an augmenting effect of CDM on sustained activity.

NEONATAL ADMINISTRATION OF MONOSODIUM GLUTAMATE SEVERELY DISRUPTS FLASH EVOKED POTENTIAL ONTOGENY. G.C. Rigdon and R.S. Dyer. USEPA/HERL/NTD, Research Triangle Park, NC.

Neonatal administration of monosodium glutamate (MSG) produces loss of retinal ganglion cells, optic nerve atrophy, and thinning of the retinal inner nuclear layer. Surprisingly, MSG treated animals exhibit a normal proportion of light responsive single neurons in the dorsal lateral geniculate nucleus. We evaluated visual function in MSG-treated rats by studying flash evoked potential (FEP) ontogeny. Animals were administered (i.p.) either 4 mg/kg MSG or vehicle daily on PND 2-9 (PND is the date of birth). FEPs were recorded from chronically implanted, awake Long-Evans rats on PND 15, 22, and 60. On PND 15, 9 of 12 control animals exhibited responses to light flashes, while only 4 of 12 MSG treated animals did so. Most animals from both groups exhibited responses to light flashes by day 22. MSG produced significant effects on all peak latencies measured (P1, N1, F2, N3) and also on peak N1 amplitude. Significant interactions between dose and age or dose and frequency of flash stimulation occurred with peak N1, P2 and N3 latencies and a dose x age x frequency interaction occurred with peak N3 amplitude. These findings indicate that the neurochemical and morphological alterations induced by neonatal MSG administration are accompanied by profound alterations in FEP ontogeny. (This work was supported by a National Research associateship Award.)

The rat visual system, which has become popular for testing agents with potential neurotoxicity, is often evaluated electrophysiologically with the flash-evoked potential (FEP). To interpret more effectively the outcomes of FEP studies involving unknown toxicants we determine the impact upon FEPs of agents with identified pharmacological actions. Aspartate is a prevalent putative neurotransmitter in rat visual cortex (e.g. Baughman and Gilbert, J. Neuroscience, 1981,4:427439). Ketamine, a cyclohexylamine, may selectively block N-methyl-D-aspartate (NMDA) receptors. We report here the effect of ketamine on FEPs. Long-Evans hooded rats with chronic skull electrodes overlying the visual cortex were treated with either 0 (vehicle), 37, 75, or 150 mg/kg ketamine hydrochloride, i.p., and placed in a chamber with either dark or light background illumination for 10 min. FEPs were averaged following 128 strobe flashes. Among the effects of ketamine were the following: Peak F1 amplitude increased by a factor of 4, in a dosedependent manner. Peak N1 virtually disappeared at 150 mg/kg. Peak P2 amplitude increased by 50% but only in the light background, and only at 150 mg/kg. These findings demonstrate separable effects of ketamine on different FEP peaks. The data provide evidence that NMDA receptors play an important role in the generation of FEPs in rats.

NEUROPHYSIOLOGIC RESPONSE OF RHESUS MACAQUE TO ATROPINE AND PYRIDOSTIGMINE USING A VAGAL TONE MONITOR. S. Birnbaum, B.G. Richardson, and J.A. Dellinger. University of Illinois, Urbana, IL.

Twelve young adult rhesus macaques received atropine sulfate (ATR) im doses of 0, 14, 44, and 140 mcg/kg in a Latin Square design. Each monkey was pretreated with pyridostigmine bromide (0.2 mg/kg im) 30 min prior to the ATR injection. The heart period variance (HPV) and an estimate of respiratory sinus arrhythmia (V) were measured using a Vagal Tone Monitor. Erythrocyte and plasma cholinesterase (ChE) activities were measured 30 min and 3 hr after the pyridostigmine (Pyr). The ChE assays were completed within 1 hr of the Pyr injection. Mean erythrocyte and plasma ChE activities were 55% and 33% inhibited respectively. V and HP increased 30 min after Pyr, and the subsequent ATR treatment reduced HP and V in a dose dependent manner. However, the vagolytic effects of ATR were attenuated following the Pyr pretreatment compared to the previous experiment using ATR alone. The E2DOSs for a 30% decrease in HP and V were 66 and 20 mcg/kg, respectively. (compared to 26 and 9 mcg/kg, respectively, for ATR alone). Therefore, twice as much ATR was needed to produce the same vagal tone effects following Pyr. The attenuated response to ATR confirms our previous results with two organophosphorus anti-ChE compounds in dogs. Supported by the U.S. Air Force.

ENHANCED SUSCEPTIBILITY TO AMPHALD M KINDLING FOLLOWING TREATMENT WITH FORMAMIDINE PESTICIDES AMITRAS AND CHLORDIMEFORM. M.E. Gilbert, Northrop Services, Inc.-Environmental Sciences, RTP, NC Sponsor: P.J. Bushnell.

Formamidine pesticides have been reported to act as monoamine oxidase inhibitors, α2-adrenoreceptor agonists, anticholinergics, and local anesthetics. Electrical kindling of the amygdala and hippocampus was used to evaluate the effects of two formamidines, chlordimeform (CDF) and amitraz (AMG), upon seizure susceptibility in the rat. Male Long Evans rats were implanted with electrodes in the amygdala or dorsal dentate gyrus, and administered CDF (40 mg/kg), AMG (50 mg/kg), or equal volumes of respective vehicles. After discharge (AD) thresholds were determined and animals stimulated twice daily at 2 and 4 hours postinjection at a standard 200 μA stimulus intensity. Both CDF and AMG produced a significant facilitation in amygdaloid kindling, and CDF also facilitated hippocampal kindling. No alterations in AD thresholds were observed. The α2-adrenergic action of these compounds may produce a facilitation in electrical kindling by reducing brain levels of norepinephrine as selective depletion of this neurotransmitter has been shown to enhance kindling rates.

FORESTOMACH CARCINOMAS IN PARTIALLY-HEPATECTOMIZED RATS INDUCED BY PHENOLIC ANTIANTIOXIDANTS. A. Hane, R. Lefevre and R. Abraham. Section of Toxicology/Pathology Albany Medical College, Albany, New York.

We had reported earlier that in partially hepatectomized rats (PH) feeding BHA (Butylated hydroxyanisole) resulted in the development of in situ carcinomas within a short period of 3 months, whereas in intact rats such tumors are seen at 2 years. The PH model developed in this laboratory was used to investigate the effects of "surgical trauma" and whether other phenolics could induce tumors as rapidly as BHA. The data to be presented reveal that PH rats fed 2% BHA for 6 months showed a 100% incidence of invasive carcinomas, whereas intact and sham operated rats fed 2% BHA had moderate or mild hyperplasia. Initially i.e. at 15 and 30 days the labeling index (LI) in the forestomach was: PH + BHA 24.5% and 33.2%; intact + BHA 12.7 and 18.7; sham 14.9 and 20.3; control 3.1%. At 180 days the LI was 22.3% (carcinomas); 29.1%; 25.8% and 3.1%. Ethylacrylate (1X) induced forestomach tumors as early as 30 days in PH rats with progressive development at 3 months, whereas tertbutylhydroquinone induced - thickening of the forestomach mucosa. The histologic data and mechanism involved in the rapid development of forestomach tumors in PH rats will be discussed.
**MORPHOLOGICAL TRANSFORMATION AND PROMOTION OF TRANSFORMATION IN C3H/10T1/2 Cl 8 MOUSE EMBRYO CELLS BY SODIUM ARSENITE. J.R. Landolph and C. Troesch. Depts. of Microbiology and Pathology and the USC Comprehensive Cancer Center, USC School of Medicine, Los Angeles, CA.**

Sodium arsenite at concentrations ranging from 6-30 μM reproducibly induced small numbers of morphologically transformed type II and type III foci in C3H/10T1/2 cells and enhanced the frequency of transformation in cells initiated with 0.1 μg/ml of 3-methylcholanthrene. Sodium arsenate and potassium arsenate did not induce morphological transformation in these cells. Sodium arsenite, sodium arsenate, and potassium arsenate did not induce mutation to ouabain resistance in C3H/10T1/2 cells over cytotoxic concentration ranges. Four foci induced by sodium arsenite were ring-cloned, cell lines were established from them, and the cell lines were biologically characterized. All four cell lines had higher saturation densities than C3H/10T1/2 cells, grew in soft agarose, and produced progressively growing fibrosarcomas when injected into Balb/c nude mice. Therefore, this study shows that sodium arsenite can induce neoplastic transformation in C3H/10T1/2 cells and can promote transformation initiated by 3-methylcholanthrene. These arsenite transformed cell lines can now be used to study unique non-mutagenic mechanisms of transformation induced by sodium arsenite.


This study of the relationship of groups of glutathione transferase-positive (GST-P+) hepatocytes to resistant hepatocytes was begun in order to develop a bioassay for initiated hepatocytes. Adult Fischer 344 male rats were given DEN at a known initiating dose of 200 mg/kg b.w. i.p. and sacrificed 2 weeks later, during selection and up to 2 weeks after selection with 2-acetylaminofluorene (2-AAF) and partial hepatectomy (PH) using the resistant hepatocyte model. In response to DEN initiation, the liver shows many (50 ± 10 groups/cm²) small groups of GST-P+ hepatocytes which do not exceed 6 hepatocytes in widest diameter. Within several days after selection larger GST-P+ foci and nodules were evident (up to 50 nodules/cm²). These results strongly support the concept that GST-P+ hepatocytes present within a few days after initiation are resistant. More direct demonstration of this cellular progression of GST-P+ cells is now under study. The ability to analyze and quantitate initiated hepatocytes in response to chemical carcinogens appears to be attainable in the near future.


This study assesses effects of 2 PCBs, 2,2',4,4',5,5'-HCBP (PB-type) and 3,3',4,4'-TCBP (MC-type) on the initiation of liver carcinogenesis and correlates these influences with responses of the endoplasmic reticulum to each agent. Both PCBs diminish the mitoinhibitory toxicity of 2-acetylaminofluorene (2-AAF) during selection for 2-AAF resistance of initiated hepatocytes using the Sopt-Farber model (JNCI 76: 683, 1986). We now examine the effect of exposure to a single dose of these PCBs on the initiating activity of several carcinogens, diethylnitrosamine (DEN) and 2-AAF. PCBs alone do not initiate resistant hepatocyte nodules. However, the PB-type PCB increased the number of nodules initiated by 2-AAF in a dose-dependent manner. The 2 different types of PCBs increase cytochromes P-450 or P-448 in the predictable manner. Specific progesterone binding was increased by exposure to PB-types of PCBs. The interpretation of the effect of the PB-type PCB to increase the initiating ability of 2-AAF is difficult.

**MORPHOLOGICAL AND CYTOGENETIC EVALUATION OF MINERAL FIBER INDUCED RAT PLEURAL TUMORS AND TRANPLANTED TUMORS. L.D. Palekar,* J.F. Eyre,* and D.L. Coffin,** *Northrop Services, Inc. **U.S. EPA/RTTP, N.C.**

Pleural tumors were induced by intrapleural injection of either erionite or UICC chrysotile. Four basic morphological patterns were observed 1) tubulopapillary, 2) fibrosarcomatous, 3) mixed-fibrosarcomatous and tubulopapillary and 4) mixed-fibrosarcomatous and condrosarcomatous. The cytogenetic analysis of the cell lines derived from these tumors revealed numerous heterogeneous chromosomal anomalies such as deletions, translocations, breaks and extensive numerical changes. The modal chromosome number varied from 40 to 92. The most frequent chromosome change involved anomalies of chromosome #1. Seven cell lines, with or without chromosome #1 anomaly were transplanted into syngenic rats. All seven tumor cell lines induced tumors after subcutaneous or intrapleural injections within two months. The morphology of the transplanted tumors was similar to the original tumors. The cytogenetic analysis of the transplanted tumors revealed that the tumors induced with intrapleural injections were also cytogenetically similar to their original tumors, however, tumors derived from subcutaneous injections had a diploid chromosome number. This abstract is prepared for a presentation and does not necessarily reflect USEPA policies.
EVIDENCE FOR LACK OF EFFECT OF DEHP AND PHENOBARBITAL ON GROWTH AND PROGRESSION OF NATURALLY OCCURRING RODENT LIVER TUMORS. J. M. Ward, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick, MD. Sponsor: M.P. Waalkes

Model systems were developed to determine the effects of nongenotoxic chemicals on the growth and progression of naturally occurring preneoplastic and neoplastic focal hepatic proliferative lesions (FHPL) in aging F344/Ncr rats and C3H/HeNcr mice. F344 rats, 18-24 months of age, with basophilic hepatic cellular foci and C3H mice, 12 months of age, with liver tumors were exposed to diets or water containing di(2-ethylhexyl)phthalate (DEHP) or phenobarbital (PB). Injections of tritiated thymidine were used to determine labeling indices (LI) of hepatocytes in lesions. Animals were sacrificed at varying intervals from 4-35 weeks. The numbers of FHPL were quantified by image analysis. Neither chemical had any effect on LI in naturally occurring FHPL or normal hepatocytes in either species. A unique type of eosinophilic preneoplastic and neoplastic lesion was induced, however, by PB during these 36 weeks. In contrast, few of these lesions could be induced in younger rodents during a similar period. These studies provide evidence that these two 'nongenotoxic' chemicals 'induce' liver tumors by mechanisms not yet described.


Groups of 60 male & 60 female Sprague Dawley CD00 rats were given 0, 0.25, 1.5 or 9.0 mg/kg/day PNA by gavage in corn oil for a period of two years. Parameters monitored included daily observations, ophthalmoscopic exams, body weights, food consumption, hematology, clinical chemistry and urinalysis. All gross lesions and over 40 tissues were examined histologically. For the reproduction study, groups of 15 male and 30 female rats, designated as F0 generation, were given PNA at the same levels for 14 weeks prior to mating, during mating, gestation and lactation. F1 parental rats received the same dose of PNA for 18 weeks prior to mating, during mating, gestation and lactation.

In the chronic study, except for a slight decrease in survival of high dose male rats late in the study, survival in all treated groups was comparable to controls. Blood methemoglobin levels were elevated in the high dosage group; slight anemia was also observed in this group. No treatment related increase in tumor incidence was observed. In the reproduction study, no consistent pattern of effect from treatment between the F0 and F1 generation was seen in mating, pregnancy or fertility indices.


The antihistamine methapyrilene (MP) was widely used in the 1970's until it was identified as a potent rat hepatocarcinogen. MP has been shown to be non-genotoxic in many short-term tests which raises questions about its possible mode of action. We evaluated the potential of MP to induce UDS, a genetic endpoint, and SPS, an indicator of cell proliferation, in hepatocyte autoradiograms following in vivo or in vitro exposure to F-344 rats and B6C3Fr mice. UDS was also evaluated in Cynomolgus monkey and human hepatocytes treated in vitro. MP failed to induce UDS in hepatocytes from any species treated in vitro or in vivo. Controls yielded < 0.3% of cells in S-phase (% S). Treatment with 225 mg/kg MP yielded 6.3% S in male rats and 1.4% S in female mice 48 hr after treatment. SDH, bilirubin, SGOT, and SOFT showed elevations of 9-, 10-, 17- and 28-fold over controls, respectively; portal parenchymal necrosis and regeneration were also observed. These results indicate that MP is not genotoxic in liver but is a potent inducer of hepatic cell proliferation by inducing toxicity and subsequent regeneration which may be an important mechanism of hepatocarcinogenesis.

EFFECTS OF CASTRATION AND TESTOSTERONE ON THE RENAL CARCINOGENICITY OF MERCURY IN MICE. M. Hiran, H. Ueda, K. Malta, and Y. Shiraeu. Inst. of Environmental Toxicology, Tokyo, JAPAN.

We found methylmercury chloride (MMC) produced renal carcinomas only in male mice. In order to investigate the effects of castration and testosterone administration on this sex difference, we conducted an 80-week carcinogenicity study with MMC in castrated mice with or without administration of testosterone propionate (TP). Fifty ICR mice/mux were allotted to one of the following 4 groups: I, basal diet; II, 10 ppm MMC diet (MNC diet); III, castration at 5 weeks of age plus MMC diet; IV, castration plus MMC diet plus TP (s.c. injection at 0.2 mg/mause/week).

Renal epithelial tumors were found in males after 56 weeks of treatment. The incidences were 0/45, 0/42, 0/45, and 0/36 in groups I, II, III, and IV, respectively. In female, 1 and 2 mice of groups I and IV, respectively, had renal tumors. Renal epithelial hyperplasia considered to be the precancerous lesion of renal tumors was observed at the rates of 0/48, 13/48, 0/45, and 8/44 in males and of 0/47, 3/45, 1/49, and 13/47 in females of groups I, II, III, and IV, respectively. These results indicated that MMC-induced renal tumors and epithelial hyperplasia were never seen after castration in males and that the epithelial hyperplasia was increased in both sexes treated with TP. In conclusion, induction of renal tumors and epithelial hyperplasia by MMC depended on androgenic hormones such as testosterone.
6-CHLOROPICOLINIC ACID: 2-YEAR DIETARY CHRONIC TOXICITY-ONCONEGENICITY STUDY IN B6C3F1 MICE. M.A. Zimmer, D.L. Eisenbrandt, F.S. Cieslak, M.A. Hanneh and W.T. Stott. The Dow Chemical Company, Mammalian and Environmental Toxicology Research Laboratory, Midland, MI.

6-Chloropicolinic acid (6-CPA) is the primary metabolite of nitrapyrin (2-chloro-6-(trichloromethyl)-pyridine), a highly selective antimicrobial which diminishes the viability of Nitrosomonas bacteria in soil to convert ammonium nitrogen to nitrite. Seventy B6C3F1 mice/sex/dose were fed diets containing 0, 100, 300 or 900 mg 6-CPA/kg BW/day for up to 2 years. Ten mice/sex/dose were scheduled for sacrifice at 6 and 12 months and 50 mice/sex/dose at 24 months. Male mice given 900 mg/kg/day had decreased body weights and a minimal microscopic renal change consisting of a loss of normal cytoplasmic vacuolation from proximal tubular epithelial cells. Female mice given 900 mg/kg/day had increased hepatic and total liver neoplasms when compared to concurrent controls. The tumor incidences were slightly higher than laboratory control ranges but were not statistically significant. These increases appear to have no biologic significance when the lack of effects on morbidity, mortality, and liver histology and the control incidences from other laboratories are considered.


Groups of 110 F-344 rats/sex/dose were fed DIINP at dietary levels of 0, 0.03, 0.3, and 0.5 (wt%) for periods up to 2 years. Intermittent sacrifice of rats at 6, 12, and 24 months with surviving animals sacrificed at 24 months. At study termination, survivorship was in excess of 60% for every group. At the mid or high dose, the following biological effects were noted indicating an appropriate dose selection for the chronic study: decreases in food consumption and body weight (4-7%), slight increase in mortality, dose-related increase in relative organ weights (liver, kidney), and some mild effects on urinalysis/hematologic/clinical chemistry parameters. No peroxisome induction was observed in livers of treated rats compared to controls. Treatment-related non-neoplastic and neoplastic lesions occurred with the exception of mononuclear cell leukemia (MCL) and changes known to be associated with this leukemia. While DIINP was associated with a marginally increased incidence of MCL in the high dose group (both sexes), this effect was judged to be non-specific and not of biological significance to the clear no observed effect level was demonstrated for all biological endpoints at 0.05 wt. %.

INFLUENCE OF VIRAL INFECTIONS ON TUMOR INCIDENCE AND SURVIVAL OF B6C3F1 MICE. G.N. Reed, J. Edmondson, D. Crawford, and W.W. Piegorsch. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Sendai virus (SV) and mouse hepatitis virus (MHV) are the common viral infections of mice. Influence of these viral infections on the incidences of liver and lung tumors and lymphoma is of concern in chemical carcinogenicity studies. The incidences of these tumors in 41 diet control groups and 28 treated groups with and without viral infections were evaluated. There were no statistically significant (p<.05) differences in the incidences of these tumors in SV positive vs SV negative and MHV positive vs MHV negative diet control groups. In the high dose female groups there were higher incidences of liver tumors in SV positive groups and lung tumors in MHV negative groups. When the high dose groups from studies with chemicals considered carcinogenic for liver were deleted, the incidence of liver tumors in SV positive female groups decreased to control levels. The survival of male and female mice in SV positive diet control and chemical treatment groups were higher than in the groups that were negative for SV.


Extensive alteration in the normal chromosome complement is a common feature of cells derived from solid tumors. The basis for these changes may originate from tissue specificities, tumor type, state of tumor progression and/or the nature of the initiating event. The question addressed in these studies is whether DNA lesions produced by carcinogens of diverse chemical classes produce different chromosomal changes. We obtained karyotypes at the earliest possible point after tumors were formed to distinguish early changes in karyotype from those related to tumor progression. The tumors examined were rhabdomyosarcomas induced by injection of NS or MCA into muscle of C3H mice. Cell lines were established and karyotypes were performed between the third and fifth passage. The major difference between the NS and MCA cell lines was the presence in NS lines of rearranged marker chromosomes. The marker chromosome was not the same in the three NS cell lines examined. In the MCA cell lines examined double minutes were present, suggesting the possibility of gene amplification induced by MCA. Each carcinogen did produce a different spectrum of chromosomal alterations but involvement of a particular chromosome was not indicated for either carcinogen. Research sponsored by NIH, ES 00260.
Previous studies in this laboratory demonstrated that zinc deficiency significantly enhanced the incidence of esophageal tumors in rats exposed to methylbenzylxinosamine (MBN), an esophagus specific carcinogen. To clarify the relationship between timing of feeding zinc deficient diet and tumor incidence, rats were placed on zinc deficient diet for six different lengths of time. Increased feeding of the zinc deficient diet produced a corresponding linear increase in tumor incidence and numbers, with critical points of major tumor incidence increases. Zinc deficient diet had to be fed for a minimum time period extending through MBN dosing to clearly increase tumor incidence. Continuing the zinc deficient diet for one month after MBN dosing increased tumor incidence to the level produced by the zinc deficient diet given for the entire course of the experiment, although the latter group had twice as many tumors, 243 vs. 116. A group fed zinc deficient diet plus 1% difluoromethylornithine in drinking water, a substance which inhibits ornithine decarboxylase, an enzyme involved in cell proliferation, reduced tumor incidence from 97% to 34% and the number of tumors from 243 to 20. These results suggest that hyperplasia induced by zinc deficiency may be critical to its tumor enhancing effects, more so after MBN dosing than when present during the period immediately preceding carcinogen exposure.


The effect of topically applied AA, AP (a lipophilic analog of AA), sorbitan monopalmitate (SMP) and palmitic acid (PA) on TPA-induced ODC activity, DNA synthesis and skin tumor promotion was evaluated in CD-1 mice. Topical application of 28 μmol AA with 2 mmol TPA inhibited TPA-induced ODC activity by 34% but did not inhibit TPA-induced DNA synthesis. In contrast, 4 μmol AP inhibited the induction of DNA synthesis by 61% and ODC induction by 76%. The most potent to least potent inhibitor of TPA-induced DNA synthesis was AP>SMP>PA>AA.

All 4 compounds inhibited tumor promotion by TPA; the most potent antipromoter was AP and the least potent was AA. Topical application of 28 μmol of AA along with 2 mmol TPA twice weekly in mice previously initiated with 200 mmol 7,12-dimethylbenz(a)anthracene inhibited the average number of tumors/mouse by 50%. Application of only 4 μmol of AP, SMP or PA together with 5 mmol of TPA twice weekly in previously initiated mice inhibited the average tumors/mouse by 95, 75 and 50%, respectively. The results indicate that AP is a more potent inhibitor of tumor promotion than AA, PA or SMP.

Recent reports from several laboratories including our own have suggested that tumor-promoting compounds, in particular barbiturates may possess broad organ specificity and promote carcinogenesis in liver, kidney, thyroid, and bladder. To confirm these findings, in the present investigation, F344 male rats initiated with FANFT, a well-known carcinogen for urinary bladder, were subsequently fed diet containing 1000 ppm of NaPB or NaBB. Both NaPB and NaBB promoted the development of transitional cell hyperplasia, papilloma and carcinoma in the urinary bladder at 52 and 68 weeks. No lesions were observed in bladders of rats exposed to NaPB or NaBB alone. Renal lesions were found only in rats exposed to NaBB with or without FANFT and included toxic degenerative tubular lesions, tubular cell hyperplasia, adenoma and carcinoma, and transitional cell hyperplasia of the renal pelvis. Thus, we confirmed that NaPB can promote bladder carcinogenesis and showed for the first time that NaBB also has bladder tumor promoting activity in addition to being a strong promoter and/or a weak renal carcinogen.


We hypothesized that simultaneous exposure to environmentally relevant dietary levels of 245-HCB and TCDD could enhance hepatocarcinogenesis. We used the Pitot protocol in female Sprague-Dawley rats. Thirty days after partial hepatectomy and diethylnitrosamine administration, groups of 24 rats were fed a basal diet or diets containing 5 ppm HCB, 10 ppt TCDD, 100 ppt TCDD, 5 ppm HCB + 10 ppt TCDD or 5 ppm HCB + 100 ppt TCDD for 20 weeks. Six rats from each group were killed at 20 weeks, 6 at 30 weeks and 12 at 60 weeks. The number of gamma glutamyl transpeptidase positive enzyme altered foci (EAF)/cm² was significantly increased at 20 weeks only in livers of rats given HCB + 100 ppt TCDD. By 30 weeks numbers of EAF were not different among groups, but more larger foci were seen in rats given HCB + 100 ppt TCDD. Hepatocellular carcinomas and hepatic nodules occurred more frequently by 60 weeks in rats given HCB + 100 ppt TCDD. Results indicate that simultaneous exposure to HCB and TCDD enhances hepatocarcinogenesis in initiated rats. Supported by USDA and Michigan Agricultural Experiment Station.

EFFECTS OF THE TIME OF ONSET OF PHENOBARBITAL (PB) ADMINISTRATION ON HEPATIC TUMOR EXPRESSION IN DIETHYLNITROSAMINE (DENA) INITIATED B6C3F1 MICE. C.M. Weghorst, J.E. Klaunig and M.A. Pereira. Dept. of Pathology, Medical College of Ohio, Toledo, OH and Environ. Health Res. and Testing Inc., Cincinnati, OH

PB has been shown to inhibit hepatic tumor formation in male B6C3F1 mice initiated with DENA during infancy. This inhibition may be caused by a feminizing effect of PB on the male mouse during its sexual development. The present study examined the effects of varying the onset of PB administration on hepatic tumor expression. Mice were divided into 8 groups. Groups 1, 3, 5 and 7 received a single ip injection of DENA (5 ug/gbw) at day 15 of age, while groups 2, 4, 6 and 8 received saline. At weaning, groups 1 and 2 received deionized drinking water (D.D.W.) for 24 weeks. Groups 3 and 4 received D.W. containing PB (500 ppm) for 16 weeks and 8 weeks of D.D.W. Groups 5 and 6 received D.W. for 4 weeks, PB D.W. for 16 weeks, and 4 weeks of D.D.W. Groups 7 and 8 received D.W. for 8 weeks and 16 weeks of PB D.W. All mice were sampled at 28 weeks of age. No hepatic tumors were observed in groups 2, 4, 6 or 8. Groups 3, 5, and 7 displayed a significant decrease in number and area of hepatic foci and adenomas compared to group 1. These findings showed that PB inhibition of hepatocellular lesions in DENA initiated B6C3F1 mice was still present even when the onset of PB treatment was delayed.

HEPATIC TUMOR PROMOTION BY DIETARY FAT IN B6C3F1 MALE MICE. J.E. Klaunig, N.E. Schultz and K.A. Crist. Departments of Pathology and Surgery, Medical College of Ohio, Toledo, OH

Elevated levels of dietary fat have been implicated in the promotion of experimental mammary, colon and pancreatic neoplasms. In the present study, high dietary levels of polyunsaturated fat (safflower oil; 17.5% w/w) or saturated fat (lard; 17.5% w/w) were examined for hepatic tumor promoting ability in male B6C3F1 mice. Mice were divided into 6 groups of 15 mice each. At 15 days of age, mice in groups 1, 3, and 5 received a single i.p. initiating dose of diethylnitrosamine (DENA) (5 ug/gbw) in saline. Groups 2, 4, and 6 received saline. At weaning, groups 1 and 2 were fed AIN-76 diet (control); groups 3 and 4 high polyunsaturated fat diet (HPUF) and groups 5 and 6 high saturated fat diet (HSF). Mice were sampled 6 months post-weaning and livers were evaluated for neoplastic lesions. No hepatic tumors were found in mice from groups 2, 4, or 6. Groups 3 and 5 displayed a significant increase in mean hepatic adenomas/liver (31.2 and 50.3 respectively) over that seen in group 1 (18.5). Tumor size was also increased in groups 3 and 5 over that of group 1. These findings suggest that both HPUF and HSF diets promote hepatic tumor development in DENA initiated male B6C3F1 mice.

Male Sprague-Dawley rats and female ICR/Ha mice were exposed to either beta-propiolactone (BPL) or dimethylamobarbitol chloride (DMCC) by inhalation (rats: 10 ppm BPL or 4 ppm DMCC, 6 h/d x 30 d), topical (mice: 40 μmoles each, 3X/wk), or subcutaneous (mice: 4 μmoles BPL or 40 μmoles DMCC, 1X/wk) exposure routes. Fifty percent of all BFL induced tumors were positive in the NIH3T3 gene transfer assay. An activated H-ras was found in a mouse aqueous cell carcinoma after a second round of transfection, and Southern blot hybridization. A Hind III restriction fragment polymorphism was detected in this gene. Oligonucleotide hybridization using probes specific for the mouse H-ras 12th codon shows no point mutations at this site. A rat nasal epithelial carcinoma with NIH3T3 transforming activity is activated by a non-ras oncogene.

None of the DMCC induced rodent tumors were positive in the NIH3T3 gene transfer assay. The nude mouse co-transfection assay yielded G418 resistant colonies that were tumorogenic in nude mice. DNA from a nude mouse tumor had no exogenous rat sequences homologous to any known oncogene. Our results suggest that oncogene activation may be specific to the identity of the carcinoma, as well as the histology of the tumor. Supported by NIH Grants CA35342, ES05563, and ES03847.


Ethynylestraadiol (EE2) is an effective tumor promoter when administered continuously following initiation with diethylnitrosamine (DEN). A wide spectrum of malignant and benign mammary and liver tumors are produced following 40 to 60 weeks of promotion by EE2. In order to further examine the mechanism of tumor formation in these tissues, the NIH 3T3 mouse fibroblast transformation assay was used. Tumors were obtained from ovariectomized, female, Sprague-Dawley rats treated with a single dose of DEN (200 mg/kg, i.p.) at 70 days of age and administered EE2 (90 μg/kg/day) continuously until sacrifice. High molecular weight DNA was isolated from tumor tissues and transfected into cultured NIH 3T3 fibroblasts. Morphologically transformed foci were induced in both 1st and 2nd cycle transfections of DNA from 1/2 hepatocellular adenomas, 0/2 hepatocellular carcinomas, 1/4 mammary adenocarcinomas and 0/6 mammary fibroadenomas. Transforming efficiencies compared to positive controls suggest that the putative oncogenes are not highly amplified in the tumor DNA. The identity of the transforming oncogenes is currently being investigated. (Supported by NIH Traineeship ES-07126).


Our overall objective is to assess the status of oncogenes in the liver of the B6C3F1 mouse, a strain exhibiting a high incidence of spontaneous hepatomas. In this study, the expression of the Ki-ras oncogene was examined in normal liver, as aberrant expression of the ras oncogenes has been associated with a variety of solid tumors, including hepatomas. The expression of the serum albumin gene was also monitored as a positive control. 32P- Labelled probes for the Ki-ras and serum albumin genes were employed to assess the following parameters: a) gene methylation (hypermethylation of a gene is necessary but not sufficient for its expression); and b) mRNA level. The serum albumin gene was found to be hypermethylated as determined by MspI/HpaII restriction enzyme analysis, and serum albumin mRNA was readily detected. In contrast, Ki-ras mRNA is apparently not synthesized, even though restriction enzyme analysis indicates that this the gene is hypermethylated. The Ki-ras oncogene is hypermethylated in the liver of the Sprague-Dawley rat (Vorce and Goodman, BBRC 126: 879, 1985), a species in which embryonic liver is a low incidence of spontaneous hepatomas. These results indicate that the Ki-ras oncogene may have the potential for expression in the normal liver of the B6C3F1 mouse, and this state may facilitate hepatoma development.


We have previously reported (The Toxicologist 6(1):5, 1986) that the presence of mammary cancer with a tumor burden of at least 2%, whether chemically-induced (DMBA) or transplanted (R3230AC), caused a significant decrease in phase I liver endoplasmic reticulum (ER) drug metabolizing capacity. The present studies investigated the morphologic status of the liver ER during the presence of mammary neoplasia (transplanted R3230AC) in female rats. By light microscopy, livers from tumor-bearing rats were indistinguishable from those of sham controls. However, quantitative stereologic analyses of liver from tumor-bearing rats revealed significant increases in volume and surface parameters of smooth ER, rough ER and total ER. The ER volume parameters were increased more than the surface parameters. These data indicated that the presence of mammary cancer is associated with the induction of a hypertrophic hypo-functional hepatic ER. Further biochemical studies established that the liver ER changes in mammary tumor-bearing rats are mediated via an indirect mechanism, probably involving changes in the steroid hormone milieu.

104
VALIDATION OF RAPID BIOASSAY SYSTEM FOR DETECTION OF CARCINOGENS. T. Shirai, H. Tsuda, T. Ogiso, T. Aoki, and N. Ito. Department of Pathology, Nagoya City University Medical School, Nagoya, Japan. Sponsor: J.R.P. Cabral.

We developed a intermediate-term (8 weeks) in vivo liver assay system using male F344 rats for early detection of carcinogenicity of test chemicals. This system is composed of a sequence of a single i.p. injection of 200 mg/kg of diethyl nitrosamine, 2 weeks with no treatment and a 6-week promotion stage coupled with partial hepatectomy. Test chemicals were given in the promotion stage. Recently glutathione S-transferase P type (GST-P) was shown to be a better positive marker enzyme for detection of putative preneoplastic liver lesions than γ-glutamyl transpeptidase. GST-P positive foci in the liver were quantitatively evaluated using an image analyzer, and the results were compared with the data for carcinogenicity and mutagenicity. 102 chemicals were tested. 11 of 19 mutagenic carcinogens and 11 of 19 non-mutagenic carcinogens were positive in this system. 21 of 24 hepatocarcinogens were positive the other 3 being, peroxisome proliferators(2) and DDPH. Only 2 of 12 non-hepatic carcinogens were positive. Twenty non-carcinogens all negative, regardless of their mutagenicities. The above demonstrated correlation between carcinogenicity data and results in our system suggests its use as a useful rapid tool for carcinogenicity detection. The demonstrated responses found for the forty-five chemicals of hitherto unknown carcinogenic potential are of interest in this respect.


Chronic feeding of dl-2-ethylhexylphosphatylate (DEHP) suppresses the ability of EGF to enhance phosphorylation of its receptor protein in isolated liver plasma membranes (DeAngelis et al., Cancer Res. 45:2654, 1985). We have used cultured hepatocytes to study the ability of MEHP to directly alter EGF function (enhancement of DNA synthesis). EGF was added to 24 or 48 hr cultures and DNA synthesis measured by incorporation of 3H-thymidine into DNA 24 hr later. EGF (100 ng/ml) stimulated DNA synthesis 1200% above control at both time periods. 10-5M insulin (in) with EGF in 24 hr cultures increased this value to 1700%. MEHP (50 uM) added at 0 hr enhanced DNA synthesis 300% over control value, and by 72 hr inhibited the ability of EGF to increase DNA synthesis much beyond its effect. Hepatocytes from rats given 1.5 g DEHP/kg bw po for 3 weeks were refractory to stimulation of DNA synthesis by 10-100 ng/ml EGF. In restored 50% of cell responsiveness to EGF. These results will serve as a basis for studies into phthalate ester modulation of EGF binding to receptor, plasma membrane receptor concentration, and receptor function. This abstract does not necessarily reflect EPA policy.

THE MODULATION OF GAMMA-GLUTAMYLTRANSPEPTIDASE (GOTase) EXPRESSION IN PRIMARY CULTURES OF RAT HEPATOCYTES BY PHTHALATE NONOSTERS. L. W. Chang, J. McSlyes and A. B. DeAngelis, U.S. EPA, HERL, Cincinnati, OH.

Mono-2-ethylhexylphthalate (MEHP), a peroxisome proliferator (PP), inhibits the outgrowth of hepatic GOTase foci in diethyl nitrosamine (DEN) treated rats. Mono-n-octylphthalate (MOP), a non-PP, increases GOTase foci. The ability of MEHP and MOP to alter GOTase activity in cultured hepatocytes was studied. Hepatocytes isolated from normal and DEN-treated rats were cultured for 6 days with and without 2 mM phenobarbital (PB) or 30 uM dexamethasone (DEX). Hepatocytes from the DEN-treated rats had a greater initial GOTase activity than those from normal rats (6.98 vs 0.42 U/mg protein). A time-related increase in GOTase activity was seen in cells from normal rats (156%) but not in DEN-treated rats. The former were also more sensitive than the latter to this induction by PB (982% vs 251%) and DEX (3397% vs 391%). The induced GOTase activity was sensitive to MEHP inhibition (50%) in normal cells, but not in cells from the DEN-treated rats. MOP could not induce GOTase activity, in contrast to the effect seen in vivo. These results will serve as a basis for studies into the mechanisms of phthalate ester modulation of GOTase activity. However, as with the case of MOP, a role for the intact animal may prove essential. This abstract does not necessarily reflect EPA Policy.

Ethynylestradiol (EE2) promotes tumor formation and GGTL-positive foci in livers of rats treated previously with diethylnitrosamine (DEN). We are conducting experiments using a combination of radiographic and histochemical techniques in order to determine if EE2 is preferentially retained in cells within these GGTL-positive foci. Female rats were ovariecetomized 56 days after birth and administered a single dose of DEN (200 mg/kg, ip) or saline (S) on day 70. Rats were then treated continuously with EE2 (50 μg/kg/day). After 5 to 40 weeks of EE2 promotion, \(^{4}\)H-EE2 + excess EE2 was administered i.p. Histochemical demonstration of GGTL activity and radiographic demonstration of \(^{4}\)H were performed in frozen hepatic sections. The volume of the liver comprised of GGTL-positive foci increased significantly between 20 and 40 weeks of promotion in DEN/EE2 rats and was significantly greater in DEN/EE2 rats than in DEN only rats at 40 weeks. Preliminary experiments after five weeks of promotion have revealed specifically labeled hepatocytes in rats receiving DEN initiation, whereas no specifically labeled hepatocytes were observed in saline controls. We are currently investigating the role that specifically labeled cells in GGTL-positive foci may play in tumor promotion by EE2.

LIVER TUMOR PROMOTING ACTIVITY OF PHENOBARBITAL IS ASSOCIATED WITH ITS ABILITY TO INDUCE PHENOBARBITAL-INDUCIBLE CYTOCHROME(S) P-450 (P-450pg-β), AMINOPYRINE N-DEMETHYLASE ACTIVITY AND LIVER HYPERPLASIA. B.A. Dwan, R.W. Mins, R.A. Luber, and J.M. Rice, National Cancer Institute, Frederick, MD and Microbiological Associates, Inc., Bethesda, MD. Sponsor: M.P. Waalkes

Although phenobarbital (PB), a well-known inducer of cytochrome P-450-catalyzed enzyme activities, promotes liver carcinogenesis in rats and mice, no such promoting effect was observed in hamster liver after chronic feeding of this drug following initiation with either diethylnitrosamine or methylazoxymethanol acetate (Dwan et al., Toxicol. Appl. Pharmacol. 86:1-10, 1986). The present study was planned to compare the ability of PB to induce the principal hepatic PB-inducible form of cytochrome P-450 (P-450pg-β), aminopyrine N-demethylase activity, and liver hyperplasia in Syrian golden hamsters, F-344 rats and B6C3F1 mice. Two male animals of each species received either 500 ppm PB or unmedicated diet for 15 days. PB increased liver weight and enhanced cytochrome P-450pg-β and aminopyrine N-demethylase activity (P < 0.05) in rats and mice but failed to induce any of these parameters in hamster liver to a significant extent. Thus, in rodents, tumor promoting activity of PB parallels its ability to induce synthesis of certain P-450 species.


Phorbol myristate acetate (PMA) and Ca\(^{2+}\) stimulate GH\(_3\) cell protein synthesis synergistically. To explore the potential role of ribosomal protein phosphorylation in this stimulation, \(^{32}\)P-labeling of post-nuclear basic proteins was determined for Ca\(^{2+}\)-depleted and Ca\(^{2+}\)-restored GH\(_3\) cells treated with or without PMA. Proteins were separated by PAGE. PMA stimulated the phosphorylation of a 32KD polypeptide (S6) of the 40S ribosomal subunit in Ca\(^{2+}\)-depleted and Ca\(^{2+}\)-restored cells. In contrast, a 30KD protein associated both with microsomes and the 60S ribosomal subunit was phosphorylated in Ca\(^{2+}\)-restored cells and dephosphorylated after brief cellular Ca\(^{2+}\) depletion. PMA did not alter the phosphorylation state of the 30KD protein. The 30KD protein was dephosphorylated after treatment with 8Q20006, a NaF 2 inhibitors of protein synthesis. Ca\(^{2+}\) enhances the phosphorylation of the 30KD protein maximally within 5 min, correlating well with maximal stimulation of protein synthesis. PMA enhances the phosphorylation of S6 gradually over the course of 1 hr. However, PMA stimulates protein synthesis maximally after 2 hr. The results suggest that separate pathways exist for the action of PMA and Ca\(^{2+}\). (Supported by AM 33930, CA 07690 and by the N.J. State Commission on Cancer Research.)

HYPEROXIA REDUCES EXPERIMENTAL LUNG METASTASIS FROM SENSITIVE CELL LINES. N.C. Margaretten, and H. P. Witzelch. Oak Ridge National Laboratory, Biology Division, Oak Ridge, TN.

We investigated the sensitivity of artificial lung metastasis to hyperoxia. Male BALB/c mice received intravenous injections of 50,000 MT-7 cells, a cell line derived originally from a mammary tumor. Exposure to 70% or 80% O\(_2\) for 3 weeks significantly reduced the number of lung metastasis and so did intermittent exposure to 100% O\(_2\). Hyperoxia also reduced the number of lung metastasis originating from tumors produced by i.c. injection of MT-7 cells. Other oxygen-sensitive cell lines were lines 498, M109, and Line-1 passage 17, derived from primary lung tumors, whereas Line-1 passage 168, Lewis lung carcinoma and the melanoma-derived 8165-F and F10 lines were insensitive to hyperoxia. The activities of the enzymes superoxide dismutase, glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione peroxidase, and total glutathione levels were variable among the cell lines and basal antioxidant defense mechanisms did not correlate with oxygen sensitivity. (Operated by Martin Marietta Energy Systems, Inc. with the U.S. Department of Energy. NCM supported by NIH Grant CA-09336.)
Hyperoxia can drastically reduce the growth of artificial lung metastasis produced by intravenous injection of certain tumor cell lines. To explore possible mechanisms we measured biochemical antioxidant defense enzymes and growth parameters of lung metastasis in vivo. The biochemical studies revealed no differences in several antioxidant defenses in tumor cell homogenates isolated from mice exposed to 100% oxygen as compared with those from air exposed animals. Likewise, no differences were seen in normal lung homogenates after hyperoxic exposure. Using the technique of labeled mitoses we found that hyperoxia retarded tumor cells in vivo from entering mitosis, producing a mitotic delay. Hyperoxia also decreased the percent of tumor cells entering mitosis. Evidence thus indicates that hyperoxia is tumoricidal by its ability to produce a G2 block in lung tumor cells in a manner similar to ionizing radiation. (Operated by Martin Marietta Energy System, Inc. with the U.S. Department of Energy. NCM supported by NIH Grant CA-09336.)

Previously we reported that the initiating activity of BaP is altered by certain complex organic mixtures (COM) and not by others. Because of these results five COM were co-administered with [3H]-BaP and the amount of radioactivity bound to mouse skin DNA was determined. In the presence of the COM the greatest inhibition of BaP-binding was with those mixtures with components most similar to BaP in molecular weight. When a COM was fractionated into classes of compounds, the PAH fraction was the most effective and the aliphatic the least effective as inhibitors. Binding of BaP was further characterized by enzymatically hydrolyzing the DNA to nucleosides and separating the non-added nucleosides from other components by LH-20 chromatography. The radiolabeled added nucleosides were then separated by HPLC and the radioactivity profiles compared to the nucleoside profile for DNA isolated from mouse skin which had been treated with BaP alone; particular attention was given to the ratio of anti-BPDE to syn-BPDE adducts. These data suggest that both DNA binding and adducts profile are important in determining the contribution of a known carcinogen to tumor development initiated by COM. Work supported by U.S. Department of Energy under Contract DE-AC06-76RL0 1830.

Five complex organic mixtures (COM) were examined for their effects on the percutaneous absorption of BaP from mouse skin, and the metabolism of BaP in vitro. The COM used were solvent-refined coal liquid distillates boiling at 300-700, 700-750, 750-800, 800-850, and 850-900°F. Each mouse received either 50 μg of [14C]-labeled BaP alone, or 50 μg of [14C]-BaP in total dose of 17 μg of COM. The absorption of BaP alone followed linear first-order kinetics, with an absorption half-life of 4.5 hours. The skin half-life of BaP was increased to 15.5 hours by the 800-850°F COM and 37 hours by the 850°F COM. The rate of disappearance of BaP from the skin was compared in normal and TCDD-pretreated mice; TCDD has been reported to increase the dermal metabolism of BaP by approximately 8-fold. TCDD increased the rate of BaP disappearance by approximately one third, indicating that the metabolism of BaP by mouse skin MFO plays a relatively small role in BaP absorption. The COM inhibit the metabolism of BaP by rat liver S9, suggesting that they may also inhibit BaP metabolism by mouse skin MFO in vivo. Inhibition of skin MFO may at least partially explain the inhibition of BaP initiating activity by COM. Supported by U.S. Department of Energy Contract DE-AC06-76RL0 1830.

Previously we reported that co-administration of two carcinogenic complex organic mixtures (COM) with radiolabeled BaP markedly decreased the amount of binding to mouse skin DNA, relative to the binding for BaP alone. Since these data suggested that a major portion of the carcinogenic activity is contributed by PAH other than BaP, we are developing methods to identify carcinogens that covalently bind to DNA. Mice were treated dermally with BaP and the DNA isolated. The purified DNA was either enzymatically hydrolyzed and the adducted nucleosides separated and quantified by HPLC procedures or the BaP-tetraol adducts were released from the purified DNA under acidic conditions, derivatized, and then characterized by GC/MS methods. Results indicate that the overall recoveries of the two methods are similar, but that quantitative data on the four stereoisomeric tetraols are lost due to racemization with the acid hydrolysis method. Further, the GC/MS data indicate that the level of detection for this method is less than 10 femtomole using single ion monitoring, demonstrating the feasibility of this approach for studying adducts in vivo after exposure to COM. Work supported by U.S. Department of Energy under Contract DE-AC06-76RL0 1830.
INHIBITION OF BENZO(A)PYRENE SKIN TUMOR INITIATING ACTIVITY BY COMPLEX ORGANIC MIXTURES.
D.D. Mahlum. Pacific Northwest Laboratory, Richland, WA.

Previously we showed that a broad boiling range (300–850°F) coal-derived complex organic mixture inhibited the skin tumor initiating activity of benzo[a]pyrene (BaP). In an attempt to segregate the materials responsible for this inhibition, we distilled the COM to give distillates with the following boiling ranges: 300–700°, 700–750°, 750–800°, 800–950°, and >950°F. All distillates boiling above 700°F inhibited BaP-initiating activity. We prepared chemical class fractions from the 750–800°F distillate to determine if a specific chemical class was inhibitory. This study showed the neutral polycyclic aromatic hydrocarbon (PAH) and the nitrogen-containing polycyclic aromatic compound fractions markedly inhibited BaP activity; the aliphatic and hydroxyl PAH fractions did not inhibit. These results indicate that tumorigenic activities of COM may be less than the sum of their parts. Moreover, the inhibition of activity by COM appears to be due to certain polycyclic aromatic classes of compounds. Work supported by U.S. Department of Energy under Contract DE-AC06-76RLO 1830.

433 GLUTATHIONE, SULFITE, AND BENZO(a)PYRENE INTERACTIONS: A MATHEMATICAL MODEL. D. A. Keller, R. H. Leung, and D. B. Mengel. Dept. of Pharmacology and Medicine, Comprehensive Cancer Ctr., Duke University Medical Ctr., Durham, NC.

A mathematical model was developed describing the effect of sulfite, a sulfur dioxide (SO2) metabolite, on glutathione (GSH), glutathione disulfide (GSSG), and glutathione S-sulfonate (GSSOS) in the human A549 lung cell line, and the effect of GSSOS on benzo[a]pyrene diol epoxide (BPDE) reactions. The model consists of coupled differential equations describing time-dependent reactions of GSH, GSSG, GSSOS, sulfite, BPDE, and DNA. Reaction rates were obtained experimentally or from the literature. Equations were solved simultaneously using an IBM PC-based Simulation Control Program (SCOP). The model predicted an increase of GSSOS, an inhibitor of the glutathione S-transferase, and a decrease of GSSG as intracellular sulfite concentration increased, agreeing well with experimental values. The GSSOS formation was predicted to increase concentrations of syn- and anti-BPDE by 55% and 30% over control, respectively, in 1 hr, resulting in 31% and 15% higher levels of syn- and anti-BPDE-DNA adducts, respectively. These predictions suggest that decreases in BPDE conjugation, as a result of GSSOS formation, may be responsible for the cocarcinogenic effect of SO2. (Supported by NIH grants ES02916, RR01693 and CAL14236, and an Electric Power Research Institute grant.)

434 TOXICOLOGICAL SIGNIFICANCE OF THE BENZYLIC DOUBLE BOND OF PRECOCENE II. S.K. Duddy and M.T.S. Hsia. Environ. Tox. Ctr. and Dept. of Entomology, Univ. of Wisconsin, Madison, WI.

Precocene II (PPI) induces DNA damage and unscheduled DNA synthesis in isolated rat hepatocytes, and hepatic centrilobular necrosis in rats in vivo. The putative toxic agent is PPI 3,4-oxide, apparently produced by cytochrome P-450 mediated monooxygenation at the 3,4-benzylidened double bond. Suspensions of rat hepatocytes were treated with PPI, 3,4-dihydro PPI (dPPI), or 3,4-dihydrodilone PPI (PPI diole) for 2 hr. Cyotoxicity was assessed by measuring leakage of glutamic-oxalacetic transaminase (GOT) into the incubation medium. The glutathione (GSH) content of cells and supernatant was also determined. PPI produced concentration-dependent depletion of GSH and significant leakage of GOT. dPPI produced slight GOT leakage and GSH depletion only at the highest concentration tested (1.0 mM, the highest concentration of PPI tested). Similar results were obtained for PPI diole. dPPI or PPI diole administered i.p. to male Sprague-Dawley rats produced no observable hepatic necrosis and no elevation in serum GOT, in sharp contrast to a comparable dose of PPI, which produced marked centrilobular necrosis and serum GOT elevation. These results suggest that the benzylidene double bond of PPI is critical for PPI-induced toxicity.

432 HUMAN MYLLOPEROXIDASE AND HORSAHADISH-PEROXIDASE CATALYZED OXIDATION OF CATECHOL. D. Ross. Sponsor: D.R. Peterson. Molecular and Environmental Toxicology Program, School of Pharmacy, University of Colorado, Boulder, CO.

Benzene induces a myelotoxic response in both animals and man, but the mechanism underlying such toxicity is not understood. After administration of benzene, its secondary phenolic metabolites — catechol and hydroquinone — accumulate in bone marrow which is rich in myeloperoxidase activity. We have, therefore, examined the metabolism of catechol by a model peroxidase — horseradish peroxidase (HRP) and by human myeloperoxidase (MPO). One major oxidation product was observed during both HRP and MPO-catalyzed oxidation of catechol utilizing HPLC analysis but its instability precluded isolation and characterization. HPLC eluate containing the unidentified metabolite was mixed with bromothiophenol directly after HPLC isolation and the resultant adduct purified by HPLC. Direct probe mass spectrometry (EI) showed this adduct was the reaction product of o-benzquinone and bromothiophenol. o-Benzoquinone was also shown to react with biological thiol such as reduced glutathione. These data show that both HRP- and MPO-catalyzed oxidation of catechol result in o-benzoquinone production.

Supported by NIH grant ES04112.
DEPLETION OF HEPATOCYTOLOGIC GLUTATHIONE (GSH) BY EXOGENOUSLY ADDED NAPHTHALEN E OXIDE (NO). M.H. Buonarati, D. Morin, and A.R. Buckholz. Dept. Veterinary Pharmacology and Toxicology, University of California, Davis, CA.

Previous studies have shown that NO generated by P450 monoxygenase mediated metabolism of naphthalene (NA) is capable of diffusing from isolated hepatocytes. The possible role of circulating NO in NA-induced bronchial necrosis is uncertain as is the ability of these unstable species to penetrate cellular membranes. Therefore, hepatocytes were incubated in the presence of 18,28- or 18,28-NA enantiomers (15, 30 and 60 μM). Depletion of intracellular GSH was concentration dependent and maximal at 5 min; at 60 μM, GSH was less than 35% of control. The formation of NA GSH adducts was dependent upon NO concentration; GSH adduct formation accounted for approximately 50% of GSH depletion. The levels of GSH adduct were highest 1 min after addition of NO and declined to 25% of this level at 30 min. In contrast, the levels of GSH adducts added to hepatocytes were relatively stable over a 120 min incubation suggesting that further metabolism of NA GSH adducts formed intracellularly is possible and that GSH adducts added exogenously cannot penetrate the hepatocyte. Significant cytotoxicity was not observed with either of the NO isomers even at 60 μM. These studies indicate that NO is sufficiently stable to diffuse into hepatocytes; approximately 5% of NO was able to reach the intracellular matrix.

PEROXIDATIVE BIOTRANSFORMATION OF CYCLIC 1,3-DIKETONES. R.A. Barter and G.A. Reed. University of Kansas Medical Center, Kansas City, KS.

Peroxidative biotransformation of the anti-inflammatory drug phenylbutazone (PB) yields a peroxyl radical and a PB hydroperoxide. The peroxyl radical may be involved in both the therapeutic and the toxic effects of PB. The site of oxidation in the PB molecule is a cyclic 1,3-diketone. This same moiety is present in indan-1,3-diones (ID). Various 2-substituted IDs have been used as oral anticoagulants and are potential hypolipidemic agents [Murphy et al., J. Med. Chem. 28: 1591 (1985)]. ID and 2-aryl-IDs are, like PB, efficiently oxidized by either horseradish peroxidase or by the peroxidase activity of microsomal prostaglandin H synthase. Of the compounds studied, however, only 2-(2-methoxyphenyl)-indan-1,3-dione (MID) oxidation is accompanied by the consumption of O₂, the required step for peroxyl radical and hydroperoxide formation. Preliminary product analysis suggests the conversion of most 2-aryl-IDs to dimeric and oligomer products, whereas MID oxidation yields oxygenated products. We conclude that the cyclic 1,3-diketone moiety is a potential site for peroxidative biotransformation, but that the subsequent formation of peroxyl radicals and hydroperoxides is dependent on the chemistry of the radical species formed by the initial oxidation. Supported by the Pharmaceutical Manufacturers Association Foundation.

TRITIUM/CARBON-14 (T/C) DUAL LABEL STUDY OF 1,2-DIBROMOBENZENE METABOLISM AND COVALENT BINDING. N. Narasimarsh and R.F. Hancil. Department of Medicinal Chemistry. University of Kansas, Lawrence, KS.

DBB, like bromobenzene, is hepatotoxic in rats presumably because of reactive metabolites which covalently modify macromolecules through covalent binding (CBV). To characterize these metabolites we have employed dual-label isotope ratio techniques and chemical trapping with N-acetylcycteine (NAC). (U)-C-14/3,5(T) dual labelled DBB (normalized T/C ratio = 1.0 pmol/mg) was incubated with PB-induced rat liver microsomes and the metabolites separated, quantitated and in most cases identified. After 60 minutes without NAC 11% of DBB was consumed (555 nmol); T/C = 1.00 for unreacted DBB. Metabolites included (nmole; T/C ratio): 3,4-dihydropicolin (350. 1.08), isomeric dihydropicolin (95, 1.12), 2,3-and 3,4-dihydromphenol (10. 0.72); CBV fraction (200, 0.47), NAC (5 mm) decreased CBV by 60% while hardly affecting the total metabolism. The yield or T/C ratio of the dihydropicolin and phenols, or the T/C ratio of the CBV fraction. The decreased yield of CBV was largely balanced by a new acidic soluble metabolite (T/C = 0.65), whose structure elucidation is underway. The high T/C ratio of the epoxide derived dihydropicolin contrasts to the low T/C ratio found in the CBV fraction, suggesting that most CBV may not arise from an epoxide. (Supported by NIHGM-21784)

FORMATION OF FORMALDEHYDE HYDRAZONE IN HYDRAZONE-INDUCED METHYLATION OF DNA GUANINE. G.E. Lambert and R.C. Shank. Dept. of Community and Environmental Medicine, Univ. of Calif., Irvine, CA.

Hydrazine (H₂) induces the formation of 7-methylguanine (7MG) and 6-methylguanine (6MG) in target organ DNA. We have suggested that H₂ reacts with endogenous formaldehyde (FM) to form a condensation product which could be metabolized to the methyating agent. Solutions prepared from 0.5 mM H₂ and FM have NMR spectra (300 MHz) consistent with the formation of formaldehyde hydrazone (FM) but not the condensation products formaldehyde azine or tetraformyltriazine. These same solutions evidencing hydrazine formation, when incubated in a system containing calf thymus DNA and rat liver postmitochondrial (S-9) fraction, resulted in methylation of DNA. Microsomal, cytosolic, and mitochondrial fractions were all active in H₂/FM induced methylation. Neither the flavin-containing monoxygenase inhibitor methimazole nor the cytochrome P450 inhibitor N- octylamine decreased H₂-induced methylation levels. At 0.1 mM both NaAzide and cyanamide inhibited DNA methylation by 1 mM H₂/FM by at least 65%. Solutions of 2 mM H₂/FM in the presence of the chemical oxidants (6 mM) HgO or NaN₃ methylated calf thymus DNA (no cell fractions included). The data suggest formation of FM as the condensation product which is rapidly transformed by various cell fractions to a methylating agent. (Supported by PHS Grants R01-ES-03726 and T32-ES-07157)
CHARACTERIZATION OF THE REACTIVITY OF METALLOTHIONEIN TOWARDS METHYL BROMIDE. E.M.K. Lu, Department of Pharmacology and Toxicology, University of Western Ontario, London, Canada.

In addition to providing metal binding sites, the 20 cysteine thiolate groups of mammalian metallothionein (MT) may play a role in the detoxification of electrophilic chemicals. To study the in vitro interaction between MT and methyl bromide (MeBr), an alkylating agent, rat liver Cd,Zn-MT-II was incubated with the chemical in phosphate buffer (pH 7.4) at 37°C for 60 minutes. In the presence of excess MeBr, MT concentration was limiting and biphasic reaction kinetics were observed. The time-dependent loss of MT bound metals as well as the decline in the maximum metal (Cd and Ag)-binding capacity of MT consisted of a fast and a slow component, each with a first order rate constant. Similar biphasic decay profile for MT thiol was observed, suggesting that the MeBr-induced reduction in metal-binding was related to the loss of protein thiol groups. Moreover, amino acid analysis of MeBr-treated MT samples showed reductions in cysteine residues, and the recovery of equimolar amounts of methyl cysteine. To assess the nature of the reaction kinetics, thiol groups of MeBr-treated MT samples were titrated with 5,5'-dithiobis-(2-nitrobenzoic acid); the result of this study suggested that terminal thiolates may be more reactive than bridging thiolates towards MeBr. Supported by MRC.


Previous studies indicate that nitrosoaromatics are metabolized to N-acetyl-(AHA) and N-glycolyl (GHA) hydroxamic acids (Corbett and Corbett, Biochem. Pharmacol. in press, 1986). To determine whether these metabolites may be involved in the genotoxicity of this class of compounds, we studied the covalent binding of AHA and GHA derivatives of nitrosoaromatic to macromolecules of rat hepatocytes. In vitro C-AHA or 14C-GHA was added to primary cultures and incubated for 4 hr. Uptake of AHA and GHA was monitored by high pressure liquid chromatography. Total nucleic acid was isolated by a standard phenol extraction technique. To further separate DNA from RNA, several nonenzymatic methods were compared. LiCl precipitation was most efficient, especially in the separation of DNA from ribosomal RNA. Our results showed that more than 95% of both compounds were taken up by the hepatocytes within 4 hr. Covalent binding to both DNA and RNA was detected. These findings suggest that production of hydroxamic acid derivatives may play an important role in the genotoxicity of nitrosoaromatic compounds. (supported by NIH grant no. OH 02027)

IN VITRO STUDY ON CARBON TETRACHLORIDE AND METALLOTHIONEIN INTERACTION. Z. Suntrés and E.M.K. Lu, Dept. of Pharmacology and Toxicology, Univ. of Western Ontario, London, Canada.

To study the role of metallothionein (MT), a cysteine-rich, metal-binding protein in the detoxification of carbon tetrachloride (CCl₄), an in vitro system was used to examine the mode of their interaction. Rat hepatic Cd,Zn-MT-II was incubated with 10 μM of CCl₄ in Tris buffer (pH 7.4) at 37°C for 30 min. Under aerobic conditions, and in the presence of hepatic microsomes and NADPH, significant reductions in the maximum Cd-binding capacity and the thiol content of MT were observed. These effects were not, however, observed under N₂, thus eliminating a direct involvement of CCl₄, which is known to be generated under both incubation conditions. Incubation with chloroform (CHCl₃) produced no effects on MT, suggesting that the CHCl₃-phosgene metabolic pathway of CCl₄ was not involved in the interaction. It was also observed that addition of promethazine to the incubation completely obliterated the CCl₄-induced lipid peroxidation (L.P.), but failed to antagonize the effect of CCl₄ on MT, suggesting a limited role of products of CCl₄-induced micromolar L.P. in the inactivation of MT. Therefore, our data suggests that metabolite(s) of CCl₄ other than CCl₃, CHCl₃ and phosgene, were involved in the interaction with MT. Supported by MRC.

SELECTIVE DAMAGE TO CLARA CELLS BY NAPHTHALENE IN THE ISOLATED PERFUSED MOUSE LUNG. S. Kanekai, C.G. Plapppe, D. Mortin, and A.R. Bucklett. Vet. Pharmacology and Toxicology, UC Davis, Davis, CA.

Pulmonary Clara cells of the mouse are highly susceptible to cytotoxicants requiring metabolic activation including naphthalene (NA), dichloroethylene and CCl₄. Accordingly, a method is described for maintaining an isolated, perfused and ventilated mouse lung (IPVL) which appears suitable for studies on the relationship between metabolism and Clara cell cytotoxicity. Light and electron microscopic (EM) studies revealed only slight alterations in the architecture of the bronchiolar epithelium after 7 hours of perfusion. There was, however, substantial edema. At 2 hrs the increase in lung wet/dry weight was 15%, at 4 hrs 44% and at 7 hrs 74%. IPVL retained 50% of the initial NA monooxygenase activity for the first hour; subsequently this decreased to 48 and 34% at 4 and 7 hours of perfusion, respectively. EM of lungs perfused with 1 μM NA for 30 min followed by 6 hours with media revealed vacuolation and pyknosis of nucleii of Clara cells; the adjacent ciliated cells remained unaltered. Thus, circulating reactive metabolites do not appear to play a primary role in NA-induced Clara cell necrosis. Moreover, these techniques may be useful in determining the relationships between metabolism and cytotoxicity in lungs of an animal model that is highly susceptible to bronchiolar cytotoxicants. Supported by Toxic Substances Research and Teaching Program, University of CA.
METABOLISM OF BENZOA(PYRENE)-7,8-DIHYDRODIOIL BY LUNG NEUTROPHIL-DERIVED OXIDANTS. M.A. Truth. Johns Hopkins Univ., Baltimore, MD

Although it has been recognized that malignancies often develop at sites of ongoing inflammation and infection, including in the lung, the relationship between these two pathologic states remain to be defined at the biochemical and molecular level. We have previously shown that through the elaboration of oxidants, human neutrophils can activate BP-7,8-dihydridodiol to an intermediate which coherently binds to DNA and elicits mutagenicity in bacteria. In this study, we have recruited neutrophils to the lungs of male C57Bl/J6 mice by inhalation exposure to Proteus mirabilis. Addition of the phorbol ester TPA to lung neutrophil preparations (>90% neutrophilic cells), elicited superoxide anion generation, as indicated by SOD-inhibitable cytochrome c reduction, and oxidant-dependent chemiluminescence (CL) from the chemilumigenc probes, lucigenin and luminol. The interaction of BP-7,8-dihydridodiol with TPA-stimulated lung neutrophils resulted in CL from BP-7,8-dihydridodiol, indicative of its myeloperoxidase-dependent oxidation to a 9,10-dioxetane intermediate. The generation of CL from polycyclic aromatic hydrocarbons corresponds to their ability to elicit bacterial mutagenesis and site chromatic exchanges in mammalian cells. Such observations suggest a mechanism as to how a pulmonary infection may enhance the risk for development of carcinogenesis in the lung.

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DECREASED SUPEROXIDE ELABORATION IN THE PRESENCE OF NORMAL SERA. D. F. Gruber and A. M. Parease. Armed Forces Radiobiology Research Institute, Bethesda, MD Sponsor: V. Bogo

Circulating polymorphonuclear (PMN) leukocytes and fixed tissue macrophages represent the host's major cellular elements of nonspecific resistance. PMNs and macrophages are activated by particulate and soluble phlogistic mediators to produce a cellular respiratory burst, including the production of superoxide (SO) and other reactive oxygen intermediates (ROI) essential to bacterial destruction. SO and other ROI are produced within the cells and elaborated. Elaboration of SO presents the possibility of localized tissue injury; therefore regulatory mechanisms must exist to protect tissues from indiscriminate ROI mediated destruction. We examined normal serum for SO regulatory capacities on isolated normal rat peripheral PMNs. SO production was quantitated for a time period of minutes in the presence of an activating agent, phorbol myristate acetate and varying concentrations of normal rat serum. Normal serum present at concentrations of 1, 2, and 10% by volume, reduced SO release by 34, 50, and 77%, respectively. Complete inhibition of SO elaboration was demonstrated at a concentration of 50%. Heat inactivation of serum added at a 10% concentration inhibited SO release by 34% versus the nonactivated 77% reduction.

COMBINED INJURY INDUCED PERTURBATIONS IN NONSPECIFIC HOST RESISTANCE. D. F. Gruber and M. M. D'Alessandro. Armed Forces Radiobiology Research Institute, Bethesda, MD Sponsor: V. Bogo

Directed migration and production of reactive oxygen intermediates by neutrophils (PMN) are imperative to host nonspecific resistance. Any functional perturbations which arise assume some degree of clinical importance. We evaluated aspects of rat nonspecific resistance by examining the isolated peripheral blood PMN's for chemotaxis (C), production of superoxide (SO) and flow cytometrically (DiOC5(3)) measured changes in membrane potential (MP). Rats received a combined injury (CI) of sublethal irradiation (500cGy at 40cGy/min) followed by a 20% full thickness thermal injury. C and SO patterns were essentially identical for 32 days. C and SO, although unaffected through day 10 decreased 60% on day 14. Both functional activities were depressed through day 21 and near normal on day(s) 28-35. The decreased capacity of the PMN for C and SO correlates well with its inability to depolarize normally. CI resulted in perturbations in MP which did not return to normal for 30 days. Significant deviation from normal depolarization occurred on days 3-28 with a nadir at day 14 also. D. We have previously demonstrated the independent effects of radiation (depressed C) and thermal injury (depressed SO). CI demonstrates the cumulative lesions of both stressors.

DISODIUM Cromoglycate (DSCG) INHIBITS THE FORMATION OF FREE RADICALS, POTENT REACTIVE INTERMEDIATES. A. J. Carmichael, C. M. Arroyo, and L. G. Cockerham. Armed Forces Radiobiology Research Institute, Bethesda, MD.

A possible mechanism by which DSCG prevents a decrease in regional cerebral blood flow and hypotension in primates following whole body gamma-irradiation was investigated. Several studies have implicated superoxide radicals (O2-) in intestinal and cerebral vascular disorders following ischemia and ionizing radiation, respectively. O2- is formed during radiolysis and dissolved oxygen. For this reason, the efficiency of DSCG to scavenge O2- and possibly prevent the formation of O2- was studied using spin trapping and electron spin resonance (ESR). eTOOH were generated by photolysis (313 nm) of K4Fe(CN)6 in aqueous solutions containing the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). DMPO reacts rapidly with eTOOH (k = 2 x 1011 M-1s-1) followed by protonation to yield the ESR observable DMPO-OH spin adduct. The photolysis of several solutions containing 0.01 M DMPO, 0.1 M K4Fe(CN)6 and varying concentrations of DSCG shows an inverse relationship between DSCG concentration and the DMPO-OH ESR signal intensity. These results indicate that DSCG is an efficient eTOOH scavenger and may possibly compete effectively with oxygen for the eTOOH produced photochemically.
Treatment of mice with single injection of the synthetic polyamionic immunomodulator, maleic anhydride-divinyl ether (MVE) copolymer, decreases hepatic microsomal cytochrome P450 (P450) content and associated drug metabolizing activities as early as 4 hr after treatment with maximum decreases occurring within 16-24 hr. The mechanism(s) for this phenomenon has not been established. Decreases in P450 are paralleled by decreases in hepatic xanthine oxidase (XO) and microsomal heme oxygenase activities suggesting that the formation of oxygen intermediates may be implicated in this process. Since hepatic GSH serves as a protective substrate against reactive species, hepatic GSH content and GSH-S-transferase (GT) activity were measured at intervals after MVE treatment. GSH was decreased 20, 42, and 41% at 2, 4, and 8 hr after MVE. GT was increased at the same intervals suggesting an early increase in GSH conjugation. However, both GSH and GT begin to approach normal values as early as 16 hr after MVE treatment, preceding the maximum decreases in P450 content. These results suggest that MVE treatment initiates an early, undetermined insult to the hepatocyte which evokes a progression of events which are responsible for the changes observed for XO, P450, and P450 heme.

**LIPOID PEROXIDATION (LP) IN PRIMARY HEPATOCYTES AFTER INCUBATION WITH PEROXISOME Proliferators.**


Peroxisome proliferators (PP) represent a novel class of hepatic carcinogens, which may cause tumors as a result of increased peroxisomal H2O2 concentration and cellular oxidative stress. We have investigated the effects of incubating primary hepatocytes with PPs on LP. Hepatocytes from male F344 rats were seeded at a density of 3 million/plate and incubated with medium containing mafenofin (NAF), clofibrate acid (CA) or monoo2-ethylhexylphthalate (MEHP). The activity of peroxisomal acyl CoA oxidase, a H2O2 producing enzyme, was 0.5 mmoles/min/mg in control cells, after 2 days of incubation; this activity was increased about 3-, 2-, and 1.5-fold respectively after incubation with 200 μM of each of these PPs. Catalase activity was increased about 10-30%. LP was determined by measuring conjugated dienes (CD) in lipids extracted from harvested cells. There was a 50% increase, relative to controls, in CD/cell after treatment with 200 μM NAF or 400 μM CA. No increase in CD was observed in cells treated with 100 or 200 μM MEHP or CA, or 100 μM NAF. When the results were expressed as CD/mg lipid, increases were significant only for the cells incubated with NAF. The results indicate that incubation of primary hepatocytes with NAF or CA may lead to peroxisomal changes and result in increased cellular LP.
SEPARATE MECHANISMS FOR PROCARBAZINE-INDUCED SPERMATOXICITY AND CHEMOTHERAPEUTIC ACTIVITY. M.G. Horstman, G.G. Meadows, and G.S. Yost. College of Pharmacy, Washington State University, Pullman, WA.

Procarbazine (N-isopropyl-α-(2-methylhydrazino)-p-toluamide) is a cancer chemotherapeutic agent which depresses spermatogenesis in a dose-dependent manner as one mode of toxicity in both man and BDF mice. Either of the antioxidants N-acetylcysteine or sodium ascorbate, when co-administered i.p. with equimolar doses of procarbazine into BDF mice, affords significant protection against this toxicity. Further, the inclusion of either antioxidant in experimental procarbazine treatment of L-1210 murine leukemia had no effect on survival times compared to procarbazine administration alone. This decrease in toxicity without diminished therapeutic efficacy has possible clinical implications, as well as indicating that separate chemical mechanisms may be responsible for the toxic and therapeutic effects of the drug. Additional spermatotoxicity studies performed with known metabolites of procarbazine have shown progressive increases in toxicity along the path from procarbazine through the azo- and azoxy-derivatives, while the hydrazone caused only minor depression. This argues against the participation of methyl radical, a species not known to be produced by either azoxy-isomer, in the spermatotoxic mechanism of this drug. (Supported by USPHS Grant #CA35763.)


Isopropylcyclohexane possesses a branched chain hydrocarbon substituent attached to a cyclohexane ring. Branched chain hydrocarbons containing 8-12 carbons are known to produce hyaline droplet nephropathy in proximal tubular epithelium of male rats; whereas cyclohexene and methylcyclohexane do not appear to affect the proximal tubule. 2,2,4- and 2,3,4-trimethylpentanes are nephrotoxic branched chain hydrocarbons which possess an isopropyl group in the molecular secondary structure and yield alcohols and acids as urinary metabolites. Metabolism of cyclohexene and methylcyclohexane produces alcohols and diols as urinary metabolites. In order to determine how the isopropyl moiety would affect the metabolic oxidation of the cyclohexane ring, male F-344 rats were orally dosed with isopropylcyclohexane (0.25 g/kg) every other day over a 14 day period. Five urinary and three kidney metabolites were identified as carboxylic acids, alcohols, and diols. Unique stereoisomerism was observed. Histopathology revealed mild to moderate, diffuse hyaline droplet nephropathy. Induction of renal lesions with isopropylcyclohexane in male rats fits the pattern of metabolism observed with trimethylpentanes whereby carboxylic acids are produced in addition to alcohols and diols.

STUDIES ON THE MECHANISM OF BALANCE CENTRIFUGAL ACUTE TOXICITY: INTERACTIONS OF DEBRIOXACENTRIFUGAL BIND WITH GLUTATHIONE (GSH) AND GLUTATHIONE-F-TRANSFERASE (GSHT) IN RATS. Ahmed E. Ahmed and Gamal J. Hassan. Department of Pathology, The University of Texas Medical Branch, Galveston, Texas

DBN, a direct acting genotoxic agent, has been found in drinking water. In a time course study, rats were treated with DBN (1 mg/kg p.o.) or DBN (75 mg/kg p.o.) and killed at 0.5, 1, 2, or 4 hrs after treatment. In a dose response study, animals were treated orally with DBN or various doses of DBN (25, 50, 75, or 100 mg/kg) and killed 1 hr after treatment. In both experiments blood and organs were collected and stored frozen. At 0.5 hr after treatment a single oral dose of DBN caused a significant decrease in hepatic (54%) and gastric (62%) GSH concentrations. Hepatic GSH depletion was maximal at 0.5 hr and rebounded to control levels by 4 hrs. In contrast, gastric GSH concentrations remained depleted at all time points. DBN caused an insignificant decrease and rebound in both kidney and blood GSH. DBN significantly inhibits GSHT activity in liver, kidney and stomach. Hepatic GSHT inhibition was maximal (33% control) at 2 hrs and minimal (80% control) at 4 hrs while in kidney and stomach GSHT activity was inhibited at 1 hr (74% control) and remained low at all times after treatment. Both GSH depletion and GSHT inhibition were dose dependent. This study indicates that GSH and GSHT play an important role in the metabolism and detoxification of DBN in rats. (Supported by NTH Grant #01671)


Tertiary-butylicyclohexane (t-BC) exists in the chair conformation with the tertiary butyl group, due to its large steric requirement, permanently locked into the equatorial position. The uniqueness of this fixed configuration permits an evaluation of the stereochemical consequences of the metabolic oxidation of the cyclohexane ring. Likewise, a comparison of the amounts of ring substituted metabolites to alkyl side chain oxidation products would yield information as to the preferred sites of metabolism. Various branched chain and/or cyclic hydrocarbons with 8-12 carbons are known to induce hyaline droplet nephropathy involving proximal tubular epithelium in male rat kidneys. To determine how the tertiary butyl group would affect metabolic oxidation and induction of renal lesions, male F-344 rats were dosed by oral gavage with 0.25 g/kg t-BC every other day over 14 days. During the first 48 hour period, the rats were placed in metabolism cages and their urine was collected. Eight urinary metabolites were identified as carboxylic acids, alcohols and diols. Histopathology revealed multifocal, mild hyaline droplet nephropathy. The induction of renal lesions appears to be associated with oxidative metabolism, perhaps linked to formation of carboxylic acids.
Acetaminophen (AA) has previously been demonstrated to reversibly inhibit NAD-linked mitochondrial respiration while leaving succinate-linked respiration unaffected. Infusion of AA into isolated perfused livers from fed rats resulted in a stable, reversible, dose-dependent inhibition of oxygen uptake. Perfused livers from rats fasted for 48 hrs. or treated with diethylmaleate (1 ml/kg), treatments known to deplete cellular sulfhydryl groups, developed a secondary inhibition of hepatic oxygen uptake following infusion of 5 nM AA. The inhibition increased for 15 minutes to approximately 20% of the total hepatic oxygen uptake and then stabilized. Cessation of the AA infusion did not result in a return of oxygen uptake to basal levels. The alleged toxic AA metabolite N-acetyl-p-benzoquinone imine (NAPQI), 10 to 920 µM, has been demonstrated to nonspecifically inhibit NAD and succinate-linked mitochondrial respiration in vitro. This suggests that NAPQI formed in the endoplasmic reticulum is capable of reaching the mitochondria and inhibiting mitochondrial respiration contributing to the hepatotoxicity observed with acetaminophen. Supported by AA03548

A method for estimating the hepatic clearance for the formation of the chemically reactive metabolite of acetaminophen in vivo. R. Chen and J.R. Gillette. NHLBI, Bethesda, MD

A pharmacokinetic model has been devised to describe the formation of the GSH conjugate of acetaminophen after the administration of subtoxic doses of the drug (1.4 mmol/kg, s.c.) to male hamsters. This model assumes: a) the concentration of reactive metabolite in liver reaches a steady-state almost instantaneously due to its rapid reaction with GSH, b) the decrease in hepatic GSH after acetaminophen administration is due solely to the formation of the GSH conjugate and c) the formation of the GSH conjugate follows second order kinetics. Equations were written to describe the kinetics of acetaminophen in the body, the kinetics that govern the concentration of GSH within the liver, and the rate of formation of GSH conjugate. From measurements of the acetaminophen in blood plasma and the GSH in liver, we were able to calculate the rate of synthesis of GSH in liver, the fraction of the dose of acetaminophen that was converted to the GSH conjugate in liver and the clearance for the formation of the chemically reactive metabolite trapped as the GSH conjugate. The intrinsic clearance for the formation of the GSH conjugate by 9000g supernatant preparations of hamster livers was virtually identical to the estimate provided by the model.

The binding of reactive metabolites of xenobiotics to macromolecules is frequently implicated in the initiation and progression of the ensuing cellular perturbations. To evaluate the significance of such covalent binding, an affinity purified antibody was produced in rabbits against the widely used analgesic, acetaminophen (APAP). The antigen was constructed by sequentially linking diazotized p-amino benzoic acid (PABA) to either carbon ortho to the hydroxyl group of APAP and then attaching the free carboxyl group of PABA to amino groups on hemoglobin. In ELISA assays the antibody was capable of detecting 17 moles/well of N-acetyl benzoquinone imine derivatized proteins that have retained the N-acetyl moiety of the metabolite. Immunofluorescence microscopy localized drug-bound proteins in the centrilobular regions of livers within 2 h of APAP (600 mg/kg) administration to mice. Analysis of electrophoretically resolved proteins from APAP treated hepatocytes in culture demonstrated that the binding of APAP to proteins was highly selective. The antibody permitted the detection of a single 42-44 kD microsomal protein as the earliest acetaminophen bound macromolecule. This represents the first demonstration of the use of an antibody to detect intracellular drug-protein complexes. (N.I.H. GM31460).

We previously reported APAP-induced inhibition of mitochondrial respiration (ADP-stimulated) both in vitro and in vivo. To determine the relationship of this inhibition to APAP-induced hepatotoxicity, respiration of purified mitochondrial was assessed after APAP administration to control or piperonyl butoxide (PBO) pretreated mice or mice of different ages. One-hour pretreatment with the MFO inhibitor, PIP (400 mg/kg, ip), protected against mitochondrial respiratory inhibition observed 4 hours after challenge with APAP (600 mg/kg, po). An age-related susceptibility to respiratory inhibition by APAP was coincident with susceptibility to hepatotoxicity as assessed in our laboratory by histopathology and plasma sorbitol dehydrogenase activity, i.e. 3-4 month-old being more susceptible than 1-2 month-old mice. These data suggest that mitochondria may be critical targets of an MFO-generated APAP metabolite and may contribute to initiation and/or perpetuation of hepatotoxicity. (Supported in part by NIH grant GM-31460).
CONTINUED ELECTROPHILE PRODUCTION DURING ACETAMINOPHEN (APAP) HEPATOTOXICITY.
The Univ. of CT, Toxicology Prog., Storrs, CT

Non-protein sulfhydryl (NPSH) depletion continues beyond the period of maximal covalent binding during APAP hepatotoxicity. To determine if this could be due to continued production of electrophile, the effects on hepatotoxicity of MFO inhibition by Piperonyl Butoxide (Pip B) given either 1 hour (hr) before or 2 hr after APAP (600 mg/kg, p.o.) treatment were compared in fasted, male CD-1 mice. Pip B was given (600 mg/kg, i.p.) in corn oil vehicle. Plasma ascorbic dehydrogenase (SDH) and hepatic NPSH levels were determined at selected times (0 to 24 hrs) after APAP. One hr Pip B pre-treatment prevented APAP-induced elevations of SDH at 12 hr and NPSH depletion between 0.5 - 4 hr. Two hr post-treatment with Pip B diminished the APAP-induced elevations in SDH and depletion of NPSH at 8, 12, and 24 hrs. The biochemical evidence of Pip B protection was supported by histopathologic examinations. The reduction in APAP toxicity when Pip B was given 2 hr after APAP suggests that continued production of electrophile, after maximal covalent binding, is relevant to the progression of the lesion. (Supported in part by NIH Grant GM31460 and a Stauffer Chem. Co. Fellowship in Toxicology to JTB.)

DISSOCIATION OF COVALENT BONDING AND OXIDATIVE EFFECTS OF ACETAMINOPHEN (APAP) BY THE USE OF DIHIDRATATED ANALOGUES. R. Birge, J. Bartalone, M. Bruno, S. Cohen, J. Wang, and E.A. Khairallah. Univ. of CT, Storrs, CT

The reactive metabolite of APAP, N-acetylbenzoquinonemine, can contribute to hepatotoxicity by acting as an electrophile via the 3 and 5 positions of the aromatic ring to mediate covalent arylation to cellular thiols or as an oxidizing agent via the quinone moiety to stimulate oxidative stress. To distinguish the two pathways, we have compared the effects of APAP to the 3,5 and 2,6 dimethyl derivatives (DMA) on cultured hepatocytes. Only 2,6 DMA was nonhepatotoxic. Protein sulfhydrys were determined by homogenization in the presence of 3H-N-ethylmaleimide (NEM). By monitoring both 1H-C-AFAP and 3H-NEM binding, we observed a decrease in PSH coincident with the loss of glutathione (GSH) but preceding extensive APAP binding. Although both derivatives depleted free GSH, only the 3,5 DMA caused significant reduction in PSH. By monitoring PSH electrophoretically in the presence of 2-acrylamido-2-methylpropane (a fluorescent sulfhydryl probe), it was noted that on prolonged exposure to APAP or 3,5 DMA, all protein bands gradually lost sulfhydryl groups and protein aggregates were generated. Since the 3,5 DMA is believed not to be capable of arylation, it is concluded that the loss in PSH and the crosslinking observed with APAP may be due to a process other than covalent binding. (NIH GM31460)

EFFECT OF HEPATIC UDP-GLUCURONIC ACID (UDP-GA) DEPLETION ON THE DISPOSITION OF ACETAMINOPHEN (AA) IN RATS. Z. Gregus, Ch. Madhu, D. Goon and C.D. Klausen. Univ. of Kansas Medical Center, Kansas City, KS

The formation of AA-glucuronide (AG) is a major detoxication reaction for AA. Galactosamine (GAL) depletes UDP-GA from liver but has little effect on UDP-GA in other tissues. Therefore, the effect of GAL (600 mg/kg, ip) on the disposition of AA (0.25-2 mmol/kg, iv, 30 min after GAL) was quantitated in pentobarbital-anesthetized, bile duct-cannulated rats to determine the role of hepatic and extrahepatic glucuronidation of AA. GAL markedly diminished the biliary excretion of AA (80-90%) whereas blood levels and urinary excretion of AA decreased only 25-50%. This suggests that the AAG in bile represents predominantly AAG formed in liver, whereas AA in blood and urine is formed in both hepatic and extrahepatic tissues. GAL treatment had little influence on the fate of other AA metabolites. The blood half-life of AA in GAL-treated rats was unchanged at the lowest AA dosage (0.25 mmol/kg) but progressively increased at higher dosages (15, 40, and 71% at 0.5, 1 and 2 mmol/kg, respectively). This correlates with the dosage-dependent increase in fractional excretion of AA as AAG in control rats (6, 12, 21 and 33% of total AA excreted in 3 hr after 0.25, 0.5, 1 and 2 mmol/kg, respectively). These observations indicate that at higher dosages hepatic glucuronidation plays an increasingly significant role in AA elimination. (Supported by USPHS Grants ES-63192 and ES-07070)
BILARY EXCRETION OF ACETAMINOPHEN-GLUTATHIONE (AACGS) AS AN INDEX FOR TOXIC ACTIVATION OF ACETAMINOPHEN (AA). EFFECT OF CYTOCHROME P-450 INDUCERS AND INHIBITORS. Ch. Madhu, Z. Gregus and C.D. Klaassen. Univ. of Kansas Medical Center, Kansas City, KS

AA is converted, presumably by P-450, to an electrophile which conjugates with GS. AACGS is excreted into bile, therefore the biliary excretion rate of AACGS may reflect toxic activation of AA. In order to test this assumption, the effect of agents thought to affect activation of AA (i.e. P-450 inducers and inhibitors) on the biliary excretion of AACGS was studied in hamsters, the most sensitive species to AA hepatotoxicity. AACGS was the main AA metabolite (90%) in hamster bile. The dose-dependent biliary excretion of AACGS followed saturation kinetics with a linear increase up to 1 mmol/kg AA iv. Hepatic GS was lowered 1 hr after AA injection at dosages above 0.5 mmol/kg; at 2 mmol/kg a 56% decrease was noted. The P-450 inducers 3-methylcholantrene and phenobarbital increased (2.8 and 1.4-fold, respectively) the biliary excretion of AACGS, while ethanol and isoniazid did not affect it. Iodoacetamide decreased it (43%). Of the inhibitors tested (cimetidine, metyrapone α-naphthoflavone, piperonyl butoxide and SKF 525-A) only metyrapone diminished AACGS excretion. These observations suggest that activation of AA is mediated by specific P-450(s). Measuring biliary excretion rate of AACGS appears to be a useful method for identifying factors important in activation of AA in vivo. (Supported by USPHS Grant ES-03192)

SPECIES VARIATION IN BILIARY AND URINARY EXCRETION OF ACETAMINOPHEN (AA) METABOLITES. Z. Gregus. Ch. Madhu and C.D. Klaassen. Univ. of Kansas Medical Center, Kansas City, KS

AA is converted to a toxic electrophile which forms a glutathione conjugate (AA-GS). In addition to the toxification pathway metabolites (TM), which consist of AA-GS and its hydrolysis products (AA-cysteinylglycine, AA-cysteine and AA mercapturate), detoxification pathway metabolites (DM) such as AA glucuronide and AA-sulfate are also formed. In order to evaluate the role of these opposing pathways in the disposition of AA in different species, the biliary and urinary excretion of AA metabolites was measured for 2 hr after AA (1 mmol/kg, iv) administration of The TM/DM excretion ratio was 2.2, 1.0, 0.25, 0.1 and 0.08 for hamsters, mice, rabbits, rats and guinea pigs, respectively. TM were mainly excreted in bile and DM were primarily eliminated in urine. The composition of TM in bile reflected hepatic γ-glutamyltranspeptidase (GGT) activity: hamsters and mice (low GGT) excreted mainly AA-GS whereas bile from rabbits and guinea pigs (high GGT) contained significant amounts of AA-GS hydrolysis products. The TM/DM excretion ratios found in the species studied were inversely related to the hepatotoxic doses of AA reported for these species suggesting that sensitivity to AA-induced liver injury is determined by the balance of toxification and detoxication. The biliary excretion of AA-GS and its hydrolysis products may be used as an index for quantitating the activation of AA. (Supported by USPHS Grants ES-03192 and ES-07079)

EFFECTS OF BCNU ON ACETAMINOPHEN BIOTRANSFORMATION AND GLUTATHIONE-S-TRANSFERASE. Y.J. Park, R.D. Smith, A.D. Combs, and J.E. Kuhns. Division of Pharmacology/Toxicology, College of Pharmacy, University of Texas, Austin, TX

Acetaminophen (APAP) hepatotoxicity is produced by an electrophilic metabolite that can be detoxified by reduced glutathione (GSH). Glutathione reductase (GR), which maintains intracellular levels of GSH, is inhibited by the cytotoxic drug, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Therefore, the combined treatment of APAP and BCNU might be expected to result in increased hepatotoxicity. Previous in vivo studies have shown, however, that BCNU may provide partial protection from acute APAP hepatotoxicity as measured by total GSH content which is not as depleted as with APAP alone. Two possible explanations are 1) an inhibition of cytochrome P-450 (P450) mediated APAP metabolism to the toxic metabolite, or 2) the inhibition of GSH utilization in detoxication. Pasted, male BALB/c mice were given intraperitoneal injections of BCNU (50 mg/kg) followed by APAP (250 mg/kg) 4 hours later. Measurements of P450 activity and contents were performed 1.5 hours after APAP administration. In BCNU pretreated animals, neither P450 content, nor activity as measured by aniline hydroxylase and aminopyrine-N-demethylase were significantly different from those from corn-oil pretreated controls. Covalent binding of [14C]-APAP (250 mg/kg x 5.0 uCi) measured after BCNU pretreatment was not significantly different from controls not given BCNU. Glutathione-S-transferase (GST), is responsible for the conjugation of GSH with electrophiles to form mercapturic acids. GST activity was measured after BCNU pretreatment. This activity was significantly decreased at 1 hour and 1.5 hours in comparison to corn-oil pretreated mice. These data indicate that BCNU may act to decrease GSH utilization by the inhibition of GST.

INCREASED TOXICITY OF ACETAMINOPHEN IN THE PRESENCE OF METHOTREXATE. J. Sinclair, J. Linden-thal, S. Howell, T. Taylor, I. Cargill, P. Sinclair. VA Medical Ctr., White River Jct., VT; Dept. of Biochemistry, Dartmouth Med. School, Hanover, NH; Lederle Labs, Pearl River, NY. Sponsor: R. Costello

Abnormal liver histology and/or liver function tests have been detected in up to 20% of patients treated with methotrexate for both malignant and non-malignant diseases. We investigated the interaction of methotrexate with acetaminophen (APAP), a widely used analgesic of hepatotoxic potential. Using primary cultures of chicken embryo hepatocytes as our system of investigation, we found that methotrexate selectively increases the toxicity of APAP in cells containing elevated levels of the form(s) of P450 that convert acetaminophen into a toxic metabolite. Increased toxicity is associated with increased binding of radiolabeled acetami-nophen to protein, but thiol adduct formation is decreased. Glutathione has a crucial role in preventing the development of hepatotoxicity from acetaminophen. Methotrexate decreased the regeneration of glutathione after exposure to acetaminophen. Formation of gluta-thione requires ATP, and methotrexate may lower ATP levels via its inhibition of dihydrofolate reductase.
PHARMACOKINETICS EFFECTS OF ACUTE ADMINISTRA-
TION OF ACETAMINOPHEN (A) IN RATS PRETREATED
WITH STYRENE (S). P. Colin1, L. Regnault1, G. Stroli1 and S. Chakrabarti2, Faculté de
Pharmacie1 and Dept. Méd. Trav. Hyg. M12, Université de Montréal, Montréal, Canada.

To study the effects of a 3 week exposure (5
days a week) of a daily dose of 500 mg/kgDbw 
of S, followed by a zero or a single dose of 750mg/kg of A, on hepatorenal glutathione (GSH)
and pharmacokinetics of A and S, 11 groups of
4 male Sprague Dawley rats were treated as follows: 6 groups received daily either corn 
meal or S for 3 weeks and one dose of A at the
end of the period, and were killed 6, 8.5 or
11 h later; 3 groups were given S without the
dose of A, and 2 groups received the vehicles 
only and were killed at 6 and 11 h, respectively. 
S and A contents in liver and kidneys were determined at 6 and 11 h, and in 
serum at 6, 8.5 and 11 h. Depletion of GSH was decreased by pretreatment with S and A at
11 h, while hepatic A and S contents were
increased. Depletion of renal GSH was
decreased at 6 h, although A was increased. 
Serum elimination half-life of A was increased by
50% by S. The renal clearance of A was
decreased by S. The data show that S could modify the disposition kinetics of A.

(Supported by CAFIR and IRSSTQ)

INFLUENCE OF ASCORBIC ACID ESTERS ON METABOLISM 
OF ACETAMINOPHEN (AAP) IN RICE. A.K. Mitra and
V.C. Ravikumar. Division of Pharmacology and
Toxicology, School of Pharmacy, Northeast Louisi-
ana University, Monroe, LA. Sponsor: P.J. Medow

AAP is a widely used non-narcotic analgesic
agent. Hepatic centrilobular necrosis due to
AAP overdoses is thought to involve an electro-
philic reactive intermediate, produced by oxida-
tive biotransformation, and converted to an
hepatic glutathione (GSH) depletion. Ascorbic acid
palmitate (AP) and stearate (AS) have been shown to
significantly attenuate acute toxicity due to
AAP (The Toxicologist 6(1): 2, 1986). In the
present studies, co-administration of 600 mg/kg
each of AP/AS and AAP, p.o. to adult, male,
Swiss-Webster mice, prevented hepatic GSH deple-
tion. The 30-minute blood level of AAP given
with AS, but not with AP, was 2.4 times higher
than in mice receiving AAP alone. However, this
represents the peak level for the AS group while
AAP levels were still increasing for the AP
group. These data are further supported by the
greater reduction of rectal temperature after
yeast-induced pyrexia in mice given AS (4P) when
compared to mice given AP (2P). While the exact
mechanism by which ascorbic acid esters provide
protection is not evident, we suggest that AS and
AAP act by facilitating reduction of the reactive
intermediate back to the parent drug. The feas-
sibility of a safer oral dosage form of AAP with
the ascorbyl ester antiprotective incorporated in it
is currently being investigated.

QUALITATIVE AND QUANTITATIVE DIFFERENCES IN THE
METABOLISM OF BENZO(a)PYRENE (BP) BY THE RAT AND
HAMSTER. V.J. Wroblewski and J.R. Olson, Dept. 
Pharmacol. and Therap., SUNY Buffalo, NY

The increasing use of the hamster as a toxico-
logical model necessitates further characterization of
BP mediated metabolism in this species. In the rat, pretreatment with TCDD and 3-MC
double the rate of hepatic microsomal BP meta-
bolism (total polar metab./mole P-450) while pheno-
barbital (PB) had no effect. In contrast, BP me-
tabolism in the hamster was more than doubled by
PB but unaffected by TCDD and 3-MC. The induc-
tion of another cyt. P-448-mediated activity, 7-
ethoxyresorufin O-deethylase, correlated with the
induction of BP metabolism in the rat but not the 
hamster. Differences in the substrate specifici-
ty of P-450s in these species was also evident
from the lack of selectivity of metyrapone and 7, 
8-benzoflavone as inhibitors of BP metabolism in
the treated hamsters. HPLC indicated that 3-hydroxy-
BP was the predominant metabolite in control rat,
while control hamster formed mainly BP-4,5-diol.
TCDD pretreated rat formed elevated levels of all
BP-diols, dienes and phenols with the major meta-
bolite being BP-9,10-diol. In contrast, the
metabolite profile from TCDD pretreated hamster 
was the same as control. PB pretreated hamster
formed elevated levels of BP-diones and phenols
with the major metabolite being BP-4,5-diol,
while BP-9,10 and 7,8-diols were not detected.
The results indicate species differences in P-
450s and the formation of toxic BP metabolites.

(Supported by NHI grant ES02693)

THE METABOLISM AND DISPOSITION OF [14C]ETHYL
3-ETHOXYPROPIONATE IN THE RAT. P.J. Deisinger,
M.L. Lyon, R.J. Boatman, and D. Guest. Health 
and Environment Laboratories, Eastman Kodak Co.,

Ethyl 3-ethoxypropionate (EEP) is a water
soluble organic solvent with potential uses in
both consumer and industrial products. This
study was conducted to determine the metabolic
rate and disposition of EEP in rats. Male rats
were administered [14C]-EEP by oral gavage at dose 
levels of 150 mg/kg or 1500 mg/kg. Radioactivity 
was eliminated rapidly in the breath and urine 
after each dose of [14C]-EEP. About 50% and 65% of
the [14C] was excreted in the urine within
24 hours of dosing at the low and high dose
levels, respectively. About 34% and 20% of the
low and high dose, respectively was recovered as
[14C]CO2, mostly in the first 8 hours. Only small
amounts of radioactivity were recovered in the
feces and tissues, or as volatile organic 
materials at each dose level. The major urinary
metabolites of EEP were monoethyl malonate and
3-ethoxypropionate, with malonic acid and the
glycine conjugate of 3-ethoxypropionate detected
as minor metabolites. Trace amounts of [14C]-EEP 
were detected in the urine after both doses. No
evidence was found for the production of 
aldehydic acid metabolites, such as those
produced by the metabolism of some low molecular
weight ethylene glycol ethers.

117

The effect of dose and length of treatment on the metabolism of lindane in the rat was investigated. Weanling female Sprague-Dawley rats received Purina lab chow fortified with 0, 130, 215, or 350 ppm lindane. Six rats from each group were sacrificed after 1, 2, 4, 8, 16, and 24 weeks. 24 hr prior to sacrifice, all rats received a single, oral dose of lindane containing [U-14C]-lindane in peanut oil and were placed in metabolism cages. Urine and feces samples were taken for analysis of radioactivity. Liver and adipose tissue were analyzed by gas-liquid chromatography for lindane content. The enzyme activity involved in the dehydrogenation of lindane and the hydroxylation of the intermediate, hexachlorocyclohexene, were determined in vitro. The effect of dose and length of treatment on the excretion and distribution of radioactivity were examined. Results of the study suggest that the metabolism and excretion of lindane in the rat is a saturable, dose-dependent or non-linear process. The results also suggest that there is an age effect on the metabolism and excretion of lindane in the rat. This is an abstract for a presentation and does not necessarily reflect EPA policy.


The metabolic fate of [U-14C-phenyl]-phenyl-3-pyrazolidinone ([14C]phenidone) was studied in male Sprague-Dawley rats. Animals were given a single oral dose (40 or 120 mg/kg) of the test compound, and urine, feces, and expired air were collected for 48 hr. Forty-eight hr after treatment with the test compound, the animals were euthanized and tissue specimens were obtained. The metabolic disposition of [14C]phenidone was determined by liquid scintillation spectrometric analysis of samples of the tissues, carcass and excreta. Fecal extracts and urine were analyzed by HPLC and MS for the identification of metabolites of [14C]phenidone. Greater than 99% of the 14C administered was excreted by 48 hr, primarily (95%) in the urine at both dose levels. The chromatographic profile of the urine showed five radioactive components. The major metabolite of phenidone in the urine was a sulfuric acid conjugate of 1-phenyl-3-hydroxyprazoles. Also found in the urine were hydroxylated 1-phenyl-3-hydroxyprazole and its disulfuric acid conjugate, 1-phenyl-3-hydroxyprazole, N-acetylphenylhydrazine, and unmetabolized phenidone. The results indicate that phenidone is rapidly excreted, primarily as sulfuric acid conjugated metabolites, after oral administration.
The objective of this study was to determine if the age-related decrease in the glucoroni- dation of TBBG, a major ruber antioxidant, would result in an enhanced toxic response. Male F344 rats, ages 2.5, 16 and 26 mos were exposed to 0.25% TBBG in their diet for 14 days. The toxicity of TBBG was evaluated by measuring various serum and urinary enzyme activities along with changes in histopathology. At the end of the exposure period, bile ducts were cannulated and 14C-TBBG (5 mg/kg) was administered iv. Total radioactivity excreted and the metabolic profile in bile was evaluated. These results showed that the ability to excrete TBBG-derived radioactivity decreased with age. Only minor changes in the clinical chemistry values were observed in treated animals. TBBG exposure did not result in any significant hematological changes. However, there appeared to be an increase in the severity of leukemia in the treated 26 month animals. Various clinical chemistry values were elevated in the animals with leukemia compared to the nonleukemic rats. Even though toxicity did not seem to be enhanced in the older animals, the observed alterations in the ability of older rats to eliminate TBBG may result in an increased sensitivity at higher doses or after longer exposure periods.

Dinitrobenzenes (DNB), intermediates in dye and plastics production, induce methemoglobinemia, and m-DNB is a testicular toxicant in rats. The metabolism and excretion of radiolabeled m- and p-DNB were studied in male Fisher-344 rats. Urine and feces were collected over dry ice for 48 hours following a single oral dose (0.15 mmol/kg) of 14C-DNB or 14C-p-DNB. Radiolabel elimination in urine was rapid, 60% or 75% of dose excreted 24 hours after m- or p-DNB, respectively. The metabolites of m-DNB, m-aminoacetanilide (42% of dose), p-hydroxyacetanilide, excreted as a sulfite (6%), 1,3-di-(N-acetylaminobenzene (7%) and the glucuronide of m-nitroaniline (4%) indicate that reduction is the major route of metabolism. In contrast, p-DNB is mainly metabolized through glutathione conjugation, with S-(2-nitrophenyl)-N-acetylcysteine as the major metabolite (The Toxicologist, 6, 253, 1986). p-DNB metabolism has similarities to both m- and m-DNB in that to two of its three major metabolites, S-2-nitroso-5-nitrophenol (35%) and 1,4-di-(N-acetylamino)benzene (15%) were excreted rapidly, while the third, S-(4-nitrophenyl)-N-acetylcysteine (13%) indicates a glutathione conjugation pathway of metabolism. These results suggest that two major pathways of DNB metabolism exist, reduction and conjugation with glutathione. The relative importance of each varies with the isomer.
DISTRIBUTION AND EXCRETION OF 3,7-DIMETHYL-2,6-Octadecenal (Citral) in Rats. G. Usha, J. Diliberto and L.S. Birnam, NIAMS, Research Triangle Park, NC

3,7-Dimethyl-2,6-octadecenal (citral) is an oxygenated monoterpene and an important constituent of many essential oils. It is present in cosmetics and detergents. Widespread human exposure to citral has created a need for a disposition study. Male P344 rats were created iv with 14C-citral at 5 mg/kg and held in individual metabolism cages for 3 days. Urine, feces, volatiles, and expired air were collected for 72 hours. Approximately 60% of the administered dose was excreted in the urine, 7% in feces, 8% via the lungs as 14CO2, and less than 1% was exhaled as unmetabolized citral. Six percent remains in the carcass with the major depots being muscle, skin, liver, fat and blood. Rates were also treated dermally with 5 and 50 mg/kg 14C-citral and the disposition examined after 3 days. Citral was poorly absorbed after dermal exposure. Absorption and elimination were linear with dose. The major tissue depots were similar to that seen after iv exposure, however, feces was the most important route of excretion in the dermal studies, followed by urine and exhalation. The production of 14CO2 suggests that oxidative decarboxylation also occurred after skin application of citral. Thus, the rapid elimination of citral-derived radioactivity from the body suggests that repeated exposure would not lead to bioaccumulation.


Nonoxylnol-9 (N-9) labeled with (14C) at the ethylene oxide (EO) units were used to study the disposition and metabolism of this spermicide after vaginal administration in barren rats. Results indicated 13% absorption of radioactivity in 6.0h and a maximum of 40% in 24h. The small and large intestines were the organs of the highest 14C-activity. The absorbed radioactivity was rapidly cleared from the systemic circulation as shown from iv experiments. The bile was seen to be the major route of excretion accounting for 50% of an i.v. dose and 4.5% of a vaginal dose in 6.0h. Urine data following an i.v. dose indicated that 25% of the administered radioactivity was excreted in 6.0h. The overall absorption of (14C)N-9 radioactivity from the vaginal vault appears to be the rate limiting step in the disposition of this spermicide. Preliminary studies show that no intact N-9 is excreted in the urine. Two (14) labeled metabolites could be detected by ion exchange columns, representing acidic and neutral components in a ratio 5:4. The data indicates the cleavage of the aryl ether linkage in the molecule.


Major metabolites of SC-1084 (Methyl-3-hydroxy-4-[4-(5'-trifluoromethyl-2'-pyridyl)oxy]phenoxypentanoate) were identified in the excreta, egg yolk, and liver of laying hens dosed with encapsulated [U-14C]-phenyl ring labeled SC-1084 for 4 days at 100 ppm. Of the total residues recovered (81% of the dose) over 98% was in the excreta, with approximately 0.05% in the egg yolk and the liver. Approximately 66% of chicken excreta was identified as 2-[4-(5'-trifluoromethyl-2'-pyridyloxy)phenoxy]propionic acid (C-3 Acid). Other major excreta metabolites included 2-(4-hydroxyphenoxyp)ionic acid (15% of excreta 14C) and 4-(5'-trifluoromethyl-2'-pyridyloxy)phenol (PHENOL -3% of excreta 14C). Nearly 90% of the recovered 14C-residue in egg yolk was accounted for by three metabolites. These included the C-3 Acid (37%), PHENOL (16%), and 3-hydroxy-4-[4-(5'-trifluoromethyl-2'-pyridyloxy)phenoxypentanoic acid (C-5 Acid - 37%). These three metabolites also represented 94% of the radioactivity isolated from chicken liver. In this tissue, the C-3 Acid accounted for 6%, the C-5 Acid 56%, and the PHENOL 33% of recovered 14C-residues. These results suggest that SC-1084 is extensively metabolized by the chicken via hydrolysis and oxidation prior to elimination in the excreta.

DISTRIBUTION OF TRANS-4-HYDROXY-2-HEXENAL AND TANDEM MASS SPECTROMETRIC DETECTION OF ITS URINARY MERCAPTURIC ACID IN THE RAT. C.K. Winter, H.J. Segall, and A.D. Jones, Veterinary Medicine Pharmacology and Toxicology and Facility for Advanced Instrumentation, University of California, Davis, CA 95616.

The distribution of trans-4-hydroxy-2-hexenal (t-4HH), a product of Tlpid peroxidation and a pyrrolizidine alkaloid metabolite, was studied following injection of tritiated t-4HH into the hepatic portal vein of male Sprague-Dawley rats. Twenty-four hours after administration, less than 2% of the tritium label remained in the liver with levels in the other major organs correspondingly lower. The majority of recovered radioactive (77-83%) appeared in the urine with 60-67% appearing within 8 hours following administration. A tandem mass spectrometry technique using negative ion fast atom bombardment (FAB) mass spectrometry in combination with mass-analyzed ion kinetic energy spectrometry (MIKES) verified that a C-3 mercapturic acid conjugate of t-4HH was produced as a urinary metabolite. This compound presumably forms via a Michael addition of glutathione at C-3 of t-4HH followed by acetylation to the mercapturic acid. While Michael additions of glutathione to t-4HH-related compounds have been observed in vitro, these results provide in vivo evidence of this mechanism.
URINARY METABOLITES OF N-NITROSOThIAZOL- LIDINE (N-NT). D. Cragin and T. Shibamoto, Department of Environmental Toxicology, U.C. at Davis, Davis, CA. Sponsor: L. Shull

N-NT is a reportedly mutagenic compound recently discovered to be in many smoked meats. Little is known about the metabolism of N-NT in vivo. Therefore, in order to more fully characterize its metabolic activity, in vivo work was initiated with 2-14C-N-NT. The 2-14C-N-NT was synthesized from 14C-formaldehyde, cysteamine hydrochloride, and sodium nitrite. Rats, gavaged with 2-14C-N-NT, excreted 75% of the total dose in the urine within the first 16 hrs. An additional 4% of the dose was recovered from the urine during the following 56 hrs. An ammonium carbonate treated aliquot of the urine from the first 16 hrs was extracted with ethyl acetate and 80% of the 14C was recovered in the nonacidic fraction. Initial analysis of the nonacidic fraction by GC-MS indicated the presence of 2 metabolites, thiazolidin-2-one and 5-hydroxy-nitrosothiazolidine. Metabolism of N-NT is, therefore, similar to that of N-Nitrosopyrrolidine.


Carbaryl (1-naphthyl-N-methyl carbamate) is one of the most widely used insecticides throughout the world. It is metabolized by cleavage of the amide bond and by oxidation processes to form metabolites which may possess anticholinesterase activity. The acute oral LD50 of carbaryl was approximately 250 mg/kg and 80 mg/kg for the rat and gerbil, respectively. While the gerbil is nearly three times more susceptible to carbaryl poisoning than the rat, preliminary findings indicate no difference in cholinesterase inhibition.

Studies were conducted to determine why the gerbil is more susceptible to the rat to poisoning by the insecticide carbaryl. The metabolism and routes and rates of excretion of carbaryl in the rat and gerbil were investigated.

EFFECT OF INTERACTION BETWEEN CARBAMAZEPINE AND STYRENE ON THEIR HEPATOTOXICITY AND METABOLISM IN MICE. E. Regnault, S. Girard, and S. Cabrera, Fac. de Pharmacie and Dep't Méd. Travaux Rég. Styl, Fac. de Méd., Univ. de Montréal, Montréal, Canada.

The interaction between carbamazepine (CEZ) and styrene (S) was studied in male Sprague-Dawley rats. The control group received 10 mg/kg of the aqueous vehicle of CEZ (NaCl 0.9%) and cytoxan cellulose 0.5%) or 2 ml/kg of the city vehicle of S (corn oil) twice a day (at 8:30 AM and 5:00 PM) for 4 days. The second and the fourth groups received intraperitoneal (i.p.) administration of 30 mg/kg of an aqueous suspension of CEZ, while the others received the vehicle of CEZ. One hour after the first dose of CEZ, on the fourth day, the third and the fourth groups received i.p. administration of 800 mg/kg of S while the others received the vehicle of S. The urines were collected for 24 h on the fifth day and the animals were then sacrificed. The contents of cytochrome P-450 and the activities of epoxide hydratase and glutathione S-transferase were not increased due to interaction between CEZ and S while those of aniline hydroxylase and aniline N-demethylase did. Thus, mixed exposure favored the formation of more reactive epoxide rather than their detoxication through hydration. Accordingly, the activities of SDH and SGPT were found to synergistically increased due to such mixed interaction, resulting in potentiation of hepatotoxicity due to S. Metabolite studies indicated that S induced more urinary excretion of metabolites of CEZ.


Male rats were dosed with either 25 (low dose) or 125 µg/kg (high dose) TCDD in 5 ml/kg corn oil, whereas corresponding ad libitum and pair-fed controls received corn oil alone. At 1, 2, 4, 8, 16 and 32 days after dosing, 5 rats from each of the 3 low and high dose groups were decapitated and heparinized blood and thymus procured. Corticosterone was determined in plasma by radioimmunoassay. In both dose groups the corticosterone levels of TCDD-treated rats were higher than control levels by day 4, but due to high biological variability the results were not statistically significant until day 32 when corticosterone levels were 2-3 times higher than controls in the low dose and 6-10 times higher in the high dose group. In both dose groups, thymic weights decreased by day 4 and remained low throughout the remainder of the study. In TCDD-treated and pair-fed groups the relative thymus weights decreased to the same extent. This decrease was inversely proportional to the increase in corticosterone levels. Corticosteroids are known to cause thymic atrophy suggesting that thymic atrophy may be due to increasing corticosterone levels, which in turn could be due to reduced feed intake.

180 male Sprague-Dawley rats were divided into 2 groups. Each group consisted of 30 ad libitum-fed, 30 TCDD-treated, and 30 pair-fed rats. Treated rats received i.p. ether 25 (low dose) or 125 ug/kg (high dose) TCDD in 5 ml/kg corn oil, whereas controls received corn oil alone. At 1, 2, 4, 8, 16 and 32 days after dosage, 5 rats from each of the 3 low and high dose groups were decapitated and both whole and heparinized blood collected. Glucagon was determined in plasma by radioimmunoassay. Free thyroxine (FT₄), total thyroxine (TT₄), free triiodothyro- nine (FT₃), total triiodothyronine (TT₃), reverse triiodothyronine (rT₃), thyroid stimulating hormone (TSH) and insulin were measured in serum using radioimmunoassays. In both dose groups, TCDD treatment had the following effects: FT₄ decreased, FT₃ and insulin; increased glucagon; mixed effects upon rT₃; and no effect on FT₃, TT₃ and TSH. Pair-feeding had the following effects: decreased TT₃; increased glucagon; mixed effects on TSH; and no effect on FT₄, FT₃, TT₃, insulin and rT₃. The endocrine response of TCDD-treated rats to starvation is different from that of pair-fed rats.

HISTOLOGIC EVALUATION OF THE EFFECTS OF DIETARY TRIIODOTHYRONINE (T₃) AND SURGICAL THYROIDECTO- MY ON 2,3,7,8-TETRACHLORIDIBENZO-P-DIOXIN (TCDD) INDUCED LESIONS IN MALE SPRAGUE-DAWLEY RATS. R.A. Leedle, L.C. Gilbert, R.H. Powers, W.L. Roth and S.D. Aust. Departments of Pathology and Biochemistry, Michigan State University, E. Lansing, MI

Liver, thymus, thyroid, and spleen from normal, hyperthyroid (T₃ in diet, 500 µg/kg), and hypo- thyroid (thyroidectomized) rats given a single dose of TCDD (25 µg/kg p.o.) were examined histologically. At 12 days after treatment, hepatocytes from TCDD treated normal and hyperthyroid rats were enlarged with large vesicular nuclei. Centrolobular and midzonal hepatocytes contained large fat vacuoles. Thyroid hormone was required for, and dietary T₃ exacerbated TCDD induced hepatic fat deposition. TCDD treated thyroidectomized rats had hepatic glycogen deposition similar to controls. All other groups had no visible hepatic glycogen. TCDD caused thymic lymphocyte depletion in normal (-60%), hyperthyroid (-80%) and hypothyroid rats (-100%). Rats given TCDD had active appearing thyroid glands with tall follicular epithelium and pale staining colloid. Several ultimobranchial follicles had undergone keratinization with desquamation into a central lumen. Thyroid glands from T₃ treated rats appeared less active regardless of TCDD treatment. Supported by NIH grants ES7146 and ES3585.

EFFECT OF THYROIDECTOMY ON 2,3,7,8-TETRA- CHLORIDIBENZO-P-DIOXIN (TCDD) INDUCED LIPID PEROXIDATION, S.J. Hermansky, S.J. Stobbs and N.A. Mohamadpour, Dept. of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE.

Female rats were treated with methimazole (MTZ, 0.50 mg/kg) or propylthiouracil (PTU, 5.0 mg/kg) IP for 10 days. Control animals received the vehicle. Other animals were sham-operated or surgically thyroidectomized (TDX) and maintained for 30 days. Half of each group were treated with TCDD (150 µg/kg) orally or the corn oil vehicle 6 days prior to sacrifice. MTZ, PTU and TDX decreased thyroxine (T₄) content of plasma. TCDD alone decreased T₄ levels and similar effects were produced by TCDD in MTZ and PTU treated animals. TCDD administration resulted in a 9% increase in plasma T₃-3. Neither anti-thyroid drug or TDX prevented TCDD-induced weight loss. TCDD administration resulted in a 300% increase in hepatic malondialdehyde (MDA) content and 60% decrease in glutathione peroxidase (GSHPxn) activity. TCDD increased hepatic MDA content by 330% in sham-operated rats and 220% in TDX rats. Neither anti-thyroid affected the TCDD-induced alterations in MDA content or GSHPxn activity, while in TDX rats the effects of TCDD were less pronounced. Hypothyroidism has a limited protective effect on TCDD induced lipid peroxidation. The results do not preclude the possibility that TCDD acts as a thyroid agonist.


Previous studies have shown that rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exhibit symptoms of hyperthyroidism and vitamin A deficiency, including depressed hepatic and elevated renal retinyl ester levels. We observed that administration of T₃ (500 µg/kg diet) in male Sprague-Dawley rats for 12 days caused a slight (<10%) decrease in total hepatic retinyl palmitate levels when compared to rats fed a control diet. Treatment of rats with a single dose of TCDD (25 µg/kg, p.o.) also decreased hepatic retinyl palmitate (30%) over the same period, and the combination of TCDD treatment and dietary T₃ caused the most severe depletion (38%). Renal accumulation of retinoids, characteristic of vitamin A deple- tion, was most severe in the animals treated with both TCDD and T₃, reaching concentrations 10.2x that of controls. TCDD treatment alone caused accumulation to 5.2x control levels, and dietary T₃ alone to 2.8x control values. The exacerbation of TCDD-caused vitamin A depletion by T₃ suggests that the changes in thyroid hor- mone status caused by TCDD may be linked to the mechanistic basis for the vitamin A depletion observed. Supported by NIH grant ES3585.
THYROID HORMONES AND THYROXINE GLUCURONIDATION IN HAMSTERS TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD). E.C. Henry and T.A. Gasiewicz. Division of Toxicology, University of Rochester, Rochester, NY

TCDD and related compounds cause a depression of serum thyroxine (T4) in rats. In contrast, hamsters, which are relatively insensitive to TCDD-induced lethality, showed a dose-dependent increase in serum T4 and triiodothyronine (T3) to a maximum level of 200% of control (ED50 = 10 μg/kg). In hamsters receiving 100 μg TCDD/kg (i.p.), serum T4 was higher than in pair-fed and ad libitum controls throughout the 52-day experiment. Serum T3 and TSH (thyroid-stimulating hormone) were also elevated, but recovered more quickly than T4. Reverse T3, like T4, was increased by TCDD in hamsters but was decreased in rats. Hepatic microsomal UDP-glucuronosyltransferase activity using T4 as substrate (T4-GT) was induced in hamsters by TCDD to about 175% of control on a whole liver basis. In rats, absolute T4-GT activity was 3-4 fold higher than in hamsters, but it was induced by TCDD by the same proportion in both species. The similarity in T4-GT inducibility by TCDD suggests that there are similar mechanisms in addition to T4-GT induction which account for the species-specific alterations in T4. Whereas many biochemical responses to TCDD in hamsters are comparable to those in rats, the hamster appears to be unique in its increased thyroid hormone levels. An understanding of the mechanism of this species difference may be helpful in unravelling the species dependence in susceptibility to TCDD.

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THE EFFECT OF DIETARY TRIOiodothyronine (T3) ON 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)-INDUCED TOXICITY IN RATS. L.C. Gilbert, M.A. Jurk, R.H. Powers, W.L. Roth and S.D. Auat. Dept. of Biochemistry, Michigan State University, E. Lansing, MI

Several studies have demonstrated that TCDD affects the thyroid hormone status of rats; these animals demonstrate symptoms characteristic of hypo- and hyper-thyroidism. Administration of T3 (500 μg/kg-diet) to male Sprague-Dawley rats for 12 days exacerbated the depression of growth rate and feed efficiency, hepatic hypertrophy, and depression of plasma T4 levels, caused by a single oral dose of TCDD (25 μg/kg). Plasma T3 concentration in TCDD-treated rats was 55% that of control rats. Treatment of rats with either T3 or TCDD produced significantly elevated levels of hepatic malic enzyme activity compared to control rats (5.2- and 3.6-fold, respectively), and the combination of TCDD and T3 produced the largest elevation of malic enzyme activity observed (15.4 fold). Dietary T3 did not exacerbate the TCDD-caused lymphoid involution as measured by the thymic weight/body weight ratio, nor did TCDD treatment exacerbate the T3-dependent renal hypertrophy observed. Supported by NIH grant ES05955


Male Sprague-Dawley rats (207 ± 2.2) were housed in wire bottom cages and adapted for 4 weeks to 4 ± 1°C. Thereafter 3 groups were formed and for 2 weeks, fed either a high fat (HF), a high carbohydrate (HC) or a high protein (HP) diet (Taklad, Madison, WI). Six rats in each group were then injected i.p. with 125 μg/kg TCDD, pair-fed controls matched by body weight received i.p. corn oil only. Body weight, feed intake and survival time were monitored daily. All 3 TCDD-treated groups of rats progressively reduced their caloric intake after TCDD dosage. In comparison to the same pretreatment interval (10 days), HC rats reduced by 25% their caloric intake whereas both HF and HP rats consumed only 15% less Kcal/MBS. TCDD-treated rats fed a high fat diet lost body weight more rapidly than their PF controls (P < 0.05) but mean time to 50% mortality and mean time to death were significantly (P < 0.05) lower in rats fed a HC diet than in the other two groups. These findings suggest that TCDD-treated cold-adapted rats can utilize glucose as a substrate of energy metabolism more readily than fat or proteins. The underlying metabolic change may contribute to the development of the wasting syndrome.


Two groups of 3 to 5 male Sprague-Dawley rats (200-290) received TCDD ip (125 μg/kg) in corn oil whereas pair-fed controls were given vehicle alone. 1, 2, 4, and 8 days after treatment, a small temperature probe was introduced into the interscapular brown adipose tissue (BAT) under urethane anesthesia. Norepinephrine (NE) was infused for 60 min through the femoral vein at a rate of 480 ng/min. Rectal and BAT temperatures were recorded. In pair-fed controls, this NE infusion increased BAT temperature by 1.67 ± 0.25°C. In TCDD-treated animals, the temperature response decreased with time, reaching 0.20 ± 0.09°C at 8 days (p < 0.05) after dosing. Two other groups of animals were treated exactly as above. BATs were removed at 1, 2, 6, and 8 days after treatment and mitochondria isolated. GDP binding was measured as an estimate of the thermogenic capacity of BAT. There were no significant differences in the GDP binding to mitochondria of pair-fed and TCDD-treated rats (207 ± 13 vs. 214 ± 27 pmol/g) at 8 days. These results indicate that TCDD decreased the thermogenic response of the BAT to NE and that this effect was not caused by a decreased availability of uncoupling protein.

123
Dietary iron, and TCDD-induced alterations in hepatic lipid peroxidation and glutathione content in rats, M.A. Shara, W.A. Al-Turk, H.A. Mohamadpour and S.J. Stohs, Dept. of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE.

The role of ferrous iron (Fe) in TCDD-induced lipid peroxidation (LP) was investigated. The effects of diets containing deficient (6 ppm), normal (35 ppm) and supplemented (120 ppm) Fe for 17, 24 and 31 days, on hepatic LP (malondialdehyde content), reduced glutathione (GSH), GSH peroxidase (FX) activity, and liver and body weights of female rats following TCDD administration were examined. Animals were treated with 40 μg TCDD/kg/day P.O. in corn oil or the vehicle on days 9, 8 and 7 prior to sacrifice. TCDD produced 3-fold increases in hepatic LP in animals on normal and Fe supplemented diets, but no increase in LP in Fe deficient animals. Dietary Fe had no effect on hepatic GSH-FX activity. Normal and Fe supplemented diets had no effect on hepatic GSH content while the Fe deficient diet resulted in 11-21% decreases in GSH content. TCDD administration produced 15-22% decreases in hepatic GSH content in animals on the control and Fe supplemented diets. No differences in body or liver weights were observed in animals on the 3 diets. TCDD treatment resulted in decreases in body weights of animals on all 3 diets. TCDD induced LP appears to be Fe dependent. However, the loss in body weight due to TCDD toxicity may not be dependent on lipid peroxidation.

TCDD produces hydromephrosis in fetal mice by inducing hyperplasia of the ureteric epithelium, B.D. Abbott, L.S. Birnbaum, and R.M. Pratt, NIEMS, Research Triangle Park, NC.

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a potent kidney teratogen. Teratology studies have not revealed the etiology of this condition. Examination of the urinary system during early mouse development shows a specific TCDD-induced alteration. Pregnant C57BL/6J female mice received a single dose of 0 or 12 μg/kg TCDD/kg by gavage on day 10 of pregnancy. Fetal urinary systems were examined on days 14, 15, 16, and 17. Dye was injected into the lumen of the bladder and the patency and configuration of the ureteric lumen were observed. TCDD treatment did not delay or prevent breakdown of the ureteric membrane which occurred between days 15 and 16 in the control. The ureteric lumina of TCDD-exposed fetuses were narrow, tortuous, and urine flow appeared to be obstructed. Sections of ureter were observed by light microscopy, and the lumina of TCDD-exposed ureters were observed to be filled with epithelial cells. As a result of hyperplasia of the ureteric luminal epithelium, hydroureter and hydromephrosis became pronounced by day 17. We conclude that the kidney abnormality induced by TCDD is true hydromephrosis, an accumulation of urine in the kidney due to obstructed outflow.

TCDD effects the basal lamina and extracellular matrix of the fetal mouse kidney, B.D. Abbott, L.S. Birnbaum, and R.M. Pratt, NIEMS, Research Triangle Park, NC.

TCDD is teratogenic in mice, producing hydronephrosis even at low levels of exposure. The effects of TCDD on the differentiating Bowman's capsule and proximal tubule of the metanephric kidney are examined. C57BL/6J female mice, given 12μg/kg TCDD, p.o., on gestation day 10 were killed on days 14, 15, and 16. Fetal kidneys were prepared for immunofluorescent localization of fibronectin and laminin. Treated and control kidneys showed the same pattern of staining for fibronectin, but TCDD-treated kidneys displayed diminished overall intensity. Deviations in the pattern of antibody binding to laminin were detected for differentiating TCDD-treated nephrons and the intensity of the fluorescence also decreased. Laminin antibody binding to the basal lamina was decreased in the parietal layer of Bowman's capsules. Analysis by TEM focused on the basal lamina of the proximal tubule and Bowman's capsule and on the abundance of extracellular matrix proteins (ECM) adjacent to the lamina. In TCDD-exposed kidneys, ECM components were less abundant, and the basal lamina of the developing Bowman's capsule had a diffuse and discontinuous lamina densa. These ultrastructural changes may alter the filtration properties of the basal lamina and contribute to excess urine production, thus increasing the severity of hydronephrosis.

Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin: Antagonism by Aroclor 1254; J.M. Haake, F. Phillips and S. Safe, Department of Veterinary Physiology and Pharmacology and Veterinary Public Health, Texas A&M University, College Station, TX.

The dose-response teratogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were determined in the C57BL/6J mouse. Administration of a single dose of 2,3,7,8-TCDD (20 μg/kg) in corn oil to the pregnant female on day 10 resulted in 70% cleft palate and 85% hydronephrosis in the fetuses. In contrast, administration of the commercial polychlorinated biphenyl, Aroclor 1254 (75 μmol/kg), to the pregnant mice did not result in any cleft palate or hydronephrosis in the fetuses. Cotreatment of the pregnant C57BL/6J mice with Aroclor 1254 (75 μmol/kg) and 2,3,7,8-TCDD (20 μg/kg) resulted in only 8% fetal cleft palate and was significantly lower than the 70% fetal cleft palate observed using 2,3,7,8-TCDD alone. These results are comparable to other studies in our laboratory which demonstrate that Aroclor 1254 antagonizes receptor-mediated responses to 2,3,7,8-TCDD. (Sponsored by N.I.H. ES03554.)
IMMUNOTOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN: ANTAGONISM BY AROCLOR 1254. D. Davis, I. Tizard and S. Safe, Departments of Veterinary Physiology and Pharmacology and Veterinary Microbiology and Immunology, Texas A&M University, College Station, TX.

The immunotoxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were determined in the C57BL/6J mouse using the splenic direct antibody plaque forming cell (PF) response after immunization with sheep erythrocytes (SRBC). Administration of 2,3,7,8-TCDD (1.2 mg/kg) resulted in a 74% decrease in the number of anti-SRBC antibody forming cells per spleen; administration of the commercial polychlorinated biphenyl, Aroclor 1254 at dose levels of 4.8 and 24 mg/kg decreased antibody production 28 and 47%, respectively. Co-administration of 2,3,7,8-TCDD (1.2 mg/kg) and Aroclor 1254 (4.8 and 24 mg/kg) resulted in a 61 and 5% decrease in splenic antibody production. These results demonstrate that Aroclor 1254 acts as a partial antagonist for the receptor-mediated immunotoxic response of 2,3,7,8-TCDD in the mouse and is consistent with other studies in our laboratory which demonstrate the activity of Aroclor 1254 as a 2,3,7,8-TCDD antagonist. (Supported by N.I.H. ES03554)


We examined the potential mechanisms involved in TCDD's ability to induce endotoxin (EN) hypersensitivity. Acute doses of 50, 100, or 200 ug/kg TCDD clearly induced an EN hypersensitive state in B6C3F1 mice as shown by increased mortality following i.v. injection of EN (E. coli, LPS). This finding was observed 7 days post TCDD exposure, but not 1 day post TCDD exposure. No correlation was observed between TCDD dosing alone and serum EN levels. However, clearance of injected EN was significantly inhibited following dosing with TCDD. Methylprednisolone antagonized the mortality associated with combined TCDD/EN exposures. Serum triglycerides were increased 2-fold in TCDD/EN mice compared to either treatment alone, suggesting synergistic detrimental hepatic effects. Studies performed on congenic mice indicated that the observed effects do not segregate with the Ah locus. In summary, while TCDD alone does not increase serum EN, it does prolong the clearance of injected EN. Methylprednisolone's reversal of the mortality associated with TCDD/EN treatment is consistent with inflammatory involvement in death. Thus, changes in hepatic handling of EN, caused by progressive TCDD induced hepatic dysfunction, may be responsible for the EN hyper-sensitivity.


TCDD and estradiol (E2) were studied in three strains of mice: CD-1 and C57B/6 (TCDD-resistant), and DBA/2 (TCDD-resistant). Immature females were treated with 0 to 2000 ng/kg/day E2 daily for 2 weeks, sc. On days 7, 9, 11, and 13, mice received 100 ug TCDD/kg by gavage. Relative uterine weight increased in mice of all three strains treated with E2 alone. Uterine inhibition was suppressed by TCDD treatment. However, this negative effect of TCDD was antagonized in a saturable dose response manner by E2. TCDD induced aryl hydrocarbon hydroxylase in liver microsomes of treated mice independent of E2 dose and strain of mouse. Only the CD-1 mice treated with TCDD and E2 showed a decrease in an electrophoretic band migrating with cytochrome P450a and epoxide hydrolase. These results suggest that the interactions of TCDD with E2 may be at the receptor level, rather than involving only increased E2 metabolism in the liver. Supported in part by USEPA (EC8312114-01-1).

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been reported to produce testicular hypoplasia and impairment of spermatogenesis in laboratory animals. Decreases in the biosynthesis of and amount of testicular heme, as well as decreases in the concentrations of both testicular microsomal cytochrome P-450 and serum testosterone have also been demonstrated after TCDD exposure. This study was undertaken to examine the concentration of intratesticular steroids in rats treated with TCDD. Male, Sprague-Dawley rats (220–240 g) received a single, oral dose of TCDD (25 µg/kg) on day 0. Concentrations of testicular steroids were determined by high performance liquid chromatography. Testicular testosterone concentrations were significantly decreased 3 and 7 days after administration of TCDD. There was no effect on testicular progesterone concentrations 3 days after TCDD treatment. However, testicular progesterone concentrations were significantly higher than control 7 days following treatment with TCDD. These results are supportive of earlier findings in this laboratory of decreased rat testicular 17- hydroxylase activity after TCDD treatment. (Supported by NIH Grant ES-02423)

INHIBITION OF TESTOSTERONE PRODUCTION IN TESTES FROM 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)-TREATED RATS. Robert W. Moore, James M. Kleeman and Richard E. Peterson. Sch. of Pharmacy and Env. Toxicol. Ctr., Univ. of Wis., Madison, WI.

TCDD-treated male rats have a pronounced androgenic deficiency despite unaltered plasma luteinizing hormone (LH) concentrations and unchanged androgen catabolism and excretion rates. Effects of TCDD on testicular steroidogenesis were therefore investigated. Seven days after 100 µg TCDD/kg, testosterone (T) production by decapsulated testes incubated with the LH analog human chorionic gonadotropin (hCG) was decreased by 40–50% at all concentrations of hCG tested. Similar results were obtained with dibutyryl-cAMP. hCG-Stimulated T production was unaltered in pair-fed control rats. The testis is therefore a target organ with respect to the TCDD-induced androgenic deficiency, and this lesion is unlikely to be due to an LH receptor defect or undernutrition. In control tests, cholesterol availability to cytochrome P-450 Santo limits steroidogenesis, but rapid T synthesis can occur without LH or hCG if 25-OH-cholesterol (a P-450 Santo substrate) or pregnenolone are provided. TCDD treatment decreased 25-OH-cholesterol-supported T production (up to 25%) at high substrate concentrations, and had no effect on pregnenolone-supported T production. The ability of cytochrome P-450 Santo to catalyze pregnenolone formation appears to be the key lesion, with impaired cholesterol availability as the major cause. (NIH ES01332 and T32 ES07015)


Activated neutrophils (PMNs) can cause tissue injury by releasing reactive oxygen species, proteolytic enzymes, and other products. PMNs infiltrate peritoneal areas of the liver in certain types of cholestasis, raising the possibility that they may be involved in the pathogenesis. Since PMNs may be exposed to bile components during cholestasis, we tested the effect of several bile acids on the ability of rat peritoneal PMNs to release O2-. Bile acids themselves did not stimulate PMNs to release O2-. However, free cholate, deoxycholate, and chenodeoxycholate enhanced O2 production by PMNs stimulated with phorbol myristate acetate (PMA). Cholate had no effect in this system. When PMNs were activated with a submaximal concentration of PMA, lithocholate (31.5 µM) stimulated PMNs to release 10-fold more O2 than PMA did alone, whereas chenodeoxycholate and deoxycholate (100 µM) caused modest enhancement of O2 production. Conjugation of cholate with taurine reduced lithocholate's ability to potentiate O2 production following PMA stimulation. These data indicate that certain bile acids can potentiate O2 release from activated neutrophils and suggest a possible role for this interaction in certain types of cholestatic liver injury. (Supported by NIH Grant ES04139.)
OZONE INHALATION DECREASES CYTOCHROME b_{45} IN RAT ALVEOLAR MACROPHAGES, J.E. Ryer, G. Witz, B.D. Goldstein and M.A. Amato. GJPT-UMDNJ-RWJ Medical School / Rutgers University, Piscataway, NJ

Laboratory animals exposed to ozone exhibit a heightened susceptibility to pulmonary bacterial infection. Our laboratory has demonstrated that alveolar macrophages isolated from rats exposed to ozone have a decreased ability to produce superoxide anion radical (O_{2}^{-}) upon stimulation. This active oxygen species, which is produced by a NADPH-dependent oxidase system, is further converted to potent bactericidal species. The present data show that cytochrome b_{45} (cyt b_{45}), which has been suggested to be an integral component in the electron transport chain in the human alveolar macrophage oxidase system, is present in rat alveolar macrophages. In addition, the results demonstrate that alveolar macrophages from rats exposed to 3 parts per million ozone for 3 hours exhibit a 35% decrease in cyt b_{45}, as measured by difference spectroscopy. This decrease was paralleled by a 57% decrease in O_{2}^{-} production. These results suggest that increases in cyt b_{45} may be, in part, responsible for the ozone-induced inhibition of O_{2}^{-} production by rat alveolar macrophages.

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OZONE INHALATION AFFECTS RAT ALVEOLAR MACROPHAGE SUPEROXIDE ANION RADICAL PRODUCTION, PHAGOCYTOSIS, MEMBRANE LIPID FLUIDITY, AND MEMBRANE SOLPHERYL GROUP STATUS, J.E. Ryer, M.A. Amato, B.D. Goldstein, and G. Witz, JGPT-UMDNJ-RWJ Medical School/Rutgers University, Piscataway, N.J.

Previous studies from our laboratory have shown that alveolar macrophages (AM) isolated from rats immediately following ozone (O_{3}) exposure are less able to produce superoxide anion radical (O_{2}^{-}), a potent species involved in bacterial killing. The present studies extend these findings to the effects of O_{3} on phagocytosis and other plasma membrane (PM) associated properties. AM isolated from rats immediately and 2 hr following exposure to O_{3} (1.4 ppm, 3 hr) exhibited a 20% and a 22% decrease in O_{2}^{-} production and a 28% and 20% decrease in phagocytosis, respectively. To determine if changes in AM function are related to other O_{3}-induced alterations in the AM plasma membrane, AM from similarly exposed rats were isolated at the same time points and examined for the number of available PM sulfhydryl (SH) groups and PM lipid fluidity. These AM exhibited a 35% and a 29% decrease in the number of available PM SH groups, respectively, and increases in fluorescence polarization indicative of decreased PM lipid fluidity. These studies suggest that O_{3} acts to compromise AM bactericidal function through alterations in the functional and dynamic properties of the AM plasma membrane.

Supported by the American Lung Association.

DEMONSTRATION OF INCREASED OXIDANT GENERATION BY DBA/2 MOUSE BONE MARROW NEUTROPHILS, L.E. Tved-
dok, S.E. Perschke and M.A. Trush, Johns Hopkins Univ., Baltimore, MD

Neutrophil-derived oxidants have been implicated in both damage to biomolecules and the metabolic activation of xenobiotics. Bone marrow, a relatively neutrophil-rich tissue with low cytochrome P-450 activity, is subject to toxicity from orally administered benzo[a]pyrene (BP) in mice with non-inducible P-450 systems. Thus, we have compared the oxidant generation of neutrophilic cells isolated from femurs of male DBA/2 and C57Bl/J6 mice, strains that are susceptible and non-susceptible, respectively, to bone marrow toxicity from BP. Oxidant generation of neutrophilic preparations was assayed by superoxide anion generation and oxidant-dependent chemiluminescence (GLO) from luminol or lucigenin. In all assays, cells from DBA mice demonstrated increased oxidant generation. Moreover, a two-fold enhancement of oxidant-dependent CL from BP-7,8-dihydrodiol was observed with TPA-stimulated neutrophilic cells from DBA mice as compared to cells from C57 mice. CL from BP-7,8-dihydrodiol has previously been shown to correlate with its ability to elicit genotoxic effects. These results suggest that the increased risk of DBA mice for BP-induced bone marrow toxicity may be related to their greater ability to generate oxidants.

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EVIDENCE FOR REACTIVE OXYGEN SPECIES INDUCING MUTATIONS IN MAMMALIAN CELLS, A.W. Bae, Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX

We have studied the mutagenicity (by selecting for mutants resistant to 6-thioguanine) and cytotoxicity (by determining cellular cloning efficiency) of physical and chemical agents in Chinese hamster ovary (CHO) cells, clone K_{1}-BH_{4}, and its radiation hypersensitive transformant, A652. A652 cells contain a single functional copy of a bacterial gene, the xanthine-guanine phosphoribosyltransferase (XGRT) gene (xgt) instead of its mammalian equivalent, the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene (hpres). We found that X-ray and neutron irradiations are equally toxic to both cell types; these physical agents, however, approximately 10-times more mutagenic to A652 than K_{1}-BH_{4} cells. If reactive oxygen species mediate the mutagenic effects of radiation and chemicals, then radiomimetic compounds such as streptonigrin and bleomycin which exert their biological effects via reactive oxygen species, and oxidizing compounds such as potassium superoxide and hydrogen peroxide, should elicit a similar differential mutagenic response in both cell types. Our results fulfill such predictions. (Sponsored by DVSP of EPA).
OXY RADICAL PRODUCTION AND ARACHIDONIC ACID RELEASE BY MURINE PERITONEAL MACROPHAGES STIMULATED BY TUMOR PROMOTERS. G. Witz and B. Czerniecki. JGPT-UMDNJ-Robert Wood Johnson Medical School/Rutgers University, Piscataway, NJ

Active species of oxygen have been suggested to play a role in the process of tumor promotion. In previous studies we demonstrated that mouse skin tumor promoters injected intraperitoneally (i.p.) stimulated the production of superoxide anion radicals (O₂⁻) in murine peritoneal exudate cells (PEC). Since enhanced phospholipid metabolism has been implicated during the process of tumor promotion, the present studies were undertaken to determine whether the stimulation of oxy radical production is accompanied by arachidonic acid (AA) release. In mice treated i.p. with 0.1μg mezerein (MEZ) or phorbol myristate acetate (PMA) prostaglandin E₂ (PGE₂) release from PEC was 2 and 3 times greater than that of control cells, respectively. I.p. treatment with the same dose of phorbol dibutyrate (PDB) or phorbol diacetate (PDA) did not stimulate PGE₂ release. The production of O₂⁻ by PEC was stimulated by i.p. injection of 0.1μg PMA, but not by similar doses of MEZ, PDB or PDA as assessed by the reduction of nitroblue tetrazolium in vitro. With the exception of MEZ, the ability of tumor promoters to stimulate in vitro the release of [³⁵]AA from resident macrophages was found to correlate with their ability to stimulate the respiratory burst in vivo. Supported by PHS grant CA33270 awarded by the NCI, DHHS and by the Harrington Foundation.

OZONE STIMULATES RABBIT ALVEOLAR MACROPHAGES TO RELEASE NEUTROPHIL AND MONOCYTE CHEMOTACTIC ACTIVITY. K. E. Driscoll and R. B. Schlesinger. New York University Med. Cntr., NY, NY

The effects of in vitro and in vivo ozone exposure on the release of leukocyte chemotactic activity by alveolar macrophages (AM) was investigated. AM were obtained from New Zealand White rabbits by lung lavage, established in monolayer culture, and exposed for 2 hr to atmospheres containing 0.0, 0.1, 0.3 or 1.2 ppm ozone (O₃). Macrophage conditioned media was evaluated immediately, 2 hr and 6 hr post exposure for leukocyte chemotactic activity using modified Boyden chambers, and rabbit peripheral blood neutrophils and monocytes as responding cells. For the in vivo study, groups of 5 rabbits were given a 2 hr exposure to air, 0.1 or 1.2 ppm O₃; sacrificed immediately post exposure and the lungs lavaged to obtain AM. The AM were cultured for 6 hr and the conditioned media tested for chemotactic activity. Neutrophil chemotactic activity was significantly increased after in vitro exposure to 0.3 and 1.2 ppm O₃; monocyte chemotactic activity was increased after in vitro exposure to 1.2 ppm, In vivo exposure to 1.2 ppm increased both neutrophil and monocyte chemotactic activity. These results demonstrate that O₃ exposure can stimulate rabbit alveolar macrophages to release chemotactic activity for peripheral blood neutrophils and monocytes. The release of macrophage-derived factors may, in part be responsible for the recruitment of inflammatory cells observed after O₃ exposure.

EFFECT OF ALPHA, BETA-UNSATURATED ALDEHYDES ON MEMBRANE LIPID FLUIDITY AND PHAGOCYTOSIS OF RAT ALVEOLAR MACROPHAGES. M. J. Lavric and G. Witz. UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

Alpha, beta-unsaturated aldehydes such as acrolein (ACR) and crotonaldehyde (CRO) are reactive toxic compounds which occur as atmospheric environmental pollutants. Similar compounds, such as 4-hydroxynonenal (HNE), are formed endogenously during lipid peroxidation. These compounds have been shown in our laboratory to inhibit the ability of rat alveolar macrophages (AM) to metabolize oxygen to superoxide anion radical (O₂⁻), a reactive oxygen species involved in bacterial killing. The present studies show that AM incubated with ACR exhibit a dose-dependent decrease in membrane lipid fluidity as indicated by an increase in the polarization of 1,6-diphenylhexatriene fluorescence from a value of 0.241 for untreated cells to 0.261 for cells incubated with 0.1mM ACR. Similar results were obtained for CRO and NON, while propionaldehyde, a saturated aldehyde, had no effect. ACR also decreased phagocytosis of latex beads by AM in a dose-related manner with an IC₅₀ of 0.063mM. These results suggest that a decrease in membrane lipid fluidity of AM as a result of exposure to reactive aldehydes may contribute to impairment of membrane-dependent functions such as O₂⁻ production and phagocytosis. Supported by NIH grant ES02510.

LEUKOTRIENES IN PULMONARY OXYGEN TOXICITY. L.M. Patton and M.D. Faiman. Dept. of Pharmacol. and Toxicol., Univ. of Kansas, Lawrence, KS

The present studies were performed to determine the role leukotrienes may play in the increased vascular permeability and edema formation after exposure of rats to 100% oxygen (O₂). Male rats were exposed to 100% O₂ for 24 or 48 hours, anaesthetized, and an isolated perfused lung (IPL) system prepared. The IPL perfusate superfused an isolated guinea-pig ileal smooth muscle (GPIMS) strip, the bioassay system used to determine leukotriene-like activity. GPIMS contraction was significantly increased by perfusate obtained from rats exposed to either 24 or 48 hours of O₂. This increase in GPIMS contraction correlated with an increase in perfusate protein and malondialdehyde, and a decrease in lung dry wt/wet wt after 48 hours of O₂ exposure, indicating pulmonary damage had occurred at this time. D-sulfur treatment of rats with BW755C (10 mg/kg, i.p.), an inhibitor of cyclo-oxygenase and lipoxygenase activity, significantly decreased the increase in GPIMS contraction and perfusate protein after 48 hours of O₂ exposure. These studies suggest that the leukotrienes may increase pulmonary vascular permeability during O₂ exposure. (Supported in part by a contract from the U.S. Air Force, #33615-82-D-0601 and by the National Institute on Alcohol Abuse and Alcoholism, grant No. 0377).
Ozone occurs in the atmosphere, both naturally and as a gaseous pollutant. Previous studies in our laboratory have shown that 0.7 ppm ozone inhalation by mice causes mediastinal lymphadenopathy, altered T-lymphocyte reactivity, and thymic involution, in addition to the characteristic pulmonary lesions. In the present study, BALB/c mice were exposed to air or air plus 0.7 ppm ozone for four days to determine if the T-lymphocyte could be directly linked to the sites of pulmonary damage. Frozen tissue sections were stained using an indirect immunofluorescence procedure. This allowed the identification of the T-lymphocyte subpopulation (Thy-1.2). In control animals, occasional Thy-1.2 cells were present in the capillary bed of the lung and a few T-cells were seen near the airways. In ozone-exposed mice Thy-1.2 cells infiltrated the peribronchial portion of the airways and had collected near small vessels. These cells were also present in alveolar ducts and in regions where ozone-mediated damage and inflammatory cell infiltration occurred. Thy-1.2 cells found in ozone-induced sites of damage were present in small clusters of cells, rather than being randomly distributed throughout the lesion. The presence of T-lymphocytes may be related to an involvement in the regulation of pulmonary damage produced during ozone exposure.

Mice exposed to ozone show an increase in the number of lymphocytes present in the mediastinal lymph node and an increased reactivity of these cells to the mitogen Con A. T-lymphocyte deficient (atrophic) mice also exhibit a greater degree of ozone-mediated pulmonary damage than immunocompetent animals. In this study we identified the lymphoid subpopulations of mediastinal lymph node cells from female BALB/c mice by the surface markers Thy-1.2, Lyt-1, Lyt-2, and IgM via indirect immunofluorescence. The number of cells per node of mice exposed to 0.7 ppm ozone for four days was 1.3 times that of control animals. A significant increase in the total number of cells for each subpopulation was found, without a significant change in the percentage of Thy-1.2 and Lyt-1, or IgM cells. The percentage of Lyt-2 (T-suppressor) cells was significantly (P<0.001) reduced (19.5% in exposed vs. 27.5% in control). This decrease in the relative percentage of Lyt-2 cells without a decrease in the percentage of Lyt-1 cells, suggests a preferential increase in the T-helper lymphocyte subpopulation (Lyt-1, Lyt-2). These increases in lymphocyte numbers and ratio of Lyt-1 to Lyt-2 cells indicate that immune enhancement, rather than suppression occurs in the mediastinal lymph node during ozone exposure.

OZONE FREE RADICAL MEDIATED ISCHEMIA- REPERFUSION DAMAGE TO GERMIL BRAIN. A. Duchon, M. Chevion, J.F. Agarvey, R.A. Floyd. Oklahoma Medical Research Foundation and Department of Pharmacology University of Oklahoma Health Science Center, Oklahoma City, OK.

Reperfusion of ischemic brain causes tissue damage. Oxygen free radicals have been implicated as mediating agents in brain damage. Trapping agents which react with oxygen free radicals to yield products that can be quantified extremely sensitively is an approach we have taken to investigate the role of oxygen free radicals in ischemia-reperfusion injury. Salicylate react rapidly with hydroxyl free radicals and yields dihydrobenzoic acid products (DHBA). These can be quantitated at the nanomolar level by high pressure liquid chromatography with electrochemical detection (LCED). Studies on ischemia reperfusion injury as assessed by behavioral changes were conducted on gerbils using salicylate and LCED methodology. DHBA increased following blood reperfusion. The level of DHBA's reached a maximum of 15 min ischemia was followed by 5 min of reperfusion. The data supports the notion that oxygen free radicals are involved in ischemia-reperfusion induced brain damage. This work was supported in part by Grants NIH (NS 23307) and Am. Heart Assoc. (OK56-G-6). A. Duchon was a Fleming Scholar in the Summer of 1986.


Tert-butyl hydroperoxide (tBH) has been shown to be cytotoxic to a variety of cell types in vitro. The present study has examined the response of isolated rabbit proximal tubule segments to tBH-induced oxidative stress. Time (0-120 min.) and tBH (0.03-1.0 mM) concentration-dependent release of phosphoglucomutase (PGM) activity, protein and alkaline phosphatase (AAP) activity was observed. Release of PGM activity in control tubules ranged from 6 to 21% of total enzyme activity over the 120 min. period. Protein and AAP activity were 3% and 3% to 5% over two hours, respectively. Intracellular glutathione (GSH) increased slightly from 27 to 32 nmoles GSH/mg protein over 120 min. in control tubules whereas GSH levels were stable at 0.03 nM tBH and decreased markedly at higher tBH concentrations (max. depletion of 85%). Release of thiorbituric acid (TBA) reactive material was unaffected at 0.03 mM tBH increased in a time and concentration-dependent manner at higher concentrations (maximum formation 4 pmole/mg protein). These results show that treatment of proximal tubule segments with tBH results in release of cellular proteins, depletion of intracellular GSH and production of TBA-reactive lipid peroxidation products.
ANTIOXIDANTS AND CYCLIC AMP AGONISTS PREVENT THE INHIBITION OF MOUSE HEPATOCYTE INTERCELLULAR COMMUNICATION BY LIVER TUMOR PROMOTERS. R.J. Ruch and J.E. Klaunig, Department of Pathology, Medical College of Ohio, Toledo, OH.

Intercellular communication via gap junctions (IC) may function in the control of cellular replication. Most tumor promoters inhibit IC between cultured cells. This effect may function in vivo to permit the clonal expansion of initiated cells. In this study, the rodent liver tumor promoters, phenobarbital (PB; 20-500 µg/ml), DDT (0.1-10.0 µg/ml), and lindane (0.1-5.0 µg/ml), inhibited IC between male B6C3F1 mouse hepatocytes in primary culture. IC was detected by autoradiography as the passage of [5-3H]arabinoside nucleotides from pre-labelled "donor" hepatocytes to non-labelled "recipient" hepatocytes. The addition of the antioxidants, superoxide dismutase (SOD; 100 U/ml), DPPD (25 µM), and vitamin E (100 µM), prevented the inhibition of hepatocyte IC by the liver tumor promoters. Hydrogen peroxide-generating glucose oxidase (GO; 0.01-0.1 U/ml) also inhibited mouse hepatocyte IC. This effect was prevented only by DPPD and vitamin E, and not by SOD. In addition, the cAMP agonists, caffeine (0.01-1.0 mM) and dibutyryl cAMP (0.001-0.1 mM), prevented the inhibition of IC by PB. The results suggest that these liver tumor promoters inhibit mouse hepatocyte IC through the generation of activated oxygen species and that increased intracellular cAMP levels can override this effect.

VITAMIN A POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY. Alaa E. El-Sissi, David L. Earnest and I. Glenn Sipes, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.

Pretreatment of rats with Vitamin A dramatically potentiates the hepatotoxicity of CCl4. Studies with 13C-labelled CCl4 indicate that Vit. A does not enhance the metabolism or covalent binding of 13C-Cl. However, Vit. A pretreatment enhances CCl4-induced lipid peroxidation as assessed by ethane exhalation. Electron microscopic examination of liver sections from Vit. A rats reveals "activated" Kupffer cells, which explains the increase in colloidal carbon clearance from plasma of Vit. A rats. Activated Kupffer cells could release active oxygen species upon challenge (i.e., CCl4), that may result in lipid peroxidation and hepatocellular necrosis. To test this hypothesis, superoxide dismutase (SOD, 10,000 IU/kg) or catalase (CAT, 40,000 IU/kg) was given I.V. 2 hr after CCl4 (0.15 ml/kg, i.p.) to control or retinol pretreated rats (250,000 IU/kg/day, p.o. for 1 week). SOD or CAT effectively blocked the potentiation of CCl4 liver injury by Vit. A pretreatment (as assessed at 24 hr by SGPT and histology). Methyl palmitate (MP, 2 g/kg), an inhibitor of Kupffer cell activity, when given I.V. 24 hr before CCl4 to Vit. A pretreated rats, also blocked the potentiation of liver injury. SOD or CAT did not influence CCl4 toxicity in control rats (0.15 or 2 ml CCl4/kg). In conclusion, the potentiation of CCl4 liver injury by Vit. A appears to be mediated by active oxygen species released from phagocytic cells activated by Vit. A. (SPA0 CA27502)

DIFFERENTIAL MECHANISMS OF Ca2+-DEPENDENT CELL INJURY. C. E. Thomas, X. Olafsdottir, and D. J. Reed, Dept. of Biochemistry & Biophysics, Oregon State Univ., Corvallis, OR.

The role of oxidative stress in cell toxicity was studied using isolated rat hepatocytes incubated in the absence of extracellular calcium. This treatment damaged hepatocytes as evidenced by LDH leakage (58% vs. 18% for controls at 5 hr). Malondialdehyde (MDA) production preceded and accompanied LDH leakage, while a decrease in cellular protein thiol levels occurred concomitant with LDH leakage. The chelating agents desferrioxamine, EDTA, EGTA; and the antioxidants vitamin E, DPPD, and promethazine inhibited MDA production and prevented both LDH leakage and the decrease in protein thiols. Cysteine, N-acetyl-cysteine, and GSH provided partial protection against LDH leakage, MDA production, and loss of protein thiols. In comparison, treatment of hepatocytes with extracellular Ca2+ (2 mM) and the Ca2+ ionophore A23187 (20 µM) also resulted in LDH leakage (90% at 5 hr). Desferrioxamine, DPPD, and promethazine had no effect on A23187-induced LDH leakage, but inhibited MDA formation. In contrast, EGTA had no effect on MDA production, but inhibited LDH leakage. These results suggest that Ca2+ is involved in cell injury in both systems. However, in the absence of extracellular Ca2+, cell injury results from peroxidative damage, while cell injury by an influx of extracellular Ca2+ occurs independent of peroxidation. (Supported by USPHS ES01978)


It is currently thought that pyrrolylation of neurofilaments may be the initiating event in γ-diketone neuropathy. The present study compares the neurotoxicity and pyrrole-forming ability of 2,5-hexanidine (2,5-HD) with perdeuterio-(D-)2,5-HD. Due to a primary isotope effect, the latter derivative was expected to form the pyrrole at a slower rate. In vitro studies confirmed that D-β-2,5-HD pyrrolylated protein at only ¼ the rate of 2,5-HD. For in vivo studies, Wistar rats were given daily IP doses of either 3.5 mmol 2,5-HD or D-β-2,5-HD/kg for 2½ wk or 2.5 mmol/kg for 5 wk. Animals receiving 2,5-HD and D-β-2,5-HD had lost 27% and 8% body weight, respectively. Moderate to severe hindlimb paralysis was noted in the 2,5-HD groups while only mild effects were present in the D-β-2,5-HD groups. Neuropathological changes were also more pronounced in the 2,5-HD groups. Pyrrole levels in serum and nervous tissue were 2- to 3-fold higher in the 2,5-HD as compared to the D-β-2,5-HD-treated rats. In contrast, tissue diketone concentrations were not significantly different, indicating a similar tissue uptake. These findings support a requirement for pyrrole adduct formation in γ-diketone neuropathy. (Supported by NH/NIOSH grant OH-01972).
SIMULTANEOUS EXPOSURE TO N-HExANE AND METHYL ISO-BUTYL KETONE (MBK) PRODUCED CROSSLINING AND DECREASED PHOSPHORYLATION OF NEUROFILAMENT PROTEINS IN HENS. M.B. Abou-Donia, C. Habig, E. Suwita, and D.M. Lapadula. Duke University Medical Center, Durham, NC.

Aliphatic hexacarbons produce a distal neuropathy in which there is an accumulation of neurofilaments. MBK has previously been demonstrated to potentiate n-hexane-induced neurotoxicity. Groups of 5 hens were simultaneously treated by inhalation with 1000 ppm n-hexane and either 1000, 500, or 0 ppm MBK (methyl iso-butyl ketone). One group was treated with 1000 ppm MBK and one group served as an untreated control. Hens receiving n-hexane and either 500 or 1000 ppm MBK showed signs of neurotoxicity. At the end of 30 days, animals were killed and spinal cords and livers were removed. Neurofilament proteins were isolated from spinal cords by axonal floatation. Liver microsomes were prepared by CaCl2 precipitation. There was a decreased protein phosphorylation evident in animals treated with MBK + n-hexane. Protein crosslinking was assessed by reactivity of high molecular weight proteins with antibodies to the neurofilament proteins. Crosslinking was observed in all animals treated with n-hexane. Hepatic cytochrome P-450 content was induced in all animals treated with MBK (375-744%) while animals treated with n-hexane alone were not significantly altered (138% of control). Supported in part by NIOSH Grant No. OH00823.

EFFECTS OF ACR, 2,5-HD, DMHD AND IDPN UPON MICRO TUBULE CYTOSKELETAL NETWORKS. DW Sickles, JA Tischfield and TG Obiak. Medical College of Georgia, Augusta, GA. SPON: BD Goldstein

We hypothesize that the microtubule is the primary site of action of neurofilamentous axonopathy-producing toxins. Effects upon the microtubule cytoskeleton by these toxins have been examined with immunofluorescence of cultured mouse Balb 3T3 cells. ACR (50mM-2hr) exposure disperses the normal tubulin cytoskeleton into an extremely fine, but dense network which, with higher doses or longer exposures (4hr), causes disassembly which produces uniform cellular staining. 2,5-HD (4mM) and DMHD (1mM) appear to produce a highly crosslinked network within an hour, whereas higher doses or longer exposures also cause disassembly. IDPN (0.1%) has no effect at 1-2 hr. At 4hr all of the toxins cause disassembly of the microtubule cytoskeleton similar to that caused by colchicine, a microtubule poison. Preliminary data suggest a protective effect by 5mM glutathione on the disassembly caused by 5mM ACR. We conclude that the microtubule is rapidly disassembled or crosslinked by toxins which produce a neurofilamentous axonopathy. Further research is required to determine whether or not this is the critical mechanism of toxic neuropathy. Supported by NIOSH #OH02020.

TOXIC ACTION OF ACR, 2,5HD AND DMHD UPON THE MICRO TUBULE: MITOSIS AND TUBULIN CROSSLINING. DW Sickles, JA Tischfield, DA Walter, AR Testino, JK Pearson and TG Obiak. Medical College of Georgia, Augusta, GA. SPON: BD Goldstein

The primary site of action of neurofilamentous axonopathy-producing toxins may be the microtubule. Acrylamide exposure of HT 1080 human sarcoma cells produced a dose-dependent (1-5mM) increase in mitotic figures. Five mM ACR for 4 hr caused a 5.2x increase in the number of mitotic cells, while a microtubule poison (colchicine @ 30uM) caused a 6.2x increase. Similarly, DMHD (2.5 mM) caused a 2.4x increase. HD appeared to be cytotoxic at the lowest dose (4mM) while IDPN had no apparent effect. Peripheral nerve proteins were preincubated with toxins for 8hr at 37°C, separated by SDS-PAGE, transferred to nitrocellulose paper and stained with monoclonal antibodies against tubulin or neurofilament proteins. 2,5-HD (200uM) and DMHD (8.5mM) preincubation resulted in the disappearance of tubulin and neurofilament bands; the protein did not leave the stacking gel. Preincubation with ACR (21uM) or IDPN (406uM) had no effect upon the protein bands compared to controls (buffer incubated). ACR and certain hexacarbons block mitosis, which is primarily dependent upon microtubules. The hexacarbons, but not ACR, may produce this effect through crosslinking of tubulin protein(s). Supported by NIOSH #OH02020.


Inhibition of enzymes of energy producing pathways has been proposed as a potential site of action of acrylamide (ACR), 2,5-hexanedione (HD), and 3,4-dimethyl-2,5-hexanedione (DMHD) in producing a peripheral neuropathy. While neurospecific isoenzyme forms of glycolytic enzymes have been shown to be inhibited by these toxins, no examination of neuron-specific effects on oxidative enzymes has been reported. Using quantitative histochemical techniques, we have examined the effects of a single acute dose of ACR, HD, and DMHD on neuronal and non-neuronal NADH-TR activity. One hour after injection of rats with ACR (50mg/kg), HD (435mg/kg) or DMHD (35mg/kg), ACR animals showed a significant decrease in NADH-TR activity of 9.5% in axons, 17.3% in Purkinje cells, and 17.8% in DRG cells; HD animals showed a nonsignificant decrease of 12.0% in DRG cells and 7.3% in Purkinje cells; DMHD animals showed a significant decrease of 16.3% in DRG cells while the Purkinje cells showed a nonsignificant decrease of 11.1%. No significant changes were observed in kidney, liver or stomach cells with these toxins. The ACR data demonstrate its neural specificity and support the energy hypothesis while the single acute doses of HD and DMHD used do not demonstrate significant neural specificity. Supported in part by grant # NIOSH OH02020.
527  NEURON-SPECIFIC INHIBITION OF LIP OAMIDE
DEHYDROGENASE (LpdH) AND CYTOCHROME c REDUCTASE
(CCR) BY ACRYLAMIDE. D.W. Sickles Medical College
of Georgia, Augusta, GA. SPON: B.D. Goldstein

Inhibition of oxidative enzymes has been
suggested as a critical site of action in
acrylamide (ACR) neurotoxicity. The specificity
of action of ACR upon LpdH and CCR activity in
neural and non-neural tissues was evaluated.
One hour after a single 50mg/kg dose of ACR, the
LpdH activity in homogenates of brain (-16%),
spinal cord (-16%), kidney (-21%) and
stomach (-12%) were reduced; the CCR activity
in brain (-14.9%) and spinal cord (-8.7%) were
significantly reduced also. In vitro
preincubation of tissue homogenates @ 37°C
for 30 minutes with 1mM ACR caused inhibition of
brain/spinal cord (-29.6%), DRG (-11%) and
stomach (-16.3%) CCR activity. LpdH was
inhibited by the preincubation and could not be
tested; no enzyme inhibition was observed
without preincubation. Neuron-enriched
fractions of rat brain, obtained by gradient
centrifugation, exposed in vitro to 1mM ACR @
37°C for 30 minutes significantly decreased
LpdH (-44.8%) and CCR (-16.9%) activities. ACR
appears to have a more significant effect upon
neural than non-neural LpdH and CCR activities.
However, the low level of inhibition and the
significant effects upon two non-neural tissues
casts doubt upon these enzymes as critical sites
of ACR action.

Supported by NIOSH #OH02020

528  ATP, CP, AND AXOPLASMIC TRANSPORT IN SCIATIC
NERVES OF ACR, 2,5-HD AND DMHD EXPOSED RATS.
D.W. Sickles and J.K. Pearson Medical College of
Georgia, Augusta, GA. SPON: B.D. Goldstein

Enzyme inhibitions by neurofilamentous
axonopathy-producing neurotoxins are believed to
compromise the energy supply and axoplasmic
transport system. ACR produced a
dose-dependent decrease in rate (14-19%) and
capacity (40-79%) of fast anterograde
transport within 3 hours of a single 50 and
100mg/kg dose, respectively. ATP and CP
levels were measured with a
luciferin-luciferase bioluminescence assay in a
Beckman scintillation counter equipped for
single photon counting. ATP and CP levels in
the nerve contralateral to the one used to
determine axoplasmic transport were
significantly increased by 21-30%, 3 hours
after injection. Likewise, the hour following
4mmol 2,5-HD or 0.25 mmol DMHD injection, the ATP
and CP levels were increased but large
variations prevented observing any statically
significant changes. We conclude that
axoplasmic transport is very significantly
inhibited by 0.7-1.4 mM doses of acrylamide;
the compromise is not caused by a depletion of
high energy phosphates. In addition,
preliminary data demonstrates that neurotoxic
hexacarbons do not significantly decrease
energy high energy phosphates.

Supported by NIOSH #OH02020

529  ATP PRODUCTION BY BRAIN MITOCHONDRIA EXPOSED
TO NEUROFILAMENTOUS AXONOPATHY PRODUCING
NEUROTOXINS. D.W. Sickles, S.R. Fowler and A.R.
Testino Medical College of Georgia, Augusta
GA. SPON: B.D. Goldstein

Enzymes of energy transformation pathways
have been implicated as the critical site of
action of certain neurotoxins producing
neurofilamentous axonopathies. Brain
mitochondrial ATP production before and after
exposure to these toxins was measured with a
luciferin-luciferase bioluminescence assay.
Absence of substrate (pyruvate) or addition of
1mM DNP completely blocked ATP production.
Acrylamide (0.73mM) preincubated with the
toxin for 30 minutes at 37°C did not
significantly affect the rate of production or
endogenous ATP levels. Brain mitochondria from
rats receiving 50mg/kg/day for 5 days showed a
reduction of 11% in ATP production. DMHD
(0.25mM) and 1DNP (0.15%v/v) exposure to
mitochondria had no significant effect. 4 mM
HD significantly reduced both the rate of
production (28%) and ATP content (54%).
Although 4mM HD inhibits ATP production, the
absence of significant effects by the other
toxins argues against the oxidative enzymes as
a common site of action in producing a
peripheral neuropathy.

Supported by NIOSH #OH02020

530  CRESELYLENZOXIDOPHOSPHORIN OXIDE (CBDP) PRE-
TREATMENT OF THE RAT ALTERS SOMAN TOXICITY AND
CHOLINESTERASE (ChE) INHIBITION IN BLOOD AND
Maxwell, K. Hodge and K. Brecht. USAMRICD,
Aberdeen Proving Ground, MD

CBDP binds preferentially and irreversibly
to carbamylcholineesterase (CaE) but not to soman (GO)
"detoxification.
Thus, in the presence of
CBDP, more GD is available to inhibit ChE
(Toxicol Appl Pharmacol, 20: 474, 1971). This
study examined GD toxicity and ChE inhibition
with or without CBDP pretreatment (preRx).
Male rats received CBDP (1.0 mg/kg, sc) in 10%
ehanolic propylene glycol (V/V) or VEH alone
followed 1 hr later by saline (SAL) or selected
doses of GD. The 24 hr LD50 for GD was
determined for both preRx groups. CaE (plasma
only) and ChE were measured in plasma, RBC,
spinal cord, whole brain and regions 30 min
after SAL or GD. CBDP preRx reduced GD's
LD50 from 116.6 mg/kg (V/V) to 20.5 mg/kg.
in both SAL- and GD-treated groups, CBDP preRx
completely inhibited plasma CaE. CBDP reduced
gd's LD50 Edward for brain regional ChE inhibition
approximately 10-fold. The data indicate that,
follow this CBDP preRx dose, 90% less GD is
required to induce 50% ChE inhibition in the
central nervous system. These data are
consistent with the hypothesis that, following
CBDP, more GD is available to inhibit critical
ChE sites, thus potentiating GD lethality.
A DEVELOPMENTAL MODEL OF ORGANOPHOSPHATE INDUCED PERSISTANT BRAIN ACETYLCHOLINESTERASE (ACHE) INHIBITION. C. Overstreet, S. Padilla and R. Veronesi, USEPA/HERL/NTD, RTP, NC.

The absence of valid experimental models to study the developmental consequences of organophosphate induced persistent cholinesterase inhibition to the central and peripheral nervous systems, has limited our understanding of these effects. In this study, we have outlined exposure parameters for perinatal, Long-Evans, male and female rat pups using disopropyl fluorophosphate (DFP). DFP in corn oil was injected (sc) into pups from postnatal day 1 to 21 at a dose of either 0.5 mg/kg per day, 1 mg/kg every other day, or an alternating schedule of 1 mg/kg on odd numbered days and 0.5 mg/kg on even numbered days. Higher concentrations of DFP or increased frequency of injections resulted in high infant mortality. Radiometric assays of whole brain ACHE activity at varying times indicated that multiple DFP injections reduced ACHE activity throughout the preweaning period by at least 25-36%, in the absence of significant brain and body weight loss. This depression was confirmed by ACHE histochemistry. Multiple doses were essential to produce the persistent inhibition of ACHE activity. These exposure parameters introduce a dosing regimen which produces persistent depression of ACHE in rat pups throughout the critical periods of neuronal migration and synaptogenesis.

CHANGES IN HIGH-ENERGY PHOSPHATE COMPOUNDS IN RAT SKELETAL MUSCLES INTOXICATED WITH DFP AND SOMAN. W-D. Settbahn and R. Caprara. Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, TN.

The quantitative changes in high-energy phosphate compounds caused by disopropylphosphorofluoridate (DFP) and pinacolyl methylphosphonofluoridate (soman) were determined using reversed phase-HPLC in rat skeletal muscles. Following a non-lethal acute toxicity-producing dose of either DFP (1.5 mg/kg, sc) or soman (0.1 mg/kg, sc), a significant decline in phosphocreatine (PC) was seen as early as within 1 hr (60 to 75% of control), with a maximum change by 6 hr (43-65%). Among the muscles studied, the hemidiaphragm was maximally affected (43%) with soman treatment. A detailed analysis of adenine nucleotides indicated a significant decrease of adenosine triphosphate (ATP) in hemidiaphragm only (72 to 78% of control), with a marked increase in adenosine monophosphate (AMP). The observed changes in PC, AMP and ATP were reversed toward their baseline values within 72 hr. It is concluded that both DFP and soman reduced the phosphocreatine in skeletal muscles, probably through enhanced activity of reversed creatine phosphokinase reaction to meet the demand of increased ATP during severe muscular fasciculations. The observed higher AMP level was possibly a result of myokinase stimulation. (ANRE?83-C-3244).

ETHYL METHYLPHOSPHONATE (YL) NEUROTOXICITY IN HENS. E.J. Olajos and H. Wall. Toxicol. Div., CRDEC, US. Army, APG, MD 21010 and Comp. Path. Br., MRICD, US. Army, APG, MD.

Although phosphinates, as a group of organophosphorus esters, are not associated with delayed neurotoxicity, the tauromerism exhibited between the pentavalent and trivalent forms of YL warrants neurotoxicologic evaluation. Hens were dosed acutely with YL (850 mg kg⁻¹, oral) or tri-o-cresyl phosphate (TCP) (600 mg kg⁻¹, oral) and observed for up to 33 days. Assessment of delayed neurotoxicity was based on morphological changes (axonal degeneration and demyelination), ataxia, and inhibition of neurotoxic esterase (NTE) activity. After dosing, cholinergic signs were evident in both YL and TCP-treated birds. Histologic examination revealed no changes in neural tissues from YL-dosed hens. Lesions, characteristic of delayed neurotoxicity, were observed in the TCP-treated birds. The YL-dosed hens did not exhibit ataxia or other behavioral signs of delayed neurotoxicity during the post-dosing observation period. Further, YL did not inhibit NTE activity at 24-h postdosing.

Based on the above findings, YL does not cause a delayed neurotoxic effect in the adult hen.

EFFECTS OF CRESELBENZIODIOXAPHOSPHORIN OXIDE (CBDP) ON TISSUE CARBOXYLESTERASE (CaE) AND CHOLINESTERASE (ChE) ACTIVITIES IN THE RAT. V.K. Hirnersha, D.M. Maxwell, Y. Hodge, K. Brecht and T.-W. Shih, USBAMCD, Aberdeen Proving Ground, MD.

CBDP, the putative cyclic metabolite of tri-o-cresylphosphate, inhibits CaE's in preference to ChE's in vitro (Maxwell et al. unpublished data). This study examines the in vivo effect of CBDP on CaE and ChE in selected rat tissues. Male Sprague-Dawley rats (200-300g) were treated sc with various doses (0.1 to 1000μg/kg) of CBDP in 10% ethanolic propylene glycol (VEH) or with VEH alone. Ninety min later CaE and/or ChE activities were analyzed in blood (plasma and EBC's), lung, liver, spinal cord (SC), whole brain (WB) and selected brain regions (brainstem, cortex, hippocampus, midbrain, cerebellum, striatum). VEH treatment alone did not affect either CaE or ChE activity in any tissue. At doses of 0.5 μg/kg or greater, CBDP inhibited CaE activity by >99% in plasma and lung, but not in liver. A CBDP dose-dependent inhibition of ChE activity was seen in plasma, EBC, SC, WB and in all brain regions only at CBDP doses exceeding 0.5μg/kg. CBDP inhibited blood esterases in the order plasma CaE >> plasma ChE >> EBC ChE. These data support the in vitro data indicating that CBDP inhibits CaE in preference to ChE.
Calmodulin (CaM) Kinase II is involved in the increased phosphorylation of microtubule and neurofilament triplet proteins in chickens treated with TOCP, an organophosphorus compound capable of inducing delayed neuropathy (OPIDN). In this report, chickens were given a single oral dose of 0 or 750 mg/kg of TOCP and killed 21 days after treatment. Brains were homogenized in 100 mM PIPES, pH 6.9, 10 mM EDTA and 10 mM EGTA and centrifuged at 100,000 x g for 1 hr. The cytosol was passed through P-100 column and eluted with NaCl to separate tubulin from the crude CaM Kinase II. Phosphorylation reactions were started by adding kinase isolated from control (Kc) or treated (Kt) to tubulin isolated from control (Tc) or treated (Tt) into a phosphorylation buffer of 10 mM PIPES, pH 7.4, 1 mM EDTA, 2 mM EGTA, 10 mM Mg2+, 50 µM Ca2+, 5 µM [γ-32P]ATP and either 1 µg calmodulin or 50 µM trifluoperazine. Proteins were subjected to SDS-PAGE and autoradiography. The autophosphorylation of CaM Kinase II in treated animal was increased. Kt was incubated with Tc or Tt and Kc was incubated with Tc or Tt. The phosphorylation of Tc or Tt in the presence of Kt was increased. These data suggest that the altered autophosphorylation of CaM Kinase II may play a role in the development of OPIDN. Supported by NIH Grant No. OH02003 and NIEHS Grant No. ES02717.

Correlation between Prolonged Inhibition of Neurotoxic Esterase by TPP and TOCP and the Rate of Absorption as Monitored by 31P Nuclear Magnetic Resonance (NMR). Clark D. Carrington, C. Tyler Burt, and Mohamed B. Abou-Doria. Duke University and NIEHS, Durham and RTP, N.C.

Single subcutaneous doses of either 1000 mg/kg triphenyl phosphate (TPP) or 1187 mg/kg tri-o-cresyl phosphate (TOCP) to adult hens was found to result in over 70% inhibition of neurotoxic esterase (NTE) in both brain and sciatic nerve. This high level of NTE inhibition persisted for several weeks. This prolonged inhibition appears to account for the inability of prior administration of phenylmethylsulfonyl fluoride to prevent the development of a neuropathy following the administration of TOCP. Absorption of both TPP and TOCP was monitored in vivo over a two week period using NMR spectroscopy and a surface coil which was placed over the site of injection. The absorption from the injection site was biphasic for both TPP and TOCP. For both compounds, the t½ for the first phase was a day or less, while the t½ for the slower phase was several weeks. However, about 75% of the TPP was absorbed during the slower phase, compared to less than 15% of the TOCP. Thus, slow absorption is likely to account for the prolonged inhibition of NTE by TPP, it appears that TOCP or it's neurotoxic metabolite must be stored elsewhere subsequent to absorption for several weeks. (Supp. by NIH grant OH00823).
A number of organophosphate esters (OPs) such as diisopropylfluorophosphate (DFP) cause delayed neuropathy (OPIDN) in chickens, humans and other sensitive animals. Some are acutely toxic and protectants such as atropine must be given. Work presented here proceeded from an observation made on a study for USAHRL (82PP2816) using inbred hens, in injections of DFP and 20 mg atropine sulphate/kg, scoring of locomotion, brain activity of neurotoxic esterase (NTE) and histopathology to study the time of onset and severity of OPIDN. In 4 experiments, hens given atropine invariably were delayed in the onset of the disorder. For example, in one experiment, 4 hens showed ataxia at 15 days without atropine and at 19 days with the protectant. Atropine inhibited brain NTE with a KI of approx. 1.5 mM, similar to its reported reversible inhibition of human AChE. The results suggest inhibition of NTE may play a role in atropine’s effect on OPIDN, but the high KI leads us to suspect that more may be involved. Histology will be presented, studying whether damage to nerves will parallel the behavioral findings. Regardless, the data show treatment with atropine is not “neutral” in the study of OPIDN and may cause an underestimate of the neurotoxicity of an OP. (Supported in part by NIH ES00202.)

Although the chicken is an animal model of organophosphorus-induced delayed neuropathy (OPIDN), manifestations of OPIDN are different in other avian species. Quail, for example, do not develop ataxia or paraplegic. Turkeys do, but without well-defined histological lesions. The present study examined the interaction of organophosphorus compounds (OPs) with neurotoxic esterase (NTE), carboxylesterase (CbxylE) and acetylcholinesterase (AChE) in brains of chickens, turkeys and quail. Activity of NTE was higher in chicken and turkey than in quail (388±22, 274±35, 217±25 nmol/15min/mg protein, respectively, mean±SE, N=9-23) and NTE was a higher percentage of the total CbxylE present (15.5%, 13.5%, 9.0% respectively). 150 concentrations of mpaxox for inhibition of NTE were, however, similar in all 3 avian species (range 8.2-9.6 μM). Activity of AChE was also higher in chicken and turkey than in quail (13.8±1.2, 13.0±0.8, 9.5±0.8 nmol/1min/g, N=6, respectively), with 150 concentration of paraoxon toward this enzyme over 5-fold higher in quail. These results indicate that, in addition to being more closely related in their response to OPIDN, chickens and turkeys are more closely related with respect to sensitivity of neural esterases to OPs. (Supported by NIENs grant 03384)

Use of a synthetic glucocorticoid (triamcinolone, Tmc) was investigated to determine if type of adrenal corticoid (mineralo- or gluco-) could affect development of OPIDN in adult white leghorn chickens. The corticoid was administered in the diet at concentrations of 0.1-10 ppm beginning 1 da before and continuing 10 da after administration of triorthotolyl phosphate (TOTP, 360 mg/kg po), phenyl saligenin phosphate (PSP, 2.5 mg/kg im) and diisopropyl phosphorofluoridate (DFP, 1 mg/kg sc). Tmc at 0.1 ppm was beneficial in delaying development of ataxia after administration of TOTP and PSP. Development of OPIDN was exacerbated in 10 ppm Tmc in chickens given any of the OP’s tested. Clinical scores, with grades of 0-4 for nonaffected to paralyzed, x250, N=4-8, were 3.6±0.4 with Tmc vs 2.8±0.2 without 21 da after TOTP, 3.5±0.2 vs 2.5±0.2 at 12 da after PSP, and 2.1±0.3 vs 1.5±0.3 at 14 da after DFP. Heterophilo-lymphocyte ratios, an indicator of stress and increased susceptibility to detrimental influences, were significantly elevated by 10 ppm Tmc and 114 cytochrome P450 levels were significantly depressed by 67% of levels in 114 from solvent-treated chickens). These results indicate that high doses of glucocorticoids are unlikely to be beneficial in alleviation of OPIDN in chickens. (Supported by NIENs grant 03384)

EFFECT OF DIETARY TRIAMCINOLONE ON DEVELOPMENT OF ORGANOPHOSPHORUS-INDUCED DELAYED NEUROPATHY (OPIDN) IN CHICKENS. M. Ehrlich, B.S. Jortner and W.B. Gross, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

DIMETHYL METHYLPHOSPHONATE (DMMP) NEUROTOXICITY IN HENS. E.J. Olivos and H. Wall: Toxicology Div., CRDEC, U.S. Army, APG, MD and Comp. Path. Br., MRICD, U.S. Army, APG, MD

A previous study (J. Toxicol. Environ. Health, 8, 619-27, 1981) reported that the adenexen exhibited no delayed neurotoxicity after daily injections of DMMP (50 mg kg-1, 1.4 for 10 days). The current study was conducted to determine delayed neurotoxic potential of DMMP in hens after dosing with DMMP near the LD50 level. Hens (57) were given single oral doses of DMMP (2.3-13.8 g kg-1). TCPO-dosed hens (15) received 1.2 g kg-1 via the oral route. The treated hens were observed for up to 48-h for cholineric signs and over a 35-day period for motor impairment. The oral LD50 dose of DMMP in hens is 1.9 g kg-1. DMMP at a dose of 9.2 g kg-1 did not inhibit neurotoxic esterase (NTE) activity compared to >80% NTE inhibition noted in TCPO-dosed hens. Histologic evaluation of the DMMP-dosed hens (2.3-5.8 g kg-1), necropsied at 35 and 38 days postdosing, revealed no DMMP-related lesions of the spinal cord and sciatic nerve. Axo- nal degeneration and demyelination were evident in neural tissues from TCPO-treated hens. Severe motor impairment (7.3 on a scale of 0-8) was noted in the TCPO-dosed hens, but marginal motor impairment (2 on a scale of 0-8) was seen in the DMMP-dosed hens (2.3-5.8 g kg-1). The data suggest neural damage tendencies in hens dosed with DMMP at levels near the LD50 value.
Previous work from our laboratory demonstrated motor impairment and neural lesions in QL-dosed adult hens. The purpose of this study was to evaluate the delayed neurotoxic potential of QL in the White Carneau strain of pigeons. The birds (8/treatment group) were given single oral doses of QL (0.9g kg⁻¹) or o-tolylphosphate (1.3g kg⁻¹) and observed for up to 34 days after dosing.

Neurotoxic esterase (NTE) activity was also monitored in pigeons (6/treatment group) orally dosed with 1.8 g kg⁻¹ of QL or with 2.4 g kg⁻¹ of o-tolylphosphate. Marginal motor incapacitation (2.0 on a scale of 0-8) was noted in the QL-dosed birds, whereas a marked degree of motor impairment (5.6 on a scale of 0-8) was observed in the o-tolylphosphate-treated group. Axonal degeneration and demyelination were apparent in the spinal cords and sciatic nerves of the o-tolylphosphate-dosed birds but not in the QL-dosed pigeons. NTE activity was not inhibited in the QL-dosed birds compared to a >90% inhibition in the o-tolylphosphate-treated pigeons. Although neural lesions were absent and NTE activity was not inhibited in the QL-dosed pigeons, perceptible motor impairment is suggestive of neural dysfunction.

**THE EFFECT OF ROUTE OF ADMINISTRATION OF ORGANOPHOSPHATE DELAYED NEUROTOXINS IN YOUNG CHICKENS.**

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Sponsor: D. Polin.

The administration of an organophosphate delayed neurotoxin via a route which eliminates absorption through the gastrointestinal tract has been suggested to result in a young animal displaying signs characteristic of organophosphate-induced delayed neurotoxicity (OPIDN). In the present study, four-week old White Leghorn chickens were administered a single dose of 500 mg/kg tri-o-tolyl phosphate (TOTP) or 100 mg/kg o-tolyl saligenin phosphate (SP) via the oral, intraperitoneal or intramuscular route. Forty-eight hours later, half the birds in each group were killed for subsequent determination of whole-brain neurotoxic esterase (NTE) activity while the remaining 5 birds in each group were observed daily through day 21 for development of OPIDN clinical signs. TOTP administered orally caused an 85% inhibition of NTE activity while the other routes resulted in inhibition in excess of 95%. SP given via the different routes resulted in a 73-74% inhibition of NTE. No birds displayed clinical signs typical of OPIDN. Thus, the resistance of the young chicken to the delayed effects of organophosphate compounds is due to factors other than poor absorption of the compound through the gastrointestinal tract.

**PREVENTION OF DEXAMETHASONE-SUPPRESSED RAT TESTICULAR REHES, CYTOCHROME P-450, 17-HYDOXYLASE AND 17,20-LYASE BY HUMAN CHORIONIC GONADOTROPIN (HCG).**

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Previous studies from this laboratory suggested decreased testosterone production in the rat following dexamethasone (DXM) treatment is a result of decreased testicular microsomal heme, cytochrome P-450 and activities of the cytochrome P-450-mediated enzymes 17-hydroxylase (17OH) and 17,20-lyase. The present study was initiated to examine the role of HCG in DXM induced suppression of rat testicular steroidogenesis. Male Sprague-Dawley rats (165-180 g) were administered HCG (100 u.i.) at 12 h intervals for 9 days. Dexamethasone (5 mg/kg) was administered s.c. during the final 3 days of HCG treatment.

Testicular heme and cytochrome P-450 levels were determined 16 hours after the final dose. Heme and cytochrome P-450 levels were decreased to 50 and 40% of control respectively in DXM treated animals; whereas DXM-HCG treated values were equal to control values. Activities of the microsomal cytochrome P-450-mediated enzymes 17OH and 17,20-lyase were decreased to 46 and 33% of control values respectively, by DXM. HCG pretreatment (HCG-DXM) resulted in prevention of decreased enzyme activities. These results indicate that HCG can prevent glucocorticoid-induced impairment of testicular steroidogenesis.

(Supported by NIH Grant ES-02423).

Salicylazosulfapyridine (SASP; CAS #: 599-79-1), an antiinflammatory drug, is metabolized to sulfapyridine, a sulfonamide. Since some other sulfonamides are goitrogenic and cause thyroid tumors in rodents, we hypothesized that SASP causes morphologic and functional departures of the pituitary-thyroid axis. SASP was administered once daily for 5 or 7 weeks by oral gavage to F344 rats and B6C3F1 mice at doses of 0, 675, 1350, and 2700 mg/kg BW (5 animals/sex/dose group). Weight gains in treated rats were depressed in a dose-dependent manner and ranged from 0 to 80% of control weight gains. Weight gains of treated mice did not differ from controls. Chemically related gross lesions included enlarged, discolored thyroid glands in rats and enlarged cecums in rats and male mice. Rat serum T3 and T4 hormones were decreased in a dose-dependent manner and this was most severe in males. TSH increased in a dose-dependent manner with high doses of SASP causing up to 8-fold increases over controls in male and female rats. There were no significant changes in these hormone levels in mice. These observations are consistent with the interpretation of a dose-dependent primary hypothyroidism in rats.

MODULATION OF ACETAMINOPHEN (AA) HEPATOTOXICITY IN RATS BY ENDOCRINE STATUS. S.D. Ray, P.Y. Lee and G. Ji, College of Pharmacy, Rutgers University, Piscataway, NJ.

Alcohol-potentiated acetaminophen hepatotoxicity (APAH) was quantitated using the AA-induced irreversible inhibition of oxygen uptake by the isolated perfused liver from rats pretreated with alcohol in vivo. In normal rats the alcohol pretreatment increased the AA (25 mM)-induced irreversible inhibition of hepatic respiration (I/IHR) from 16 ± 11 % of the basal respiration inhibited per hour to 73 ± 11 % / hr. (mean ± SEM, n = 4 – 5). Hypophysectomy and thyroidectomy abolished APAH completely (the rates of AA-induced I/IHR ranged from 21 ± 6 to 29 ± 15 % / hr. hour regardless of the alcohol pretreatment). In contrast, adrenalectomy alone enhanced AA-induced I/IHR (53 ± 9 % / hr.). Alcohol pretreatment further increased the rate of AA-induced I/IHR to 120 ± 39 % / hr. These observations led us to postulate that (1) the acute alcohol pretreatment released thyroid hormones mediated by TSH, (2) thyroid hormones enhanced the conversion of AA to N-acetylbenezoinone imine in the liver, and (3) glucocorticoids inhibited the effects of thyroid hormones on APAH through the Mank-Osprey mechanism of glucocorticoid actions. Supported by AA05848.


Sponsor: J.H. Monneret.

Salicylazosulfapyridine (SASP) was administered by gavage in corn oil to male and female F-344/N rats for 12 days at 0, 675, 1350, and 2700 mg/kg body weight. Enlargement of the thyroid was observed grossly in high dose male and female rats and in male rats only at 1350 mg/kg. Microscopically, the thyroid follicles observed in treated animals varied in size and contained less colloid than controls. The follicular epithelium showed evidence of hypertrophy and hyperplasia. A few mitotic figures were present. Ultrastructural examination of the thyroid from high dose male and female rats revealed distortion of rough endoplasmic reticulum (RER) with colloid decreased numbers of cytoplasmic dense bodies and cytoplasmic colloid droplets, fusion of apical microvilli, increased numbers of dense secretory granules, and increased non-RER associated ribosomes when compared to controls. These morphologic changes in the thyroid are consistent with increased functional activity.


Iodinated isomers of aromatic and heteroaromatic amino acids were previously shown by indirect evidence to be generated in vivo in the gastrointestinal tract during ingestion of chlorine based disinfectants. Mono and di-iodo isomers of tyrosine, histidine, tryptophan and p-aminobenzoic acid have been studied in an enzyme inhibition assay in which release of 125I from labelled thyroxine by hepatic and renal microsomal preparations was measured using ion exchange chromatography. Mono and diido forms of histidine, tryptophan and PABA partially blocked the deiodinase activity at 10⁻⁶M. These findings imply that the ingestion of chlorine based disinfectants via e.g., drinking water, and the associated formation and proteolysis of iodinated proteins in the gut-tract leads to the absorption of iodinated amino acids which affect thyroid metabolism. These observations provide a partial mechanistic explanation for the thyroid effects of disinfectants previously reported. (This abstract does not necessarily reflect EPA policy).
THE EFFECT OF CHLORINE DIOXIDE ON IODIDE UPTAKE AND ORGANIZATION BY THYROID CELLS IN CULTURE. R.M. Harrington, H.C. Shertzler, and J.P. Berczil. Toxicology and Microbiology Division, HERL, U.S. EPA, Cincinnati, Ohio, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio

Previous studies in this laboratory showed that ingestion of the drinking water disinfectant, chlorine dioxide, resulted in a decrease in circulating radioiodide levels in both rats and monkeys. In this study rat thyroid cells in culture were used to evaluate the effects of ClO₂ upon iodide uptake and organization. To measure the effect of ClO₂ upon iodide uptake, thyroid cells were plated into dishes containing 125I- and various concentrations of ClO₂. After equilibrium was reached the medium was aspirated, measured and the cells were removed and weighed. The ratio of cpm/g cells: cpm/ml media was calculated. To measure the effect of ClO₂ upon iodide organization, cells were grown in various concentrations of ClO₂. 125I- was added and allowed to incubate for 24 hrs. Iodide incorporation was then measured and expressed as 125I incorporation per mg DNA. ClO₂ did not inhibit iodide uptake or organization, suggesting that the suppression of radioiodide levels may involve other aspects of thyroid function or regulation. (This abstract does not necessarily reflect EPA policy).

CHRONOPHARMACOLOGY OF PHENYL BUTAZONE: INFLUENCE OF MELATONIN. N.S. Dhani, N.A. Keranyi, D.C. Holley, and G. Seuer. Palmer College of Chiropractic, Davenport, IA, U.S.A.; Banting Institute, University of Toronto, Toronto, Ontario, Canada; Sunnybrook Medical Centre, Toronto, Ontario, Canada; and Department of Biological Sciences, San Jose State University, San Jose, CA, U.S.A.

It is well documented and generally accepted that body function and constituents of plasma and urine demonstrate pronounced circadian rhythmology. However, the effect of the time of drug administration and its influence upon pharmacokinetic parameters and drug biological effects have received scant attention. We therefore, studied the pharmacokinetics of phenylbutazone (PB), and the effects of PB administration to rats pretreated with melatonin (M). Male Wistar rats (200–250 g) were pretreated (i.v., 16:00) for 14 d with M (100 μg/kg) to induce melatonin rhythm for an additional 6 d with PB (i.v., 16:00). On the day subsequent to the last PB injection blood samples were taken at 00:00, 06:00, 12:00 and 18:00 and analysed for Hot., total WBC, urea, and aspartate aminotransferase. Rats receiving M showed statistically significant differences for all parameters at all time points except at 12:00. This indicates that rats receiving M and presumably desynchronised, show altered time dependent sensitivity to the effects of PB. In a separate study rats were given PB (i.v., 16:00) at 12:00 for 6 d and on d 7 sampled to determine the circadian rhythm of plasma corticosterone (B). M treatment resulted in dose dependent lower B and considerable dampening of the rhythm amplitude. PB treatment resulted in lower B at 06:00 and 00:00 and a much greater amplitude. The half-life of elimination (min.-s.E.M.) of i.v. administered PB (100 μg/kg) ranged from 4.0±14 at 12:00 to 11.5±5 at 00:00 (11.0±2, 12:00). These observations emphasize the necessity of considering changes in pharmacokinetics and -dynamics of commonly used drugs when given to patients undergoing profound disturbances in their biological timing mechanism.


Studies were conducted to determine the effects of immobilization on the serum levels of corticosterone, aldosterone, electrolytes and osmolality. Male rats were immobilized in Broome-style restrainers for 0, 10, 30, 60, and 240 minutes. Radioimmunoassay (RIA) of aldosterone resulted in a mean value of 8.3 ng/dL at time 0, a peak value of 25.6 ng/dL at 30 minutes and a decline to 15.1 ng/dL at 240 minutes. Corticosterone RIA resulted in a mean value of 9.6 μg/dL at time 0, a peak value of 54.7 μg/dL at 30 minutes and a decline to 18.8 μg/dL at 240 minutes. Statistically significant (p < 0.05) increases in aldosterone and corticosterone levels at 10, 30 and 60 minutes suggest an acute response to immobilization. The decrease in hormone levels at 60 and 240 minutes suggests animal acclimation to the restraint tube. Significant changes in electrolyte concentrations include elevated sodium levels at 60 and 240 minutes, increased chloride at 60 minutes and decreased calcium levels at 240 minutes. Osmolality levels did not vary significantly. Results of this study suggest that immobilization may bias the interpretation and value of some physiologic parameters.


Hexachlorobenzene (HCB) exposure has been shown to alter the normal concentrations of parathyroid hormone and vitamin D₃ in rats and to result in osteoporosis in man. Experiments were undertaken to investigate the effects of HCB on the homeostatic mechanism of calcium metabolism and determine its effect on bone in rats. Fischer 344 rats were dosed with 0, 0.1, 1.0, 10.0 or 25.0 mg HCB/kg body weight 5 days/wk for 5, 10 or 15 wks. Rat body wts were not affected at any dose level or length of exposure. Liver weights were significantly elevated above control values at the 3 higher dose levels at all 3 time periods. Kidney wts were significantly elevated at the 2 higher dose levels at 10 and 15 wks. Parathyroid hormone and 1,25-dihydroxy-vitamin D₃ were measured in the 5 wk exposure group and found to be significantly elevated in the 3 higher dose levels. Wet femur wt was not affected at any of these dose levels. The femur volume was significantly decreased at the 5, 10 and 15 wk time periods while femur density was significantly increased with HCB exposure. Dry femur density was also increased in the cases where wet femur density was increased. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
ACTH - INDUCED ADRENAL ADENOMAS IN RATS TREATED WITH TRILOSTANE. B.A. Mayes, R.J. Fabian, J.D. Frantz*, L. Rapp and H.P. Drobeck. Sterling-Winthrop Research Institute, Rensselaer, NY and *Rohm and Haas Co., Spring House, PA.

Trilostane (Modrastane\textsuperscript{TM}, Win 24,540) is a reversible inhibitor of adrenal steroidogenesis (AS) which acts through competitive inhibition of the 3β-hydroxysteroid dehydrogenase enzyme system. Reduced AS lowers circulating corticosteroid levels and, consequently, increases pituitary secretion of ACTH which produces adrenal hyperplasia (AH) and adenomas in rats after chronic high doses of trilostane. The present study was designed to further substantiate the mechanism of these effects. Trilostane was administered by gavage in 1% gum tragacanth (1% G.T.) at dose levels of 50 and 100 mg/kg to 30 normal (intact) and 30 hypophyseotomized (hypo) male rats/group. Controls received 1% G.T. All medications were administered as 5 ml/kg/daily for 6 months. A satellite group of 12 intact rats was medicated for 9 days at 100 mg/kg for determination of plasma ACTH levels. Controls received 1% G.T. All intact rats responded to trilostane with AH including cortical adenomas in 6/30 at 100 mg/kg. As expected, plasma ACTH levels were higher ($p<0.001$) in treated rats than in controls. In hypo rats, adenals were hyperplastic and hypophyseal did not further alter the morphology. Thus, the mechanism of trilostane induced AH and adenoma formation is clearly a hyperstimulation of the adenals by the tropic hormone ACTH and not a direct trilostane effect.


The influence of prolactin on the porphyrin secretion in the Hardierian gland of mice was examined. The Hardierian gland of mice treated orally with butyrophennones, timiperone and haloperidol, appeared grossly to be dark tanish in color and diffusely speckled with dots of pigments. Microscopical examination revealed an increase in amount of porphyrin in the alveolar lemm. It is well known that butyrophenones elevate the blood prolactin level in rodents, and also that pituitary isografts cause long lasting elevation of the blood prolactin level. When mice were grafted with four pituitaries under the kidney capsule, the porphyrin pigment in the Hardierian gland was increased. The results suggest that the increase in porphyrin deposition in the Hardierian gland induced by timiperone and haloperidol treatment was attributed to the elevation of the blood prolactin level. Spectrofluorometric analysis of the porphyrin content of the Hardierian gland in male mice revealed that both pituitary grafts and timiperone increased the porphyrin content of the gland. However, the administration of dopamine agonist, bromocriptine (CB 154), to male mice which had been grafted with pituitaries or treated with timiperone prevented the increase of the porphyrin content of the gland. These data suggest that prolactin accelerates the secretion or synthesis of porphyrin in the Hardierian gland of mice.


Carbendazim (MBC) is a systemic fumigant in wide agricultural use. Subchronic dosing has marked effects on testicular parameters at levels above 150-200 mg/kg/d. The purpose of this study was to explore the influence of MBC on pituitary (pit) and brain components of the male reproductive system. At 21 d, Long-Evans rats received daily peroral doses of 50, 100, 200 or 400 mg/kg MBC for 11 wks. Controls received corn oil vehicle. Serum and anterior pits and hypothalami were analyzed for LH, FSH, prolactin (Prl), TSH and hypophysial gonadotropin-releasing hormone (GnRH). No changes were seen in serum LH, Prl, TSH or testosterone. At 200 and 400 mg, serum FSH increased. Prl FSH, Prl and TSH were unchanged. Prl LH was increased at 200 and 400 mg. No effects on GnRH were found in mediobasal hypothalamus, which contains the region from which GnRH is normally released. But, in the GnRH cell-body-containing rostral area, there was an initial rise followed by a dose-dependent fall. The data indicate higher doses of MBC affect certain endocrine parameters. The nature of the changes suggest that the effect of MBC on the testes is a primary one, but that sustained elevations in FSH could affect Sertoli cell-linked spermatogenesis.

Increased body burdens of heavy metal cations such as Ni²⁺, Cd²⁺ and Zn²⁺ are known to adversely affect reproductive function in several species. The effects of these metals on gonadal function are well documented. In contrast, little is known about their possible direct pituitary (PIT) effects. The purpose of this study was to determine, in vitro, the effect of Ni²⁺, Cd²⁺ and Zn²⁺ (50 μM) on both basal and KCl-stimulated LH, TSH and Prolactin (Prl) release. PIT fragments from adult male Long-Evans rats were perfused in a control buffer for 2 h. This buffer was then replaced with a similar buffer containing one of the metal ions. Basal and stimulated LH release were unaffected by Ni²⁺ and Zn²⁺, however, Cd²⁺ caused an increase in basal LH. Basal Prl release was decreased by Ni²⁺ and Zn²⁺, while Cd²⁺ resulted in increased Prl release. KCl-induced Prl release was reduced by Zn²⁺, but unaffected by Ni²⁺ and Cd²⁺. Similarly, basal TSH release was reduced by Zn²⁺, but unaffected by Cd²⁺ or Ni²⁺. Following exposure to Zn²⁺, a rebound effect was noted for Prl, LH and TSH in that both basal and KCl-stimulated secretion were increased when the metal buffer was removed. These results show that the metal cations tested do have a direct effect on PIT hormone release and that this effect varies depending upon the metal and hormone being evaluated.


DNB treatment has been shown to cause testicular atrophy. It is not known whether these changes are due to a direct effect of this compound on the testis or the pituitary. This study evaluated the endocrine status of male rats following a single oral dose of DNB (32 mg DNB/kg). Rats were sacrificed 3 h, 1, 7 or 14 days later. Serum and pituitary LH, FSH and prolactin concentrations were determined. Testosterone (T) and androgen-binding protein (ABP) concentrations in serum, caput epididymis, interstitial fluid (IF) and seminiferous tubule fluid (SNF) were also determined. In vitro hCG-stimulated T release was determined in the decapsulated testis. Pituitary hormone concentrations were unaffected at any time after treatment. Serum FSH was elevated at day 14. There was a transient decrease in serum T at 1 day and a significant increase at days 7 & 14. IF, SNF and caput T were increased at 7 & 14 days. hCG-stimulated T release was increased at 1, 7 & 14 days. ABP levels were increased at 7 & 14 days in serum and IF. ABP in SNF was increased at 1 & 7 days, but returned to control levels by day 14. Caput ABP was unaffected at any time. These data demonstrate significant changes in testicular endocrine function with little or no change in pituitary hormone secretion.

561 DEVELOPMENTAL TOXICITY OF RHODAMINE 123 IN DROSOPHILA MELANOGASTER W.W. Zhang and R.D. Hood. Department of Biology, University of Alabama, Tuscaloosa, AL. Sponsor: R.S. Filler

Rhodamine 123 (Rh 123) is a tarotogenic cationic dye that inhibits mitochondrial energy metabolism. Rh 123 was tested for developmental effects in D. melanogaster (Oregon-R) to complement mechanistic studies of rhodamine dyes in mammals. Larvae were reared in vials containing 1 g diet plus 5 ml H₂O. Six dosage groups were used (0.0-1.0 mg Rh 123/vial, mixed with the diet medium). Larvae (130 or more/group) were kept on test diets until metamorphosis. Eclosion timing, mortality, sex ratio, and adult morphology were examined. Rh 123 treatment resulted in dose-related increases in larval lethality (ranging from 10% for controls to 30% at the high dose), eclosion delay (up to 48 hr), and external defects of adults (P < 0.05). There were no differences in incidence of humeral defects, but other bristle defects (missing, extra, doubled, bent, stubby, split, or colorless) were the main anomalies seen (from 3% for controls to 12% at the high dose). At the high dose, Rh 123 induced 7.6% wing defects (missing, notched, or distorted) plus 1.3% other malformations, while no such defects were seen in controls (P < 0.05). Sex ratio was unaffected. Such data suggest that D. melanogaster may be useful as a model system for studies of mitochondrial-mediated developmental toxicity.

562 APPLICATION OF POSTIMPLANTATION Rodent EMBRYO CULTURE SYSTEMS TO IDENTIFY PRENATAL TOXIC COMPONENTS OF HAZARDOUS WASTE SITES. T Rick Irvin, R Roop and A Akgerman, Lab of Toxicology, Vet Anatomy Dept (TRI) and Chem Eng Dept (RR,AA), Texas A&M, Coll Sta, TX. Sponsor: A. Ray

We have employed in vitro rodent embryo culture to assess the developmental toxicity of creosote, a wood preservative containing a large percentage of toxic waste sites in the US. Whole Sprague-Dawley concepti (day 10 embryos) were cultured in 50% Wamsley's media and 50% fresh rat serum supplemented with rogent hepatic S-9 fractions. After 24 hours of culture over a 32-fold dose range, embryotoxic effects of creosote included: decreased crown-rump length, deformities of the telencephalon, and absence of red blood cell circulation through the yolk sac. Creosote was subsequently fractionated by size exclusion chromatography, and each fraction was tested for in vitro embryotoxicity. Creosote fractions accounting for less than 20% by weight were isolated which retain over 90% of the developmental toxicity of the unfractonated sample. These findings document the usefulness of this system to assay complex mixtures of hazardous waste and more precisely pinpoint those creosote components which, through targeted reclamation processes, may be eliminated. Supported by TEES Engineering Toxicology Division (Pub 86-4).
Waipuro acid (VPA), cytochalasin D (CD), and 7-hydroxy-acetylaminofluorene (7-OH-AAF) cause abnormal closure of the anterior neuropore in rat embryos in vitro. We attempted to determine whether VPA status was a common modulating factor in the dysmorphogenesis produced by these compounds. Each compound increased the incidence of ONT; VPA (11%), CD (50%), and 7-OH-AAF (67%). VPA and 7-OH-AAF also produced other abnormalities. Only CD altered GSH levels, differentially reducing embryonic (E) levels by 50% compared to 20% in the visceral yolk sac (VYS). Depletion of GSH with L-buthionine-S,R-sulfoximine (BSO) increased the incidence of ONT in CD-treated conceptuses (80%) and elicited an additive increase in GSH. 2-2oxothiazolidine-4-carboxylate decreased the incidence of ONT with CD (30%) but did not restore GSH levels. The incidence of ONT was not affected by VPA. 7-OH-AAF although addition of BSO resulted in further decreases in E- and VYS-GSH for both compounds and a 20% increase in mortality with VPA. The data indicate that although the modulation of intracellular GSH alters the incidence of ONT in rat embryos in culture, the mechanism by which dysmorphogenesis occurs and the roles thiols play in determining the etiologic or physiologic outcome may differ for each teratogen. NIH grants ES-04041 and ES-03157.


We have employed in vitro embryo culture to identify prenatal toxic components of Iva angustifolia, an abortive range plant toxic when consumed by cattle during the second to third trimesters of gestation. Whole Iva plants were air dried and extracted with ethanol or chloroform [mixture ratio, 1:30 (w/v)]. Solvent extracts were fractionated by size exclusion chromatography; fractions were dried under nitrogen for in vitro embryotoxicity analysis. Embryotoxic effects were restricted to 17% by weight of total Iva extracts with direct toxicity observed in the absence or presence of rat hepatic S-9 culture supplementation. Over 100-fold dose ranges of some toxic fractions identified, prenatal toxic indices observed included loss of yolk sac circulation, decreases in somite development, and decreased crown-rump length. These findings support the first resolution of those Iva components responsible for animal disease and document the usefulness of this system to rapidly identify toxic constituents of complex plant toxinant mixtures. Supported by TAE Project H 6735.

Chick Embryo Retina Cell Culture as an In Vitro Teratogen Screen. G.P. Dauston and J.E. Yonker Procter & Gamble, Cincinnati, Ohio.

We have applied the chick embryo retina cell culture developed by A.A. Moscona (Exp. Cell Res., 22:955) as an in vitro screen for teratogens. During the first day of culture, cells dissociated from the neural retina of day 6 chick embryos form aggregates of a specific diameter and histological organization. Over the next several days the aggregates grow and the cells differentiate morphologically and biochemically according to a developmental timetable. The culture system undergoes many fundamental processes of development - including cell-cell interactions, migration, growth and division, gene expression (differentiation) - and should be sensitive to a wide variety of teratogens. Measurable endpoints include number and size of aggregates formed after the first day in culture; and growth (protein or DNA content), histogenesis and biochemical markers of differentiation after several days in culture. The screen is affected by substances which interfere with cell-cell communication (TPA); alter gene expression (bromodeoxyuridine); inhibit cell division (colchicine); are cytotoxic (mercuric chloride); and are pharmacologically active (sodium selenioate). These agents alter different endpoints, and it is therefore possible to gain information on teratogenic mechanism from the assay.


Primary Sertoli-germ cell co-cultures isolated from 28-30 day old male rats were treated with CdCl2; dibromochloropropane (DBCP) and two presumptive metabolites: 1,3 dibromopropane (1,3 DBP) and 1,2 dibromopropane (1,2 DBP); methoxy ethanol (ME) and ethoxy ethanol (EE) along with their major metabolites methoxyacetic acid (MA) and ethoxyacetic acid (EA), respectively.

Toxicity was assessed by measuring germ cell release from the Sertoli cell substratum as described by Gray et al. (Toxicol. Appl. Pharm. 79, 490(1986)). Co-cultures treated with CdCl2 exhibited germ cell detachment between 0.5 and 4 μM. DBCP and 1,3 DBP induced germ cell release at 100 μM while 1,2 DBP was ineffective at 1000 μM. Neither ME nor EE caused an increase in germ cell detachment at 100 μM. However, MA and EA showed germ cell release at 10 μM

MA and EA showed a stage specific release of germ cells (pachytene spermatocytes were conspicuously absent). The other chemicals tested showed non-specific germ cell release. The coculture method gives good correlation of dose with cytotoxicity. Although mechanistic aspects of cytotoxicity cannot be easily addressed from the results of this experimental system, it may be a sensitive initial screening test for suspected testicular toxins. NIEHS R0104141.
LACTATE AND PYRUVATE PRODUCTION AS SPECIFIC INDICES OF ALTERED SERTOLI CELL FUNCTION IN VITRO AFTER THE ADDITION OF TESTICULAR TOXICANTS J Williams**, D.C.H McBrien*** and P.M.D Foster*, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK* and Brunel University, Uxbridge, Middlesex UK**.

Sponsor: E.A Lock

Lactate (L) and pyruvate (P) are hormonally stimulated secretory products of the Sertoli cell (SC), essential for germ cell survival. 1,3-dinitrobenzene (DNB) and mono-(2-ethylhexyl) phthalate (MEHP), are compounds whose testicular target is the SC. Studies were conducted to ascertain if L and P production by SC in vitro could be modified by toxicant addition at doses which did not induce cytotoxicity. DNB produced a dose-related (10^-6^-10^-4M) increase in L (maximally 3 fold) and P (6-8 fold) over a 24hr period. In the same period, MEHP (10^-6^-10^-4M) only affected L (2-3 fold). Using 10^-4M DNB or MEHP the earliest significant increases could be observed in L production at 1hr for DNB and 2hr for MEHP and P Production at 2hr for DNB. The inactive isomer of DNB (1,2- and 1,4-) did not produce this effect nor did methoxyacetic acid, a germ cell toxicant. The measurement of L and P may offer a sensitive and specific method for assessing altered SC function in vitro.

EFFECT OF ME AND MA ON RAT SERTOLI-GERM CELLS CO-CULTURE DNA SYNTHESIS IN VITRO. S. Favittiranon and M.J. Brabec. Program in Toxicology, The U. of Michigan and the Reproductive Toxicology group, Dept. of Chem., Eastern Michigan U., Ypsilanti, MI.

Methoxyethanol (ME) reduces fertility and testis weights in rat and rabbits. The toxic metabolite of ME, methoxyacetate (MA) causes the preferential loss of pachytene spermatocytes in rat testicular cell co-cultures and IN VIVO. Inequality in DNA synthesis may play a major role in this process. We examined the effects of ME and MA on mature rat testicular co-culture DNA synthesis. Co-cultures isolated from 28 day old rats were exposed to ME and MA, 1, 5 and 10 mM for 24 hr. DNA and 3H-thymidine incorporation were measured on TCA-precipitated fractions. Neither ME nor MA had significant effects on DNA contents. However, 3H-thymidine incorporation was decreased to 65±11% and 59±0% by 5 and 10 mM MA. ME had no effect. These results suggested that MA inhibits DNA synthesis without measurable change in DNA. The effect was specific for the spermatocyte population because Sertoli cell DNA synthesis is inactive in adult animals. The results also confirm the lack of toxicity of ME.

Supported by NIEHS #ES04141 and The General Motors Corporation.

EFFECT OF MONO-(2-ETHYHEXYL)PHTHALATE (MEHP) ON THE HORMONAL RESPONSIVENESS OF RAT SERTOLI CELLS IN VITRO P.M.D Foster and S.C Lloyd, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: E.A.Lock

Certain phthalate esters (e.g., MEHP) are known to damage the testis with the Sertoli cell as the primary target. Moreover, these compounds exhibit an age sensitivity in their ability to produce testicular damage, with immature animals being more affected. The Sertoli cell is the principal testicular target for the action of follicle stimulating hormone (FSH), which is known to be of lesser biological importance in mature animals for the maintenance of spermatogenesis. The effects of MEHP on FSH-stimulated production of cAMP in immature rat Sertoli cell cultures was studied. When MEHP was added to cultures maximally stimulated with hormone, a significant, dose-related diminution of response occurred in the range 10^-7^-10^-4M, doses which did not affect the viability of the cells as estimated by ATP content. The effect of MEHP was demonstrated within 8 hours over the whole FSH dose-response curve. MEHP did not interfere with the assay of cAMP nor directly with FSH. The toxic effects on Sertoli cell function were obtained in vitro at low concentrations of MEHP. These effects may play a role in the specific cellular toxicity and age-dependant changes observed with MEHP in rat testis.
DITHIOCARBAMATE INHIBITION OF CADMIUM-INDUCED CHANGES IN SPERMATOZOA ChOLINE ACETYLTRANSFERASE. S. G. Jones, M. M. Jones, Dept. of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN, C. Dwiwi and S. S. Kumar, Dept. of Medical Genetics, Meharry Medical College, Nashville, TN and E. M. Walker, Jr., Dept. of Laboratory Services, J. L. McClellan Veterans Administration Medical Center, Little Rock, AR

The administration of sodium N-methyl-D-glucamine dithiocarbamate subsequent to the administration of cadmium chloride to male rats, inhibits the cadmium-induced decreases in spermatozoan choline acetyltransferase (CHAT) levels. The protection obtained was most pronounced in the cauda, was somewhat less noticeable in the corpus and least in the caput. In the caput, under the conditions used, protection was only detectable up to 48 h. Histological examination of the tissues largely confirmed these results and indicated partial protection, though the enzymatic levels were more sensitive indicators of damage than histopathological indicators.


The chromat of mammalian sperm nuclei is stabilized and possibly protected by disulfide bonds. Interspecies differences in sperm nuclear stability may have relevance in reproductive risk assessment. To compare the structural stability of 5 species of sperm, we induced sperm nuclear decondensation in vitro with the disulfide reducing agent DTT, and SDS. As the sperm swelled we quantified changes in light scattering properties by flow cytometry (Ortho 50H) and in physical dimensions by microscopy. The time required for thiol-induced decondensation varied by species with human<chinchilla<mouse<hamster<rat. We also microinjected sperm nuclei into hamster oocytes to determine the time course of decondensation in vivo. The time required for oocyte-induced decondensation varied by species in a manner similar to that seen in vitro: human<mouse<chinchilla<hamster<rat. Human and mouse sperm decondensed within 15 to 30 min of injection, while chinchilla and hamster sperm did so within 45 to 60 min. In contrast, none of the rat sperm nuclei decondensed within 60 min. All but the rat sperm nuclei transformed into male pronuclei within 3 hr. We conclude that these species differences in sperm nuclear stability are related to the extent and/or differential release of disulfide bonds.

NITOREDUCTION OF 1,3-DINITROBENZENE BY RAT TESTICULAR CELL CULTURES AND ITS RELATIONSHIP TO TARGET ORGAN TOXICITY. P.M.D Foster, S.C. Lloyd and M.S. Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: E.A. Lock

1,3-Dinitrobenzene (DNB) will produce testicular damage in rats, following oral administration with the Tertol cell as the initial target. The morphological effects can be reproduced in Sertoli-germ cell co-cultures by addition of DNB (5x10^6 M). Experiments with [14C] DNB (10-4-10^-6 M) were conducted in rat testicular cell cultures to determine if metabolism was involved in this toxicity. Radiolabel was taken up by plated cells, maximally 13% of applied dose. Analysis of culture media from both Sertoli and Sertoli-germ cell cultures revealed the production in 24 h of 2 major metabolites. These metabolites were isolated and identified as m-nitroaniline and m-nitroacetanilide. When these compounds were tested in vitro, (up to 10^-4 M) they were not toxic to Tertol. Preliminary evidence suggests that m-nitroaniline production occurs similar in vivo, effects to DNB at approximately 1/5th of the dose. This is the first report of xenobiotic metabolism by Sertoli cell cultures and we postulate that a reactive intermediate produced in situ may be responsible for the target cell toxicity observed in vivo.
Intrauterine position relative to male siblings appears to render offspring of polytocous rodents more sensitive to the teratogenic, embryolethal or growth-retarding effects of common developmental toxins (e.g., ethanol, butamediol, aspirin, Dieldrin). To determine the effect of intrauterine sibling contiguity on the endpoints of any glycol ether developmental toxicity doses (0, 3, 30 or 300 mg/kg) of 2-phenoxethanol (POE) were given by gavage to Long-Evans rats from day 6 to 15 of gestation. POE caused a significant increase in fetal anomalies (hydromyelia, and variant ossification patterns of the skull and sternum) considered to be suggestive of aterated growth. The expression of anomalies was significantly (P < 0.05) correlated to the prevalence of males within a litter (r=0.40). A significant birthweight depression was detected among male pups at the highest (300 mg/kg) dose; and male pups which were contiguous to two male siblings were preferentially and most severely affected (absolute and relative birth weights were decreased 3.24 and 2.29 g, respectively, which was 88% of control weights). Doses of up to 300 mg/kg of POE had no observable or significant effect on feed consumption or maternal body weight gains during gestation. These data support the concept that intrauterine position, particularly with reference to contiguous male siblings, affects the expression of developmental toxicity in polytocous rodent species. Supported by March of Dimes grant 15-51.

The toxicity of DEHP during lactation to dams and their suckling pups was assessed after five daily oral doses (2 g/kg) of DEHP to lactating rats on days 2-6, 6-10, or 14-18 of lactation. Decreased body wt was observed in dams and pups, and increases in palmityl Co A oxidase and carnitine acetyltransferase activities in pups (5-8 fold) and pups (2-fold) were not due to decreased food consumption by the mother. Six hrs after the third dose of DEHP (2 g/kg), rat milk contained 220 ± 20 µg/ml DEHP and 236 ± 10 µg/ml MEHP (mean±SEM). The rat milk contained 11 µg/ml DEHP and 76±12 µg/ml MEHP (n=9). The high milk/plasma ratio (>200) for DEHP indicates very efficient excretion of DEHP into rat milk. DEHP and MEHP were not detectable in the pups' plasma. Mammary gland wt was reduced by 25% in DEHP-treated rats. Milk lipid and solids were increased compared to control and pair-fed rats, while both DEHP-treated and pair-fed rat's milk had increased protein and decreased lactose concentrations compared to ad lib fed controls. Therefore, DEHP dosing during lactation leads to the transfer of large amounts of DEHP into rat milk and causes changes in mammary gland and milk composition.


Because of 4-NPI's structural similarity to a known human teratogen, and developmental effects in rabbits, a rat teratology study was conducted. 4-NPI was administered to pregnant Sprague-Dawley rats by gavage in carboxymethyl cellulose at dose levels of 0, 10, 50, 200 mg/kg/day on gestation Days 6-15. On Day 8, Vitamin A (100,000 IU, Aqusol A) was used as a positive control. Developmental toxicity, including malformations, mortality, and growth retardation, was seen in the Vitamin A group. At 200 mg/kg/day, 4-NPI produced maternal toxicity as evidenced by remarkable clinical observations and lower mean body weight gains and food consumption. Developmental toxicity was also seen in the form of fetal mortality and growth retardation. Four fetuses had small/missing pinnae with no inner ear involvement. One fetus had a short tail. Slight maternal toxicity (lower body weight gains) was seen at 50 mg/kg/day; however, no developmental toxicity was seen. At 10 mg/kg/day, 4-NPI produced no maternal or developmental toxicity. A single malformation, anophthalmia, was seen in the control group.


Di-n-propylphthalate (DPP), di-n-propylphthalate (DPP), and di-n-octylphthalate (DOP) were tested by the "Fertility Assessment By Continuous Breeding (FACB)" protocol using CD-1 mice. FACB consists of 4 tasks: dose finding, continuous breeding, identification of the affected sex, and offspring assessment. At the conclusion, animals are necropsied and fixed for histopathological examination. The route of administration was feed. DPP was tested at 0, 0.5, 1.25, and 2.5% dose levels, while DPP and DOP were tested at 0, 1.25, 2.5, and 5.0% dose levels. DPP severely affected fertility and reproduction at all dose levels in both sexes. Males in the 2.5% DPP group revealed degeneration of seminiferous tubules, testicular interstitial cell hyperplasia and reduced epididymal sperm counts. DPP was less potent than DPP. Pairs exposed to 5% DPP for 13 weeks delivered no litters. Interestingly, females previously exposed to 5% DPP then mated to control males had 0% fertile matings vs. 67% fertile matings when control females were mated with 5% males. The corresponding value for control males vs. control females was 72%. DPP administered in feed at up to 5.0% did not affect fertility or reproduction in 2 generations.

Butyl Benzyl Phthalate at 0.03, 0.09, 0.28, 0.83 or 2.5% in the diet was administered for 26-weeks to male F-344 rats in order to determine its toxicity. In a concomitant experiment butyl benzyl phthalate was administered at 0.03, 0.28 and 2.5% in feed to male F-344 rats, who were allowed a two day recovery period before being co-housed with virgin female rats for seven days to determine the effect of BBP on the ability of male rats to breed and reproduce. Concurrent controls for both studies were fed the basal diet alone. Toxicity, compared to controls, was associated with only the highest (2.5%) dose level of BBP in both studies. Weight gain was depressed for the high dose groups of animals throughout the duration of the studies. Other toxic effects of feeding 2.5% BBP in the diet to male F-344 rats included decreased testicular size and abnormal morphology, histopathological evidence of virtual aspermia, total suppression of male reproductive capacity and alterations in hematology values.


DEHP (CAS 117-81-7), a widely used plasticizer, has previously been identified as a reproductive and developmental toxicant. In the present study, P sub-0 dams were exposed to DEHP throughout gestation (days 0-20) at dietary levels of 0%, 0.25%, 0.50% or 1.00% DEHP resulted in average daily doses of 0, 164, 313 or 575 mg/kg/day, respectively. F sub-1 pups were examined for body wt., viability, physical development, locomotor activity, and reproductive performance, including F sub-1 growth and viability through postnatal day (pnd) 4. No adverse effects were observed at 0.25% DEHP. Adverse effects at 0.50% and 1.00% included maternal toxicity (reduced food intake; reduced wt. gain), and reduced pre- and neo-natal viability of the F sub-1 offspring. On pnd 1, a dose-related reduction in litter size and pup wt. were observed, and pups in the 1.00% group weighed significantly less than controls. No residual or delayed effects were observed on maternal status (pnd 4-28), or F sub-2 growth, viability, development or reproductive performance (pnd 4-128). Supported by NTP/NCTR/NIHES Contract No. 222-80-2031(C).

FERTILITY EFFECTS OF ETHYLENE GLYCOL MONOMETHYL ETHER (EGME) IN THE MALE MOUSE. A.R. Nikura, D.P. Wattle, and J.D. Zaneveld, University of Illinois at Chicago, and Rush-Presbyterian-St. Luke's Medical Centre, Chicago, IL.

Glycol ethers are known male reproductive toxins in rats and men.

Groups of at least eight proven male CD-1 mice received p.o. either distilled water or EGME at one of three dose levels (100, 300, or 500 mg/kg per day) for two or four weeks. Each male was paired with two females during the last eight days of dosing. The males were then euthanized, and the testes, epididymides, seminal vesicles, liver, kidneys, and spleen were removed and weighed. The motility, forward progression, and concentration of cauda epididymal spermatozoa were evaluated. The females were necropsied at day 10 of pregnancy.

Epididymal sperm parameters deteriorated, and testes weights decreased significantly with increasing EGME doses after both two and four weeks of exposure. No significant differences in seminal vesicle, liver, kidney, and spleen weights, or fertility were observed between any of the groups.

These results suggest that EGME exhibits toxic effects on the male reproductive system of mice mainly at the level of the testes, when administered at the tested dose levels and durations of exposure.


Sprague-Dawley COBSO® rats were used to assess the potential adverse effects on the reproductive capability of the F0 generation and the development of the offspring through weaning. Three groups (30 males, 30 females) of parental animals were exposed to Diethylene Glycol via the drinking water at dosages of 0, 156, 500 and 1500 mg/kg/day for 73 days prior to pairing and continuing to termination. Fifteen females per group received a Caesarean section on gestation day 20 and fetuses were examined for skeletal and soft tissue abnormalities. The remaining females were allowed to deliver. The gonads, kidneys and liver were weighed from F0 animals and from 15 randomly selected F1 pups/sex/group at weaning. Administration of Diethylene Glycol did not adversely affect parental survival, general clinical condition or water intake. F0 female body weights at all dose levels were comparable to controls. A treatment-related decrease in body weight gain occurred during the initial week of treatment in the mid and high dose females. Statistically significant increased relative kidney weights suggest a potential treatment-related effect on the F0 males and the F1 male offspring at the high dose level. Diethylene Glycol had no adverse effect on the reproductive capability of the F0 generation or the development, survival and growth of the F1 generation to weaning.
2-Methoxyethanol (ME) was applied on the backs of Sprague-Dawley male rats, at dose levels of 5.0 and 2.5 g/kg on occluded sites, and 5.0 g/kg on uncovered sites. Because deaths occurred among rats with occluded test sites, dosing of this group was discontinued following 2 exposures to 5.0 g/kg or 8 exposures to 2.5 g/kg; among rats with uncovered test sites, no deaths were observed at 5.0 g/kg. Reproductive effects were evaluated by mating trials with untreated females and by examination of male reproductive tissues. Caudal epididymal sperm counts (ESC) and morphology (ESM), testicular spermatid count (TSC), and reproductive organ weights were evaluated on weeks 4, 7, 10 and 14. Fertility was reduced in males treated at 5 g/kg, with or without covering of the treatment site. Other treatment-related effects included a decline in ESC and TSC, a reduction in weights of testes and epididymides, and an increase in the number of sperm with abnormal morphology in rats exposed to ME, either with or without occlusion. Studies on reversibility of these effects are in progress.

The reproductive toxicity of concentrated tea infusion (CTI) and CTI combined with methylmethanesulfonate (MMS) was evaluated in male BALB/c mice. CTI was made from Chinese green tea by vacuum distillation of tea infusion (65-75°C, 320 mmHg). Compared with no treatment, a low dose of CTI alone (0.5 g/kg/day x 5, p.o., equivalent to an average human daily intake) had no effect. A high CTI dose (4.0 g/kg/day, 65% of the LD50 in mice) induced sperm abnormalities and decreased sperm motility (p < 0.05). Histological examination revealed swollen mitochondria, vacuolated smooth endoplasmic reticulum, and abnormal acrosomes in germ cells. CTI also had a clastogenic effect on primary spermatocytes and inhibited lactate dehydrogenase isozyme X (LDH-X) activity in sperm (p < 0.05). CTI did not affect fertility or sperm morphology of F1 offspring of treated males. Combined with MMS (30 mg/kg/day x 5, i.p.), even the low CTI dose promoted the gonadotoxic effects of MMS, inhibiting parental fertility and causing F1 sperm defects (p < 0.05). Thus, Chinese green tea appears to contain components that could influence spermatogenesis, but effects were seen only at a dose that was generally toxic.
The effect of repeated opiate compound administration on spermatogenesis and reproductive tract morphology were investigated. Male Charles River CD rats were administered daily intravenous injections of fentanyl citrate (10, 40, 212 μg/kg/day) or morphine sulfate (2, 13.5, 71.6 mg/kg/day) for 14 consecutive days. Sperm concentration, motility and morphology were evaluated. Histological examination of sections from testes and epididymides (head and body) was performed. Each dosage level of both opiate compounds produced adverse effects on male spermatogenic parameters. Decreased sperm concentration and decreased sperm motility as well as increased abnormal sperm morphology were observed in treated groups. No test article related histological effect was found in the testes, however, luminal debris which appeared to be composed of degenerating heads of spermatozoan or late spermatids were observed in the epididymal ducts.

Pre-implantation losses induced in F-344 rats by methyl chloride (MeCl) are caused by its cytotoxic rather than genotoxic effects on sperm. This study examined whether the cytotoxicity is due to the testicular or germ-cell toxicity of MeCl. Groups of 18 males were exposed to 3000 ppm MeCl 6 hr/day for 5 days, with and without concurrent treatment with the anti-inflammatory agent BW755C (10 mg/kg, i.p. 1 hr pre- and post-exposure); BW755C was known to inhibit the induction of epididymal inflammation by MeCl. Six males from each group were killed weekly for 3 weeks. Toxic effects of MeCl on the testis were shown by histopathology (weeks 1-3), decreased relative organ weight (week 3), and decreased daily sperm production (weeks 1-3); these effects were not prevented by BW755C. In both the MeCl and MeCl-BW755C treatment groups, tubules devoid of sperm were observed in region 5 of the epididymis at week 2, and in regions 6A and 6B at week 3. Sperm in the vas deferens of both groups at week 3 showed decreased numbers, decreased motility and increased morphologic abnormalities. In conjunction with known epididymal transit times for F-344 rat sperm, these data indicate that the induction of pre-implantation loss by MeCl at weeks 2 and 3 post-exposure is likely to result from cytotoxic effects on sperm located in the testis at the time of exposure.

A single dose of DNB has been shown to cause testicular toxicity in the rat primarily through an effect on the Sertoli cell. The consequences of continued dosing were examined in a 90-day oral dosing study in rats using dose levels of 0, 0.1, 0.5, 2.0 and 5.0 mg/kg/day. Histological examination revealed severe testicular atrophy in the rats dosed with 5.0 mg/kg/day, with a similar but less consistent effect at 2.0 mg/kg/day. There were no effects in rats dosed with 0.5 or 0.1 mg/kg/day. Another group of animals, dosed with DNB for 90-days at the same dose levels, was maintained untreated for an additional 9-days and then killed and their testes subjected to histological evaluation. The rats dosed with 5.0 mg/kg/day DNB showed substantial recovery, but approximately half the rats dosed with 2.0 mg/kg/day showed widespread tubular atrophy. These results indicate that substantial testicular damage may be associated with more complete recovery than that which may be seen following an initially less severe effect.

DNB has been shown to cause testicular toxicity in the rat primarily through an effect on the Sertoli cell. A study was carried out to investigate the effects of such testicular toxicity on fertility in the rat. Groups of male rats of proven fertility were dosed with 0, 5 or 10 mg DNB/kg/day for 5 days. Subsequently, at weekly intervals, 5 rats per group were mated with 2 untreated females per rat, killed and their testes subjected to histopathological evaluation. There was a decline in fertility as assessed by numbers of viable implants in the females, in the rats treated with either 5 or 10 mg DNB/kg/day which was apparent from weeks 3-8 after dosing. These reductions in fertility correlated with reductions in testis weight and epididymal sperm counts. There was a complete recovery in fertility 16 weeks after dosing. Changes in testicular histology showed a widespread testicular atrophy which was more severe at 10 mg DNB/kg/day, but paradoxically there was more complete recovery at 16 weeks after dosing in the rats treated with 10 mg/kg/day than at 5 mg/kg/day. The prolonged nature of the infertility reflects the pivotal role in the Sertoli cell in all stages of spermatogenesis.
In previous studies, 48 mg m-DNB/kg produced marked reproductive effects in young adult male rats and preliminary data suggested that slightly older rats might be more susceptible. In the present study, young adult (YA) and adult (A) male Sprague-Dawley rats, 75 and 105 days of age, respectively, were concurrently gavaged with a single dose of 0, 8, 16, 24, 32, or 48 mg m-DNB/kg. Cystic fibrosis was observed in both ages at 16 mg/kg or higher; lethality and signs of neurotoxicity were observed in A but not in YA at 48 mg/kg. At 14 days posttreatment, testicular spermatic counts, cauda sperm reserves, and testis and epididymal weights were decreased at 16 mg/kg and higher in A and at 24 mg/kg and higher in YA. Effects on sperm morphology and motility were not pronounced at 48 mg/kg as at 24 and 32 mg/kg suggesting that sperm transit time may have been affected at the higher dosage. Fertilizing ability (tested at 0 and 48 mg/kg) was lost by posttreatment wk 5 and 6 in YA and A, respectively. At 5 mo posttreatment, 3/12 YA and 1/8 A had not regained fertility. These measurements indicated a no effect level of 8 mg/kg for male reproductive toxicity and that A may be somewhat more susceptible than YA to both the general and reproductive toxicity of m-DNB.

The experimental chemical N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N-phenylurea (EDU) is known to be an effective protectant against acute and chronic follicular injury due to ozone when sprayed on intact leaves or supplied to the plants through soil application. Since many of the toxic effects of ozone are thought to be mediated, in part, by the formation of free radicals, we hypothesized that EDU might be acting as a free radical scavenger. When EDU was incubated with human neutrophils stimulated with either phorbol myristate acetate (PMA) or zymosan, a dose-dependent decrease in superoxide (O$_2^-$) production was observed. However, when EDU was tested in two cell-free O$_2^-$ radical generating systems it was not able to inhibit the O$_2^-$-mediated reduction of cytochrome c. These results indicate that EDU is not acting like a superoxide dismutase and that it is not a scavenger of O$_2^-$ radicals. The observed inhibition by EDU of O$_2^-$ production by neutrophils may be related to EDU-mediated changes in the activity and/or activation of the oxidase responsible for O$_2^-$ production.

The gene coding for the delta endotoxin of Bacillus thuringiensis was inserted into the chromosome of 2 Pseudomonas fluorescens bio-types. To investigate their potential pathogenicity, mice were given a single dose of 10$^7$ CFU/animal of the GEMP or its parental isolate. Five mice/sex were administered test microbes by one of 4 routes of exposure (PO, IP, IV, IT). Controls were administered 10$^6$CFU of test microbes by the same exposure routes. All phases of the study were carried out under physical containment conditions. After dosing, mice were held for 28 days. One mouse/sex/route was sacrificed 2, 7 & 21 days post dosing. Remaining animals were killed at 28 days. Tissues and fecal samples were cultured in selective media to detect the presence of viable test microbes. Gross lesions noted at necropsy were also cultured. Viable test microbes were recovered from only 3/160 dosed mice, 2 of those 3 mice were dosed with parental microbes. No treatment related adverse effects were observed in mice. Pathogenicity studies were also carried out in bobwhite quail. The experimental design was similar to studies in mice except that only the IP route was used. No treatment related adverse effects were observed and no viable test microbes were recovered from quail tissues.

Halocacetamines (RAN) which are genotoxic agents are found in drinking water as disinfectant by-products. Human exposure to halocacetamines might represent a genotoxic hazard. The available information on the acute toxicity and lethality of halocacetamines is meager. The objective of this study was to investigate the oral toxicity and lethality of various halocacetamines. Rats were randomly assigned to 25 dosage groups, 5 rats each. Various doses of each halocacetamine were administered orally. The animals were observed for signs of toxicity or mortality for 7 days. Then, rats were sacrificed and organs (liver, lung, kidney, esophagus, stomach and duodenum) were removed, weighed and fixed in buffered formalin for histological studies. There was an eight-fold increase in LD$_{50}$ values of the most and the least toxic compounds. The LD$_{50}$ values (mg/kg body wt) for these compounds were: Monobromacetaminde 25.8, Dibromacetaminde 96.9, Monochloracetaminde 152.8, and Dichloracetaminde 202.4. Signs of toxicity included gasping, salivation, macroscopic hemorrhage, urination, cyanosis, and at higher doses, convulsions and death within 48 h after administration. Histological examinations showed severe necrosis in the submucosal layer of the gastro-oesophageal junction. Our study indicates that the toxicity of halocacetamine is a function of the type and number of halogen substitutions. (Supported by NIH Grant #1871)
THE EFFECT OF PARAGUAT ON MICROosomal OXYGEN REDUCTION AND ANTIOXIDANT ENZYMES IN THE HEPTOPANCREAS OF TWO MID-ATLANTIC BIVALVE MOLLUSCS. R.J. Wenning, R.T. DiGuilio. Ecotoxicology Laboratory, Duke University, Durham, NC. Sponsor: M.B. Abou-Denia

The role of active oxygen species and lipid peroxidation in the toxic effects of the herbicide parquat were examined in vivo and in vitro in the hepatopancreas of the wedge clam Rangia cuneata and the ribbed mussel Modiolus demissus. For in vitro analysis, the hepatopancreas was excised and homogenized in phosphate buffer and the microsomal fraction obtained by differential centrifugation. For in vivo experiments, bivalves with a 3-inch shell length were exposed in 5-L aerated, static aquaria to initial doses ranging between 0.05-5.0 mg parquat for 96 hours. In the presence of a microsomal activation system, consisting of hepatopancreas microsomes, cytochrome c, and NADPH, the ability of parquat to generate activated oxygen species and the reduction of the levels of these species by superoxide dismutase was determined. Results indicate a dose-related increase in superoxide generation to a maximum concentration of 4 mM. Baseline levels of three cellular antioxidant systems, catalase, superoxide dismutase, and glutathione will also be reported. At the present time, in vivo work is under way to characterize these three antioxidants and the stimulation of lipid peroxidation in Rangia cuneata and Modiolus demissus by parquat.

HISTOPATHOLOGY AND EVALUATION OF THE REPRODUCTIVE STATUS OF SELENIUM-EXPOSED FRESHWATER FISH. E.M.B. Sorensen and Peter Thomas. Department of Pharmacology/Toxicology, University of Texas, Austin, TX and Marine Science Institute, University of Texas, Port Aransas, TX.

Selenium contamination from particulate combustion wastes from low grade coal is known to cause reproductive failure in endemic telost species in reservoirs built for water supplies for electric generating stations. In the late 1970's, Texas Utilities Generating Company first released selenium-contaminated wastes into Martin Lake from the Martin Lake Electric Generating Station. At that time histopathological evaluation of gonadal tissue showed numerous atretic follicles and concomitant elevations of selenium levels. In 1986, a group of two-year-old sunfish from Martin Lake and a reference lake (Lake Tyler) were examined for preliminary measurements of testosterone and estradiol titers, as well as the reproductive stage of each fish. Instrumental neutron activation analysis measurements indicated that the hepatic concentration of selenium was elevated, but that the levels had been reduced slightly since the first studies. Female sunfish with the highest levels of selenium in the liver had pronounced morphological alterations within ovaries. Atretic follicles, abnormally shaped follicles, reduced yolk mass, and asynchronous gamete production were observed. These histopathological alterations in Martin Lake female sunfish accumulating the highest levels of selenium were considered diagnostic of loss of reproductive function in these fish and were consistent with changes observed in earlier studies. The steroid titers of females and males appeared to be altered in Martin Lake sunfish compared to Lake Tyler reference sunfish. Testosterone levels were elevated in selenium-exposed females but reduced in selenium-exposed males compared to controls. Data interpretation was complicated somewhat by variable reproductive status of individual sunfish.

MIXED FUNCTION OXIDASE ACTIVITY IN BROWN BULLHEAD FROM A CONTAMINATED NEUSE RIVER ESTUARY. D. Gallagher and R.T. DiGuilio. Ecotoxicology Laboratory, Duke University, Durham, NC. Sponsor: M.B. Abou-Denia

It has been suggested that induction of mixed function oxidase components could be indicative of sublethal contaminant stress in fish. Certain hepatic MFO components (cytochrome P-450, ethoxyresorufin O-deethylase, EROD) in brown bullheads (Ictalurus furcatus) from a chemical and metal contaminated creek and in bullhead from a relatively uncontaminated site were compared to determine if MFO components in fish from the contaminated site were induced. For comparative purposes, pond-reared channel catfish (Ictalurus punctatus) were also studied, with one group receiving an acute dose of the PAN 5,6 benzoazafe (BNF) by i.p.

Microsomal cytochrome P-450 content in bullheads from the contaminated creek was significantly lower than those sampled from the unpoluted creek. However, metabolism of 7-ethoxyresorufin did not differ significantly between the two groups. Neither cytochrome P-450 content nor EROD activity differed significantly between summer and late fall sampling periods. Administration of 5,6 benzoazafe by i.p. to channel catfish resulted in significant increases in both P-450 content and EROD metabolism of bullheads from the two field sites along with channel catfish data suggest that MFO components measured during this study were not reliable as indicators of creek contamination or contaminant stress in bullhead from the sampled areas. Factors influencing the activity of hepatic cytochrome P-450 dependent mixed function oxidases along with biomonitoring considerations are discussed.
COMPARATIVE CYTOTOXICITY OF AQUATIC POLLUTANTS TO BLUEGILL (BF-2) AND FATHEAD MINNOW (FHM) CELL LINES. H. Babich. Rockefeller University, LARC, New York, N.Y. Sponsor: D.M. Stark

Cultured fish cells were used to evaluate the comparative acute cytotoxicities of aquatic pollutants. The comparative potency of the toxicants was determined by the neutral red (NR) cytotoxicity assay, using BF-2 (a fibroblastic cell line, maintained at 26 C) and FHM (an epithelioid cell line, maintained at 34 C) cells as the targets. The FHM cell line was more sensitive than the BF-2 cell line to the agents tested. This greater sensitivity to the toxicants of the FHM cells was apparently due, in part, to the higher temperature of exposure. The relative potencies (based on midpoint toxicities - NR so values) to the FHM cells of a series of chlorinated pesticides was 4,4'-DDE chlorodane > aldrin > heptachlor > 2,4'-DDT > endrin; of polycyclic aromatic hydrocarbons it was acenaphthylene > 3-methylcholanthrene > benzo(a)pyrene; of polychlorinated biphenyl mixtures it was Aroclor 1242 > 1232 > 1016 > 1260; and of organometals it was methylmercuric chloride > diethyltin dichloride > dimethyltin dichloride. When based on NR 50 values in ug/ml, the sequence of toxicity of these agents to the FHM cells was similar to that to the BF-2 cells (correlation coefficient of 0.958), although the latter cell line was more resistant in absolute concentration of test agent.

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Rotenone is an extensively used chemical in the aquatic environment. Yet, few studies involving the toxicokinetics of rotenone in fishes have been conducted. In spite of the specific toxic action of rotenone, the overall toxicity of rotenone is quite variable among different species of fishes. Thus, the rate and/or route of the biotransformation of rotenone may be important in determining its overall toxicity to fishes. Treatment with β-naphthoflavone (BNF) or piperonyl butoxide (PBO), an inducer and inhibitor, respectively, of microsomal monooxygenases, significantly altered the toxicity of rotenone to rainbow trout in flow-through toxicity tests. To assess the role of biotransformation of rotenone on its toxicity, in vitro incubations of 14C-rotenone by hepatic microsomal preparations from rainbow trout were studied. The products of these incubations were analyzed by TLC and HPLC and included two major and several minor metabolites. Differences in the metabolic profiles of rotenone in similar in vitro incubations using hepatic microsomal preparations from rainbow trout pretreated with BNF, PBO, and phenobarbital were evaluated. (Supported by NIOf grant ES 01080)

DEVELOPMENT OF A FISH HEPATOCYTE MODEL FOR THE INVESTIGATION OF INTRACELLULAR METAL REGULATION. S.M. Bakal, J.M. Vosar and J.M. Frazier. The Johns Hopkins University, Baltimore, MD

In order to investigate the mechanistic basis for metal metabolism in fish an isolated fish hepatocyte culture system was developed. Isolated fish hepatocytes were prepared by an adaptation of the standard technique of Seligar (1977) for rats. Major modifications included retrograde perfusion, inclusion of 1 mM EGTA in a HEPES buffer for the first perfusion at room temperature and perfusion with collagenase at 30 C. Under these conditions approximately 200 x 10^6 cells per liver were obtained with 93% viability by trypan blue exclusion. Isolated hepatocytes were incubated at a density of 3 x 10^6 cells/ml in RPMI 1640 media at room temperature. After a 6 hour incubation, 90% of the cells were viable. The system was used to determine metallothionein (MT) induction in response to cadmium, a primary inducer of MT in rat hepatocytes. At a concentration of 1 pppm cadmium MT was induced to a lesser extent in fish hepatocytes than in rat hepatocytes. These data indicate that the isolated fish hepatocyte system can be a useful tool in the investigation of cellular regulation of metal metabolism.


Inducers on the levels of translatable mRNA (t-mRNA) was examined in rainbow trout. Levels of t1-mRNA were compared with the levels of ethoxyresorufin-O-deethylase (EROD) and ethoxyconarin-0-deethylase (ECOD) activities at 0, 2, 6, 18 and 48 hrs. following i.p. administration (100 mg/kg) of beta-naphthoflavone (β-NF). For the Lmβ 1s isozyme t1-mRNA was maximally increased (20-fold) at 18 hrs. and decreased almost to control levels by 48 hrs. post-β-NF. EROD and ECOD were significantly increased (46- and 8-fold, respectively) at 48 hrs. post-β-NF, whereas pretreatment i.p. with 2,4,5-2',4',5'-hexachlorobiphenyl (HCB) (150 mg/kg) produced no change in enzyme activity or in t1-mRNA levels. Micromesos from β-NF pretreated animals competitively inhibited binding with the anti-Lmβ IgG more efficiently than did micromesos from control animals. Lm2 could not be detected under the same conditions using anti-Lm2 IgG. These results suggest that the Lmβ mRNA is less stable than the enzyme for which it codes and that the Lm2 mRNA is either more easily degraded during isolation, or that it is present in small copy numbers and therefore is not detectable using these methods. (Supported by ES 01080, ES 01985)
HEPATOXICITY OF ACETAMINOPHEN IN THE BROWN BULLHEAD CATFISH (ICHTALURUS NEBULOSUS). J.A. Hampton and J.E. Klauing. Department of Pathology, Medical College of Ohio, Toledo, OH.

The brown bullhead catfish is a bottom dwelling freshwater teleost previously shown to be a sensitive indicator species to environmental contamination. However, little data exists characterizing mechanisms of hepatotoxicity in teleosts. The present study was undertaken to evaluate pathologic response of teleost liver to the hepatotoxic acetaminophen. Fish (N=36/dose) received a single IP injection of acetaminophen (either 5,25,50, or 100 mg/kg body wt) in corn oil. Controls (N=36) received corn oil only. Six fish each were sampled at 1, 2, 3, 4, 7 and 10 days post treatment. At sampling, serum was analyzed for LDH, SGPT and SGOT enzymes and livers were processed for histopathologic evaluation. Serum enzyme levels of LDH, GPT displayed a rapid increase (over control) after 1 day, that peaked on day 2. Serum enzyme elevation was proportional to acetaminophen dose. Following perfusion fixation of the hepatic vascular bed, histopathology revealed areas of necrosis which increased in size up to day 3 post treatment and were proportional to the acetaminophen dose. Additionally, areas of necrosis were localized to hepatocytes adjacent venous drainage. In the bullhead liver, these results indicate that a potential hepatocyte heterogeneity exists to the toxic effects of acetaminophen.


While studies on the effects of heavy metals and pesticides on rat brain ATPases have been reported from time to time, that on fish are scanty. The current study provides data on the in vitro effects of mercury on brain ATPases of the channel catfish and Sprague-Dawley rat. The fish and rat brains were removed, P2 fractions prepared and the brain ATPase activities were measured in the presence and absence of different concentrations of heavy metal, mercuric chloride. The fish brain ATPase was significantly lower than that of rat brain. The levels of Ca2+– and Na+/K+–ATPases remained similar in fish brain as compared to rat brain. The IC50 for mercury for Ca2+–ATPase in fish brain (3.6 μM) was nearly 4.8 times higher than that of rat brain (0.8 μM). Such differences were also observed in the IC50 levels for Na+/K+–ATPases in the two species. These studies suggest that mammalian brain ATPases are more sensitive to mercury than fish brain. (Supported by NIH/MBRS #808047).


The medaka (Oryzias latipes), a small aquarium fish, was shown to possess the capacity to rapidly activate AFB1 in vivo at 25°C to intermediates that bind to DNA. The dose-response for in vivo AFB1-DNA binding was linear over the range 70-850 μgAFB1/kg body weight. Maximum binding occurred within the first 24 hr after i.p. injection of [3H]AFB1, followed by a rapid loss of adducts. Aflatoxins (AF1 and unreacted AFB1) were found by HPLC analysis to be the major products excreted into water after AFB1 exposure, with excretion of AFL as early as 2 hours after AFB1 injection. These studies show that medaka possess enzymatic systems similar to rainbow trout (Salmo gairdneri) for biortransformation of AFB1 to the epoxide and to other phase I and phase II metabolites. These studies were supported by USPHS grants ES03850 and ES00541.

THE POSSIBLE ROLE OF THE LIVER RUDIMENT IN THE TOXICITY OF DIOXINS AND DIBENZOFURANS IN THE JAPANESE MEDAKA EMBRYO. J. Wisk, K.R. Cooper, Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

The results from studies in our laboratory indicate that the Japanese Medaka embryo is very sensitive to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) with both lethality and lesions occurring below concentrations of 1 ng/L (ppt). The lesions include: severe hemorrhage in the caudal veins, liver and brain region followed by collapse of the yolk sphere resulting in the development of heart defects. Embryo exposed continuously from day zero, develop no visible lesions until the formation of the liver rudiment. Embryos exposed immediately prior to and during the development of the liver rudiment develop the normal sequence of lesions. Embryos exposed after the appearance of the liver rudiment, develop no visible lesions and the animals hatch normally. It is postulated that 2,3,7,8 TCDD and 2,3,7,8 TCDD are interfering with one or several important synthetic or metabolic pathways that occur during this time of development. (Supported by USGS 24248)
Previously reported studies demonstrated the lethal effect of exposure to CCl₄ on the embryonic development of the Japanese medaka. Current studies investigate the ability of the embryo to metabolize CCl₄, and the role of this metabolism in the observed toxicity. In vivo exposure to non-lethal concentrations of CCl₄ (60 µM) resulted in covalent binding of 0.3 µmol of [³⁰Cl]carbon tetrachloride equivalents (CTE)/mg protein. In vitro incubation of an embryonic S9 fraction in the presence of CCl₄ (2 µM) resulted in 27.1 µmol of CTE bound/mg protein. Increasing the O₂ tension has been shown by others to decrease CCl₄ metabolism. Increasing the percentage of O₂ in the rearing medium from 24% to 55% did not significantly alter CCl₄ toxicity. Exposure to low levels of CCl₄ in an attempt to destroy the metabolizing enzyme, followed by lethal concentrations of the chemical also had no effect on either the time course of lesion development or extent of lethality. Our results have shown that metabolism, and consequently bioactivation, of CCl₄ by embryonic tissue does occur. This bioactivation may play an important role in the development of sublethal toxicity. However, it does not significantly contribute to lethality in the developing medaka. (Supported by USGS 24248).

The present investigation was designed to compare the rates of spontaneous reactivation and aging of brain acetylcholinesterase (AChE) from rats, mice, fathead minnows, and rainbow trout which had previously been inhibited with an I₉₀ of either paraoxon or malaoxon in vitro. The reactivation of AChE was estimated from the time-dependent increase in enzyme activity during dialysis of the 9000g supernatant of brain homogenates. Enzyme aging was calculated from the increase in Z-piraldoxime-insensitive AChE activity recovered from the dialysis. With the exception of trout, the rates of reactivation of AChE were significantly slower following inhibition by paraoxon compared to malaoxon. The trout enzyme failed to reactivate with either inhibitor. Furthermore, the rate of inactivator enzyme recovery from fish was consistently less than that for rodents for both inhibitors. In contrast to the more rapid rate of aging of the paraoxon-inhibited enzyme in rodents, fish brain AChE aged more rapidly than rodent following inhibition by malaoxon. The data suggest that despite their relative resiliency to acute poisoning, fish may be more susceptible than rodents to long-term exposure to anticholinesterase compounds. (Supported by EPA CR-810963).


Liver microsomes from juvenile male rainbow trout were solubilized with 1% CHAPS and 0.2% cholate and resolved into five peaks (A₁ to A₅) containing cytochrome P-450 by DEAE-Sepharose chromatography. The major isozymes in peaks 3 and 5 were further purified by hydroxylapatite and CM-Sepharose chromatography. SDS gel electrophoresis of the major peak 5 isozyme revealed a protein band with the same molecular weight (54,000) as cytochrome P-450 LM₀, a constitutive form previously purified from S-naphthoflavone (SNF) fed fish. Peak 3 isozyme was immunologically indistinguishable from LM₀ on a western blot. The major peak 3 isozyme (M.W. = 50,000) had a slight cross-reactivity with LM₀ polyclonal antibody. None of the two major P-450s or other isozymes in the DEAE-Sepharose peaks cross-reacted with polyclonal antibodies against the SNF-inducible LM₀ form of P-450 from rainbow trout. These results suggest that certain antigenic epitopes are shared by some constitutive P-450 isozymes and there is no immunological relatedness between LM₀ and the constitutive forms of P-450 from juvenile male trout. (Supported by NIH grants ES00060, ES00210, and ES3850).

Sulfadimethoxine Disposition in the Lobster, Homarus americanus. M.O. James and M.G. Barron. Whitney Marine Research Laboratory of the University of Florida, St. Augustine, FL.

Sulfadimethoxine and other antibiotic drugs have been proposed for use in aquaculture, and therefore information is required on drug disposition in crustacea. In the present study, groups of four mature male and female lobsters (450-600g) maintained at 14°C were injected intrapericardially (iv) with a solution of sodium sulfadimethoxine, 42 mg/kg. Separate groups of lobsters were force-fed with shrimp containing sodium sulfadimethoxine or sulfadimethoxine, 42 mg/kg. Hemolymph samples were taken at intervals after the dose and analysed for sulfadimethoxine. Data from the iv study fit a two compartment model with an alpha phase elimination half life of 2 ± 0.1 hr (mean ± S.D. n=8) and a beta phase half life of 77 ± 3 hr. No sex difference was observed, so data from males and females was pooled. Total body clearance was 14 ml/hr/kg and the steady state volume of distribution was 940 ml/kg. After oral administration, absorption was slow, with peak hemolymph concentrations reached at 1 to 2 days (Na salt) or 2 to 3 days (free drug) after the dose. Oral bioavailability (by area under the curve) was 50 ± 2% for both dosage forms. Compared with mammalian species, the elimination of sulfadimethoxine was very slow in the lobster. Supported by FDA Center for Veterinary Medicine.
Complex interactions with soil may greatly alter the way which a chemical subsequently interacts with the body when exposure to soil-adsorbed versus pure form of chemical occurs. Toluene alone or with 0.5g of clay or sandy soil was suspended in 5% gum acacia. A pure tolune or tolune-soil suspension was immediately administered by gavage to groups of fasted male rats. The highest peak plasma concentration of radioactivity was produced by pure toluene followed by clay then sandy soil treatments. The plasma elimination half-lives (hrs) were as follows: sandy (130.6)>pure(93.2)>clay(82.7). The tissue concentrations of radioactivity 2 hours post-administration were highest in stomach>skin>fat>in the pure group, stomach>fat>pancreas in the sandy group and fat>stomach>intestinen in the clay group. Sandy soil increased the bioavailability of toluene in stomach, livern and bone marrow while clay soil did so in the lung compared to the pure compound. 48 hour studies revealed that radioactivity was excreted primarily in the urine and to lesser extents in expired air than in feces for all treatment groups. Hippuric acid was the primary urinary metabolite detected in all treatment groups. Supported by NSF/Industry/University Cooperative Center for Research in Hazardous and Toxic Substances.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants which are known to be biologically active in mammalian systems. PAHs also have been reported to stimulate growth in whole plants and to induce morphogenesis in plant tissue cultures. To investigate the growth altering abilities of PAHs in plants, polypropidaceous fern gametophytes were grown under sterile conditions on media containing the PAH benzo(a)pyrene (BP). The growth pattern characteristic of these plants enables alterations in growth and morphogenesis to be observed at the cellular level in a whole plant system. Doses of BP in the range 0.1-3.2 μg/ml enhanced the onset of morphogenesis. The low and high doses of BP were found to accelerate and inhibit growth, respectively. Analyses utilizing ELISA (enzyme-linked immunosorbent assay) techniques indicate that BP-treated fern gametophytes have altered levels of the growth regulators, auxin and cytokinin. Metabolism studies using high performance liquid chromatography techniques indicate that polar metabolites of BP are formed. The data indicate that BP and/or its metabolites may have an impact on normal growth patterns of terrestrial plants.

Supported by U.S. EPA grants 808459, 811209 and Sigma Xi Grant-in-Aid of Research Award.

Dehydroamino acids are found in many bioactive peptides and are consistent components within the structure of the potent hepatotoxins produced by blue-green algae. A purified hepatotoxin from the blue-green algae Nodularia spumigena was isolated and determined to contain the dehydroamino acid N-methyl-o-amino-butyric acid. This moiety was selectively hydrogenated with sodium borohydride and the toxicity of the resultant derivative was compared with the parent toxin. A dehydrogenated toxin derivative was formed which was one fourth as toxic as the parent compound. Mice that died after being given either the parent toxin or dehydrogenated toxin had large dark blue-black livers. Mice which survived parent toxin had no gross lesions but those surviving the dehydrogenated compound exhibited enlarged tan and red mottled livers. The dehydroamino acid thus plays a key role in the natural toxicity of this cyclic peptide toxin.

There has been recent concern about radon and its decay products as major environmental health hazards. Polonium-210, an alpha emitter, is one of the longer life decay products of radon-222. Polonium-210 is believed to combine in vivo with thiols and thiol-containing proteins. Since dimercaptometal binding agents compete in vivo with many thiol moieties for heavy metals, and because of this laboratory's interest in these water soluble dimercaptocompounds, DMPA, DMSA, DMPS, as well as N-acetyl-L-cysteine have been investigated as to their activity for protecting rats against the lethal effects of 210Po and for mobilizing tissue 211Po. Rats given 210Po (36 μC/kg) ip had a median survival time (nst) of 39 days. The nst was increased to 106 days when any of the three dimercaptans was administered sc beginning immediately after 210Po (p<0.002). After 21 days, kidney levels of 210Po in rats given 210Po (0.4 μC/kg) sc, followed one hour later by DMPA, were 28% of the untreated controls and significantly lower than those receiving DMSA, DMPS, N-acetyl-L-cysteine, or WR2721 under the conditions of the present experiments. Because of its benzene ring, DMPA may have some unique properties as compared to the other water soluble dimercaptometal binding agents. (Supported by CHE2185)
DECONTAMINATION STRATEGIES FOR
HEPTACHLOR CONTAMINATED LIVESTOCK
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During early 1986, contaminated
feedstuffs from a gaechol plant in
Van Buren, Ark. resulted in
violative residues of heptachlor
epoxide, oxychlordane, methoxychlor
and aflatoxin M1 on several farms
in SW Missouri. As a direct
consequence, more than 50 dairies
were prohibited from selling milk
and thousands of animals were
destroyed. We evaluated decon-
tamination strategies including
activated charcoal, feeding
management, mineral oil and
inducers of phase II metabolism in
dairy, beef and swine. Activated
charcoal and mineral oil did
without effect on heptachlor
epoxide and oxychlordane body
burdens. In feeder pigs there was
a slight statistical increase in
rate of elimination after BHA,
however, none of the treatments
were clinically useful.

EFFECTS OF ACRYLAMIDE AND 2,5-HEXANEDIONE
ON MITOCHONDRIAL RESPIRATION. C. Medrano
and R. M. LoPachin. University of Houston, College of
Pharmacy, Houston, TX

Several lines of evidence suggest that the distal axonopathy
caused by acrylamide (ACR) and 2,5-hexanodione (2,5-HD) is
due to an inhibition of specific glycolytic enzymes and/or
oxidative metabolism. We have shown previously that ACR
and 2,5-HD do not produce marked changes in brain
glycolytic flux (LoPachin et al., Neurotoxicology 5:253-36,
1984). Therefore, we have examined the in vitro and in vivo
effects of these chemicals on rat brain mitochondrial
respiration. Whole brain mitochondria were isolated according
to a modification of the Meredith and Fishtam method (Analyt.
consumption was measured polarographically with a Clark's
oxygen electrode. Acute in vitro addition (1 mM final
concentration) of ACR or 2,5-HD to the mitochondrial
incubation media did not affect the ADP/O ratio, the respiratory
control ratio (RCR), nor rates of state 3, state 4 or FCCP-
uncoupled respiration. Incubation of mitochondria for 1 hr in
media containing 1 mM 2,5-HD caused a significant decrease
in RCR (5.1±0.5 vs 3.4±0.5) which was associated with an
increase in state 4 (31.5±2.6 vs 44.3±2.5 nmol O2/min
protein/min) and a decrease state 3 respiration (171.5±33.4 vs
229±23.8 nmol O2/min protein/min). The ADP/O ratio and
FCCP respiration were not affected by 2,5-HD incubation.
Similar incubations with ACR and 1,6-hexanediol had no
effect on these parameters. Chronic intoxication of rats with
either 2,5-HD (400 mg/kg/day x 31 days) or ACR (30
mg/kg/day x 10 days) did not influence whole brain
mitochondrial respiration. These results suggest that 2,5-HD
but not ACR has a direct effect on mitochondrial function.
Studies are underway examining the neurotoxic effects
of mitochondrial respiration in selected brain regions.
Supported by NIH grant ES0 3830-01.

LEAD-INDUCED ALTERATIONS IN ENERGY METABOLISM IN
ISOLATED RAT RETINA. D. A. Fox and S. D.
Rubinstein. College of Optometry, University of
Houston, Houston, TX.

Our previous studies established that development-
mental Pb exposure causes longterm selective rod
photoreceptor deficits. Photoreceptors have high
metabolism (23% cortex) which probably accounts
for the majority in retina. Bull et al. (1975)
demonstrated an inhibition of K+-stimulated respi-
ration (K-SR) in rat brain slices by Pb with no
change in nonstimulated rate (NSR). To examine
effects of Pb on retinal energy metabolism
(oxygen uptake) we used isolated whole adult rat
retinas. Our control rates (1.62 nmol/min/g dry wt/
hr) are in agreement with published values. In
controls, K-SR (5 to 50 mM) exhibited small (33%),
brief increases followed by large (~24%),
prolonged decrease (inhibition) 18-75 uM Pb inhibi-
ted, in dose-response manner, NSR 15-25%. Initial
K-SR exhibited dose-response increases (3-25X)
with no change in inhibition. Adult retinas from
developmental Pb exposed rats had 35% inhibition of
NSR. Similar to 75 uM Pb in vitro, there was a
25% increase in initial K-SR, but only a 5% inhibition.
In contrast to the cortical data (see Bull), the in vitro and in vivo results demons-
strate an inhibition of NSR and an enhancement of
initial K-SR with only the in vitro treatment
exhibiting the increased inhibition. The results suggest
an uncoupling of energy metabolism with
greater effects occurring in vivo. Supported by
NS 03183, EY 07088 and Sigma XI.

ENHANCED NEUROTOXICITY OF 1-METHYL-4-PHENYL-
1,2,3,6-TETRAHYDROPTERIDINE (MPTP) IN C57B1 MICE
BY PRETREATMENT WITH DIETHYLDITHIOCARBAMIC
ACID (DDC). D. B. Miller, J. P. O'Callaghan, and J. P.
Reinhart. 1 U.S. Environmental Protection Agency,
RTP, NC and 2The Wellcome Research Laboratories,
RTP, NC.

MPTP is neurotoxic to the mouse as evidenced by
depletions in dopamine (DA), elevations in the
dopamine-specific protein, glial fibrillary
acidic protein (GFAP), and alterations in motor
function. DDC, a superoxide dismutase inhibitor,
in combination with MPTP enhances the
depletion of DA in striatum. Here the neuro-
toxicity of the DDC+MPTP combination was further
characterized by measuring GFAP and catechola-
mines in striatum and hippocampus, as well as motor
function, in mice that had received
MPTP (35 mg/kg, i.p.) only or 0.5 hr after DDC
(400 mg/kg) and were killed 2 days after the 5th
daily injection. DDC+MPTP resulted in more
deaths and greater losses in body, thymus and
spleen weight than MPTP. Catalysis and akinsia
were more pronounced and lasted for longer
periods. In striatum, DA depletions were greater
than 98% while GFAP was elevated to 410% of
control. The elevation of GFAP and decrease in
noradrenaline levels in hippocampus only after
DDC+MPTP argue for damage of this structure as
well as striatum. These data indicate that both
the general toxicity and the neurotoxicity of
MPTP are augmented by pretreatment with DDC.

The neurotoxic effects of TBN were assessed following its administration to developing rats. L-E rats that received TBN (2.3, or 4 mg/kg, i.p.) or saline on postnatal day (PND) 5 were killed on PND 13, 22, or 60. Brain weights and light microscopy were used as morphological indices. The neuron-specific protein, neurofilament (NP) 200 and p38 (a synaptic vesicle protein), and the glia specific protein, glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP), were used as biochemical indices. Body, thymus and spleen weights were included as indices of overt toxicity and immunosuppression. At all time points TBN caused dose-dependent decreases in wet weight of forebrain, hippocampus (HiP) and cerebellum (CBM); CBM was most affected, HiP the least. Light microscopic evaluations were equivocal. Body weight was reduced only after 4.0 mg/kg; transient reductions in thymus weight but not spleen weight also were observed. On both a per structure and per mg protein basis, time- and dose-dependent reductions in NP-200, p38 and MBP were observed in all brain regions; CBM was most affected. GFAP increased in CBM on PND 22, a finding indicative of gliosis. Collectively, the data indicate that TBN is neurotoxic to the developing rat.

PRECONVULSANT AND ANTICONVULSANT MEMBRANE FLUIDITY EFFECTS. C. Lebel, M. Gill and R. Schatz. Toxicology Program, Northeastern University, Boston, MA.

Cerebral methylation of membrane phospholipids and proteins has been linked to neurosecretion and nerve signal transduction. Alterations in membrane composition and lipid mobility may affect cell function. We use the chemovoluslant L-methionine sulfoximine (MSO, 170 mg/kg, 3h before sacrifice) and the anticonvulsant diazepam (DZ, 5 mg/kg, 1.3h before sacrifice) and the anticonvulsant diazepam (DZ, 5 mg/kg, 1.3h before sacrifice). Mouse brain S-adenosyl-L-methionine (AdoMet) and S-adenosyl-L-homocysteine (AdoHcy) levels were determined and the AdoMet/AdoHcy ratio used as a methylation index, where increases suggest increased methylation rate. MSO decreased AdoMet 47%, AdoHcy 61% and increased the ratio 58%. MSO also increased histamine-N-methyltransferase (HMT) 15%, catechol-O-methyltransferase (COMT) 8%, protein carboxymethylation (PCM) 56% and phospholipid methylation (PLM) 25%. Unexpectedly, DZ increases the AdoMet/AdoHcy ratio, does not affect COMT and HMT, and markedly increases PLM (50-300%) and PCM 8%. MSO and DZ decreased cerebrocortical membrane fluidity (27), suggesting no simple relationship exists between MSO seizures and DZ protection. Similar alterations seen in fluidity suggest MSO decreases may facilitate an excitatory neuronal pathway while DZ decreased fluidity facilitates an inhibitory neuronal pathway. Supported by Am. Epilepsy Found., GE Plastics Div., BSG-9462-21 and N.E. Pathology Services.

THE EFFECTS OF L-METHIONINE- d,1-SULFOXIMINE ON CEREBRAL SULFUR CONTAINING COMPOUNDS. T. Aucoin, and R. Schatz. Toxicology Program, Northeastern University, Boston, MA.

The long latency convulsant L-methionine-d,1-sulfoximine (MSO, 3h prior to sacrifice) has been proposed to increase methylation flux. MSO decreases levels of the methyl group donor S-adenosyl-L-methionine (AdoMet) 47% and its demethylated product S-adenosyl-L-homocysteine (AdoHcy) 61%. L-methionine, which also decreases with MSO administration, and AdoMet have been used to protect cellular components against electrophilic attack. In order to further elucidate the effects of this pathway, the levels of homocysteine and reduced and oxidized glutathione were determined using a glassy-carbon LL/EC detection technique. MSO decreased levels of homocysteine (Hcy) 142 and oxidized glutathione (GSSG) 47% but did not affect levels of reduced glutathione (GSH). Alterations in the ratio (GSSG/GSH) and/or turnover of these compounds could potentially affect neurotransmitter release, protein synthesis, adenylate cyclase activity and microtubular function. In spite of the fact that levels of sulfur containing amino acids and nucleotides involved in GSH synthesis are decreased after MSO, brain GSH levels were unchanged. Further, although these compounds of the methylation pathway are decreased, methylation flux appears to, in fact, be increased in the MSO epileptogenic brain indicating the possibility that GSH turnover may be altered. This latter possibility is under investigation.


It has been reported earlier that chlorocone produces neurotoxicity by modulating the Na+ pump in adult rat brain. The present studies were initiated to investigate its effect on maturing rat brain ATPases. Brain P- fractions were prepared from 1-50 day old rats. Na+-K+ oligomycin-sensitive (O.5) and -insensitive (O.1) Mg2+ ATPases were determined in the absence and presence of different concentrations of chlorocone. All three ATPases were increased with age up to day 15. The sensitivity of these enzymes during their maturation to chlorocone differed considerably. For example, Na+-K+ and O.5 Mg2+ ATPases were more sensitive to chlorocone during day 1-5 as compared to 20-50 days old rat brain enzymes. O.1 Mg2+ ATPase was insensitive to chlorocone at all ages. Ca2+ ATPase was not increased with age, however, it was more sensitive to chlorocone at early age. These results suggest that the Na+ pump, Ca2+ ATPase and ATP synthesizing enzymes are more sensitive to chlorocone during postnatal development. (Supported by NIH/MBRS Grant #08047.)

Previous studies showed a decreased radiolabelled dopamine (DA) turnover and altered DA responsiveness to d-amphetamine in rats exposed to inorganic lead (Pb) from birth, suggesting Pb-induced changes in DA synthesis. A primary form of DA synthesis regulation is the inhibition exerted on tyrosine hydroxylase activity via dopaminergic autoreceptors. This study assessed the functional status of this mechanism. At parturition dams received 0.2% Pb acetate in the drinking water while control dams received distilled water. Pups were weaned and maintained on the same solution given their dam until sacrifice at 125 days. 40 min before sacrifice rats were given saline or a DA agonist (3-20 mg/kg ip), 6,7-dihydroxy-2-dimethylaminotetralin (TL-99), followed 10 min later by 750 mg/kg ip of gammabutyrolactone (GBL). Nucleus accumbens (NA) and caudate-putamen (C-P) were analysed for content of DA and its metabolites. The ability of TL-99 to prevent the GBL-induced increase in DA content was significantly diminished in NA of exposed rats compared to controls. A similar effect in C-P was not statistically significant. These findings suggest that chronic Pb exposure impairs receptor-mediated regulation of synthesis in mesolimbic DA neurons. (Supported by ES03382).

PYRETHROIDS AND THE STRIATAL DOPAMINERGIC SYSTEM IN VIVO. J.O. Doherty1 N. Mori1, K. Nishimura2, N. Kurihara2, and T. Fujita2 1HED (TS-759) EPA, Washington, D.C. and 2Kyoto University, Kyoto JAPAN.

If Type I and II pyrethroids differentially affect the striatal dopaminergic system this may relate to the characteristic behavioral symptoms expressed by each class of pyrethroids. To assess for potential dopaminergic effects, rats were dosed with near lethal doses of type I (permethrin and allethrin) and II (cypermethrin and fenvalerate) pyrethroids and the striatal dihydroxyphenylacetic acid (DOPAC) content was determined by High Performance Liquid Chromatography as an index of striatal dopaminergic activity. All of the pyrethroids caused a similar slight to moderate increase (23-38%) in DOPAC. Higher brain levels of type I pyrethroids (2.64 to 5.82 ug/gm) compared with the type II pyrethroids (0.4 to 0.6 ug/gm) were present when behavioral responses were maximal. Other insecticides with similar proposed modes of pharmacological action were also tested and caused either slight to moderate increases or no effect on DOPAC levels. The differences in behavioral effects of the type I and type II pyrethroids in rats were not shown to be related to a specific effect on the striatal dopaminergic system. [Note: We thank Dr. K. Suzuki, Mr. T. Hiroconi, Mr. J. Ohnishi and Dr. M. Miyamoto of the Sumitomo Chem. Co. for their expert assistance.]

624 METHYLMERCURY (MeHg) INDUCED DOPAMINE RELEASE FROM SUPERFUSED RAT STRIATAL SYNAPTOSOMES. D.J. Minnema, Dept. Envir. Hth., U. Cinti. Cincinnati, OH. Sponsor: E.J. O’Flaherty

MeHg is well recognized as a neurotoxicant of environmental concern. Previous studies have suggested that MeHg is a potent inducer of dopamine (DA) release from CNS tissue preparations (Komulainen & Tuomisto, 1982; Bondy et al., 1979). In the present study, "purified" synaptosomes prepared from rat striatum were preloaded with [3H]-DA, layered on cellulose-acetate filters, and superfused at 2 ml/min with a physiological Krebs-HEPES buffer. Superfusate fractions were collected at 15 sec intervals. Addition of MeHg (1,3,5, and 10 µM) to the superfusing buffer during fractions 5-10 produced a marked dose-dependent increase in the spontaneous DA release. This MeHg-Induced DA release was not dependent on the presence of external calcium (Ca). It does not appear that MeHg induces DA release by increasing intracellular Ca++ since MeHg induced minimal increases in 45Ca efflux from 45Ca preloaded synaptosomes. Lowering the external sodium concentration from 120 to 12 mM did not alter the magnitude of the MeHg effect. These results confirm that MeHg is a potent inducer of spontaneous DA release. The mechanisms by which MeHg produces this effect do not appear to involve membrane depolarization or Na,K-ATPase inhibition. Supported by ES03399.

625 BIOGENIC AMINE ALTERATIONS IN DIFFERENT BRAIN REGIONS OF MICE EXPOSED TO BENZO(a)PYRENE. S. Jayasekara, D.B. Brown and R.P. Sharma. Center for Environmental Toxicology, Utah State University, Logan, UT.

Benzo(a)pyrene (BaP) is a product of incomplete fossil fuel combustion, a well known pollutant, and an established carcinogen. In the present study male CD-1 mice were exposed to ip injections of 0, 5, 25 and 100 mg/kg body weight BaP - twice every week. Endogenous levels of brain biogenic amines and their selected metabolites: norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT), vanillylamandelic acid (VMA), dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (HIAA), were measured using HPLC and electrochemical detection. The brain regions studied were cortex, striatum, hypothalamus, midbrain, medulla oblongata, and cerebellum. BaP treatment caused an increase in the levels of NE, DA, 5-HT in the hypothalamus and striatum. Increased levels of DOPAC and HIAA were noticed in the same brain regions. 5-HT level was higher in the hypothalamus. Significantly higher levels of NE and DA were observed in the midbrain and medulla oblongata respectively. 5-HT levels in these two regions were higher but not statistically significant. The results suggest that exposure to BaP caused an elevation of steady state levels of biogenic amines in different regions of the mouse brain.
EFFECTS OF DIETARY COPPER ON CADMIUM, SELENIUM, COPPER, ZINC, CALCIUM AND IRON IN THE EYE OF CADMIUM-TREATED RATS. F.S. Janais and H. Roque, St. John's University, Jamaica, N.Y.

Weaning Sprague-Dawley rats were fed a diet containing 0.5 ppm selenium (Se) with 0, 10 ppm or 50 ppm copper (Cu). Rats were treated with either 5 mg Cd (as CdCl2) via osmotic minipumps (Alzet 2002) (Cd-OP) or fed 50 ppm Cd in their feed (Cd-D) for 7 weeks. Cd-treated rats had approximately 0.300 mg Cd/g tissue, wet weight. Cd-D resulted in a linear increase in eye Cu levels whereas Cd-OP resulted in a 100% increase in eye Cu in the low-Cu rats but showed no significant alterations in rats fed 10 ppm or 50 ppm Cu. Feeding rats 50 ppm Cu resulted in a 90% increase in eye calcium (Ca) levels. Cd-D or Cd-OP markedly reduced eye Ca levels. Iron (Fe) levels increased by 140% in Cd-OP rats fed the low-Cu diet but was unaffected in rats fed 10 ppm Cu and reduced by 42% in rats fed 50 ppm Cu. Cd-OP rats exhibited a 34% increase in eye Fe in rats fed 10 ppm Cu and a 50% decrease in rats fed 50 ppm dietary Cu. Zinc (Zn) levels were not significantly altered by either Cd treatment. (Supported by NIH Grant ES03370)

EFFECTS OF DIETARY SELENIUM ON CADMIUM, COPPER, ZINC, CALCIUM AND IRON IN THE EYE OF CADMIUM-TREATED RATS.

Male, weaning Sprague-Dawley rats were fed a low-selenium (Se) diet containing 50 ppm copper (Cu) and supplemented with 0, 0.1 ppm or 0.5 ppm Se. Rats were treated with either 5 mg Cd (as CdCl2) via osmotic minipumps (Alzet 2002) or fed 50 ppm Cd in their feed. Animals were necropsied at the end of 7 weeks of treatment. Eyes were excised, weighed and wet-ashed for metal analysis by atomic absorption spectroscopy. Se was analyzed fluorometrically. Rats fed 50 ppm Cd in their feed showed a linear increase in Cd and Se levels in the eye with increasing dietary Se. Calcium (Ca) levels also showed a linear increase with increasing dietary Se. Cd treatment either via osmotic minipumps or via the feed showed greater than 50% reductions in eye Ca levels, independent of dietary Se levels. Cd-treated rats had 30% higher eye iron (Fe) levels in rats fed the low-Se diet. However, in rats fed 0.1 ppm or 0.5 ppm Se, both Cd-treatments resulted in 40% to 50% reductions in Fe levels. Eye Cu levels were not significantly altered. (Supported by NIH Grant ES03370)

IMAGING MANGANESE IN THE PRIMATE BRAIN WITH MAGNETIC RESONANCE. M.C. Newland, J.H. Kordower, T.L. Ceckler and B. Weiss. University of Rochester School of Medicine and Dentistry, Rochester, NY

The paramagnetic properties of manganese should permit in vivo imaging of manganese-rich tissues with magnetic resonance techniques. To test this hypothesis, a macaque fascicularis monkey was exposed to 30 to 60 mg/m2 MnCl2 aerosol 4 days a week for about 3 months. While under light anesthesia, the monkey's brain was imaged in sagittal and coronal sections that included the basal ganglia. With relaxation (TR) and echo (TE) times of 300 and 15 msec three separate regions were distinguished clearly: caudate, a region corresponding to putamen and globus pallidus, and one corresponding to subthalamic nuclei, substantia nigra, and ventromedial hypothalamus. The brain of an exposed control monkey was also imaged using identical parameters, but basal ganglia were not highlighted. The highlighted areas seen in the exposed monkey probably represent manganese-rich tissue because: 1) manganese shortens relaxation times such that contrast with manganese-poor tissue should be enhanced at TR:TE = 300:15 msec; and 2) these highlighted regions are those that have previously been reported to accumulate manganese, and 3) these regions were not highlighted in the unexposed monkey. (Supported by ES01247, ES01248, AA05178, CA40699, General Electric Co.)


Bismuth(Bi)-induced encephalopathy, a rarely observed phenoma, is associated with elevated brain Bi levels. The relationship between circulating Bi, brain Bi and neurotoxicity has not been well characterized, partly because of the highly insoluble nature of Bi salts under physiological conditions and low oral absorption of Bi. Water-soluble sodium bismuth subgallate (NaBiSG) and bismuth tartrate (BT) were utilized to characterize the accumulation of Bi in the brains of male and female mice, male rats and male ferrets. Animals received i.p. dosages of NaBiSG up to 100 mg/kg weekly or BT up to 200 mg/kg weekly for periods up to 24 weeks. Interim and recovery sacrifices were taken to obtain brain and other tissues for determination of Bi content. Brain Bi levels increased over time in a linear, dose-dependent manner in all animals during treatment with either NaBiSG or BT. Additionally, the apparent rate of brain Bi accumulation associated with each NaBiSG and BT dosing regimen linearly related over the range of doses examined in each species. Comparison of all brain Bi data, regardless of species, sex or Bi salt administered, showed all data to fail on a single, straight line describing an apparent unique relationship between brain Bi level and amount of systemically administered Bi (slope = 2.52 ppm brain Bi/mg Bi/kg). These results suggest soluble forms of Bi can be useful in the study of Bi-induced neurotoxicity by allowing the generation of predictable brain Bi levels in rodent and non-rodent mammals.
The effect of various thiol-containing amino-acids and the tripeptide glutathione (GSH) on MeHg uptake by the brain was measured. In the rat, fifteen seconds and three minutes after intra-arterial and intravenous injection respectively, with MeHg-saline, MeHg-cysteine, MeHg-cysteine-methionine, and MeHg-GSH the relative amount of Hg in the whole brain was measured by gamma scintillation spectrometry. Brain Hg content was significantly increased in animals injected with MeHg-cysteine and MeHg-GSH (p<0.05) when compared to controls and animals injected with [14C]-sucrose; an inert substance that was used to determine radioactivity accounted for by the brain circulatory system. No change in brain Hg uptake at both fifteen seconds and three minutes was evident in rats injected with MeHg-cysteine-methionine. In MeHg-saline treated animals, following its first microcirculatory passage through the brain, the binding of MeHg to -SH groups was rapid. Greater than 99% of labeled MeHg was found to be non-diffusible. Post-priori analysis revealed increased percentage of diffusible Hg in rats injected with MeHg-cysteine and MeHg-GSH compared to controls (p<0.05). There was no significant difference in diffusible Hg between MeHg-cysteine, MeHg-GSH and MeHg-cysteine-methionine treated animals. Therefore, the endogenous transport of MeHg into the brain may not be explained by increased diffusible blood Hg.

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

Effector of TCDD on the hepatic and testicular distribution of iron, zinc and copper in rats, Z.Z. Wahba, Z.A.F. Al-Bayati and S.J. Stohs, Dept. of Pharmaceutical Sciences, University of Nebraska Medical Center, Omaha, NE

The distribution of iron, zinc and copper in subcellular fractions of hepatic and testicular tissues of male rats and hepatic tissues of female rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was determined. Animals received 40 μg TCDD/kg/day for 3 days P.O. or the vehicle and were killed 7 days post-treatment. Iron, zinc and copper were determined by atomic absorption spectroscopy. The iron content of liver from female animals was greater than male animals. TCDD administration increased iron content of mitochondria in female and male rats and decreased iron content of microsomes of both sexes. TCDD produced no changes in the zinc content of the hepatic subcellular fractions of either sex. Significant increases occurred in the copper content of whole liver, mitochondria and cytosol of male rats, and in whole liver and cytosol of female rats following TCDD treatment. Zinc and iron distribution were unaltered by TCDD in the subcellular fractions of testes. The copper content of whole testes and mitochondria decreased, while the copper content of cytosol increased. Thus, TCDD produces differential effects with respect to the subcellular distribution of iron, zinc and copper in liver and testes.

Clearence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from body fat of rhesus monkeys following chronic exposure R. E. Bowman, S. L. Schantz, N. C. A. Weerasinghe, J. Mehan, J. Pan, M. L. Gross, K. M. Boehm, J. P. Van Miller, and P. C. Welling. Harlow Primate Laboratory, University of Wisconsin, Madison WI and Chemistry Department, University of Nebraska, Lincoln NE.

Sponsor: R. E. Peterson

Four adult female rhesus monkeys were fed a diet containing 25 ppt TCDD for four years. Mesenteric and subcutaneous fat samples were obtained from each monkey via laparotomy at 0 and 6 mo. post-exposure, and at approximately 3 mo. intervals from 6 to 36 mo. post-exposure. Samples were analyzed for TCDD content via gas chromatography/high resolution mass spectrometry. Since there were no significant or consistent differences between the TCDD half-lives in the two fat compartments, the values for subcutaneous and mesenteric fat at each time point were averaged. For each monkey, average TCDD concentration was plotted as the natural logarithm vs. time. For the least squares linear regression lines, the Pearson Product Moment Correlation Coefficients ranged from .86 to .98. Half-lives for the four monkeys were 204, 578, 630 and 408 days, with a mean of 455 ± 96 (S. E.) days. The data are consistent with first order kinetics and a single compartment model. (Supported by EPA Grant B-811895)


Significant interactions between TCDD and estrogens have been reported in mice. We report here the effect of TCDD treatment on relative uterine weight and steroid UDPGT in young female guinea pigs and hamsters (a resistant species). Animals were treated with 2.5 μg TCDD/kg ip, and sacrificed 5 or 15 days after treatment. Relative uterine weight was significantly reduced in TCDD treated guinea pigs but was unaffected in hamsters. TCDD did not induce steroid UDPGT (determined by disappearance of labelled estrogen) in either species. Guinea pigs, however, had steroid UDPGT levels that were 5-10 times higher than levels in hamsters. Because of the importance of estrogens in regulating many physiological functions, this difference in steroid UDPGT levels between a susceptible and a resistant species may be relevant in explaining the differences in species susceptibility. Supported in part by USEPA (#CR812114-01-1).

Plasminogen activator activity is frequently observed in existing malignant cells or mammalian cells transformed by viruses or altered by promoters of carcinogenesis. The human breast adenocarcinoma derived cell line MCF-7 expresses both urokinase-like (u-PA) and tissue plasminogen (t-PA) activators. Exposure to physiological concentrations of 17β estradiol (E2) results in enhanced t-PA activity but not u-PA activity in MCF-7 cells. Exposure to very low (10^{-10} M) concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) does not increase t-PA activity but suppresses E2 enhancement of this activity. 2,3,7,8-Tetrachlorodibenzo-p-dioxin is a weaker antagonist while 2,6-dichlorodibenzo-furan is without effect. These structure-activity relationships parallel those seen with other in vivo and in vitro biological markers of dioxin-like activity, and are of interest because of previous observations that chronic administration of TCDD to female rats, while inducing tumors in some tissues, resulted in a reduction of age-related spontaneous tumors of the breast, uterus, and pituitary. (Supported in part by NIH grants AM2307505 and ES05561.)


The Love Canal Leachate contains more than 100 organic compounds including 2,3,7,8-TCDD at 3 ppb. To determine whether the teratogenic effects of TCDD could be resolved from this complex mixture of compounds the leachate was administered to two mouse strains during gestation. 821 C57BL/6 fetuses which express a high affinity receptor for TCDD were examined. At cumulative maternal doses of 0, 1, 3, 5, and 7 g leachate/kg the cleft palate incidence was 0%, 0%, 20%, 64%, and 89%, respectively, and the hydrophthalmia in 412 fetuses was 0%, 7%, 7%, 7%, and 92%, respectively. Maternal mortality was 5% at the highest dose. In 234 DBA/2 fetuses, which do not express the receptor, the cleft palate incidence at 0, 0.5, 1, and 2 g leachate/kg was 0%, 0%, 0.04%, and 0%, respectively, and the hydrophthalmia was 0.5%, 11%, 3%, and 0%, respectively. Maternal mortality was 0%, 7%, 7%, and 36%, respectively. Thus, a dose of TCDD caused a 20% reduction of cleft palate incidence only in C57BL/6 mice, comparable to an equivalent quantity of pure TCDD. These results indicate that the effects are primarily from the TCDD in the sample and that the other components of this complex mixture did not interfere with these receptor mediated effects.

ADDITIVE TERATOGENIC EFFECTS OF POLYCHLORINATED DIBENZOFURANS (PCDFs). L.S. Birnbaum, M.W. Harris, and R. Morrissey. Systemic Toxicology Branch, NIEHS, Research Triangle Park, NC.

PCDFs are highly toxic environmental contaminants which have been involved in several incidents of human poisoning. Two isomers, 2,3,4,7,8-pentachlorodibenzo-furan (4-PCDF) and 1,2,3,4,7,8-hexachlorodibenzo-furan (HCDF) have been shown to persist in the tissues of victims from Japan and Taiwan. The teratogenicity of these compounds, both alone and in combination, was assessed in C57BL/6N mice. Pregnant mice were treated with 100 ml/kg corn oil containing no PCDFs, 4-PCDF, HCDF, or a combination of the two on gestation days (gd) 10-13, followed by necropsy on gd 18. Maternal and fetal toxicity was assessed and soft tissues were examined for abnormalities. Both chemicals caused hydrophthalmia and cleft palate in the absence of any overt toxicity. The dilated kidney occurred at doses approximately 5-fold lower than those causing cleft palate. Combination of 4-PCDF and HCDF caused an incidence of clefts not significantly different from an additive interaction as predicted by probit analysis. In addition, the interaction of 2,3,4,5,3',4'-hexachlorobiphenyl, a related compound also present in PCDF poisoning victims, and 4-PCDF appears additive. Thus, these chemicals, which cause toxic effects similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin, interact in an additive manner in the induction of fetal anomalies in the mouse.


The Love Canal Leachate contains more than 100 organic compounds including 2,3,7,8-TCDD at 3 ppb. To determine whether the teratogenic effects of TCDD could be resolved from this complex mixture of compounds the leachate was administered to two mouse strains during gestation. 821 C57BL/6 fetuses which express a high affinity receptor for TCDD were examined. At cumulative maternal doses of 0, 1, 3, 5, and 7 g leachate/kg the cleft palate incidence was 0%, 0%, 20%, 64%, and 89%, respectively, and the hydrophthalmia in 412 fetuses was 0%, 7%, 7%, 7%, and 92%, respectively. Maternal mortality was 5% at the highest dose. In 234 DBA/2 fetuses, which do not express the receptor, the cleft palate incidence at 0, 0.5, 1, and 2 g leachate/kg was 0%, 0%, 0.04%, and 0%, respectively, and the hydrophthalmia was 0.5%, 11%, 3%, and 0%, respectively. Maternal mortality was 0%, 7%, 7%, and 36%, respectively. Thus, a dose of TCDD caused a 20% reduction of cleft palate incidence only in C57BL/6 mice, comparable to an equivalent quantity of pure TCDD. These results indicate that the effects are primarily from the TCDD in the sample and that the other components of this complex mixture did not interfere with these receptor mediated effects.

2,3,4,7,8-PENTACHLORODIBENZOFURAN (PCDF): TOXICOKINETICS AND METABOLISM IN THE RAT. D.W. Brewer and L.S. Birnbaum. NIEHS, Research Triangle Park, NC.

PCDF is a highly toxic environmental contaminant which accumulates in human tissues. Its disposition was studied in the male Fischer rat after oral or iv administration. Greater than 70% of an oral dose was absorbed. Absorption and elimination were linear between 0.1 and 1.0 µmol/kg. PCDF was rapidly eliminated from the blood and was distributed mainly to the liver and adipose tissue. Skin and muscle were found to be minor depots. Route of administration had little influence on tissue distribution of PCDF. TLC analyses indicated greater than 99% of the radioactivity accumulating in the fat and liver was unmetabolized PCDF which was eliminated very slowly (t½ = 193 and 69 days, respectively). Excretion occurred primarily via the feces; no radioactivity was detected in expired air and less than 0.02% ever appeared in the urine. The whole body half-life was calculated to be 64 days. Pretreatment with 500 µg PCDF/kg for 3 days doubled the excretion of polar metabolites compared to nonpretreated animals. Enzymatic hydrolysis of bile had little effect on chromatographic profile. It is concluded that PCDF is readily absorbed, concentrates primarily in the liver, and is slowly eliminated from the body as polar metabolites. Consequently, chronic low level environmental exposure could cause bioaccumulation at critical target sites.
TOXICITY AND DISPOSITION OF 2,3,4,7,8-PENTA-
CHLORODIBENZOFURAN (PCDF) IN THE Rhesus Monkey.
D.W. Brewster and L.S. Birnbaum. NIEHS, Research
Triangle Park, NC

Three male Rhesus monkeys were administered PCDF, a
highly toxic environmental contaminant, at 34
µg/kg, iv. PCDF was eliminated from the blood
within 20 min. and accumulated in the liver, skin,
adipose, and muscle tissue. Excretion occurred
primarily via the feces. Preliminary data indicate a whole body elimination rate be-
tween 40 and 100 days. Within 7-14 days after
administration, the hematocrit and serum trigly-
eceride and bile acid concentrations were signi-
ficantly increased while serum cholesterol, pro-
tein, and albumin concentrations were decreased
relative to pretreated levels. Thyroid hormone
levels were also altered. After 28 days, 2 of
the monkeys began exhibiting hair loss, slough-
ing off of fingernails, chloracne like lesions, and
loss of 45% of their body weight. Depressed
cage behavior was evident with huddling, loss of
appetite, and diarrhea following. Similar symp-
toms of toxicity were observed in the third ani-
mal 58 days after PCDF administration. Two of
the animals died (day 40 and 48) but the third
recovered. Pathological results indicated the pre-
ence of hyperplastic gastric mucosal lesions and
hyperplastic and metaplastic changes in the
Meibomian glands of the eye and the caruncular
glands of the ear. It is concluded that PCDF
produces dioxin-like toxicity in the monkey at
levels similar to those reported for TCDD.

AROCOLOR 1254 AS A 2,3,7,8-TRIHALODIBENZO-P-
DIOXIN ANTIGENIST IN 5TBL/6J AND DBA/2J MICE
EFFECTS ON ENZYME INDUCTION AND THYMIC ATROPHY:
R. Bannister and S. Safe. Veterinary Physiology
and Pharmacology, Texas A&M University, College
Station, TX.

The effects of Aroclor 1254 as a 2,3,7,8-tri-
chlorodibenzo-p-dioxin (TCDD) antigenist were
determined in genetically imbedded 5TBL/6J and
DBA/2J mice. The effects of 2,3,7,8-TCDD and
2,3,7,8-TCDD plus Aroclor 1254 as inducers of the
hepatic microsomal, aryl hydrocarbon hy-
droxylase (AHH) and ethoxyresorufin-O-dehy-
dethylase (EROD), were determined using differ-
ent molar ratios of Aroclor 1254 (antigenist) to
2,3,7,8-TCDD (agonist). Aroclor 1254 antigen-
ized the induction of AHH and EROD by 2,3,7,8-
TCDD (200 nmol/kg) in DBA/2J mice when the
agonist/agonist ratios were 3750:1 and
7500:1. Aroclor 1254 antagonized the induction of
hepatic microsomal AHH and EROD by 2,3,7,8-
TCDD (15 nmol/kg) only at dose levels of 25, 75 and
150 µmol/kg (19-23% inhibition) which re-
spected molar antagonist/agonist ratios of
1667:1, 5000:1 and 10,000:1 respectively.
Aroclor 1254 did not antagonize the development
of thymic atrophy by 2,3,7,8-TCDD in 5TBL/6J
mice at an Aroclor 1254/2,3,7,8-TCDD molar
ratio of 10,000:1, whereas at the same ratio
Aroclor 1254 provided significant protection from
2,3,7,8-TCDD-mediated thymic atrophy in
DBA/2J mice. (Supported by N.I.H., ES03554.)

4-METHYL-1,3,8-TRICHLORODIBENZOFURAN (MCDF)
AS A 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
(TCDD) ANTIGENIST IN RATS - ANH INDUCTION:
B. Astroff and S. Safe, Veterinary Physiology
and Pharmacology, Texas A&M University,
College Station, TX.

The competitive binding affinity EC50 value
for MCDF to the rat hepatic cytosolic recep-
tor protein was 4.9 x 10^-6 M (hydroxylapatite
assay) which was only 49-fold lower than the
EC50 value for 2,3,7,8-TCDD. In contrast,
MCDF was a poor inducer of aryl hydrocarbon
hydroxylase (AHH) and ethoxyresorufin-O-
deethylase (EROD) in rat hepatoma cells and
in Long Evans rats. At a dose level of 400
µmol/kg, MCDF elicited a submaximal (50%)
induction response for both hepatic microsoma-
al AHH and EROD. Cotreatment of Long Evans
rats with 2,3,7,8-TCDD (4.5 µg/kg) plus MCDF
(400, 200 and 50 µmol/kg) resulted in a sig-
nificant antagonism of the AHH/EROD induction
activity of 2,3,7,8-TCDD at all dose levels of
MCDF. The results indicate that MCDF is a
partial antagonist for the 2,3,7,8-TCDD re-
ceptor in the rat. (Supported by the National Institutes of Health, ES03554.)

INDUCTION OF THE HEPATIC MONOOXYGENASE SYSTEM BY
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) AT
DOSES ASSOCIATED WITH ACUTE TOXICITY. E.S. Shen,
and Pathol., SUNY, Buffalo, NY.

The ability of TCDD to produce toxicity and the
induction of aryl hydrocarbon hydroxylase segre-
gate with the Ah locus, with 5TBL/6J (C57) mice
being responsive and DBA/2J (DBA) mice nonrespon-
sive to this induction. In concordance with re-
ported studies, maximal ethoxyresorufin-O-dehy-
dethylase (EROD) induction appeared to occur at 3 µg
TCDD/kg (ip) for C57 and 30 µg/kg for DBA mice
with respective activities of 2656±129 and 1659±
100 pmol/min/mg cyt. P<4.50. However, at doses
approaching the LD50 (150 µg/kg for C57; 600 µg/
kg for DBA), EROD activity was greatly increased
in both strains (4643±429 for C57; 3348±346 for
DBA). To further characterize this phenomenon,
hepatic microsomal cyt. P<4.50 content, benzo(a)-
pyrene metabolism and EROD and cytochrome c
reductase activities were measured 1, 3 and 7 days
after TCDD administration to C57 (3 and 150 µg/
kg) and DBA (30 and 600 µg/kg) mice. Maximal
responses occurred in both strains at 3 days for
all doses with the exception of reductase activity
which was unaltered in either strain by the lower
TCDD doses. The induction of cyt. P<4.50
activities observed at LD50 doses of TCDD may be
related to the induction of reductase activity and/or specific cyt. P<4.50 isozymes. These
responses may play a role in the expression of
toxicity. (NIR grants ES02693 and GM07145).
TOXICITY OF POLYCHLORINATED DIBENZO-\(p\)-DIOXINS AND RELATED COMPOUNDS IN GUINEA PIGS - STRUCTURE-ACTIVITY RELATIONSHIPS: M. Holcomb, C. Yao and S. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

The dose-response effects of 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) and several related compounds were determined in the immature male Hartley strain guinea pig. 2,3,7,8-TCDD was a potent inducer of hepatic microsomal aryl hydrocarbon hydroxylase (AH) and ethoxyresorufin O-deethylase (EROD) and caused a 4.4 and 22-fold increase in these enzymes at dose levels which elicited a maximum induction response. The \(E_{50}\) values for body weight loss, AH and EROD induction were \(5.6 \times 10^{-3}\), \(1.1 \times 10^{-3}\), and \(1.94 \times 10^{4}\) umol/kg. The in vivo structure-activity relationships for several polychlorinated dibenzo-\(p\)-dioxins and dibenzofurans were also comparable to those reported for these compounds in rats, mice and rat hepatoma H-4-II-E cells in culture; moreover, there was an excellent linear correlation between the in vivo-\(10\log E_{50}\) values (guinea pig) and the in vitro AH induction-\(10\log E_{50}\) for the compounds used in this study. (Supported by the United States Environmental Protection Agency.)

ARYL HYDROCARBON HYDROXYLASE (AH), ETHOXYRESORUFIN O-DEETHYLASE (EROD), AND CYTOCHROME P-450 (\(c\)-P-450) IN THE GUINEA FIG AND HAMSTER IN RESPONSE TO TCDD TREATMENT. T. H. Umbreit, E. J. Hesse, and M. A. Gallo, UMNJ/Rohto, W. Johnson Medical School, Piscataway, NJ.

Induction of AH has been associated with TCDD toxicity. One argument against this theory has been the reported inability of TCDD to induce AH in guinea pigs. We examined AH in liver microsomes from young female guinea pigs and hamsters (one of the most resistant species) treated with a single dose of 2.5 \(\mu\)g TCDD/kg, \(1\) p (ca. 5x the \(L_{50}\) of guinea pigs); animals were sacrificed on day 5 and 15 after treatment. In guinea pigs, TCDD induced AH between 2.2 and 3.8 times control levels, and induced total \(c\)-P-450 between 1.71 and 2.5 times control. In hamsters, TCDD treatment induced \(c\)-P-450 ca. 1.8 times control, but reduced AH activity about half. EROD was identical in both species regardless of TCDD treatment. These results support the hypothesis that AH induction (at high doses of TCDD) may be related to toxic response in the guinea pig. Supported by USEPA (#CR812114-01-1.)

EVALUATION OF SUBCHRONIC EXPOSURE TO OCTACHLORODIBENZODIOXIN (OCDD). L.A. Couture and L.S. Birnbaum. NIEHS, Research Triangle Park, NC

OCDD is a common contaminant of chlorinated phenols (components of bactericides, defoliants, wood preservatives, etc.) and as a result, widespread environmental contamination has been detected. To assess the significance of repeated exposure, male Fischer 344 rats were treated with 50 \(\mu\)g/kg 14C-OCDD by gavage for 10, 20, 40, or 65 times (once a day, five days/week). Animals dosed \(10\)X were sacrificed on days 1, 3, 7, 14, 28, 42, 56, 84, and 112 post exposure and tissue half-lives were calculated. OCDD is slowly eliminated from the liver, skin, and adipose (t/2 = 222, 163 and 165 days, respectively). As the number of doses increased, the OCDD accumulated in the liver > adipose > skin > blood. Of the total body burden, 97% is in the liver. Most of the hepatic OCDD is associated with the microsomal fraction of the parenchymal cells. Treatment-related increases in ethoxyresorufin-O-deethylase (\(S\)) and aryl hydrocarbon hydroxyoseline (\(3\)) activities were also observed. In addition, there was a dose-related increase in the liver:body weight ratio accompanied by a mild centrilobular cytoplasmic vacuolization in the liver. Bile acid levels are also elevated. A mild, non-regenerative anemia, typical of a chronic non-infectious inflammation, is probably due to changes in the liver. Thus, OCDD appears to be accumulating in the liver with multiple exposures resulting in some hepatic alterations.
ROLE OF THE Ah RECEPTOR IN THE DOWN REGULATION OF UTERINE AND HEPATIC ESTROGEN RECEPTOR LEVELS IN RATS: M. Bomkes, J. Pliskorska-Pliszczynska and S. SAFE, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

Administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (30 μg/kg) to 21 day old female Long Evans rats results in a 54% and 41% decrease in uterine and hepatic estrogen receptor (ER) levels. Administration of estradiol (15 mg/kg) resulted in a 19% and 155% increase in cytosolic and uterine ER levels and a 42% increase in uterine wet weight 48 hours after treatment. Pretreatment of the rats with 2,3,7,8-TCDD (80 μg/kg) prior to administration of estradiol (15 μg/kg) completely blocked the estrogenic effects of estradiol with respect to uterine wet weight increases and increase in ER levels. The comparative effects of 2,3,7,8-TCDD, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 1,3,7,8-TCDD and 1,2,4,7,8-PCDD on the down regulation of uterine and hepatic ER levels were structure-dependent. There was an excellent correlation between the binding affinities of these congeners for the Ah receptor and their activity as antiestrogens and the results support a role for the Ah receptor in the down regulation of uterine and hepatic ER levels in the rat. (Supported by the Texas Agricultural Experiment Station.)

THE ROLE OF THE Ah RECEPTOR IN HEXACHLOROBENZENE (HCB) PORPHYRIA: STUDIES IN CONGENIC C57BL/6 MICE. M.E. Hahn, T.A. Gabiak, P. Linko, and J.A. Goldstein*. Division of Toxicology, University of Rochester, Rochester NY, and NIHES, Research Triangle Park, NC.

HCB and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) produce a similar hepatic porphyria and induce cytochromes P450 and P2450 in mice. Since the effects of TCDD are thought to be mediated by its binding to the Ah receptor, we examined the possible involvement of this receptor in HCB porphyria. We used two congenic strains of C57Bl/6 mice that differ at the "Ah locus". Female B6-Ah<sup>H</sup> (Ah receptor: -50 fmol/mg) and B6-D2-Ah<sup>B</sup> (Ah receptor undetectable) mice were pretreated with iron (500 mg/kg) and then fed a diet containing 200 ppm HCB. Mice from the two strains consumed similar amounts of HCB. Urinary excretion of porphyrins increased after 7 weeks (wk) of HCB treatment in the B6-Ah<sup>B</sup> mice, and after 15 wk was 250-fold higher than that of mice given iron only. In the B6-D2-Ah<sup>B</sup> mice, porphyrin excretion did not increase until after 13 wk; after 15 wk, the levels were only 5-fold higher than those of iron-only controls. Similar differences were seen in 15-wk hepatic porphyrin levels (B6-Ah<sup>B</sup>: 1110 ± 393; B6-D2-Ah<sup>B</sup>: 17.6 ± 14.5 nmol/g). Induction of cytochromes P450 and P2450 was also measured, using antisera raised against the homologous rat isozymes. HCB induced a protein recognized by anti-P-450c (P450c) in B6-Ah<sup>B</sup> mice, but not in B6-D2-Ah<sup>B</sup> mice. A protein recognized by anti-P-450d (P2450) was induced in both strains. The results suggest that the Ah receptor is involved in HCB porphyria in mice, possibly via the sustained induction of cytochrome P450.

LONG-TERM KINETICS OF THE Ah RECEPTOR IN HAMSTERS ADMINISTERED 3H-2,3,7,8-TETRACHLORODIBENZOFURAN (TCDD). T.A. Gabiak and G. Ruud, Division of Toxicology, University of Rochester, Rochester NY.

The E<sub>50</sub> values for the increase in hamster hepatic cytochrome P-450 and ethoxyccoumarin O-deethylase activity range from 0.5-1.0 μg TCDD/kg, and at 10 μg/kg these remain maximal for up to 35 days (Gabiak et al., Biochem. Pharmacol. 35, 2739, 1986). In this study, we examined the long-term kinetics of the Ah receptor under conditions of sustained, maximal enzyme induction. Golden Syrian hamsters were administered a single ip. dose of 10 μg 3<sup>H</sup>-TCDD/kg ± 10-fold excess of unlabeled TCDD. Within 24 hours following 3<sup>H</sup>-TCDD treatment, total (3<sup>H</sup>-TCDD-occupied and unoccupied) cytosolic receptor decreased to approximately 60% of the total control (untreated) level and remained near this level for up to 35 days. By day 4, 3<sup>H</sup>-TCDD-occupied cytosolic receptor was barely detectable. Salt (1 M KCl)-extractable nuclear receptors peaked within 6 h at approximately 30% of the total (cytosolic) control level and then declined with a half-life of 13 days. Total detectable receptor (total cytosolic plus salt-extractable nuclear) declined to approximately 70% of the control value within 4 days and remained near this level for 35 days. This study suggested that maximal nuclear uptake of the TCDD-receptor complex was not necessary for sustained maximal enzyme induction. Salt-unextractable complexes in the nucleus may be important in maintaining this induction. (Supported by NIH Grant ES01515 and Center Grant ES02417.)

PHYSICOCHEMICAL PROPERTIES OF THE HUMAN AND RODENT Ah RECEPTOR. J.C. Cook and W.F. Greenlee. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Many of the actions of 2,3,7,8-tetrachlorodibenzo-p-dioxin on human and rodent target cells are initiated by interaction with a specific intracellular receptor protein designated the Ah receptor. Characterization of the physicochemical properties of the Ah receptor from cultured human thymic epithelial (HuTE) cell lines indicates that this protein is different from the receptor species present in rodent liver: (1) The human receptor is less sensitive to heat inactivation. (2) The human receptor sediments similarly to the rodent receptor on sucrose gradients when incubated at 0°C, but unlike the rodent receptor, it undergoes a temperature-dependent conversion to a species sedimenting at 12.4 S when incubated at 20°C. This conversion is prevented by inclusion of 20mM molybdate in the homogenization buffer. (3) Molybdate prevents the conversion of the human receptor to a lower S value (5.0 S) in 0.4 M KCl; the rodent receptor undergoes partial conversion in the presence of molybdate. Mixing HuTE cytosol with hepatic cytosol from C57Bl/6 mice revealed the presence of a heat-labile inhibitory factor in HuTE cells. This factor may account for the lower total specific binding detected in human cytosol incubated at 20°C or prepared in the absence of molybdate. The toxicologic significance of these differences in the physicochemical properties of the human and rodent Ah receptor is not known.
The effects of substituents on the in vivo and in vitro activities of thirteen 2-substituted-3,7,8-trichlorodibenzo-p-dioxins (TCDD) were determined in the immature male Wistar rat. The competitive receptor binding affinities of these analogs were determined using $[^3H]2,3,7,8$-tetrachlorodibenzo-p-dioxin (TCDD) and rat hepatic cytosol; the results were analyzed by multiple linear regression analysis to give Equation 1 where

$$\text{pEC}_{50} = 7.196 + 0.600 \Delta E_g + 0.255 \Delta E_s + 1.683$$

HB

Table: HB represents substituent lipophilicity, van der Waals volume and hydrogen bonding capacity. The 2-CF$_3$-TCDD analog exhibited the highest binding affinity for the receptor (pEC$\text{_{50}} = 8.50$) and was more toxic than 2,3,7,8-TCDD. All of the 2-halo-3,7,8-TCDD analogs were highly toxic, whereas most of the other analogs were comparatively inactive. (Supported by the National Institutes of Health, ES03594.)

[3H]-2,3,7,8-TETRACHLORODIBENZOFLURAN (TCDF) - SYNTHESIS AND CHARACTERIZATION AS A RADIOIODINATE FOR THE Ah RECEPTOR: K. Farrell, L. Safe and S. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

[3H]-TCDF was prepared by chlorination of 1,4,9-[3H]-dibenzo[4,1,9]fluoran followed by purification by HPLC (purity > 98%, specific activity: 57 Ci/mmol). The binding of [3H]-TCDF to the cytosolic Ah receptor resembles that of [3H]-TCDD. The affinity of binding as determined from the Scatchard plot is slightly less for [3H]-TCDF (K_d = 0.95 nM) than for [3H]-TCDD (K_d = 0.60 nM). The mechanism of binding each radioligand to the Ah receptor is similar and occurs in the absence of detectable positive cooperativity. However, for quantification, the use of [3H]-TCDF is distinctly advantageous over [3H]-TCDD because the ease of dissociation of the [3H]-TCDF-Ah receptor complex is much greater than that of the [3H]-TCDD-Ah receptor complex. There were several other significant differences between the [3H]-TCDF and [3H]-TCDD radioligands including the relatively higher levels of non-specific binding by the latter substance. The significance of these results will be discussed. (Supported by the Environmental Protection Agency and the Texas Agricultural Experiment Station.)

APPLICATION OF GAP JUNCTIONS IN NORMAL LIVERS AND CHEMICALLY-INDUCED HEPATIC NODULES OF RATS: M. G. Evans, E. A. Evans, Department of Pathology, Michigan State University, E. Lansing, MI.

Gap junctions permit cell-cell exchange of certain metabolites and ions. Progression of tissue toward malignancy is often associated with decreased numbers of gap junctions. We hypothesized that decreased surface area occupied by gap junctions occurred in hepatic "nodules", as these lesions are assumed precursors to hepatocellular carcinomas. Female Sprague-Dawley rats were partially hepatomecimized, given diethylnitrosamine (10 mg/kg body wt.), and fed selected PCB or PB congeners. After 150 days, some rats had hepatic nodules. These were hemisected, and half of each nodule was fixed in formalin for light microscopy to confirm that the tissue was consistent with the histologic appearance of hepatic nodules. The other half was fixed in 4% gluteraldehyde, infiltrated with 23% glycerol, and Congo-red-stained. A total area of 4200 μm$^2$ was examined from normal liver and hepatic nodules. The ratio of hepatocytic membrane area occupied by gap junctions was 2.8% in normal liver and 1.1% in hepatic nodules. These results suggest there are fewer gap junctions in chemically-induced hepatic nodules than in normal liver.

APPLICATION OF THE "FRAP" ASSAY TO MEASURE INHIBITION OF INTERCELLULAR COMMUNICATION BY 2,2', 4,4', 5,5'-HEXABROMODIPHENYL IN WB CELLS: M. G. Evans, J. E. Trusko, Depts. of Pathology and Pediatrics/Human Development, Michigan State University, E. Lansing, MI.

Inhibition of gap junction-mediated cell-cell communication might be a mechanism of tumor promotion. The chemical 2,2', 4,4', 5,5'-hexabromodiphenyl (HBB) is a known tumor promoter in vivo and blocks cell-cell communication in vitro. F344-NB (rat epithelial) cells were plated at low density, exposed to non-toxic concentrations of 1, 5, 20, or 40 μg HBB/ml medium while grown overnight in 5% CO$_2$ at 37°C, rinsed with Ca/Mg PBS, and stained with 6-carboxyfluorescein diacetate for 20 minutes. One cell in pairs of touching cells in each treatment group was excited for Fluorescence Redistribution After Photobleaching ("FRAP") using Anchored Cell Analysis and Sorting (Meridian Instruments). The results indicate an inverse correlation between the degree of fluorescence redistribution in photobleached cells and the concentration of HBB. These results provide further evidence for the ability of HBB to inhibit gap junction-mediated cell-cell communication in vitro in a dose-dependent manner.
DOSE-DEPENDENT INHIBITION OF METABOLIC COOPERATION IN RAT EPITHELIAL (WB-F344) CELLS BY HEPATIC TUMOR PROMOTER 2,2',4,4',5,5'-HEXABROMOBIPHENYL. L. G. Evans and L. E. Troshko, Dept. of Pathology and Pediatrics/Human Development, Michigan State University, E. Lansing, MI

Inhibition of intercellular metabolic cooperation (NC) is a possible mechanism of tumor promotion. The chemical 2,2',4,4',5,5'-hexabromobiphenyl (245-HBB), is a known hepatic tumor promoter in rats. 245-HBB was tested in vitro in an MC assay with rat liver (F344-WB) epithelial cells. In the MC assay, cells able to metabolize 6-thioguanine (HGPRtm cells) transfer the resultant lethal product via gap junctions to adjacent mutant cells lacking the enzyme needed for toxic conversion (HGPRtm cells). Compounds which block transfer of the lethal product permit HGPRtm cells to live. At noncyotoxoidal concentrations of 5, 20, and 40 µg/ml medium, there was a 3-fold increase in recovery of HGPRtm cell survival from 5 to 40 µg/ml medium, indicating that 245-HBB inhibits cell-cell communication in a dose-dependent manner. These results provide further support for the ability of certain environmental toxins to block metabolic cooperation in vitro. (Supported by NIHES Grant ES07146).


The V79 metabolic cooperation assay is responsive to various classes of chemical toxicants including carcinogens, tumor promoters, teratogens, and neurotoxins. The adaptation of this or any in vitro assay system to permit the evaluation of volatile chemicals is of great importance since many human toxicants fall into this category. The assay was adapted for volatile chemicals by using sealed culture flasks and that had been filled with medium prior to the addition of test chemical. The optimal 6-thioguanine sensitive cell seeding density was increased from 4.0 to 7.5 x 10^5 cells per 25 cm² culture vessel. Preliminary data show that trichloroethylene, 1,1-dichloroethane, 1-naphthylamine, acrylonitrile, and cyclophosphamide produced positive responses in the assay when evaluated at nontoxic concentrations. These studies show that the V79 metabolic cooperation assay is responsive to carcinogens and can be adapted to evaluate volatile toxicants. (Supported by U.S. EPA contract #68-02-4032.)

INDUCTION OF HEPATIC PEROXISOME PROLIFERATION IN MALE BgC3F1 MICE FOLLOWING HIGH FAT DIETARY EXPOSURE. P. J. Schafer, J. E. Klausing, and J. A. Hampton. Department of Pathology, Medical College of Ohio, Toledo, OH

Thirty male BgC3F1 were fed either a semipurified control diet (AIN-76; 5% corn oil) (15 mice) or a high fat diet (modified AIN-76; 17.5% menhaden oil) (15 mice) highly enriched in omega 3 fatty acids for 12 weeks. The high fat diet was supplemented with 0.2% α-tocopherol and 500 µg vitamin K/kg diet. Livers were removed at necropsy, fixed in 3% glutaraldehyde, stained with 3-3'-diaminobenzidine, post-fixed with 1% OsO₄, dehydrated in ethanol and embedded in Spurr's resin. From electron micrographs (final magnification 18,520X) of mouse hepatocytes, the volume density, Vₚ (percentage of hepatocytes cytoplasm occupied by peroxisomes) and numerical density, Nₚ (number of peroxisomes profiles/mm² cytoplasm), were determined using standard morphometric techniques. Differences between control and treated groups were determined by analysis of variance and all values were expressed as mean ± standard error. The mean Vₚ (5.12 ± 1.53%) and Nₚ (3.19 x 10^5 ± 0.75 x 10^5) of peroxisomes from animals fed menhaden oil were significantly greater than values obtained from control animals (Vₚ=1.88 ± 0.69% and Nₚ=1.16 x 10^5 ± 0.27 x 10^5). Menhaden oil diet, high in omega 3 fatty acids, therefore, was a potent peroxisome proliferator in the male BgC3F1 mouse liver.


The uricosuric drug benz bromarone (3,5-dibromo-4-hydroxyphenyl)-(2-ethyl-3-benzofuranyl)-methane was administered in the diet at 1000 and 2000ppm to male F344 rats for seven weeks. Various parameters related to hepatic peroxisome proliferation were measured in the benz bromarone-exposed groups, in a group exposed to the peroxisome-proliferating lipid-lowering drug clofibrate (5000ppm) and in a group fed the basal diet. Benz bromarone produced a significant reduction in weight gain, which was related to the dose. Reduced weight gain was also observed in the clofibrate-exposed group. Hepatomegaly and increases in the activities of catalase, ascyl CoA oxidase, malate dehydrogenase and glycerol-3-phosphat dehydrogenase were produced by benz bromarone and clofibrate. Benz bromarone and clofibrate both induced similar histologic changes and ultrastructural changes in the liver, including induction of peroxisomes. Benz bromarone appears to be a peroxisome-proliferating agent in rats with a chemical structure and pharmacological properties dissimilar to other known peroxisome proliferating agents.
Comparative Effects of the Peroxisomal Proliferating Agents, Di(2-Ethylhexyl)phthalate (DEHP) and Wy-14,643 (Wy) in Rat Liver. D.S. Marsman, R.C. Cattley, and J.A. Popp, Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Sponsor: J.E. Gibson.

Because of the contrasting hepatocarcinogenic potencies of DEHP, a plasticizer, and Wy, a hypolipidemic drug, peroxisomal induction by these two compounds was compared. Male P3H4 rats were fed DEHP (1.5%) or Wy (0.1%) in NIH07 diet for 1, 2, 4, 8, 10, or 39 days. Carnitine acetyltransferase (CAT) and cyanide-insensitive palmitoyl-CoA oxidase (PCO) were assayed in supernatants of liver homogenates. CAT was elevated at every interval with Wy 2-fold higher than DEHP. The induction of the peroxisome-specific PCO by Wy paralleled CAT, reaching 10-fold elevations by 8 days. PCO was not increased by 1 day of DEHP, and was increased to only half of Wy at days 2 and 4. By 18 days the PCO induction by DEHP increased to the level of PCO induction by Wy. Based upon electron microscopy at 39 days, the peroxisomal volume fraction was increased by DEHP and Wy (4.4- and 5.9-fold, respectively). Increases in absolute liver weight were not different between DEHP and Wy. In summary, the difference in the peroxisomal response between the two compounds was more remarkable at the early time points. If peroxisomal induction is important in the hepatocarcinogenicity of these two agents, a critical event may occur early in the course of treatment.

Effect of Dietary Clobibrate on Hydrolytic Enzymes. M.S.A. Ashour, B.L. Finley, D.E. Moody, and B.D. Hambrock. Departments of Entomology and Environmental Toxicology, University of California, Davis, CA.

The effect of the peroxisome proliferator clofibrate (ethyl chloroenoxy isobutyrate) on microsomal carboxylesterases, cholesterol epoxide hydrolase and the cytosolic epoxide hydrolase was studied. Treatment with 0.5% w/w dietary clofibrate for 4 days induced carboxylesterase activities on five substrates namely, clofibrate, malathion, diethylsulfate, diethyl phthalate, and p-nitrophenylacetate in liver and kidney microsomes of mice and rats. The induction was substrate, tissue, and species dependent. The esterase activity was induced from 1.2 to 2.2 fold in mouse liver depending upon substrate used. Analogous values from rat liver ranged from 1.0 to 1.4 fold. A series of substituted trifluoroketone inhibitors of carboxylesterases were used to indicate that changes in carboxylesterase activity following clofibrate treatment were both quantitative and qualitative. Apparent induction of carboxylesterase activities were compared with induction of the cytosolic and cholesterol epoxide hydrolase activities in several species following dietary exposure to clofibrate.

The Effect of Phenobarbital Pretreatment and Oxygen Concentration on Menadione Toxicity in Isolated Rat Hepatocytes. W.S. Utley and H.M. Mehendale. Dept. Pharmacol and Toxicol, Univ. Medical Center, Jackson, MS.

Presumably phenobarbital (PB) pretreatment and high oxygen (O,.) levels facilitate menadione (M)-induced cytotoxicity. Since data supporting these assumptions are sparse, we wished to verify these assumptions. Microsomes prepared from PB pretreated male S-D rats showed an increase in cytochrome c reductase activity and menadione stimulated superoxide production vs control. Hepatocytes isolated from PB pretreated and naive animals were incubated under one of three oxygen conditions (0, 20% or 95% oxygen) for three hours. During this time samples were drawn and assayed for LDH release and trypan blue exclusion. Neither parameter indicated any significant difference in M-induced cytotoxicity between naive, PB treated, 20% and 95% O,., Consistent with the oxy-radical hypothesis of M-induced cytotoxicity, hepatocytes incubated under 0% O, (95% N, + 5% CO,) did not exhibit any M - cytotoxicity.

The failure of PB pretreatment to increase M-induced cytotoxicity in hepatocytes may indicate that a cytotoxic mechanism is induced as well. (Supported by ES-07045 and EPA grant R-811072.)

Lipid Peroxidation and Cellular Damage Caused by the Pyrrolizidine Alkaloid Senecionine, the Alkaloid Trans-4-0H-2-Hexenal, and Related Alkaloids. D.S. Griffin and H.J. Segall. VM/Pharmacology and Toxicology, University of California, Davis, CA.

Recently our lab isolated trans-4-OH-2-hexenal (t-4OH) from the hepatic microsomal metabolism of the macrocyclic pyrrolizidine alkaloid (PA) senecionine. This alkalen has been shown to produce cytotoxicity and genotoxicity in primary hepatocyte cultures and hepatic necrosis in vivo similar to that observed with senecionine. The exact mechanism of cellular injury and death may involve alkylation of cellular macromolecules, perturbation of cellular calcium homeostasis or lipid peroxidation. In this study, lipid peroxidation was examined as a possible mechanism for cell injury by the PA senecionine, t-4OH, and related alkaloids in isolated rat hepatocytes. Each compound elicited a positive dose response for peroxidation of cellular lipids as measured by the formation of thiobarbituric acid-reactive products. The addition of the anti-oxidant N,N'-diphenyl-p-phenylenediamine (DPPD) inhibited the production of thiobarbituric acid-reactants. However, DPPD had no protective effects on the cell membrane integrity as evidenced by the leakage of lactate dehydrogenase from the cells into the surrounding media. These results suggest that lipid peroxidation which occurs in the presence of the PA senecionine, t-4OH, or related alkaloids, is not entirely responsible for the cellular damage in isolated rat hepatocytes.

The use of the anti-neoplastic agent doxorubicin is limited by the cardiotoxicity and myelosuppression associated with this agent. Doxorubicin cell toxicity may be mediated through free radical generation and lipid peroxide formation. This study quantitated the direct effect of Adriamycin on aortic 6-keto-PGF_{1α} and platelet TXB_{2} formation in male Sprague Dawley rats (300-350 gm). Aortic rings and washed platelets were suspended in oxygenated tyrode solution containing 5.5mM glucose (pH 7.4). Aortic rings and platelets were incubated 40 min with 0, 50 or 100 μM doxorubicin at 37 and 26°C, resp. An aliquot of the incubant was removed to quantitate basal prostaglandin release by RIA. Prostaglandin release was stimulated by the addition of 5 μM A23187. Addition of 50 or 100 μM doxorubicin diminished aortic 6-keto-PGF_{1α} levels to 68% and 72% of control (P<0.05), resp. Pre-incubation with 50 or 100 μM doxorubicin diminished platelet TXB_{2} formation to 48 or 38% of control (P<0.05), resp. Doxorubicin directly inhibits vascular PG, and platelet TXB_{2} formation. (Supported by NIH RR 00870).

ASSESSMENT OF MALONALDEHYDE (MDA) FORMATION INDUCED BY ISOBULBUCYCLIN COMPLEXES IN ISOLATED SPERMATOGENIC CELLS. H.P. Angiolli, J. Ramos, I. V. Rosenblum. Philadelphia College of Pharmacy and Science, Philadelphia, PA.

Bleomycin (BLM), an antitumor antibiotic, is commonly used in the treatment of testicular carcinomas. The therapeutic and cytotoxic activities of BLM have been attributed to the degradation of DNA and lipid peroxidation (LP). Ferrous ion is known to be a critical cofactor in reactions catalyzed by BLM in vitro; however, little is known regarding the importance of iron in the expression of drug activity in intact cells. The purpose of the present investigation was to evaluate the role of iron in the responses of isolated rat spermatogenic cells to BLM. MDA formation was used as an indicator of BLM-induced DNA and lipid degradations. Promethazine (PZ) was used to distinguish the source of MDA. High concentrations of drug (30 and 150 µg/ml) were required to induce MDA formation in the absence of added iron. PZ (1µM) inhibited all stimulatory responses except those induced by high BLM concentrations. Reduced iron (1µM) alone or complexed with BLM (1.5 µg/ml) stimulated MDA production. Treatments with oxidized iron (1µM) and ferric-BLM had no effect. Ascorbic acid (0.5-2mM) inhibited the effects of reduced iron, but increased the amount of MDA formed in the presence of BLM or ferric-BLM. Deferoxamine (1µM), a specific iron chelator, inhibited MDA production regardless of treatment. These results suggest that MDA formation promoted by BLM (1.5 µg/ml) is mediated by iron-induced LP, whereas the MDA induced by drug alone is associated with DNA degradation.

PARACETAMOL-INDUCED CYTOSKELETAL INJURY IN CULTURED LUNG EPITHELIAL CELLS. Wande Li, Yinhzi Zhao, and J.W. Chou. Dept. of Pathol., Nongh China Coal Medical College, Tangshan, PRC and Dept. of Microbiol., Boston University School of Medicine, Boston, MA. Sponser: A.R. Rogers

Paracetamol (PO), a broad spectrum heptoid, produces a selective pulmonary toxicity in humans and animals. To understand the mechanisms of PO-induced lung injury, we have studied the effects of PO on DNA synthesis, the cytoskeletal organization, particularly, microtubules (MT) and microfilaments (MF), and the cytoskeletal protein synthesis in cultured Fischer rat lung epithelial cells (resembling type II alveolar cells in vivo). Exposure of cells to PO resulted in a dose-dependent inhibition of DNA synthesis (ID₅₀ at 135 μM) as well as marked alterations in the cytoskeletal organization and cell shape characterized by MT aggregation and bundling, and MF redistribution forming cord-like structures in the cell periphery. At high dosage, PO caused a severe loss of MT and MF. In addition, PO treatment also induced a dose and time dependent inhibition of cytoskeletal protein synthesis, in agreement with microscopic observations. However, no damage to MT and MF occurred upon adding PO directly to in vitro detergent-extracted cytoskeletons. These results suggested that cytoskeletal injury may be important in the mechanism of development of PO-induced pulmonary lesions.


It is well established that cyanide inhibits cytochrome oxidase at a site to produce histotoxic hypoxia. However, the subsequent events resulting in cellular damage and death remain to be characterized. In the present study, conjugated diene generation in brain lipids was observed following administration of varying doses of KCN to male mice. Conjugated diene production was dose and time dependent; 10 mg/kg KCN produced detectable levels of conjugated dienes at 30 min post cyanide, whereas 15 mg/kg produced elevated levels of conjugated dienes over a 10 to 60 min period after KCN. Pretreatment of mice with either diltiazem (600 µg/kg, iv), a calcium channel blocker, or allopurinol (25 mg/kg, iv), a xanthine oxidase inhibitor, blocked the generation of conjugated dienes by cyanide. These results suggest that lipid peroxidation of neuronal membranes play a role in cyanide intoxication and this action may be related to altered regulation of calcium homeostasis and subsequent activation of xanthine oxidase. (Supported in part by PHS grants ST322507039 and ES04140).

Silica-treated AM have increased bactericidal activity. Since O$_2^-$ is an important bactericidal product of AM, we examined the effect of SI incubation with AM on f-nle-leu-phe (FNLP) and phorbol dibutyrate (PDB) stimulated O$_2^-$ production and FNLP stimulated phosphatidyl inositol turnover (PI). AM (10$^6$/ml) were incubated with 250 (LSI) or 500 (HSI) ug/ml SI at 37 C for 2 hrs. HSI caused a slight decrease in AM viability (81% to 70%), while LSI had no effect on AM viability. LSI and HSI decreased FNLP stimulated O$_2^-$ production by 25% and 54% respectively, while PDB stimulation was not affected. PI was examined by quantitating the labelling of phosphatidyl inositol 4, 5 bisphosphate (PIP$_2$) and phosphatidic acid (PA) in AM prelabelled with 32P$_{32}$O. FNLP-stimulated label of PA increased 73% and 25% for cells incubated with LSI and HSI respectively. Labelled PIP$_2$ decreased by 23% one min following FNLP stimulation in control AM and 41% for AM treated with LSI. These results suggest: 1) the site of SI action proceeds protein kinase C (PKC) activation, 2) although FNLP-stimulated O$_2^-$ production was inhibited by SI, FNLP-stimulated PI appeared to be enhanced. Therefore, SI may be altering the metabolism of the PI intermediate dicylglycerol affecting PKC activation.


Misonidazole (MISO), a nitroheterocyclic radiosensitizer, is selectively toxic under hypoxic conditions. Colony-forming assays were used to determine survival of cells treated with MISO and alkaline elution techniques were used to assess DNA damage. Survival of EMT6 mouse mammary tumor cells was decreased by 80% after a 2-hr exposure to MISO at 5 nM in hypoxia, whereas survival ofoxic cells decreased 30%. MISO produced dose- and time-dependent single strand breaks in DNA of hypoxic cells but not in oxic cells. Diethyl malate (DEM) and buthionine sulfoximine were used to deplete cells of glutathione. The cytotoxicity of MISO was potentiated in hypoxic cells with decreased glutathione concentrations but not in oxic cells. Glutathione depletion also enhanced the induction of DNA single strand breaks. Cells treated with MISO at 5 nM for 6 hrs, for example, accumulated approximately 300 rad-equivalent single strand breaks, whereas DEM pretreated cells showed a similar amount of damage when treated with MISO for 2 hr. Reoxygenated EMT6 cells were able to repair MISO-induced DNA damage efficiently. Repair of drug-induced damage under uninterrupted hypoxia, however, was slowed. (Supported by an Emlie McCrulty Grant from the American Cancer Society and NCI CA36946.)

667 ABSENCE OF ROLES FOR OXIDATIVE STRESS AND LIPID PEROXIDATION IN MPTP-INDUCED CYTOTOXICITY M.S. Sandy, D. Di Monte, G. Ekström and M.T. Smith, School of Public Health, University of California, Berkeley, CA

The parkinsonism-inducing compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is metabolized by monooamine oxidase B to its putative toxic metabolite 1-methyl-4-phenylpyridinium ion (MPP$^+$) via 1-methyl-4-phenyl-2,3-dihydropyridinium ion (MPPD$^+$). MPP$^+$ is similar in structure to the herbicide parquat (PQ$^+$), and it has therefore been postulated that MPP$^+$, and hence MPTP, induced cytotoxicity may be due to oxidative stress via the production of active oxygen species. The toxic effects of PQ$^+$ in isolated hepatocytes are markedly potentiated by pretreatment with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and are extensively delayed by antioxidants and the iron chelator, desferrioxamine. None of these treatments alters the cytotoxicity of MPTP or MPP$^+$ in hepatocytes. PQ$^+$ cytotoxicity is also accompanied by extensive peroxidation and vitamin E depletion. In contrast, MPP$^+$ produces only a slight increase in lipid peroxidation above control levels in hepatocytes, while both MPTP and MPPD$^+$ are potent antioxidants. MPTP and MPPD$^+$ completely inhibit chemically and radiation-induced lipid peroxidation in rat liver microsomes with MPPD$^+$ being the most effective. Oxidative stress and lipid peroxidation are therefore unlikely to play any role in MPTP toxicity.

669 INDUCTION OF OXYGEN TOLERANCE IN MICE. N.C. Margaretten, and H.P. Witschi. Oak Ridge National Laboratory, Biology Division, Oak Ridge, TN

It is generally assumed that rats are the only species which can be made resistant to the lethal effects of 100% O$_2$. We report that it is possible to induce oxygen tolerance in mouse lung by producing diffuse lung damage followed by repair. Male BALB/c mice, 6 to 8 weeks old, were treated with a single ip dose of 400 mg/kg of butylated hydroxytoluene (BHT). Control mice injected with corn oil and placed into 100% O$_2$ all died between day 4 and 5 in 100% O$_2$ (LT50 4.5 days) and no-d BHT-treated animals placed into 0, 2 days after BHT. However, mice placed into 100% O$_2$ one week after BHT survived for a full week in 100% O$_2$ (LT50 10 days). In the lungs of the pretreated animals, the activities of glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase (G6PDH) and superoxide dismutase were higher if calculated per lung; however, calculated on a per mg of protein basis, G6PDH was the only enzyme to show increased activity. It is concluded that replacement of most of the alveolar lining cells by "young" type I cells derived from recently divided type II cells conveys oxygen tolerance to adult mice. (Operated by Martin Marietta Energy Systems, Inc. with the U.S. Dept. of Energy. NCM supported by NHT Grant CA-09336.)
METABOLISM OF Methotrexate IN HEPATIC CELLS.
C. Kruger-McDermott, M. Rhee and J. Galivan.
N.Y.S. Health Labs, Albany, NY
Sponsor: L. Kavarnsky

Methotrexate (MTX) is used clinically as a chemotherapeutic agent. Low dose MTX has been reported to cause hepatic toxicity. MTX is metabolized differently by hepatocytes and hepatoma (H35) cells which play a role in its toxicity. Consequently, the extent of formation of these metabolites in each cell type was studied. After a short term low dose MTX pulse, cellular MTX derivatives were evaluated by HPLC. H35 cells have 5x the glutamyltransferase capacity of hepatocytes under optimal conditions and generate more longer length (G4 & G5) polyglutamate (PG). Conversely, 7-OH-MTX is the major product of MTX metabolism in hepatocytes. This product is also glutamylated. Low dose MTX pulse leads to the formation of longer chain length PG compared to high dose MTX pulse in both cell types. 7-OH-MTX is less toxic than MTX PGs, however, 7-OH-MTX PG has a longer cellular retention time than 7-OH-MTX which might contribute to toxicity (L.Fahre et al. Cancer Research 43, 4648-52, 1983). Toxicity to hepatocytes after long term, low pulse MTX exposure may not be dependent on inhibition of dihydrofolate reductase since liver is in a G0 state. The increase in MTX PGs after low dose may be a contributing factor. MTX PGs may enhance MTX toxicity in cells due to their greater retention time as compared to the parent compound.

HISTONE PHOSPHORYLATION DURING DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMA HL-60 CELLS INDUCED BY A PHORBOL DIESTER. D.G. DeBord and C.S. Baxter. Univ. of Cincinnati Coll. of Med. Cincinnati, OH.

Human HL-60 promyeLOCYTIC leukemia cells can be induced to differentiate along either the granulocytic or monocytic pathway and provide a model system for studying the biochemical mechanisms regulating this process. Phorbol diesters such as 12-0-tetradecanoylphorbol-13-acetate (TPA) induce differentiation to monocytic cells. When HL-60 cells were treated with TPA phosphorylation of histone H2B was induced in a time and dose dependent manner, while that of the other histones remained unchanged. Phosphorylation was quantitated using two-dimensional gel electrophoresis of 32p labelled histones. In the presence of 100 nM TPA, phosphorylation of histone H2B was increased after 1, 2 and 4 hours by 61, 73 and 130% and with 10 nM TPA by 35, 64 and 97% relative to controls. The role of histone H2B phosphorylation in the process of differentiation has been further investigated. The granulocytic inducer of HL-60 cells, DMSO, showed no changes in the phosphorylation of the histones. Supported by USPHS Grants CA34446 and ES07073.

Depts. of Entomology & Environmental Toxicology, University of California, Davis, CA.

Human term placenta offers a readily available source of human tissue for studies of toxicologically important enzymes. The specific activity of cytosolic epoxide hydrolase (C11H) in human placenta crude cytosol, using trans-stilbene oxide as substrate, was 50 pmol/min/mg protein. We have purified human placental C11H >800-fold by affinity chromatography using benzyl mercaptan or 7-methoxycyclomethyl thiol coupled to epoxy-activated Sepharose CL-68 for the selective adsorption of the enzyme and 4-fluorochalcone oxide (a C11H inhibitor) for its selective elution (Toxicologist 6:273, 1986). The purified enzyme was characterized by SDS-polyacrylamide gel electrophoresis and isoelectric focusing. The electrophoretic patterns differed from that observed with C11H from mouse liver. The purified C11H from human placenta should permit a detailed comparison of the rodent and human enzymes, important for validating the rodent models in current use.


Human lymphocytes isolated over a 1.080 Percoll gradient were incubated at 37°C with 2,2'-dichlordodiethyl sulide (sulfur mustard, HD). The cellular toxicity induced by HD was both time- and concentration-dependent. At 10^-4 M HD there was no significant cell death at 4 hrs, but by 16 hrs, greater than 30% of the cells were dead. By 24 hrs there was only 50% survival of the lymphocytes as measured by trypan blue dye exclusion. There was no significant increase in cell death when the incubation was continued for 48 hrs. Lymphocytes were incubated with varying concentrations of HD for 24 hrs. Control cells and cells incubated with HD at <10^-4 M or lower exhibited no detectable toxicity. At concentrations greater than 10^-4 M HD, there was a linear relationship between HD added and the observed cellular toxicity. The NAD levels of the samples were examined and there appeared to be a time- and concentration-dependent decrease in NAD. The relationship between HD exposure and decreases in NAD levels in both cytosolic and cellular NAD was as predicted by our hypothesis, which links DNA alkylation with activation of poly (ADP-ribose) polymerase, resulting in NAD depletion and cell death. This model should enable us to study the biochemical mechanism of HD-induced pathology at the cellular level.
Intercellular communication is imperative for differentiation of MGC and successful spermatogenesis. Message translation in the seminiferous tubule remains largely unknown, especially between developing germ cells. Previous investigations in our laboratory have elucidated a membrane-bound AC, which is responsive to GTP in a dose-dependent manner. The purpose of this study was to assess the effects of the phorbol ester, tetradecanoylphorbol-13-acetate (TPA), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on AC activity. Isolated MGC were exposed to TPA and TCDD (1.7 nM) for 1 hr at 33°C, and plasma membranes were purified through ultracentrifugation. The addition of these membranes with ATP (0.5 mM) and GTP (0.1-100 μM) generated cAMP, which was quantitated by RIA. Preliminary data indicate that TPA enhances GTP-stimulated AC activity, while TCDD has a slight inhibitory or no effect as compared to vehicle controls (0.5% DMSO). These data suggest that AC activity in MGC is modulated by hormonal agents, however TPA and TCDD may act via two distinct mechanisms.

Organ differences in stimulation of microsomal NADPH oxidation by bipyrindyl herbicides.

L. Zychlinski, P. Raska-Enery, and M. R. Montgomery. Univ. of South Florida, Coll. of Public Health and VA Hospital Research Service, Tampa, FL

The effects of paraquat (Pq) and diquat (Dq) (0.1-1.0 mM) on NADPH oxidation rates were determined in vitro using rat lung, liver, and kidney microsomal preparations (mics) in the absence or presence of the mixed function oxidation (MFO) substrates, ethylmorphine (Em) or benzphetamine (Bz). NADPH oxidation was stimulated by both herbicides in the absence or presence of both substrates in a concentration-dependent manner. For all situations, Dq consistently produced a greater stimulation of oxidation rates than Pq. At the highest conc. of Pq or Dq, stimulation of NADPH oxidation in lung mics was equal in the absence of MFO substrates and in the presence of Bz; however, in liver mics, stimulation of NADPH oxidation was equal in the presence of both substrates, Em and Bz, but greater in the absence of substrates. In kidney mics, stimulation of NADPH oxidation by bipyrindyls was equal in all three situations; in the absence of MFO substrates, and in the presence of Em or Bz. Pq and Dq are potent stimulators of in vitro NADPH oxidation in microsomal preparations from several organs; however, the addition of MFO substrates produces results for which a consistent pattern cannot be identified. Supported by NIH Grant ES03340 and VA Medical Research Funds.

Phenotypic modulation of aortic smooth muscle cells in culture.

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Smooth muscle cells (SMC) isolated from rat aortae and grown in primary culture are capable of expressing a range of phenotypes. As a function of the culture conditions, these cells may modulate between a primarily contractile and synthetic phenotype. The synthetic state is associated with an increase in proliferative capabilities characteristic of atherosclerotic smooth muscle cells. Phosphoinositol (PI) turnover has been implicated in the regulation of cellular growth and proliferation. The present studies were conducted to assess PI turnover and phenotypic modulation in SMC. Synthetic SMC had a greater amount of phosphatidylinositol (27%), phosphatidylcholine 4-phosphate (129%), phosphatidylinositol 4,5-bisphosphate (74%), and phosphatidic acid (35%) than contractile SMC (n=3). The addition of dibutyryl cAMP (0.2 mM) and theophylline (0.1 mM) to synthetic SMC for 72 hr resulted in morphological changes that may be associated with phenotypic modulation of the cells. These changes occurred concomitantly with a two-fold decrease in the levels of all phosphoinositides (n=3). These data suggest that the cascade of cellular events initiated by PI turnover may be an important regulator of the phenotypic stability of SMC. The atherogenic effect of toxicants such as allylamine may be mediated by alterations in the phenotypic expression of SMC.

Effect of trimethyltin (TMT) on rat hepatic glutathione transferase activity in vivo and in vitro.


The glutathione transferases (GST) occur as a family of dimeric isozymes, their activity determined by specific subunits present. Organells have been reported to inhibit GST activity in rat liver. The effect of TMT on several GST isozymes was examined. GST activities toward 1-chloro-2,4-dinitrobenzene (DCNB), androstene-3,17-dione (AN), trans-4-phenyl-3-buten-2-one (TPB), ethacrynic acid (EA) and dinitrochlorobenzene (DNB) were used as markers for isozyme activities. Adult male Sprague-Dawley rats were dosed ip with 7.0 mg TMT/kg or saline and sacrificed after 4, 24, or 48 hrs. Livers were homogenized in buffered sucrose and cytosol was prepared. GST activities were assayed using published methods. For in vitro experiments control cytosol was used and TMT (0.01-10 mM) added. TMT (10.0 mM) inhibited: DCNB, 97%; EA, 39%; AN, 30%; TPB, 151; and DNBC, 14%. Inhibition by 0.10 mM TMT was: DCNB, 48% and EA and DNBC, 14%. The 3-3 isozyme is thus the most susceptible to in vitro TMT inhibition. No inhibition was seen after in vivo exposure, but concentrations of TMT in in vivo assays were similar to those used in in vitro assays. Detoxication mechanisms may protect GST from the in vivo effects of TMT and account for differences seen after in vitro exposure.

Chloroform (CHCl₃), 1,2-dibromoethane (DBE), 1,1,2-trichloroethane (TCE), and dib(2-ethylhexyl)phthalate (DEHP) are common contaminants in drinking water and in waste water sludge. The effect of these non-genotoxic chemicals on cholesterol synthesis in cultured rat hepatocytes was investigated in an attempt to correlate cholesterol synthesis with cell proliferation. Hepatocytes were cultured in the presence of the test chemicals for 48 hrs at which time cholesterol synthesis was determined by measuring the incorporation of 2-3H-acetate into cholesterol. All four chemicals tested stimulated cholesterol synthesis: 50–100 μM CHCl₃, 4%; 50–100 μM TCE, 34%; 0.5–1 μM DBE, 3%; and 20–50 μM DEHP, 57%. Phenobarbital (1.5 mM), a well studied non-genotoxic carcinogen, stimulated cholesterol synthesis 140% above the control. DEHP treatment in vivo is known to inhibit cholesterol synthesis in rats. In the present study, DEHP stimulated cholesterol synthesis in vitro. We are now investigating the effects of these chemicals on the activity of 3-hydroxy-3-methylglutaryl CoA reductase, a rate limiting enzyme for cholesterol synthesis and hepatocyte DNA synthesis. This abstract does not necessarily reflect EPA policy. T.V.R. is a recipient of an NR-ERS Fellowship.

VITAMIN E SUCCINATE PROTECTS HEPATOCYTES FROM CHEMICAL TOXICITY. M.W. Fariss. Environmental Toxicology, Department of Pathology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA

Recent studies have shown that in the presence of vitamin E succinate, rat hepatocytes incubated with Ca²⁺-free medium are protected against the toxic effect of chemicals unlike cells incubated with 3.5 mM Ca²⁺ (Fariss et al. Science 227:751, 1985). To examine if the cytoprotective capacity of α-tocopherol succinate is dependent on the absence of extracellular Ca²⁺, rat and canine hepatocytes in suspension were incubated in the presence and absence of α-tocopherol succinate (25 μM) and extracellular Ca²⁺ (0, 0.8 and 3.5 mM) and exposed to ethyl methane-sulfonate (50 mM) or Ca²⁺ ionophore A23187 (5 μM) for 6 hours. Cells incubated with 0 or 0.8 mM extracellular Ca²⁺ and α-tocopherol succinate were completely protected (as measured by LDH leakage and the loss of intracellular K⁺) against the toxic effects of these chemicals. In contrast, by increasing the extracellular Ca²⁺ concentration to 3.5 mM the protective effect of α-tocopherol succinate was dramatically reduced or eliminated. These experiments demonstrate that under physiological conditions (0.8 mM Ca²⁺) α-tocopherol succinate is a potent cytoprotective agent against chemically induced toxicity.

INFLUENCE OF DIETHYL MALATE ON GLUTATHIONE METABOLISM IN RATS PRETREATED WITH HIPPURIC ACID. C. McGowan. Food Science and Human Nutrition Department, University of Florida, Gainesville, FL.

The administration of hippurate (HA) to mice has been shown to inhibit kidney γ-glutamyl transpeptidase activity in vivo. Thus, influences on the turnover of glutathione (GSH) as normally controlled by the γ-glutamyl cycle are evident. Data on the effects of HA in the rat are limited. The objective of this study was to investigate whether HA pretreatment of rats affects the diethyl malate (DEM)-mediated effects on GSH. Male Sprague-Dawley rats (175–225g) were injected intraperitoneally (ip) with 0 or 10 mmole/kg HA followed in 12 hours by ip injections of 0 or 1 ml/kg diethyl malate (DEM). Rats were killed 3 hours after DEM injection and samples (liver and plasma) were analyzed for GSH and cysteine (CYS) content and γ-glutamyl transpeptidase (GGTP) activity was assayed in the kidney. Pretreatment of rats with HA and no effect on liver and plasma GSH and CYS nor did it influence GGTP activity. Hepatic GSH but not CYS was significantly (p<0.05) lowered by DEM and a DEM × HA interaction for hepatic GSH approached significance (p<0.07). These data suggest that 10 mmole/kg HA does not inhibit rat renal GGTP activity and that further investigations of the possible DEM × HA interaction for hepatic GSH is indicated.

IN VITRO INACTIVATION OF PLASMA α1-PROTEINASE INHIBITOR BY EPICIDES AND 1,2-DIHALOETHANES. C.A.S. Awari, Jose C. Guan and Brian K. Barton, Division of Biochemistry, The University of Texas Medical Branch, Galveston, Texas

Inactivation of plasma α1-proteinase inhibitor (α1-PI) as well as inhibition of proteinase activity in whole plasma has been explored as a potential biological marker of the chemical exposure. Epicides (styrene oxide, ethylene oxide, propylene oxide and dihaloethanes, 1,2-dichloroethene and 1,2-dibromoethene) were used as model compounds. All these compounds have shown dose-dependent inactivation of α1-PI and also proteinase inhibitory activity of plasma when measured against elastase and trypsin, using synthetic substrates, o-N-succinyl-(ala)₅-p-nitroanilide and o-N-benzoyl-(ala)₅-arginine-p-nitroanilide, respectively. Styrene oxide was found to be the most potent inhibitor of α1-PI followed by ethylene oxide. Propylene oxide and 1,2-dihaloethanes were relatively less effective in inhibiting the activity. The loss of proteinase inhibitory activity correlated well with the disappearance of free amino groups as measured by the 2,4,6-trinitrobenzene sulfonic acid method, suggesting that lysine residues of α1-PI are important for its biological function. The present data also indicates that proteinase inhibitory activity in whole plasma can be used as a biological marker of the chemical exposure. (Supported by NIOSH Grant No. OH-02149).
Kidney tissue from male Sprague-Dawley rats was subcellularly fractionated for the purpose of determining which cellular components the radiolabel was associated with. The methods involved homogenization and ultracentrifugation. A series of experiments were conducted in order to determine the optimal condition for the isolation of nuclei, mitochondria, lysosomes, and microsomes. In these experiments, the ratio of tissue to homogenizing medium, homogenization speed, filtration of homogenate, ultracentrifugation speed, and a wash step for nuclear pellet purification were individually tested. The purity of the various cellular fractions was evaluated using transmission electron microscopy. Based on the results of these experiments, a method for kidney subcellular fractionation was developed. Using this method, kidney tissue from rats dosed orally with radiolabelled 14C glucose was fractionated and the percentage of radiolabel determined as follows: purified nuclear fraction (Pellet I) 0.2%, cellular debris fraction 6.4%, heavy mitochondrial fraction (Pellet II) 7.0%, light mitochondrial-lysosomal fraction (Pellet III) 3.2%, microsomal fraction (Pellet IV) 2.0%, and soluble fraction 81.3%.

The peoralsens, when activated by ultraviolet light (UVA, 320-400 nm), are potent modulators of epidermal growth and differentiation. When photostimulated, these compounds act directly on the cell surface membrane. We found that these compounds are potent inhibitors of EGF binding to mammary cells in culture. In B16 cells, inhibition of EGF binding by 8-methoxypterolens (8-MOP) was rapid and dependent on the dose of 8-MOP (0.01-1.0 mM) and UVA light (0.5-2.0 J/cm²). High doses of UVA light alone (2.0-6.0 J/cm²) were also inhibitory, indicating that peoralsens potentiate the UVA-induced inhibition of EGF binding. Two populations of receptors with differing affinities for EGF were identified in the cells. UVA and F UVA were found selectively inhibit binding to higher affinity receptors. The kinetics of binding inhibition were similar to that of the phorbol esters, indicating that the peoralsens inhibit EGF binding by an indirect mechanism. Peoralsens do not compete directly for EGF or phorbol ester binding suggesting that they inhibit EGF binding by a distinct mechanism.

In a series of studies investigating the effect of 1 h of anoxia on isolated enterocytes, the cells made anoxic were as viable as indicated by trypan blue exclusion as those incubated in air. Attempts to show a difference in viability using LDH leakage were also unsuccessful. Despite no apparent difference in these two indicators, both groups of cell had lost 75% of their high energy phosphate stores and NAD⁺. There was also a massive leakage of GSH into the supernatant during the incubation period for both groups of cells reducing the intracellular GSH content. When the utilization of uniformly labeled 14C glucose was studied, the anoxic cells were not able to utilize glucose after reoxygenation while the cells incubated aerobically continued to utilize glucose. Although both Trypan blue exclusion and LDH leakage will continue to be used as standards for viability, significant biochemical changes indicating cell injury may exist and not be detected by these gross measurements.

A method to determine the concentration of covalently bound HCHO in DNA following inhalation exposure of rats to HCHO and H⁺CHO has been developed. Exploratory experiments were performed using rat hepatic nuclei incubated with H⁻CHO (0.36 mM, 37°C, 1.5 hr, pH 8). The DNA cross-linked to proteins by HCHO was isolated by hydroxyapatite chromatography, heated (60°C) overnight, enzymatically hydrolyzed, then chromatographed by reversed-phase HPLC. The H⁺CHO was quantitatively recovered in a peak eluting before the nucleosides, indicating that it had been released by hydrolysis. This method was applied in three experiments to rat DNA exposed by inhalation to HCHO and H⁻CHO (2 ppm, 6 hr; 4 rats per experiment). The DNA was isolated from the respiratory mucus, and the concentration of covalently bound H⁺CHO in DNA was calculated from the H⁺CHO ratio of the DNA (RAP 76, 26 (1984)) and from the amount of 14C in the early eluting peak. The results were: (H⁺CHO method) 0.02 ± 0.03 nmole/mg; (HPLC method) 0.12 ± 0.02 nmole/mg. The ratio of these estimates (1.86) is explainable by an isotope effect (1.02 ± 0.11), shown to occur in the oxidation of H⁺CHO and H⁻CHO.
CHARACTERIZATION OF CROSS-LINKS BETWEEN DNA AND LYSINE PRODUCED BY FORMALDEHYDE. T.R. Fennell, P.H. Deal, and J.A. Swenberg. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Although formaldehyde is known to cause DNA-protein cross-linking both in vitro and in vivo, the nature of the cross-links has not been determined. We have investigated the reaction of lysine with calf thymus DNA, deoxyadenosine (DA) or deoxyguanosine (DG). Extensive binding (1 lysine residue per 8 nucleotides), measured following dialysis at 40°C, was obtained on incubation of lysine (10 mM) with DNA (3 mg/ml) and formaldehyde (600 mM) at pH 7.4 for 8 h at 37°C. However, on HPLC analysis of digested DNA, only normal nucleotides and N'-hydroxymethyl-DA were found, with no indication of cross-linked adducts. On incubation of DA (10 mM) with lysine (10 mM) and formaldehyde (100 mM) at 37°C for 24 h at pH 7.4, no evidence of cross-linking was found, but under similar conditions DG reacted with lysine and formaldehyde to produce two cross-linked adducts which could be resolved by reverse phase HPLC. Both were unstable in aqueous solution, with half lives of 30 and 120 min at 37°C, and both were found to contain DG, lysine and formaldehyde by proton NMR. Characterization of the positions of substitution is currently being carried out by NMR spectroscopy. These data suggest that the secondary and tertiary structure of DNA are needed to stabilize cross-links.

CHOLESTEROL AND PHOSPHOLIPID PEROXIDATION IN MEMBRANE BILAYERS. E. Kim and A. Sevianian, Institute for Toxicology, Univ. of Southern California Los Angeles, CA.

Small unilamellar vesicles (liposomes) were used to examine phosphatidylcholine(PC)/phosphatidylethanolamine(PE) and cholesterol oxidation(CO). Liposomes were composed of PC:PE(5:1 molar ratio) and the cholesterol/phospholipid molar ratios ranged from 1:450 to 1:4. Lipid peroxidation(LP) was initiated by autoxidation, superoxide radical generation(xanthine-xanthine oxidase), cumene hydroperoxide-hematin, or gamma-irradiation. The extent of LP was measured via thioarbituric acid reacting products(TBAR), and the products of fatty acid and cholesterol were identified and quantified by HPLC. Elevated cholesterol in membranes correlated with decreased LP via all oxidation systems. Cholesterol was most effective at inhibiting LP by cumene hydroperoxide system, was less effective for superoxide generating system, and least effective for gamma-irradiation and autoxidation. The degree of CO is influenced by its content and its lipids and may relate to the unsaturation index of component lipids. Propagation of LP is influenced by the degree of lipid unsaturation and limited by the availability of unsaturated fatty acids. Cholesterol reduces lipid unsaturation and membrane fluidity and in its presence the extent of propagation reactions is more limited than that of initiation reactions. The nature of oxidation products are also markedly altered under these circumstances.


Human hepatocytes (HH) are routinely isolated and characterized in our laboratory for viability and functional capability prior to conducting metabolism and toxicity studies. To eliminate the unpredictable timing of experiments with HH, practical techniques for cryopreserving and storing liver cells for later use need to be developed. Two convenient methods of freezing and several variables suggested in the literature for rat hepatocytes (RH) have been compared: 1) Fast freeze by direct immersion of the cryovials into liquid nitrogen (LN) and 2) Two-step freeze with the cryovials first placed in dry ice for 20 min, then immersed in LN. All cells were then stored at -135°C. Two-step freezing was superior to fast freeze in both human (52% viability, 21% recovery vs 14% viability, 7% recovery) and rat (82% viability, 91% recovery vs 28% viability, 20% recovery). Preincubating the cells for 1 hr in a gently shaking incubator at 37°C prior to freezing them also improved viability and recovery in HH. Preliminary studies suggested slight differences in cryoprotectant effectiveness, with 10% DMSO giving improved protection for RH and 10% glycerol for cynomolgus monkey hepatocytes used as an HH model. Although viability and recovery with HH were below values considered optimal, results of viability greater than 50% are encouraging. Supported by NIH contract ES-55109.


The covalent binding of the activated forms of several aflatoxins to the N-7 of guanine on purified DNA has been studied. Binding studies using tritiated aflatoxins indicate that chloro-peroxybenzoic acid-activated AFM1 and AFBI bind with approximately the same frequency to DNA. AFL binds almost twice as well as AFM1 or AFBI whereas AFR-M1 binds the least. These reactions produce alkali-labile sites which can be identified using a simple variation of the Maxam-Gilbert sequencing procedure. Two DNA fragments were exposed to each aflatoxin, and the reaction intensities at 33 guanine residues were determined. Data indicate that none of the aflatoxins had identical reaction profiles. We interpret these results to indicate that the various adducts cannot be considered biologically equivalent for tumor initiation. The frequency of occurrence of modifications at particular sites for AFBI was also compared with empirical "rules" established for AFBI by Misra et al. [Biochemistry (1983) 22, 3551-59]. The results indicate that binding intensity rules based on nearest neighbor nucleotides do not reliably predict guanyl-AFBI binding frequencies.
INHALATION FERTILITY AND REPRODUCTION STUDIES WITH 0.0'-DIMETHYLPHOSPHORODITHIOATE. W.F. Heydens, J.M. Kronenberg, D.C. Thake and S.M. Shoemaker. Monsanto Company, St. Louis, MO

Fertility and reproduction studies of 0,0'-dimethyl phosphorodithioate were conducted in rats. Males were exposed 5 days/week for 11 weeks, mated with 2 untreated females (1:1), retained during a recovery period (114 days), and re-mated; their mates were examined at mid-gestation to assess fertility. Females were exposed 5 days/week for 11 weeks, mated with untreated males, and again exposed on gestation days 0-20. Some females were examined at mid-gestation, and others were allowed to litter. FIA animals were necropsied for histological examination, or mated to assess fertility. Mean analytical exposure concentrations for the studies were approximately 4.5, 26, and 170 mg/cubic meter of air. Exposed high level males were infertile after exposures, and there appeared to be little or no recovery from this effect. The fertility of low level males was unaffected, but equivocal results were obtained at the mid level. In addition, histopathologic lesions were observed in testes from other low and mid level animals exposed concurrently. No unequivocal reproductive effects were detected in exposed FO females. No lesions or reproductive effects were observed in FI males and females.


F-344 rats were exposed to DMEA (CAS No. 108-01-0) vapor by inhalation on gestational days (gd) 6 through 15, 6 hr/day, at 0, 10, 30 or 100 ppm (analytical mean values 0, 10.4, 29.8 and 100 ppm, respectively). At sacrifice (gd 21), fetuses were examined for external, visceral, and skeletal alterations. Maternal toxicity was observed: reduced weight and weight gain during and after exposures at 100 ppm and ocular changes at 30 and 100 ppm (minimal and transient at 100 ppm). Histologic examination of eyes indicated no treatment-related lesions. There were no differences among groups for pre- or postimplantation loss or fetal sex ratio. Fetal body weights/litter were increased at 100 ppm. There were no treatment-related increases in individual or total malformations, in individual or total external or visceral variations, or in total skeletal variations. Of 98 fetal skeletal variations observed, the incidence of one, bipyramidal cervical centra, increased at 100 ppm. Exposure to DMEA vapor during organogenesis in F-344 rats resulted in maternal toxicity at 30 and 100 ppm and possible minimal fetotoxicity at 100 ppm. No embryotoxicity or teratogenicity was observed at any exposure concentrations employed. Copyright © 1986 Union Carbide Corporation.


The U.S. EPA, Office of Solid Waste has reviewed health and environmental effects of CS2 to support Listings under the RCRA. Concentrations of 12-13 ppm CS2 have been associated with reproductive dysfunctions in males and females (Cal and Bao, 1981; Lancerjan, 1981); a review of this literature, however, indicated that sensitivity of the human reproductive process to CS2 exposure is difficult to assess. In animal studies, exposure to CS2 has been associated with malformations and developmental effects. Tabacova et al., 1983 studied teratogenicity of CS2 in FI and F2 progeny born to gestationally exposed FO and FI mothers. This study suggested effects of CS2 (0.1ppm) in the F2 but not in FI pups, using fetus as the unit; terata per litter (s) were not reported. In other teratogenic studies (Choudhury and Manson, 1981, Hardin et al., 1981, Jones-Price, 1984), no morphological alterations were observed in rats or rabbits exposed to much higher levels of CS2 (40ppm). Based on the weight of evidence presented in the above studies, CS2 exposure may pose a reproductive/developmental risk to humans. However, the threshold at which adverse effects may occur has not been clearly identified due to lack of adequate continuous multigeneration studies.
694 REPRODUCTIVE EFFECTS IN LONG-EVANS RATS EXPOSED PERINATALLY TO CHLORIN DIOXIDE. B.D. Carlton, A.H. Basaran, L.E. Mezza, M.K. Smith Battelle Columbus Division, Columbus, OH and USEPA, HEEL, Cincinnati, OH.

Long-Evans rats, 4-6 weeks of age, were dosed with 0.0, 2.5, 5.0, or 10.0 mg/kg chlorine dioxide (ClO₂) in deionized water (10 ml/kg) for up to 73 days. Males were exposed 56 days, and females for 14 days prior to breeding, and throughout the 10-day breeding period. Males were killed and evaluated for sperm parameters and reproductive tract histopathology following the breeding period. Females were dosed throughout gestation and lactation until weaning on lactation day 21, when both dams and selected pups were necropsied. Neither clinical signs of toxicity nor adverse effects on any reproductive parameter examined were observed in the parental animals. Sperm motility, morphology, and swimming velocity in F₀ males were not different between groups. Litter size, pup viability, and pup weight were unaltered by chlorine dioxide exposure. F₀ reproductive tract organ weights and F₁ organ weights for testis, epididymis, uterus, and ovaries were not different between groups, but vaginal weight was significantly decreased (p<0.03) for female weanlings in the high dose (10.0 mg/kg) group relative to controls. Supported by EPA project no. CR810862. This abstract does not represent the policy or opinions of the USEPA.


Soman (pinacolyl methylphosphonofluoridate) is an organophosphate with potent anticholinesterase properties. Lack of developmental toxicity data prompted the initiation of this study. Gravid CD rats were gavaged with 0, 37.5, 75, 150 or 165 μg/kg/day of Soman on gestational days (GD) 6 – 15. Dams were observed daily for signs of toxicity and sacrificed on GD 20. Mean maternal weight changes, fetal implant status, fetal weight and fetal malformations were compared among groups by analysis of variance after transformation to normalize the data. Soman caused 4% maternal mortality at the 165 μg/kg/day dose level. Significant (p < 0.05) dose related effects were observed on maternal weight changes during gestation indicating maternal toxicity at doses above 75 μg/kg/day. Laporotomy revealed no significant differences among groups in the incidence of implantation sites, viable fetuses, resorptions, dead or malformed fetuses, or in average body weight of live fetuses per litter. These results show no evidence of developmental toxicity in the CD rat following exposure to Soman during embryonic differentiation and organogenesis, even at a dose which produced significant maternal toxicity. [This research was supported by the USAMRDL under ARMY project number 83PP3812.]

696 TERATOLOGICAL EVALUATIONS OF AMETRYN TECHNICAL, A TRIAZINE HERBICIDE, IN RATS AND RABBITS. R. Infruma, E. Yau and V. Traîna, Ciba-Geigy Pharmaceuticals Division, Research Department, Safety Evaluation Facility, Summit, N.J. and L. Wetzel and J. Stevens, Ciba-Geigy Agricultural Division, Safety Evaluations Department, Greensboro, N.C.

Ametryn Technical was evaluated for its embryotoxic, fetotoxic, and teratogenic potential in rats and rabbits. The compound was orally administered at doses of 0, 5, 50, or 250 mg/kg/day (MD) to groups of rats on gestational days 6-15, while rabbits were administered doses of 0, 1, 10, or 60 MD on gestational days 7-19. Treatment-related maternal effects were observed at doses ≥ 50 MD in rats and at the 60 MD dose level in rabbits. In rats these effects included reductions in feed consumption, body weight gain, and an increase in the incidence of clinical signs. Maternal effects in rabbits were limited to reductions in feed consumption and body weight gain. An increased incidence of minor peripheral skeletal variations were observed in fetal rats derived from the intermediate and highdose groups. There was no evidence of developmental toxicity in rabbits. The maternal NOEL was estimated to be at least 5 MD in rats and 10 MD in rabbits. The developmental NOEL was estimated to be at least 5 MD in rats and 60 MD in rabbits. These data indicate that Ametryn Technical was not teratogenic in either species.

697 MATERNAL AND DEVELOPMENTAL TOXICITY OF DERMALLY APPLIED CLARIFIED SLURRY OIL IN RATS. H.H. Feuston, S.L. Kerstetter, E.J. Singer, M.A. Mehlman Mobil Oil Corporation, Princeton, NJ

Clarified Slurry Oil (CSO), the residual hydrocarbon fraction from the Fluidized Catalytic Cracker, was applied on gestation days 0-19 to the backs of collared, pregnant rats at doses of 0, 4, 6, 30, 125, and 250 mg/kg/day. Signs of maternal toxicity including vaginal bleeding, death, reduced weight gain and reduced food consumption were observed at levels as low as 8 mg/kg. On gestation day 20, dams were necropsied and the fetuses evaluated for normal development. In the dams increased liver weights (relative) and atrophy of the thymus were observed at 250 mg/kg. There were no effects on normal development. The number of resorptions increased with dose at 30 mg/kg and above with no surviving fetuses at 250 mg/kg. At dose levels of 30 mg/kg or greater fetuses were smaller in length and weight than the controls. Abnormal external development was observed in a few fetuses from CSO-treated dams. Additional testing would be necessary to determine whether the small number of anomalies seen in the surviving pups was a direct action of CSO on the offspring or an indirect effect of maternal toxicity.

1,1,1-Trichloroethane (TCEN) is an industrial solvent that has been found in contaminated water supplies. Cardiac malformations have been reported in 21-day old rats born to dams exposed prenatally and throughout lactation to 10 ppm TCEN in the drinking water (Dapson et al., Teratology 29, 25A, 1984). In the present study, male and female CD rats (20/group) were given deionized/filtered water, 0.05% Tween 80 with 0.9 ppm 1,4-dioxane (vehicle control), 3, 10, or 30 ppm TCEN for 2 weeks prior to mating and during mating. Sperm-positive females were treated at the same dose levels throughout gestation and lactation. TCEN blood levels were determined in the F0 males and females after pre-mating and mating, and in the F0 females after lactation. Pups were evaluated for visceral malformations and for TCEN blood levels on postnatal day 21. There was no evidence of an increase in cardiac or other visceral malformations in the pups at any dose level of TCEN. These results are in contrast to the published preliminary findings noted above. Supported by NTP/NIHES Contract No. N01-ES-55080.


Phenylethyl alcohol (PEA) is an important and widely distributed fragrance ingredient due to having the characteristic odor of roses. It is also a natural constituent of roses. A study of the potential effects of dermally applied PEA on pregnant rats was conducted by applying undiluted PEA under 24 hr/day occlusion in doses of 0.14, 0.43 or 1.4 ml/kg daily during days 6 to 13 of pregnancy. The high dose resulted in maternal toxicity accompanied by a broad spectrum of fetal effects including morphological abnormalities in virtually all fetuses. The mid-dose did not produce maternal toxicity or fetal effects other than very small cervical rib buds in 30/129 fetuses. The low dose did not produce any definitive evidence of adverse effect. The 0.14 ml/kg dose is about 250 times higher than the calculated 90% exposure dose from the use of PEA in fragranced products. Comparative rat/human blood level concentrations increase this safety factor to approximately 10,000.

PEA is used as a food additive and a flavor ad- juvant and is a natural constituent of fruit, bread, cheese and alcoholic beverages. Microen- capuleated PEA was administered in the diet to nulliparous Sprague-Dawley rats at levels of 1000, 3000 and 10,000 ppm during the period of major organogenesis (d. 6-15 post coitum). Effe- cts in the dams were limited to a transient suppression of maternal food consumption resulting in slight weight loss during the first two days of treatment at the high dose level only. Effects in the offspring were minimal with malforma- tions limited to a total of 5 pups ( 3 in Control and 2 in the mid-dose). The number and type of visceral anomalies were comparable be- tween treated and control. Of fetuses exhibit- ing skeletal anomalies, an increased incidence of incompletely ossified was noted only in the 10,000 ppm group. This was considered to be a possible consequence of the earlier impairment of maternal weight gain. There were no obvious differences between control and treated groups for skeletal variants, number of live young, em- bryolethality, implants, litter weight, mean fetal weight or sex ratio.


Groups of rats (30/sex/generation) were fed diets containing 0, 0.1, 0.5, or 1.0% DSS for at least 10 weeks. FO, F1, and F2 animals were then mated to produce F1, F2, and F3 litters, respectively. There were no effects on the reproductive function of either sex in any gen- eration and no treatment-related antemortem or macroscopic observations. At the highest dose, body weights (BW) were lower during the pre- mating phase for males in all generations and for F1 and F2 females. Premating BWs were also low for F1 and F2 animals at the mid dose. Pup BWs were low on lactation Day 0 for the high dose during the third generation and on Day 21 for mid and high doses for all generations. Pup survivability ranged from 95-100% for controls and 91-100% for treated animals. In conclusion, DSS administered in the diet to three successive generations of rats did not interfere with reproductive performance at doses up to and including 1.0% DSS. The only effect seen was a reduction in BW at levels of 0.5% and above.

ABSENCE OF TERATOGENIC RESPONSE IN RATS AND RABBITS GIVEN A DETERGENT BUILDER. R.S. Nair, F.R. Johannsen, Monsanto Co., St. Louis, MO, and R.E. Schroeder, Bio/dynamics Inc., East Millstone, NJ.

Builder U (alkali metal polycarboxylate), a new detergent builder developed by Monsanto, was administered by gavage to four groups of 24 mated Sprague Dawley CD® rats at dosages of 0, 250, 1000 and 2500 mg/kg/day in distilled water on gestation days 6 through 15 or four groups of 18 mated New Zealand white rabbits at dosa- ges of 0, 50, 250 & 500 mg/kg/day on gestation days 7 through 19. All animals were observed daily. All rats were sacrificed on gestation day 20 and fetuses examined for external malfor- mations, and one half of the fetuses in each litter evaluated for visceral or skeletal malfor- mations. All rabbits were sacrificed on gestation day 30 and all fetuses examined for external, visceral and skeletal malformations. In the rat, no maternal toxicity was observed up to 2500 mg/kg. The incidence of external, visceral and skeletal malformations and varia- tions in all treated groups was comparable to controls. In the rabbit, maternal toxicity (significant weight loss and abortions) was observed at the high dose. Embryotoxicity (increased resorptions) and fetotoxicity (increased ossification variations) was also observed at the high dose. However, no increased incidence of terata were seen. Builder U is not teratogenic in either the rat or rabbit.


Alkylate 215 is a mixture of decyl-, undecyl-, and dodecylbenzenes used in the manufacture of alkylbenzene sulfonate detergent products. Groups of 24 mated Charles River® CD female rats were given 0, 125, 500 or 2000 mg/kg/day of Alkylate 215 in corn oil by gavage on days 6 through 15 of gestation. Dams were sacrificed on gestation day 20 and fetuses were examined for external, soft tissue and skeletal defects. No treatment related mortality occurred in the dams. Reduced maternal weight gain was observed in dams treated with 500 and 2000 mg/kg/day. No treatment related effects were observed on uterine implantation data, fetal body weights, and gross or soft tissue defects. In the high dose group, an increase was observed in the following parame- ters: incidence of fetuses with at least one ossification variation, incomplete ossification of several cranial bones, incomplete ossification of certain vertebral elements, and rudimentary rib structures. In the mid dose group there was an increased incidence of fetuses with rudimentary rib structures. Therefore, maternal and fetal toxicity was observed with Alkylate 215 at doses of 500 mg/kg/day and above. Alkylate 215 was judged not to be teratogenic to rats in this study.
TERATOLOGY STUDIES OF VANCOMYCIN (V) ADMINISTERED INTRAVENOUSLY (IV) TO RATS AND RABBITS.
R.A. Byrd, M.K. Buening, and C.L. Gries. Toxicology Division, Lilly Research Laboratories, Greenfield, IN.

V is a glycopeptide antibiotic widely used in the treatment of potentially life-threatening infections caused by certain gram-positive organisms. The use of V is supported by three decades of laboratory and clinical experience. Studies in rats and rabbits were conducted to evaluate the teratogenic potential of V. Pregnant rats and rabbits were dosed IV once daily on gestation days 6-15 and 6-18, respectively. Doses for rats were 0, 40, 120, and 400 mg/kg; doses for rabbits were 0, 40, 80, and 120 mg/kg. Cesarean sections were performed on rats and rabbits on gestation days 20 and 28, respectively. Mild regenerative nephrosis was produced in maternal rats given 120 and 200 mg/kg. Maternal food consumption and weight gain and fetal viability, weight, and morphology were not affected. Cortical tubular nephrosis was produced in maternal rabbits given 80 and 120 mg/kg. Maternal food consumption and weight gain and fetal weights were depressed at 120 mg/kg. Fetal viability and morphology were not affected. The no-observed-effect level (NOEL) for maternal toxicity in both species was 40 mg/kg; no teratogenic effects were seen at 200 mg/kg in the rat and 120 mg/kg in the rabbit. The highest doses tested in each species. Based on these data, the embryo/fetal NOEL exceeded the maternal NOEL in rats and rabbits.


Virginiamycin, suspended in 1.0% (w/v) carboxymethylcellulose, was administered orally via gavage once daily on days 6 through 15 of gestation to 100 mice (n=25). Virginiamycin was given at dosages of 0, 25, 160 or 1000 mg/kg/day, using a dosage volume of 10.0 mL/kg, adjusted daily for body weight changes. Body weights and clinical signs were evaluated daily. Mice were Caesarean-sectioned on day 18 of gestation, and the uterine contents were evaluated using standard developmental toxicity techniques. Histological sections of the fetuses in each litter were sectioned (Wilson's technique) or alizarin Red S stained for evaluation. The 1000 mg/kg/day dosage group gained slightly, but significantly (P<0.01), more weight than the control group on days 6 to 9 g. During the postadministration period (days 16 to 19 g) the 1600 mg/kg/day group gained slightly less weight than the control group. These data recapitulated results of the pilot study. A small increase (not significant) in the litter incidence of fetuses with a reversible developmental delay, slight dilatation of the pelvis of one or both kidneys, occurred for the 1000 mg/kg/day dosage group. No other maternal or developmental effects occurred. Both the maternal and developmental NOEL's were 160 mg/kg/day.


This study was designed to assess the long-term effects of Virginiamycin administered in the diet through two generations (Fo, Flb) of CD® rats. Each adult generation (25 animals/sex/dose group) was mated to produce two litters, and Flb adult generation animals were selected from the Flb litters. Dose levels were 0, 25, 65 and 300 mg/kg/day (MPK). Due to increased pup mortality, noted in a concurrent study with an in-uterine exposure at 300 MPK, the high-dose level was reduced to 100 MPK for mating, gestation and lactation of the Flb litter and the F2a and F2b litters. All adults and weaned pups received a gross postmortem examination at sacrifice. No adverse effects of treatment were seen at the low- or mid-dose levels. In the high-dose males (Fo, Fl), lower weights were seen during the pre-mating growth period, mating and post-mating period. During the Fla litters, high-dose females (300 MPK) had lower weights at Day 20 of gestation and at lactation Days 4 and 19. Pups in the high-dose litters (Fla) had lower weights at Days 14 and 21. During the Flb, F2 and F2a litter intervals with high-dose females receiving a lower dose level (100 MPK), no adverse effect of treatment was evident from reproductive indices, litter data or offspring data (weight, survival).
STUDY OF THE TERATOGENIC POTENTIAL OF GUAR GUM.

Despite the widespread use of guar gum as a food binder, thickener, and stabilizer, very few studies have been done to test its possible reproductive or teratogenic effects. Following 13 weeks of treatment, Osborne-Mendel rats were mated within dose levels (0, 1, 2, 4, 7.5, or 15% dietary content) and used in this teratology study. The animals ate the test diet throughout mating and pregnancy. The amount of food consumed per pregnant female during days 0-20 was less in all treated groups than in the control group but the decrease was not dose-related. Ingestion of guar gum had no effect on fertility, implantation efficiency, fetal development, or sex distribution. No terata were seen. Under the conditions of the study, guar gum appeared to be non-teratogenic.

TOXICITY STUDY OF AN IODINE CONTAINING FEMALE STERILIZATION FORMULATION (ICF). D. P. Walker, T. Parmley, A. Martin, M. R. Starley, C. D. Fakroddin and A. Goldsmith, University of Illinois at Chicago and Northwestern University Medical School, Chicago, IL and Johns Hopkins University, Baltimore, MD.

A new ICF for the Femcept R delivery system is being developed for transcervical sterilization of women. This study evaluated the effects of the ICF on the uterus and the peritoneal cavity. The ICF was prepared by Bionexus, Inc. (Raleigh, NC) and contained I 3, KI, glycerol, Vascoray R and gum tragacanth. Fourteen cynomolgus monkeys were divided into four groups of at least three animals. One group was administered vehicle and another ICF transcervically until the uterine cavity and the fallopian tubes were filled with the dosing medium. Other groups were treated intraperitoneally with vehicle (0.5 ml) and ICF (0.5 ml). Blood was drawn from the animals before and during the experiment. All animals were necropsied 45 days after dosing and tissues examined histologically. No changes in the peritoneal cavity, the uterine tissues or blood parameters, including T4 and TSH were noted in any of the test groups compared to control. Histological changes were limited to fibrotic lesions in the lamina propria of the oviducts of monkeys administered ICF transcervically. These studies demonstrate the lack of toxicity of the ICF on the uterus and peritoneal cavity. Supported by the Program for Applied Research on Fertility Regulation (Agency for International Development: PARFR-385).

EFFECT OF AN EXTRACT OF SENEcio VULGARIS AND SENEcONINE ON RAT FETUSES. N.A. Nuzzo, A. Hall, A. Martin, R.J. Molyneux and D.P. Walker. PCRPS, University of Illinois at Chicago, Chicago, IL and USDA, Albany, CA.

Pyrrolizidine alkaloids are known to cause liver damage in animals and man. The present study investigates the effects of a Senecio vulgaris extract containing pyrrolizidine alkaloids and seneconine, the major pyrrolizidine alkaloid in S. vulgaris, on the fetus. Pregnant rats were orally dosed with S. vulgaris extract (920 mg/kg) or seneconine (20 mg/kg) on days 1 thru 10 of gestation or sc with seneconine (2 and 5 mg/kg) on days 4-16 of gestation. On day 16 of gestation the animals were euthanized and the number of implantation sites and normal fetuses counted. The fetuses from the sc treated animals were weighed and crown-to-rump length determined. In addition, the fetal livers were removed and weighed. The number of normal fetuses decreased after oral administration of the plant extract and seneconine. Seneconine also caused a decrease in the fetal liver/body weight ratio after sc dosing. These data demonstrate an effect of a pyrrolizidine alkaloid containing extract and seneconine on pregnancy outcome. The decrease in the fetal liver/body weight ratio suggests the observed fetal effects are mediated by direct action on fetal liver.


Exposure of male rats to ACR produces significant pre-implantation loss at weeks 1-4 after treatment. This research was designed to assess the factors which may contribute to pre-implantation loss, i.e., copulatory dysfunction and spermatotoxicity. Adult, male, Long-Evans rats received 0, 15, or 45 mg/kg of ACR (p.o.) for 5 consecutive days. Males were mated at Weeks 1-4 after exposure and copulatory behaviors evaluated. Females were assessed for location of sperm in the vagina or uterus and uterine sperm motility. At Week 1, only 60% and 20% of the females mated to males receiving 15 mg/kg and 45 mg/kg of ACR respectively, showed sperm in the uterus. At succeeding weeks most females mated to treated males had sperm in the uterus. Male copulatory behavior and motility of sperm found in the female reproductive tract were comparable for control and treated males throughout the study. Males were dosed in the same manner to examine spermatotoxic effects of ACR. Reproductive organ weights, epididymal sperm count, and epididymal sperm motility showed no significant differences from controls. The role of other factors such as fertilization failure is now being evaluated. (This abstract does not represent the policy or opinion of the USEPA).
TERATOLOGIC EVALUATION OF NITROFURAZONE (NF) IN


NF, an antibiotic widely used in human and veterinary medicine, was administered to timed mated CD-1 mice during major organogenesis [gestational days (gd) 6-15]. In a pilot study, 120 mg/kg/day (0.075% in the diet) produced 15% maternal mortality/morbidity. Thus, 0%, 0.0038, 0.0075%, 0.0250% or 0.0500% NF in the diet were administered in the final study (0, 6, 14, 41 and 82 mg/kg/day, respectively). Dams were sacrificed on gd 17 (20-26/group), and each live fetus was examined for external, visceral and skeletal malformations. No treatment-related effects were observed on maternal mortality, food and water intake, body wt., wt. gain, liver wt. or gravid uterine wt. Maternal clinical signs of toxicity were observed at the high dose. Embryo/fetal toxicity included a dose-related increase in late fetal death (0.7%, 0.7%, 1.1% and 2.0%), and a dose-related decrease in avg. fetal body wt./litter which was significant at the high dose. The % malformed live fetuses/litter was not affected by treatment. In summary, NF produced fetal growth retardation and an increased incidence of late fetal death at exposure levels which were minimally toxic to the dams. Supported by NTP/NIH Contract No-1-ES-55080.


The teratogenic potential of Dezaguaine, an antimetabolite purine analog, was evaluated in Charles River CD female rats and Dutch Belted female rabbits. Dezaguaine was administered intravenously as a single daily dose to rats at dosages of 0.6, 3.6 and 7.5 mg/kg on Days 6-15 of gestation and to rabbits at dosages of 0.5, 1.5 and 4.0 mg/kg on Days 6-18 of gestation. Cesarean sections were performed on Days 20 and 28 for the rat and rabbit, respectively, and fetuses examined for external, visceral and skeletal malformations. In the rat, increased early fetal death and suppressed maternal weight gain were seen only at 7.5 mg/kg while retarded fetal growth and increased developmental variations were seen at both 7.5 and 3.6 mg/kg. No teratogenic effects were observed in the rat at the doses evaluated. In rabbits, increased early fetal death, retarded fetal growth and suppressed maternal weight gain occurred at 4.0 mg/kg. Malformations noted at the high dose included renal, pancreatic and gall bladder agenesis, cardiovascular defects, diaphragmatic hernia and thoracogastro/-gastrochisis. Gall bladder agenesis was also evident at 1.5 mg/kg. In summary, Dezaguaine was fetotoxic but not teratogenic in the rat, whereas it was both fetotoxic and teratogenic in the rabbit.

TERATOLOGIC EVALUATION OF BETA(3,4-EPOXYCYCLO-
HEXYL)ETHYLTRIMETHOXYSILANE (ECMS) IN RATS AND


ECMS(CAS No. 3388-04-3) is an industrial intermediate, shown to be mutagenic and weakly carcinogenic. F-344 rats and NZB rabbits were dosed with ECMS by gavage on gestational days (gd) 6-15 at 0, 2.5, 10 or 25X (v/v in corn oil), 10 ml/kg (rats), and on gd 6-18 at 0, 5, 25 or 75X, 1 ml/kg (rabbits). At sacrifice (gd 21 rats; gd 29 rabbits), fetuses were examined for external, visceral, and skeletal alterations. In rats, maternal toxicity was observed at 10 and 25X reduced body weight (BW) gain and food consumption, clinical signs, reduced BW corrected for gravid uterine weight, and elevated liver weight (as % BW). There were no effects on pre- or postimplantation loss, fetal BW/litter, or incidence of malformations. Minimal fetotoxicity (reduced ossification) was observed at 25X. In rabbits, maternal mortality (2/20) was observed at 75X and kidney weight (as % BW) was elevated at 75X. There were no effects on pre- or postimplantation loss, fetal BW/litter, or incidence of malformations. Minimal fetotoxicity (reduced ossification) was observed at 75X. No embryo toxicity or teratogenicity was observed in either species at any dosages employed. Copyright© 1986 Union Carbide Corporation.


We are evaluating a number of endpoints for inclusion in a protocol to screen compounds for reproductive toxicity. In this study rats were exposed to carbendazim (C) at 5, 10, 100, 200, or 400 mg/kg/d from weaning through pregnancy and lactation. Results indicate that puberty and estrous cyclicity were not affected. During breeding, females had sperm or plugs and all became pregnant or pseudopregnant. At > 200 mg/kg/d some had no implants, but some of those that became pregnant had bloodysmear due to fetal wastage. Postnatal viability was also reduced and hydrocephalic pups were found at 100. In the males, liver, kidney, adrenal, pituitary, and seminal vesicle weights were not affected. Body weight was down 10% at 400, testis and caudal weights, caudal and testicular sperm counts and sperm motility and morphology were reduced at 200 and 400. Broken sperm and debris were found at 50 and above. Serous, testicular, hypophysis, and testicular hormonal content was determined but these endocrine parameters were not markedly affected. No organ weight changes were found in the females. In conclusion, C altered the semen; and caused fetal death and malformations but did not affect pubertal or endocrine parameters.

Recent reviews of published data imply that insufficient attention is given to the maternal component when assessing developmental toxicity. Whilst this conclusion may be valid within the context of published data, this presentation hopefully will help to modify this impression. Studies performed at Huntingdon over the last 10 years, consisting of approximately 150 rabbit and 150 rat 'teratology' studies together with approximately 75 peri- and post-natal studies and 125 single or multiple generation studies will be assessed for the attention given by the authors to the maternal component in the conduct and reporting of these studies.

MATERNAL TOXICITY AND FETAL MALFORMATIONS OF RODENT - RABBIT SPECIES.

Specific malformations in rats and rabbits have recently been interpreted to be a possible consequence of maternal toxicity per se.

Data generated from approximately 100 rat and 100 rabbit 'teratology' studies performed at Huntingdon over the last 5 years will be examined to determine the extent to which our information supports this interpretation.


As part of the Tier I Assessment Program of the Environmental Criteria and Assessment Office of the U.S. Environmental Protection Agency, preliminary health summary and assessment documents were prepared on monochloroethane, maleic anhydride, phthalic anhydride, and methyl methacrylate. Humans exposed to monochloroethane have exhibited symptoms such as central nervous system depression and dizziness. Maleic anhydride is a potential teratogen and in humans has marked irritant properties. Phthalic anhydride is also a strong irritant and may cause reproductive toxicity. Methyl methacrylate can cause contact dermatitis in humans and in animal studies has demonstrated developmental toxicity.

REPRODUCTIVE ASSESSMENT OF MALE WASTE WATER WORKERS. G.K. Lenasters, K. Hansen, H. Zenick*, C. Meyer, V. Hertzberg, S. Clark, Department of Environmental Health, University of Cincinnati, Cincinnati, OH. U.S. Environmental Protection Agency, Washington, DC*

This study was designed to examine the reproductive risks in male workers with multiple chemical waste exposures. Two strategies were employed: 1) a retrospective study examining the relationship between work and reproductive histories and 2) semen evaluations. The data to date have been evaluated employing a dichotomous exposure model (ever-exposed, never exposed). Survey endpoints analyzed have included fetal loss, fertility rates, and measures of the periods of time during which the couple were actively trying to conceive. No significant differences have been observed on these measures. Preliminary semen evaluations have assessed sperm concentration, viability, motility, and morphology. Exposed workers had significantly lower sperm concentrations with a greater proportion having sperm concentrations <40 million/ml. Percent viable, motile, and normal forms were not different. The contribution of a number of modifying factors to these semen outcomes remains to be determined. (March of Dimes Grant 15-60).

Five levels of potential reproductive risk are assigned to drugs by the FDA (A, B, C, D, and X). Although these categories do not involve the use of quantitative safety factors, therapeutic ratios specific to reproductive effects can be derived to serve as measures of acceptable safety factors. Of 569 USP DI drug monographs reviewed, 96 provided sufficient data to calculate the ratio of lowest dose causing reproductive effects in humans or experimental animals to the therapeutic dose. The ratios were then tabulated by FDA pregnancy category. Therapeutic ratios of less than 20 were found for over 50% of drugs in category A, 75% in category B, 75% in category C, and 100% in category D. More than half (56%) of the 96 drugs reviewed had therapeutic ratios of 10 or less. Seven of the 96 drugs had therapeutic ratios of less than 1; 2 of the 7 were category D drugs (demonstrated to have caused birth defects in well-controlled human studies). Only 15% of the drugs reviewed had therapeutic ratios of greater than 100. These ratios stand in contrast to safety factors of 10 to 1000 traditionally applied to toxic chemicals. Reproductive safety factors for drugs provide one possible benchmark for setting safety factors for toxic chemicals, recognizing differences in risk/benefit considerations for drugs and other environmental agents.

EMBRYOTOXICITY and GLUTATHIONE (GSH) DEPLETION ELICITED BY INHIBITION OF Y-GLUTAMYL TRANSFERASE (GGT) by ACIVICIN and ANTI-GGT ANTIBODIES. K.L. Stark, C. Harris, and M.R. Juchau. Dept. of Pharmacol., Sch. of Med., Univ. of Washington, Seattle WA.

GGT is a key enzyme in GSH metabolism and thiol-based cellular defense systems. The effects of GGT inhibition by Acivicin or anti-GGT IgG on cultured day 10-11 rat conceptuses were examined. Acivicin and IgG each produced embryotoxicity, malformations, and altered GSH levels, although each produced a unique spectrum of effects. Acivicin, after 24-hr, produced decreased yolk sac vasculature, embryonic (E) and Ys- protein content, E-GSH levels, and Ys-GGT activity. After 3-hr exposures, only decreased Ys-GGT activity was observed. IgG produced no apparent effects on Ys-vasculature or Ys-protein after 24-hr exposures, even though inhibition of Ys-GGT was equal to that of Acivicin (30%). IgG did significantly decrease E-protein after 24-hr, and depleted embryonic GSH after both 3- and 24-hr. Effects produced by each inhibitor on the Ys indicated that Acivicin acted partially via mechanisms other than GGT inhibition. The capacity of the IgG to elicit malformations and decrease GSH in the embryo while acting only on Ys-GGT emphasizes the ability of the Ys to modulate embryonic development during organogenesis. Supported by NIH grants ES-04041 and ES-03157.
The excretion of radiocarbon from 14C-nifluridine into milk of lactating rats was compared utilizing two different methods of milk sampling. Lactating rats received 10 mg/kg 14C-nifluridine orally. Method 1 employed the analysis of stomach contents from pups that had suckled treated dams 1, 4, 7, 10, 13, 25 and 49 hours after dose administration. Method 2 employed the use of a vacuum milking device to obtain milk samples from lactating rats 4, 25 and 49 hours after dosing. Both methods demonstrated that radiocarbon from 14C-nifluridine was excreted into milk of lactating rats. Milk-to-maternal plasma concentration ratios for method 1 rose and remained constant between 4 hours (0.66) and 13 hours (0.75), then fell to 0.25 and 0.19 at 25 and 49 hours, respectively. Milk-to-maternal plasma concentration ratios for method 2 were identical to those of method 1 at 4 hours (0.68), but were higher at 25 hours (0.61) and 49 hours (0.51). This study demonstrates that both methods of milk sampling result in comparable milk excretion data. However, method 1 enables the elimination of anesthesia, allows for the sampling of consistent milk aliquots and provides a means for analyzing frequent time intervals.

Chemical Health Assessment Methodology (CHEM) is a standard procedure for assessing hazardous properties of airborne toxic contaminants. CHEM evaluates substances for four health effects: carcinogenicity, mutagenicity, reproductive/developmental toxicity and systemic toxicity. Three elements are considered in the assessment: weight of evidence, potency and severity of effect. The product of the assessment is a relative score, A to E, plus ND designation for no data, in each health effect category for each chemical. Scores in the health effect categories are conceptually equivalent to a geometric mean of the three elements of assessment. The Methodology represents a combination of two extreme approaches to assessing hazards of chemicals: comprehensive health assessment document and a ranking system. It produces a profile of toxic properties of chemicals which preserves their unique multidimensional character and highlights data gaps. CHEM is a versatile risk assessment tool. In addition to improving our conceptualization of hazards, the simplicity of the four-letter code allows for relative ranking of chemicals, as well as grouping hazards into categories. At the same time the complex underlying data base can be used to develop numerical criteria for human exposure.

A sequential testing procedure is derived to estimate the NOEL-dose, the highest experimental dose for which the true response rate does not exceed background rate, for incidence data from animal studies. The method assumes that the true response rates are nondecreasing as dose levels increase. It conditions on the total number of responses observed, and is "exact" in the same sense as Fisher's exact test. The probability distribution of the estimator of the NOEL-dose and its properties, e.g. expected value and standard deviation, are obtained as a function of the true response rates. When used with a set of data, the NOEL-dose is estimated, and estimates are obtained for the probability distribution and its properties. The latter estimates are equivalent to the bootstrap estimates that would result from repeated simulations using the maximum likelihood estimates of the true response rates. The test procedure is currently operational for a control group paired with two dose groups of up to size 20 each. Extension to more groups or to larger sample sizes is straightforward.

Guidelines for exposure to various metals in ambient air, including cadmium (Cd), are currently being developed by regulatory agencies. Adverse health effects associated with inhalation exposure of humans to Cd compounds include both renal dysfunction (the most sensitive effect) and respiratory impairment. Three risk assessment approaches were used to develop ambient air criteria for Cd. A traditional approach incorporated the use of an uncertainty factor over the no-observed-effect level (NOEL) estimated from studies in occupationally exposed workers. The second approach involved the use of probit and logit regression models on concentration-response data from occupational studies to estimate the air level of Cd associated with a 0.1% probability of renal dysfunction. The final approach utilized target-organ dose-response data from both human and animal studies correlating concentration of Cd in renal cortex with renal dysfunction. A renal cortex concentration on which to base a criterion was determined and metabolic models (Friedberg et al., 1974) were used to estimate a corresponding daily exposure to Cd in ambient air. The three risk assessment methods resulted in comparable ambient air criteria ranging from about 10 to 40 ng Cd/m$^3$.


This simplified approach integrates estimates of chemical toxicity (type and degree) and extent of human exposure (frequency and severity) to yield a preliminary human health hazard assessment. Based on the ratio of the estimated toxic dose to the anticipated human exposure, chemicals may be classified as presenting "no significant hazard" (if the ratio is very large)...or as presenting a "potentially serious hazard" (if the ratio is less than an established "trigger" value). If the ratio falls between these "triggers", the hazard is classified as "intermediate." If the available data are inadequate to support one of the above classifications, the hazard is characterized as "indeterminate", and a recommendation for additional testing/data-gathering follows. Skilled professional judgment is critical to estimating the output values and to applying the output from the assessment procedure.


Adverse health effects of the industrial chemicals, methyl bromide, chlorinated styrenes, and brominated diphenyl ethers, have been reviewed under the Chemical Hazard Information Profile program of the USEPA. Concerns regarding these halogenated compounds vary. Methyl bromide, with a large production volume, is a systemic toxicant, is mutagenic to microorganisms, and is a suspected carcinogen in rats. Chlorinated styrenes, by-products of industrial processes, are released into the aquatic environment. Octachlorostyrene (OCS) is biocorrupted via the aquatic and marine food chains; it has non-ocenocogenic chronic effects in laboratory animals and is non-mutagenic in microorganisms. Of the ten brominated diphenyl ethers reviewed, only decabromodiphenyl ether has a significant production volume; it is carcinogenic to rats and has adverse effects on rat fetuses. A commercial formulation of octabromodiphenyl ether causes fetal malformations. The deca-, octa-, and pentabromodiphenyl ethers were not genotoxic in limited studies. *Operated by Martin Marietta Energy Systems, Inc., under contract No. DE-AC05-840R21400 with the U.S. Department of Energy.)

ASSESSING THE PREDICTIVE VALUE OF ANIMAL TOXICITY TESTS FOR MAN. CE Lumley and MW Walker, Centre for Medicines Research, Carshalton, Surrey, UK. Sponsor: S. D. Gargiulli

A preliminary study has been completed to examine the relevance of animal toxicity tests for man. Five pharmaceutical companies in Europe have provided information from repeated-dose toxicity tests, clinical trials and post-marketing spontaneous reports and surveillance studies (where applicable) for 17 compounds (12 marketed). These include 6 anti-infectives, 5 non-steroidal anti-inflammatories, 4 drugs acting on the central nervous system (CNS) and 2 cardiovascular system (CVS) drugs. All compounds were investigated in both rats and a non-rat species (12 in dogs, 7 in primates) species. Five body systems (CVS, gastrointestinal, urinary, liver, CNS) and specific effects within them were examined, using a methodology based on that of Schein et al (1970). The addition of the non-rat species decreased underprediction in all systems but increased overprediction in all except the CNS. The animal toxicity tests were good at identifying compounds with potential clinical effects on the liver or urinary system, but with considerable over-prediction. However, prediction was not as good when specific effects were considered. This approach may be valuable in improving toxicity tests, eliminating those that are unnecessary and allowing better prediction of potential problems.


Nonmonotonic dose-response functions are not uncommon in toxicology and may provide useful information about thresholds and interactions. However, one type of nonlinearity may pose difficulties for our understanding of toxic effects. We refer to U-shaped dose-response relationships that describe an apparent improvement or enhancement in function at intermediate dose levels. (Either a U or an inverted U may describe such relationships, depending on how the dependent variable is represented.) For example, studies of children have shown that blood lead levels of about 10-25 µg/dl are associated with evoked-potential latencies that are decreased by comparison to latencies at lower or "baseline" exposure levels (<10 µg/dl). At higher levels (>25 µg/dl), latencies are longer, as expected. Thus, there appears to be some degree of facilitation of neural conduction associated with an intermediate level of toxic exposure. The neurotoxicology of lead provides additional examples of such seeming paradoxes, but other toxicological and health endpoints also show similar U-shaped dose-response relationships. This paper describes the features of these phenomena, and discusses various explanations that might account for such curvilinearity, along with their implications for regulatory decision-making.

THEORETICAL FOUNDATIONS OF TOXICOLOGY. S. Ji Dept. of Pharmacol. & Toxicology, Rutgers University, Piscataway, N.J.

To investigate the molecular mechanisms underlying chemical cytotoxicity, it is essential to have theoretical models for cells and multicellular organisms. Such models have been formulated recently for the living cell (the Propulator: J. theor. Biol. 112, 399 (1985)) and the human body (the Piscatawaytor: Fed. Proc. 45, 701 (1986)), based on the conformational strains of biopolymers carrying free energy and genetic information) theory of enzymic catalysis and Prigogine's theory of dissipative structures (dynamic chemical gradients in space and time supported by dissipation of free energy; e.g., membrane potentials). These models suggest that the ultimate targets of chemical cytotoxicity are not the stable, isolatable cellular constituents modified by cytotoxicants but rather the dynamic dissipative structures of Prigogine. The new theory of chemical cytotoxicity appears to provide a rational basis for resolving the paradoxes that, although implicated in some cell injury mechanisms, none of the following mechanisms appear to serve as the universal, common pathway of chemically induced cell injuries: covalent binding of "reactive metabolites, increase in cytosolic Ca", lipid peroxidation, free radical formation, and production of reactive oxygen species. Supported by A05848.


The range of possible values for a given acceptable daily intake (ADI) depends in part on the probability distribution of its underlying uncertainty factors. Published data illustrate this concept with three of the traditional areas of scientific uncertainty associated with ADIs, that of interspecies variability to the toxicity of a chemical, subchronic exposure to chronic exposure extrapolation, and a low-effect to no-effect dose extrapolation. Unpublished data regarding interspecies variability in toxicity is also shown. Based on both sets of data, it is proposed that an analysis of an observed excursion above a given ADI, should be based in part on the composite probability density that results from different combinations of probability density of individual uncertainty factors. Implications of this proposition are discussed in light of current efforts to address such excursions, for example, margins of safety. Areas of research are also identified.

MECHANISM OF INDUCTION OF CYTOCHROMES P-450 BY XENOBIOTICS. S. Ji, Dept. Pharmacol. Toxicol., College of Pharmacy, Rutgers University, Piscataway.

Based on the postulate that cells have evolved to utilize even radical chemistry to their survival advantage, a hypothetical, radical-mediated molecular mechanism for the induction of cytochromes P-450 by xenobiotics has been formulated. The mechanism can act as a "xenobiotic sensor" whose role it is to remove all lipophilic xenobiotics (XH) from the intracellular space by inducing P-450 systems that can recognize XH with varying specificities. When the intracellular concentration of XH is decreased, the mechanism automatically reverts back to the pre-exposure state. There are nine key steps; (1) entrapping of XH by the endoplasmic reticulum due to the lipophilicity of XH, (2) perturbation of the conformation of P-450 enzymes, (3) formation of the superoxide anion in cyt. P-450, (4) conversion of O2 to HO, (5) enzymic or non-enzymic interaction between HO and XH to form XH, (6) partial conversion of XH to oxidized products for excretion, (7) covalent interaction of XH with DNA to turn on the gene expression for the m-RNA, with specificity for XH, (8) synthesis of m-RNA, and (9) synthesis of P-450 systems. The model predicts that radical scavengers will inhibit the induction of cytochromes P-450 by xenobiotics. Supported by A05848.
USEPA guidelines were used to calculate water quality criteria for the following munition compounds: hexahydro-1,3,5-trinitro-1,3,5-triazine, nitrocellulose, nitroglycerin, 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, and white phosphorus. Data for calculating an aquatic criterion must meet strict requirements regarding numbers and types of species tested as well as the types of tests conducted. A criterion maximum concentration and a criterion continuous concentration are calculated based on acute and chronic aquatic toxicity data, respectively. In order to calculate a human health criterion, priority is given to human and/or animal carcinogenicity data that is used to estimate a criterion based on concentrations of a chemical related to risks of $10^{-7}$, $10^{-6}$, and $10^{-5}$. For noncarcinogenic chemicals, the criterion is related to threshold toxicity data derived from human or animal chronic studies and is based on an acceptable daily intake. Water quality criteria for the seven munition compounds and pertinent data are presented.

*Operated by Martin Marietta Energy Systems, Inc. Under contract DR-AC05-840821400 with the U.S. DOE.

The human health significance of exposure to mercury in fish was evaluated for seven fish species surveyed in Clear Lake, Lake County. Fish contained geometric mean mercury levels of 0.13-0.82 ppm. Mercury level correlates with fish size. Larger specimens of top trophic species (Largemouth Bass, Channel Catfish, and Crappie) tended to exceed the action level of 1 ppm. Most of the mercury in fish is methylmercury, which affects the nervous system. Fish is the chief source for human exposure to methylmercury. A daily intake of 0.3 mg methylmercury would result in the lowest-observed-clinical-effect level of 200 ng Hg/ml blood in non-pregnant adults. The findings suggest that consumption of no more than 2-20 meals per month, depending upon fish species and size, would protect non-pregnant adults from methylmercury toxicity. The developing fetus is about four times more sensitive to methylmercury. Pregnant women, nursing mothers, those who may soon become pregnant, and children under age six should avoid the fish. Given the wide variations of mercury concentrations in various locations, consumers should vary their fish consumption, not only for sportfish in this study but also for commercial fish.

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A methodology was developed for assessing the potential risks associated with the ocean disposal of municipal sewage sludge. A long-term equilibrium model was used to predict concentrations of toxic organic chemicals in the ocean resulting from an input of 20,000 metric tons/day of sewage sludge at the 106-mile site on the continental slope of the East Coast. Contaminant levels in edible fish were determined from these predicted, ambient water column concentrations, bioconcentration data, and estimates of exposed fish populations, based on an analysis of their geographic/seasonal/depth distribution. Commercial fish landings data and human consumption rates were used to determine ingestion of contaminated and uncontaminated fish. This exposure information was used in risk assessment procedures to examine the potential for health and environmental impacts from sludge disposal.

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The purpose of this study was to measure percent ozone uptake (O$_3$ UP) of the total respiratory tract in various small animals to establish criteria for making quantitative comparisons between animals and man. Three strains of rats (Fischer-344, Sprague Dawley, and Long Evans) and Hartley guinea pigs of similar size, but not age, were evaluated during exposure to 0.3 ppm ozone (O$_3$). Fractional uptake was measured by sampling O$_3$ before and after passing the animal's nose during nose-only exposures. Each animal was individually evaluated while sealed inside a body plethysmograph so that respiratory signals and uptake could be measured simultaneously. O$_3$ UP was not different among the 3 strains of rats as well as between rats and guinea pigs (Kt50 = 43.821.9%). O$_3$ UP was also unaltered by exposure to 0.6 ppm O$_3$ (41.321.8%) in Fischer rats. Despite minor differences in tidal breathing between rat strains and obvious differences between rats and guinea pigs, the data indicate that total respiratory uptake of O$_3$ is approximately 42%. These data suggest that differences in sensitivity between these two species can not be explained by differences in delivered dose. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
CRITICAL TIME FOR POST-EXPOSURE EFFECT OF LUNG GLUTATHIONE ON OZONE-INDUCED FIBROSIS IN MICE. D.T. Kirkpatrick, J.D. Sun, J.A. Pickrell, J.R. Harkema, and R.F. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Exposure to 1 ppm ozone (14 days, 23hr/day) causes pulmonary fibrosis (PF) in mice treated for 90 days after exposure with the GSH decreasing agent, buthionine sulfoximine (BSO) (The Toxicologist 6: 515, 1986). To study the time period during which GSH affects development of PF, male B6C3F1 mice were exposed to O3 as described above and given 30mM BSO in drinking water for 7, 14, 21, or 90 days after exposure. Lung nonprotein sulphydryl (NPSH), as an estimate of GSH, decreased by 50% in all BSO treatment groups, compared to identically exposed, water drinking groups. A 100% O3-induced increase in lung NPSH was depleted to 75% of normal by a 7 day BSO treatment. 03-exposed, water drinking mice had no fibrotic changes at 90 days post-exposure, but all mice that received BSO for 7, 14, 21 or 90 days after exposure had areas of interstitial, alveolar duct-associated fibrosis. GSH decreases caused by BSO treatment as short as one week post-exposure led to histologically observable fibrosis. The first 7 days following exposure to ozone appears to be a critical time for the observed GSH effect on fibrosis. Increased alveolar macrophage (AM) numbers (lavage AM count 3.7x control at 7 days post-exposure) may be involved in this effect. (Research supported by U.S. DOE Contract No. DE-AC04-76EV01013.)

CHANGES IN COLLAGEN METABOLISM AND PROTEINOSYSIS AFTER REPEATED INHALATION EXPOSURE TO OZONE. J. A. Pickrell, F. F. Hahn, A. H. Reber*, D. A. Horoski, and R. F. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; *VHI Department, Purdue University, Lafayette, IN.

To study the changes in collagen metabolism that occur in the development of pulmonary fibrosis, female rats were exposed to 0, 0.57 and 1.1 ppm ozone for 19 hours/day for 11 days and sacrificed 12 or 60 days after initiation of exposure. The lungs of rats sacrificed at 12 days after initiation of exposure to 1.1 ppm had (1) interstitial pneumonia characterized by a mixed inflammatory cell infiltrate, type II cell hyperplasia, and fibroplasia, a proliferation of the collagen producing cells, (2) increased cathespun D and macrophage elastase activity, indicating macrophage-induced proteinolysis, (3) a decreased rate of intracellular degradation of newly produced collagen before its secretion, and (4) increased lavage fluid hydroxyproline, indicating turnover of extracellular collagenous matrix. Reduced intracellular collagen degradation correlated directly with increased net collagen production and fibroplasia by 12 days, and preceded increased total lung collagen and the development of modest fibroplasia in the alveolar duct regions by 60 days after the 1.1 ppm exposure was initiated. (Research Supported By the U.S. Department of Energy Contract No. DE-AC04-76EV01013.)


A chronic study is in progress examining various biological endpoints following 1 week, 3 weeks, 3 months, 12 months, and 18 months of exposure. Fisher 344 rats are exposed 22 hours per day to O3 and NO2 with a 2 hour down time for service and animal care. The exposure regimen includes 13 hours of O3 at a background level of 0.06 ppm with a 9 hour spike reaching a maximum concentration of 0.25 ppm. The NO2 regimen consists of 16 hours exposure at a background level of 0.5 ppm with a 6 hour spike to a maximum concentration of 1.5 ppm which is held for 2 hours. Both O3 and NO2 are held at background levels during the weekend. Following 3 months of exposure regimen, effects have been noted in lung LDH and lavage cells acid phosphatase, 5'-nucleotidase, and leuinc aminopeptidase (all values decreased for O3 and NO2). The LDH iso-enzyme pattern in the lung showed a diminution of bands LD4 and LD5 and a large increase in band LD1 for both O3 and NO2 vs air controls. Total glutathione peroxidase was significantly increased in the lungs of rats exposed to O3 and superoxide dismutase significantly decreased. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
The effect of oxidant insult on pulmonary uptake of the antioxidant vitamin E was investigated. Male Long Evans rats were exposed to 0, 10, 20, or 40 ppm of NO2 for four hours. Immediately after exposure, serum-associated tritiated alpha-tocopherol (vitamin E) was introduced into their circulation. The tritium content of lung was measured at 30 and 240 minutes after injection. At 30 min, lungs of NO2-exposed animals contained about 20 percent less tritium than controls at all doses. No significant differences were seen at 240 min at any dose of NO2. These results suggest a small inhibition of vitamin E uptake shortly after NO2 exposure. Normal uptake is apparently resumed within four hours. (Supported by DOE Contract No. DE-FG02-85ER60282 and NIH Training Grant 5T32 ES07026.)

The increase in antioxidant enzyme activity in whole lung and specific lung cells was studied following a 4 day exposure of rats to 20 mg NO2/m3. Isolated, cultured alveolar macrophages (AM) and type II pneumocytes (II) from control and NO2-exposed rats were additionally examined for their sensitivity toward an in-vitro NO2 exposure. Glucose 6-phosphate dehydrogenase, glutathione reductase and glutathione peroxidase activities in whole lung tissues were enhanced by 50% respectively compared to unexposed rats. However, only GSH-Px was induced in AM and II, being more pronounced in II (54%). AM or II from NO2-exposed rats were morphologically indistinguishable from respective cells isolated from control rats. In-vitro NO2 exposure of AM and II from NO2-exposed rats resulted in comparable changes in phagocytosis and/or viability features as observed after in-vitro exposure of cells from unexposed rats. These data indicate that induction of pulmonary antioxidant enzyme activity may be partly reflected in specific lung cells. However, this cellular increase does not necessarily result in a decreased NO2 sensitivity.

The toxic effect of NO2 on lung tissue is via oxidation of lung lipids. Knowledge of the rate of this free radical reaction is necessary for the development of a mathematical model of NO2 dosimetry in the lung. Liposomes of dilauroyl phosphatidylcholine (18:2) and dilauroylenoloy phosphatidylcholine (18:3) were exposed to 0-10 ppm NO2 in air. Peroxidation was measured by diene conjugation at 233 or 235 nm. Peroxidation of 18:3 liposomes also produced malonaldehyde, but 18:2 liposomes did not. The rate constant for peroxidation of 18:2 and 18:3 liposomes was $-10^{-10}$ M⁻¹s⁻¹. In the absence of NO2, 1 mM sodium nitrite did not initiate peroxidation of the liposomes. Thus, initiation of PUFA peroxidation does not proceed by the formation of nitrite, and the slow rate constants derived here are consistent with a mechanism of allylic hydrogen abstraction. Malonaldehyde arises from α,β unsaturated cyclic peroxides. (Supported by EPA Cooperative Agreement CR809715 and NIH Grants RR01693 and CA14236.)

Oxidation of lung components is thought to be the primary mechanism of pulmonary toxicity from NO2. Using liposomes as a model membrane system, we have studied NO2 peroxidation of polyunsaturated fatty acids and protective effects of α-tocopherol and ascorbate. Liposomes were prepared from dioleoyl phosphatidylcholine (18:2) with 2 mole % α-tocopherol in the membrane or from dipalmitoyl phosphatidylcholine (16:0) which were brought to 50 μM with ascorbate. When 18:2 and 16:0 liposomes were exposed to 10 ppm NO2, α-tocopherol decreased with a concomitant increase in α-tocopheryl quinone. The rate of oxidation of α-tocopherol varied with time with a maximal rate of -1.1 X 10⁻³ M⁻¹s⁻¹. When 18:2 liposomes in 50 μM ascorbate were exposed to 10 ppm NO2, ascorbate was oxidized at a rate of -6.7 X 10⁻³ M⁻¹s⁻¹. These results contrast with fatty acid oxidation of -10⁻¹⁰ M⁻¹s⁻¹. This data indicates that α-tocopherol protects the membrane against NO2 by preferential oxidation. However, ascorbate alone does not protect membranes. (Supported by EPA Cooperative Agreement CR809715 and NIH Grants RR01693 and CA14236.)
Nitrogen dioxide (NO₂) is an oxidant pollutant arising from both automobile engine and stationary source emissions. A synergistic interaction occurs between ozone and respirable acidic aerosols (ammonium sulfate, sulfuric acid [H₂SO₄]), but not neutral aerosols (sodium chloride [NaCl], sodium sulfate), in rats as evaluated by increases in apparent rates of lung collagen synthesis and in protein content of bronchoalveolar lavage fluid. We now report, using these assays, a synergistic interaction between NO₂ and respirable aerosols of acidic and neutral pH. Significant increases above control values were observed after exposure of rats to 5 ppm of NO₂ alone. Values from groups exposed to NO₂ plus either NaCl or H₂SO₄ aerosol (1 mg/m³) were significantly elevated above values from rats exposed to NO₂ alone. The synergistic interaction between NaCl aerosol and NO₂, but not ozone, suggests that a different mechanism may occur between these two oxidant gases. It is known that nitrosyl chloride, may form in atmospheres containing NO₂ and NaCl. Although we have not identified the biochemical mechanisms that result in synergistic lung damage, it is possible that the formation of nitrosyl chloride may contribute toward lung damage caused by exposure of rats to NO₂ with NaCl aerosol.

Inhalation toxicology evaluations of complex mixture atmospheres containing carbon monoxide must consider the contribution of carbon monoxide to the toxicological response. Sprague-Dawley derived male rats were anesthetized by intraperitoneal injection of pentobarbital sodium and the right external jugular vein of each subject was catheterized with 0.025 inch i.d. allisitic tubing. The cannula was exteriorized at the dorsal surface caudal of the scapula and secured. Following a minimum of 48 hours post surgery, the subjects were exposed to steady-state concentrations of carbon monoxide in air at either 527, 1091 or 1800 ppm for a period adequate to produce steady-state carboxyhemoglobin (COHb) concentrations of 40, 59 and 68%, respectively. A minimum of six blood samples was used to describe each phase of the CO kinetics. The absorption halftime (t½) for the equilibrium COHb concentrations were 17.2±1.7, 12.6±1.1 and 9.0±0.8 minutes, respectively. The corresponding elimination halftime were 42.3±7.9, 32.6±3.9 and 29.7±3.1 minutes. Thus, the dynamics of COHb concentration in the rat are of such duration that they must be considered in the interpretation of total integrated dose, particularly in exposures of less than six hours.

Inhalation toxicology evaluations of complex mixtures which contain significant concentrations of carbon monoxide must consider the contribution of carbon monoxide to the toxicological response for proper interpretation. Changes in minute ventilation result in a different total delivered dose even under conditions of fixed atmosphere concentration and exposure duration. The influence of carbon monoxide alone, on pulmonary function in juvenile Sprague-Dawley derived rats was assessed during repeated nose-only inhalation exposures to 0, 527, 1091 or 1800 ppm CO. Tidal volume, respiratory rate and minute volume were calculated from respiratory airflow measurements acquired by whole-body plethysmography. Five exposure averages of tidal volume, respiratory rate and minute volume were normalized to their associated baseline conditions. The percent of baseline minute volume performance of the control, 527, 1091 and 1800 ppm exposure was 90.3±3.5, standard error of the mean (SEM), 74%±4.1 SEM, 65%±3.1 SEM and 72%±8.9 SEM, respectively. The changes in minute volume were related primarily to changes in respiratory rate. Tidal volume did not change as a result of CO exposure.

The acute toxicity of lethal concentrations of carbon monoxide (CO) is well characterized in man and experimental animals. However, little information is available on repeated administration of sublethal concentrations. CO was presented by nose-only inhalation one hour per day for 14 consecutive days to groups of 20 male and 25 female Sprague-Dawley derived rats. CO concentrations in air were 0, 527, 1091 and 1800 ppm. Blood samples were taken from the retro-orbital sinus under CO₂ anesthesia at 50-60 minutes of exposure on days 1, 7 and 14 for analysis of hematocrit and carboxyhemoglobin (COHb). Blood COHb averaged 33, 49 and 50% in low, medium and high dose groups, respectively. Animals were necropsied 24 hours after their last exposure. Organ weights were determined for heart, brain, lung, liver, spleen, adrenals, and kidneys. The only dose-related effect was a significant increase in heart weight in both sexes, both for absolute weights and for weights relative to body weight or brain weight. Heart weights for females averaged 1.02, 1.10 and 1.30g; for males the weights were 1.35, 1.46 and 1.67g. Heart weights from sham, air-exposed animals averaged 0.88g in females and 1.26g in males. Cardiomegaly is considered to be a response to increased cardiac work secondary to tissue hypoxia induced by CO.

Juvenile Sprague-Dawley rats were exposed nose-only to 0, 527, 1091, and 1800 ppm of carbon monoxide (CO), one hour per day for each 14 consecutive days. The hearts, brains, and respiratory tracts (including nasal passages) were examined histopathologically. There were no differences between the groups in terms of the histopathology produced in the respiratory tracts and in the brains. In heart tissue there was a dose-related increase in multi-focal chronic inflammation in the cardiac muscle. Microscopically, the lesion consisted of scattered interstitial aggregates of lymphohistiocytic cells. These aggregates were found most frequently in the ventricular walls and septum. In a few instances, the inflammatory infiltrations were accompanied by minimal amounts of muscle fiber atrophy and necrosis (the latter occasionally accompanied by fibrosis). These lesions resemble age-related cardiomyopathy, a common finding in older animals of this strain. The changes were associated with pronounced ventricular enlargements.

ETHYL CHLORIDE (EtCl): 11-DAY CONTINUOUS EXPOSURE INHALATION TOXICITY STUDY IN B6C3F1 MICE. T.D. Landry, K.A. Johnson, J.E. Phillips and S.K. Weiss. The Dow Chemical Company, Mammalian and Environmental Toxicology Research Laboratory, Midland, MI.

An inhalation exposure study was conducted to evaluate the potential toxicity of EtCl under nearly continuous exposure conditions. Groups of seven mice/sex were exposed for 23 hrs/day to 0, 250, 1250, or 5000 ppm EtCl for eleven consecutive days. Exposures were discontinued for approximately one hr/day to observe care for, and periodically to weigh the mice. On the day following the last exposure, a neurobehavioral observation battery was performed; samples were obtained for clinical chemistry and hematology, and necropsies were performed. Extensive sets of tissues from the 0 and 5000 ppm exposed mice were examined histologically. Sections of the brain, liver and kidneys were evaluated in the intermediate exposure groups.

Exposures to EtCl were well tolerated. The only effects observed were in the livers of 5000 ppm exposed mice. There were increased relative liver weights and a slight increase in hepatocellular vacuolation (glycogen or fat). These effects were not considered to reflect significant toxicity. The no-observable effect level was 1250 ppm.

EFFECTS OF INHALED ETHYL CHLORIDE ON FETAL DEVELOPMENT IN CF-1 MICE. T.R. Hanley, Jr., B.H. Scorticini, K.A. Johnson, and J.J. Momany-Pfrunder. Mammalian and Environmental Toxicology Research Laboratory, NIEHS, The Dow Chemical Company, Midland, MI.

Ethyl chloride (EtCl), a colorless, flammable gas, was evaluated for teratogenic potential in pregnant mice. Groups of 30 bred CF-1 mice were exposed for 6 hr/day on days 6-15 of gestation via inhalation to concentrations of 0, 500, 1500 or 5000 ppm (0.13, 4.0, or 13.2 mg/l air) of EtCl vapor. These exposure concentrations were selected based on the results of a probe study in which decreased weight gain was observed in pregnant females at 5000 ppm and above. No significant effects on maternal body weights, body weight gains, liver weights, reproductive parameters or fetal body weights were observed at any of the exposure levels tested. Examination of the fetal mice for external, visceral, and skeletal malformations failed to indicate any evidence of a teratogenic response. A small increase in the incidence of foramina of the skull bones, suggestive of at most very slight fetotoxicity, was observed only at the high exposure level (5000 ppm). Thus, exposure to ethyl chloride at concentrations up to 5000 ppm during the period of major organogenesis did not produce any evidence of a teratogenic response in fetal mice.


Male and female B6C3F1 mice and F344 rats were exposed to 0, 312, 625, 1250, 2500, or 5000 ppm tetrafluoroethylene (TFE) 6 hrs/day/5 days/week for 13 weeks. Compound related mortality, clinical signs, reproductive effects, and significant biological hematology changes were not observed in exposed rats or mice. TFE-induced reduction in rate of body weight gain was observed in both sexes of both species. Increased urine volume, glucose, and protein were observed in exposed rats (more predominant in males). Exposed mice demonstrated increased urine volumes with decreased urine specific gravity and urinary glucose and protein. Mild karyomegaly of renal tubular epithelial cells was observed in treated mice except at the lowest exposure concentration. Increased renal cortical tubular changes of degeneration and regeneration were observed in treated rats except at the lowest concentration with male rats being more affected than females. Pancreatic acinar cell necrosis was observed only in male and female rats exposed to 5000 ppm TFE. In summary, exposure to TFE for 13 weeks produced toxic effects on the kidney with rats being affected more than mice and males being affected more than females. In addition, TFE-related effects were observed in the rat pancreas.
Groups of male (19) and female (19) Sprague-Dawley rats were exposed via nose-only to 0.0, 0.16, 1.0 or 2.2 mg of propylene glycol/l of air for 6 hr/day, 5 days/week for 13 weeks. Although respiratory rates and minute volume decreased during aerosol exposure, these changes were not dose-related. Tidal volume remained unchanged. The mean terminal body weights were not significantly different than controls for any group of male animals. The mean female body weights for the 2.2 mg/l group were significantly (p < 0.01) less than control female rats beginning on day 50 and continuing throughout the study. This effect in female rats was consistent with a decrease (p = 0.05) in feed consumption for the high dose female rats beginning on study day 43. Due to the lack of consistent significant effects as a function of dose and sex, changes in selected clinical pathology parameters and organ weights were not considered biologically significant. The nasal passages in 7 of 19 females at the 2.2 mg/l dose exhibited a significant (p < 0.01) increase in the number of goblet cells. Exposure to the 2.2 mg/l dose may affect the nasal passages by acting as an astringent to the respiratory epithelium in rats; however, these changes were transient and are not considered adverse effects.

Male and female F-344 rats were exposed at 0, 25, or 250 ppm triethylamine (TEA) vapor, 6.5 hr per day, 5 days per week for 24 weeks in order to assess cardiac and other organ system toxicity. Rats were weighed biweekly and scheduled sacrifices were performed following 30, 60, and 120 days of exposure. No statistically significant treatment-related effects on organ weights, hematology, clinical chemistry, or electrocardiographic indices were observed. Body weight gain was not affected by TEA treatment. No evidence of cardiototoxicity was seen in rats exposed to either TEA concentration for up to 24 weeks. No gross or histopathologic lesions were noted in any of the organs examined including the nasal passages. This latter finding is in marked contrast to previously reported findings from this laboratory in which squamous metaplasia, suppurative rhinitis, and lymphoid hyperplasia were found in the respiratory epithelium of F-344 rats exposed to the structurally related chemical, diethyline, under the same conditions as this study (PAT 6:559-565, 1986).

Subchronic inhalation toxicity of partially hydrogenated terphenyls (Thermolin® 66 heat transfer fluid) was evaluated using groups of 15 male and 15 female Sprague-Dawley rats exposed (whole body) to target atmospheric concentrations of 0, 10, 100 and 500 mg/m³ for 6 hr/day, 5 days/week for 13 weeks. The study design included observations for clinical signs, body weights, ophthamalic exams, hematology and clinical chemistry, major organ weights, and gross- and histopathology. No treatment-related effects were noted in the ophthamalic exams, clinical chemistry, hematology, and gross- or histopathology. Body weights were slightly depressed in high-dose males. Liver weights and liver/body weights were increased in treated males in a dose-related fashion, although the changes at the lowest dosage were not significant. Treated female liver weights were similar to controls. Histopathological examinations revealed no lesions related to terphenyl administration and no pathological correlation to the increased liver weights. The no adverse-effect level is considered to be at least 98 mg/m³, the mean concentration of the 100 mg/m³ target level.

Peroxidase (PER) has been shown to be carcinogenic in the mouse but not rat liver (NTP, 1986). In this study F344 rats and B6C3F1 mice were exposed to PER by inhalation (400 ppm, 6h/day) for 26 days. Proliferated peroxisomes were observed in the livers of mice exposed to PER and peroxisomal β-oxidation was increased up to 4 fold. Peroxidase proliferation was not seen in rat liver or in the kidneys of either species. Trichloroacetic acid (TCA), a known metabolite of PER and peroxisome proliferating agent, was measured in the blood of rats and mice during and for 48h after a single 6h exposure to 400ppm PER. Peak blood levels of TCA in mice were 13 times higher than in rats. Over the course of the experiment determination of AUC showed that mice were exposed to 6.2 times more TCA than rats. The difference in the metabolism of PER to TCA in mice and rats leads to the species difference in hepatic peroxisome proliferation and is probably the basis of the species difference in hepatocarcinogenicity. Peroxidase proliferation does not appear to play a role in the induction of tumours by PER in the rat kidney.
This study was conducted to evaluate the inhalation toxicity of perchloromethyl mercaptan (PMM) in rats at vapor concentrations above and below the current TLV for PMM (0.8 mg/m³). PMM is a chemical intermediate and it produces severe respiratory irritation and lacrimation following acute inhalation exposure. Fifteen male and female Sprague-Dawley rats per group were exposed to PMM vapor at cumulative mean air concentrations of 0.013, 1.0 or 8.7 mg/m³ for 6 hr/day, 5 days/week for two weeks. Exposure to PMM did not result in treatment-related mortality. Clinical changes observed at 8.7 mg/m³ included haircoat stains, labored breathing, tremors and decreased body weight gain. Necropsy observations were restricted to the lungs (evidence of edema, increased lung weights and increased mucus secretion at 8.7 mg/m³). Histologic pulmonary changes included alveolitis, interstitial fibroplasia and peri-vascular edema (8.7 mg/m³). Mild nasal epithelial changes were noted at 8.7 and 1.0 mg/m³. This study demonstrated a respiratory irritant effect of PMM vapor at 8.7 and 1.0 mg/m³ but not at 0.13 mg/m³ following multiple exposures.

Male and female Sprague-Dawley rats were exposed to vapors of TBA at mean concentrations of 0.19, 0.50, and 2.0 mg/l over a 4-week period (20 exposures, 6hr/d, 5d/wk). High level animals had decreased mean body weights throughout the study and one male died. High level rats of both sexes had inflammation of the nasal passages and conjunctiva upon microscopic examination. In a 13-week study (62 exposures) at the same levels, TBA-related effects included: 4 spontaneous and 7 in extremis deaths, marked decreases in body weight and activity, poor physical appearance and ocular and respiratory irritation at the high level; in both sexes at the mid level and in males at the low level, ocular and respiratory irritation and hypoactivity were seen less frequently. Liver weights were decreased in males and high level females. Enzymes associated with hepatotoxicity were elevated in high level rats, however, no corresponding gross or microscopic lesions were found. Severe, purulent, chronic inflammation of the respiratory tract and proliferation of the submucosal mucous glands in the high level were observed microscopically. After 13 weeks of exposure to TBA, 20 mg/l appears to be a "no effect" level for female rats and a "minimal effect" level for male rats.

This study was designed to assess the toxicity of Sevoflurane when administered to 24 cynomolgus monkeys (12/sex) via inhalation for 3 hrs/day, 3 days/wk. for 8 wks. at 1.0, 1.6 and 2.5 MAC (minimum alveolar concentration). Physical observations, ophthalmology, body weights, food consumption, clinical laboratory studies, serum and urine fluoride levels and urinalysis were performed pretest and at selected intervals during the treatment period. End-tidal Sevoflurane and carbon dioxide concentrations were monitored during the last exposure period of wks. 1, 2, 4, 6 and 8. All surviving animals were sacrificed and post mortem examinations were performed. Selected organs were weighed and organ/body weight ratios calculated. Microscopic evaluations were performed for all animals. SGOT, SGPT, LH and CPK were elevated following the first week of exposure to MAC levels of 1.0 and 1.6. These levels returned to control level at the second week of measurement. The pattern was similar for the 2.5 MAC level of exposure except enzyme values did not return to control levels until wks. 4, 6 or 8. Sevoflurane did not produce any evidence of unexpected, adverse toxicological or pathological effects under the conditions of this study.

Two fourteen-week inhalation toxicity studies of 0.0'-dimethyl phosphorodithioate were conducted in rats. In the first, mean analytical exposure concentrations were 16, 100, and 321 mg/cubic meter. Reductions in body weights (>5%) occurred in mid and high level rats. Brain cholinesterase levels were decreased 15-26% in high level males and all groups of females. The following lesions were observed; nasal mucosal hyperplasia/metaplasia, pituitary and thyroid follicular hypertrophy/hyperplasia, and testicular and skeletal muscle degeneration/atrophy. A second study was performed to further evaluate these effects; mean analytical exposure concentrations were 4.4, 27, and 160 mg/cubic meter. Animals were necropsied either after exposures or following a 6-week recovery period. Body weight reductions occurred only in the high level. Brain cholinesterase levels were decreased in some high level animals. Histopathologic lesions like those observed in the first study were seen in the nose and skeletal muscle (mid and high levels), taste (primarily high level), and thyroids (high level only) after both the exposure and recovery periods. In addition, ocular (corneal) lesions were noted at all exposure levels; they appeared to reverse only in some low level animals.
A 2-WEEK SUBCHRONIC INHALATION STUDY ON 3,4-DICHLOROANILINE IN RATS L. A. Kinney, T. W. Stone, Jr. and C. L. Kennedy, Jr. E. I. du Pont de Nemours and Company, Newark, DE

3,4-Dichloroaniline (3,4-DCA) is a crystalline solid with a moderate vapor pressure. Groups of 20 male Crl:CD BR rats were exposed nose-only, 6 hr/d, 5 d/wk for 2 weeks to 0, 10, 45 or 200 mg/m³ of 3,4-DCA in air. Ten rats per group were given clinical pathology and pathology examinations after the 10th exposure and after 14 days of recovery; 10 rats per group were used to monitor methemoglobin formation. 3,4-DCA caused dose-dependent methemoglobinemia in all exposed rats (2,5-, 5,0- and 40-fold increases over control values, respectively). However, no biological changes in response to the methemoglobinemia were observed at 10 mg/m³. At 45 and 200 mg/m³, rats had accumulations of hemosiderin in spleen. At 200 mg/m³, rats had elevated spleen weights and extramedullary hematopoiesis in spleen. These rats also had depressed erythrocyte count, hemoglobin concentration and hematocrit and elevated platelet count and mean corpuscular volume. These changes are suggestive of increased erythrocyte destruction at 45 and 200 mg/m³, with a resultant compound-related anemia and enhanced erythropoiesis at 200 mg/m³.

LONG-TERM EFFECTS OF HYDROGEN CHLORIDE ON PULMONARY FUNCTION AND MORPHOLOGY IN NONHUMAN PRIMATES. A. Anzuetp, W.G. Switzer, H.L. Kelsen, & R.K. Hinderer. 1) Southwest Research Institute, San Antonio, TX; 2) UT Health Science Center, SA, TX; 3) B. F. Goodrich, Akron, OH

A previous study (Toxicologist, 5, 52, 1986) reported the effects of a 15-min exposure of baboons to HCl (500, 5000 and 10,000 ppm nominal concentrations) on respiratory response during exposure and on pulmonary function and CO₂ challenge response at 3-days and 3-months post exposure. This study extends the observation of these animals to one year post exposure. Pulmonary function tests and CO₂ challenge responses were conducted at 5 months and 1 year post exposure. The right medial lobe of the lung was removed from one control and three 10,000 ppm exposed animals for histopathological examination. Sequentinal mucosal biopsies from the upper airway to a segmental bronchus also were obtained. Exposure to HCl did not produce any long-term effects in the 500 and 5000 ppm animals. Exposure to 10,000 ppm caused chronic nasal obstruction and mouth breathing, hypoxemia at six months and one year post exposure and increased lung functional residual capacity at one year. Focal lung fibrosis was present in only one of the three 10,000 ppm HCl exposed animals. These studies showed that in primates exceedingly high levels of HCl are required to produce any significant long-term pulmonary effects.

(Clinical Aspects and Causalism of Acute Induction by Inhalation in Humans. J.R. Weiser, Department of Internal Medicine, Medical University of Lübeck, FRG. Sponsor: C.-P. Siegers.

The purpose of this report is to discuss treatment, symptoms, course and morphology of NH₃ poisoning. 8 workers were poisoned by the sudden breakage of a coolingsystem pipe, 2 out 8 put. were exposed to about 3000 ppm of NH₃. The other intensive-care pat. suffered of cardiopulmonary arrest of 1 to 3 min. Cutaneous lesions showed third degree burns over about 20 % of the body surface. He was treated with epinephrine, atropine, NaHCO₃, prednisolone (600 mg/d), desamethasone (1 mg/kg/d) and later cephalosporin without success. After 13 days he died from therapy-resistant bronchopneumonia with collagen breakdown. The pathologic status showed signs of glottis edema and toxic edema of the lungs. The other intensive-care pat. who had second degree burns over 10 % of his body, was given 3 l of O₂ and 7 l of acetic acid by insufflation through the endotracheal tube. X-rays showed prominent pulmonary vascular markings, no traces of which could be detected in later X-rays controls. Continuous control or blood gas analysis showed a decrease down to 60 %, (sorr) after 8 h. Thus the major problem of acute NH₃ intoxication is the complication of the respiratory tract, especially inflammation of the esophagus and bronchial system. However, through endotracheal insufflation of acetic acid the pulmonary complications could be reduced.

A COMPARISON OF ACUTE INHALATION TOXICITY OF A SERIES OF CHLOROSILANES WITH HYDROGEN CHLORIDE. G.B. Kolesar, W.J. Siddiqui, and E.J. Robbe. Dow Corning Corporation, Midland, MI

Chlorosilanes are used as intermediates in a variety of industrial applications. Acute inhalation studies were conducted to aid in evaluating the health hazards associated with these materials and the data were compared with the toxicity of hydrogen chloride. Groups of 10 (5/sex) Sprague-Dawley rats were exposed for one hour in a three-liter, nose only chamber. The one-hour L₅₀ for HCl was 9,530 ppm in rats. The chlorosilanes evaluated were methyltri chlorosilane, dimethylchlorosilane, and trimethylchlorosilane. The L₅₀ values for chlorosilanes were significantly higher than the data reported in the literature. Corneal opacity and respiratory difficulty were the main clinical signs following exposures. Necropsy of the animals that died as a result of exposure to chlorosilanes showed pulmonary edema and significant focal hemorrhage in lungs. Based on HCl equivalents, these materials had L₅₀ values which range one-third to one-half of the L₅₀ of HCl. The clinical and gross pathological observations indicate the different times of action for the chlorosilanes and HCl and may suggest a possible explanation for differences in L₅₀ values.

CLINICAL ASPECTS AND CASUISTRY OF ACUTE INDUCTION BY AMMONIA INHALATION IN HUMANS. J.R. Weiser, Department of Internal Medicine, Medical University of Lübeck, FRG. Sponsor: C.-P. Siegers.

The nasal mucosa protects the delicate lower respiratory tract by warming, moisturizing, and cleaning inspired air. The nose also contains the receptors for olfaction and is an important target site for inhaled toxic materials. For use in future dose-response studies of inhaled gases, morphometric analysis of the primate nasal cavity has been undertaken to determine nasal cavity surface area and volume of the Rhemus monkey and to quantify the surface areas of squamous, respiratory and olfactory epithelia. Measurements were made on 20, 4 μm thick, plastic, step cross-sections (3.89 mm apart) through the nasal cavity and naso-pharynx of an adult male Rhemus monkey. Surface area and airway volume measurements of the nasal passage and maxillary sinus were determined using a Videoplan II image analyzer. Data points were collected every 0.05 mm around the perimeter of each section. The nasal cavity surface area was 81.97 cm² and the volume was 8.25 cm³. The surface area and volume of the maxillary sinus were 10.67 cm³ and 1.92 cm³ respectively. The information provided by this study, once coupled with data from more animals, will provide useful baseline values for ongoing regional dosimetry studies of inhaled formaldehyde in monkeys.

TEMPORAL CHARACTERISTICS OF LATE BRONCHIAL AND EARLY ALVEOLAR CLEARANCE IN RABBITS. B.D. Naumann and R.B. Schlesinger. New York University Medical Center, Institute of Environmental Medicine, New York, NY.

Clearance of inhaled particles during the early post-exposure period can be altered by ambient pollutants at relatively low levels. Detection of slight, though possibly significant, alterations in clearance requires a sensitive, well characterized, measurement system. To further refine our system, this study was designed to evaluate any measurable changes in late bronchial (BR) or early alveolar (ALV) clearance in normal rabbits that may occur during the course of a typical study involving repeated measurements. Rabbits were exposed to a Sz-85 tagged polystyrene latex aerosol and clearance was followed for 2 weeks (Run A). Both late BR (12-48h) and early ALV (2-14d) clearance were measured. The same rabbits were reexposed to the tracer aerosol 2 months after the first exposure and clearance was again measured (Run B). The entire experiment was repeated once. Both late BR and early ALV clearance were found to be significantly slower in Run B compared to Run A in both experiments. Based on the results of this study it appears that temporal changes in late bronchial and early alveolar clearance are similar. Consideration must be given to these changes when interpreting the results of studies involving repeated exposures.

A MORPHOMETRIC ANALYSIS OF RABBIT AIRWAYS AFTER REPEATED EXPOSURES TO SULFURIC ACID AEROSOL. J.M. Gearhart and R.B. Schlesinger. New York University Medical Center, New York, NY.

Many inhaled toxic materials are hypothesized to initially affect the smaller airways of the lung, before evidence of functional alterations. This study determined the effects of repeated exposure to sulfuric acid mist, an irritant that is both an environmental and occupational pollutant, on the morphometry of the respiratory tract of rabbits. Twenty rabbits inhaled 250 μg/m³ sulfate aerosol (MMD=0.3 μm) for 1 hr/day, 5 days/week, with sacrifices at 4, 8, and 12 mo. Some animals were allowed an additional 3 mo recovery to study resolution of damage. Control groups of animals received a water aerosol. Tissue samples from each lung were embedded in plastic, sectioned at 3 μm, and stained with H & E or Alcian Blue/PAS. All airways were sized with a filter eyepiece, and mean cell density (SCD) was determined under high magnification. Acid exposure changed airway sizes compared to control. Except for the followup group, all acid animals had a shift to smaller airways, which was most pronounced in the 8 mo group. Acid exposure caused increases in SCD in small airways as early as 4 mo, that was unresolved by 3 mo after exposure ceased. There was a significant shift from PAS to Alcian Blue cells at different time points. Some recovery of airway damage occurred after irritant exposure, but recovery was not complete.

EFFECTS OF INTERMITTENT EXPOSURE TO SULFURIC ACID AEROSOL ON MUCCOCILIARY CLEARANCE IN RABBITS. J.M. Gearhart and R.B. Schlesinger. New York University Medical Center, New York, NY.

This study was designed to determine the quantitative and temporal alteration in tracheobronchial muccociliary clearance (TBC) from the lungs of rabbits due to daily exposures to an atmospheric pollutant, submicrometer-sized sulfuric acid mist. Twenty rabbits were exposed to 250 μg/m³ sulfuric acid mist (MMD=0.3 μm) for 1 hr/day, 5 days/week, with sacrifices for histopathology at 4, 8, and 12 mo. Some animals were allowed 3 mo recovery to study resolution of clearance changes. Control animals received a water aerosol. At intervals of 2 to 4 weeks, animals inhaled a tracer aerosol (MMD=4.5μm) tagged with Te 99m, and the clearance of activity from the chest was monitored for 4 hrs. TBC was slower the first month of exposure and this slowing was maintained throughout the exposure period for all exposure groups, with periodic return of clearance to near control levels. After cessation of acid exposure, clearance became extremely slow and did not return to normal by the end of the followup period. Chronic exposure to sulfuric acid mist at a level the TLV causes slowing in TBC at times very early in the exposure protocol, when other functional changes are rarely observable. It is also apparent that the effects of long term irritant exposure on clearance are not necessarily progressive but adaptation may occur early in the exposure process.
Peracetic acid is supplied commercially as a 35% solution in acetic acid (40%), hydrogen peroxide (72%) and water (18%). Groups of 10 male Crl:CD:BR rats were exposed nose-only, 6 h/day, 5 d/wk for 2 weeks to 0, 5.7, 23, or 72 mg/m³ of commercial peracetic acid solution in air.

Clinical pathology and pathology examinations were done after the 10th exposure and after 13 days of recovery. At 23 and 72 mg/m³, rats had depressed body weights and were observed to be pulling back into their restrainers to avoid the test material during exposures. At 72 mg/m³, rats had loud noise and labor breathed.

Several depressed organ weights were observed that were mainly attributed to the depressed body weights. Compound-related lesions in the nasal respiratory epithelium were observed at 23 and 72 mg/m³, including acute inflammatory cell infiltrate, squamous metaplasia and foci of epithelial ulceration. The lesions were less severe after 13 days of recovery. The 5.7 mg/m³ exposure concentration caused no adverse effects. At higher concentrations, peracetic acid caused general systemic effects and focal lesions in the respiratory epithelium indicative of its corrosive nature.

THE PULMONARY RESPONSE OF GUINEA PIGS EXPOSED TO A COMBINATION OF ZNO-SO₂ AEROSOLS FOLLOWING THE ‘WORK WEEK’ EXPOSURE REGIME

Both SO₂ and ultrafine metal oxides are emitted to the atmosphere during industrial processes such as welding, smelting or fabrication of molten metals. Some of these aerosols can convert SO₂ to irritant sulfur compounds in the work place, chronic, low-level exposures to these process-generated airborne pollutants are unavoidable. Previously, we have demonstrated that ZnO at concentrations below the recommended TLV (5 mg/m³) in the presence of 1 ppm SO₂ resulted in the formation of an irritant aerosol that caused changes in lung function. At low-level exposures to 1 mg/m³ ZnO and 1 ppm SO₂, lung volumes (total lung capacity and vital capacity) and diffusion capacity (DLCO) were significantly decreased on the 4th day during a 5-day, 3 hr/day exposure regime. In the present study, we simulated the human workweek, where guinea pigs were exposed (1 mg/m³ ZnO, 1 ppm SO₂, 3 hr/day) for 5 days, rest over the weekend and then reexposed. Results indicate that various lung function parameters that were lowered after 5 days of exposure, recovered during the weekend rest. However, the next exposure dropped it back to the same level as before the weekend rest period. The data show that low level exposure to ZnO/So₂ could sensitize the airway to the next insult. The nature of the response is under further investigation. Supported by NIEHS P01-ES04249.

RESPONSE OF GUINEA PIGS UPON EXPOSURE TO SULFUR OXIDES ON THE SURFACE OF FRESHLY GENERATED ULTRAFINE ZNO AEROSOLS

L.C. Chen, H.F. Lam, J. Guty, G. Mohiuddin and M.O. Amund

Ultrafine aerosols and SO₂ are emitted into the atmosphere together during combustion and smelting of ores. Interactions between these components can produce irritant aerosols. Previous studies in guinea pigs had shown that when freshly formed ZnO and SO₂ were mixed at room temperature and high humidity, irritant aerosols were produced that caused reversible changes in pulmonary flow resistance and compliance. Sulfite was detected on the surface of these aerosols. When SO₂ and water were added to the furnace the irritant aerosols produced caused prolonged changes in resistance and compliance. These slow irreversible changes in lung mechanics are consistent with the response of sulfuric acid. Sulfite was the predominant species on the surface of the aerosols. In addition, changes in lung volumes and DLCO were also observed following exposures to these aerosols. In the present study, we assessed quantitatively the relationship between the formation of surface coated sulfur species and changes in pulmonary function upon exposures to these aerosols. Changes in lung mechanics, volumes, and DLCO were measured. Sulfur species on the surface of the ZnO aerosols were analysed by ion chromatography, flame photometric detector system, and ESCA. The results were compared with those observed following exposures to pure sulfuric acid droplets and sodium sulfite aerosols. Supported by NIEHS P01-ES04249 and RO1-ES-1939.
DEGENERATION AND REGENERATION OF THE OLFATORY EPITHELIUM FOLLOWING INHALATION EXPOSURE TO METHYL BROMIDE. W.J. Hutt, P.A. Working, and K.T. Morgan, CIIT, Research Triangle Park, NC.

Inhalation exposure of rats to methyl bromide (MeBr) has been shown to induce severe olfactory epithelial degeneration. The present study was conducted to further characterize this degenerative process and to determine whether the olfactory epithelium is repaired following exposure. Adult, male F-344 rats were exposed to 0 or 200 ppm MeBr 6 hr/day for 5 days (first exposure = day 1). Animals were sacrificed on day 1, 3, 5, 10, 30, and 72. Extensive destruction of the olfactory epithelium was evident in animals killed directly after a single exposure to MeBr. By day 3, in spite of continued exposure, there was replacement of the olfactory epithelium by a squamous cell layer that increased in number and basophilic cytoplasmic staining by 5 days. At 10 days (5d post exposure), the epithelial region was covered by a layer of polygonal, basophilic cells, and at 30 days these cells had extensively organized to reform the original olfactory epithelial pattern. By day 38, 75-80% of the olfactory epithelium was repaired morphologically. These findings demonstrate that the olfactory mucosa is highly sensitive to the toxic effects of MeBr and that olfactory epithelial regeneration occurs rapidly following exposure. Therefore, acute inhalation exposure of rats to MeBr may provide a useful model for investigating olfactory epithelial regeneration.


Platelet activating factor (PAF), a biologically active phospholipid has been implicated as a mediator of pulmonary inflammatory reactions. It is synthesized and released by cells within the lung such as basophils, neutrophils, mast cells, eosinophils and alveolar macrophages. This substance may play an important role during exposure to airborne chemicals which may induce its synthesis and release. Outbred English short-hair male guinea pigs were exposed to an aerosol of PAF by aerosolizing a solution of PAF in distilled water containing 0.01 to 1 percent guinea pigs serum albumin. The exposure duration was 30 minutes at concentrations from 2 to 8 mg/m³. Flow-volume loops were obtained in each animal during air breathing and during challenge with 10% CO₂ in 20% O₂ and 70% N₂. Flow-volume loops were highly abnormal following PAF and the effect was long-lasting. The flow-volume loops indicated that severe airflow limitations were present during exposure and following inhalation of PAF. Supported under NIEHS Grant No. 1 ROI ES07274.

RESPIRATORY ALLERGY; SUBCHRONIC GUINEA PIG INTRATRACHEAL TEST SYSTEM. E.R. Fletcher, N.L. Ritz, and P.J. Danneman. The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH. Sponsor: Michael J. Murray

A respiratory tract (intratracheal, IT) test system was developed for evaluating bacterial enzyme proteins for their relative allergenic potency. Results obtained in the IT test system are used to assess the occupational safety of new enzymes and ingredients in enzyme-containing laundry products. In developing the model, animal species, sex, and age were evaluated. Guinea pigs were determined to be more useful than rats. Female guinea pigs responded more consistently with allergic antibodies (IgE) than males. Four-week old guinea pigs produced greater allergic and precipitating antibody responses, and were more susceptible to anaphylactic-type responses than 14 week old guinea pigs. Weekly IT exposures were sufficient to produce allergic and precipitating antibody responses and allergic respiratory tract responses within 10 weeks. Tomato juice detergent ingredients, when dosed simultaneously with allergen, resulted in enhanced allergen-specific antibody responses and concomitant allergic respiratory responses.


Platelet activating factor (PAF), a biologically active phospholipid has been implicated as a mediator of pulmonary inflammatory reactions. It is synthesized and released by cells within the lung such as basophils, neutrophils, mast cells, eosinophils and alveolar macrophages. This substance may play an important role during exposure to airborne chemicals which may induce its synthesis and release. Outbred English short-hair male guinea pigs were exposed to an aerosol of PAF by aerosolizing a solution of PAF in distilled water containing 0.01 to 1 percent guinea pigs serum albumin. The exposure duration was 30 minutes at concentrations from 2 to 8 mg/m³. Flow-volume loops were obtained in each animal during air breathing and during challenge with 10% CO₂ in 20% O₂ and 70% N₂. Flow-volume loops were highly abnormal following PAF and the effect was long-lasting. The flow-volume loops indicated that severe airflow limitations were present during exposure and following inhalation of PAF. Supported under NIEHS Grant No. 1 ROI ES07274.

IRRITATION RESPONSE TO BISPHENOL-A (BPA) AEROSOL IN MICE AND RATS. W.K. Steinbargen, W.W. Harrington, and K.L. Kong, CIIT, Research Triangle Park, NC

BPA (α,α'- Isopropylidenediphenol) is used in the manufacture of polycarbonate and epoxy resins. The purpose of this study was to evaluate the irritant potential of BPA. Male B6C3F1 mice and P-384 rats were head-only exposed for 15 minutes to BPA aerosol concentrations ranging from 30 to 820 mg/m³, determined gravimetrically. Particle size (MMAD) was 0.72 to 1.13 μm. Respiratory rate and waveforms were recorded. Mice exposed to 60mg/m³ or above showed a brief period of sensory irritation, as characterized by a pause in expiration. This was usually followed by rapid breathing. At 152 mg/m³ or above, rapid breathing was replaced by combined sensory and pulmonary irritation (pause in inspiration) with intermittent apneic rate. The rate responded similarly except for the major component of the irritation was pulmonary. The combined irritation led to a concentration-dependent decrease in respiratory rate with the slope being significantly different from zero for both species. Calculated RD₉₀ values were 689 and 959 mg/m³ for mice and rats, respectively. The response of mice and rats to BPA differed from classical sensory irritants. The predominance of pulmonary irritation probably resulted from the insolubility of BPA in water, i.e. mucous fluid, and a particle size favoring lung over nasal deposition.
ONE-MONTH INHALATION STUDY WITH CUMENE.
C.L. Bechtel, R.D. Short, W.E. Ribelin, and M.V. Roloff. Monsanto Company, St. Louis, MO.

To determine the potential inhalation toxicity of cumene, male and female Sprague-Dawley rats were exposed to analytically-determined mean concentrations of 0, 105.1, 300.1 and 599.3 ppm cumene in air, six hours per day, five days per week over an approximate four-week period (minimum of 20 exposure days). No animals died during the study. Indications of a slight irritant response to the nose, eyes, and mouth were seen as a result of exposure. No changes were seen in mean weight of treated animals compared to controls. Clinical, gross, and microscopic pathology findings showed no remarkable differences between treated and control animals. Relative to terminal body weight, increases in the weights of the liver and/or kidneys occurred in all test groups and the absolute weights of these organs followed a similar pattern. The significance of these changes, however, is unknown, especially considering the lack of pathology findings.

ACUTE INHALATION STUDIES WITH HEATED 10,10'-OXYBISCHLOROPHENOXARSINE. W.E. Balbey, T. Roy, E.J. Singer, T. Oseltz, and C. Hardy. Mobil Oil Corporation and Huntington Research Centre.

10,10'-oxybischlorophenoxarsine (OBPA) is a biocide incorporated into many plastics and other products. However, no information was available on the toxicity of vapors which might arise from OBPA heated above 200°C as part of industrial processes. The composition of an aerosol arising from OBPA heated to 275°C was determined and animals were exposed to this airborne aerosol. During initial acute exposures of rats, no toxicity was observed except for signs of moderate irritation during exposure to the highest level, 13 μg/L. Because more severe effects were expected on the basis of published data, additional acute exposures were performed with 3 other species of animals in addition to the rat. Overt toxicity was observed in all species exposed to 85 μg/L for 6 hours, with sensitivity in the following order: mouse > rat > guinea pig > rabbit. Lung was the primary target organ. Lastly, in a sensory irritation assay with mice, the LOAEL of the aerosol was 7 μg/L. From these data, the threshold of irritation in man was estimated at 0.07 μg/L, markedly below levels which resulted in overt toxicity with acute exposure.


Three groups of eight male guinea pigs were repeatedly exposed to three paraquat aerosol concentrations. The paraquat concentrations achieved were 0.1, 0.4, and 0.8 mg/m³. Over eighty percent of the particles were found to be under 0.65 μm for all three paraquat aerosol concentrations. Each group of animals were exposed for six hours a day, five days a week for three weeks. Respiratory parameters of each animal were measured prior to exposure and every day for the duration of the experiment. The respiratory parameters were measured during air and 10% CO₂ using a whole body plethysmograph fitted with a head chamber. Respiratory rate increases and tidal volume decreases during air and CO₂ were detected during the three weeks for the 0.4 and 0.8 mg/m³ paraquat aerosol concentration groups. This rapid shallow breathing pattern reached a maximum for these two concentration groups on days three and four. Following this maximum, the differences seen between the respiratory parameters of the control groups and the 0.4 and 0.8 mg/m³ groups decreased although some of the parameters never returned to control values. Repeated paraquat exposures at 0.4 mg/m³ are therefore too high to be considered safe. Supported by NIHES I R01-ES02747.


Groups of Crl:CDER rats (12M, 12F) were exposed for 6 hr/day for 10 days to Mancozeb dust at 0, 23, 138, or 519 mg/m³. Each group was equally subdivided into whole-body (W-B) and nose-only (N-O) exposure subgroups and simultaneously exposed in the same chamber. The aerosol had a MSD of 4.1 μm, a GSD of 2.4, and a reposable fraction of 44%. W-B exposure produced a greater toxic response, both in degree and incidence, than N-O exposure for all parameters evaluated. W-B exposure to 138 mg/m³ produced decreased body weight gain and thyroid function (T3, T4), while N-O exposure at the same concentration produced no effect. At 519 mg/m³, W-B exposure produced decreased body weight gain and thyroid function, increased lung weights, and inflammatory changes in the lungs, nasal turbinates, and perilobular lymph nodes. N-O exposure at 519 mg/m³ produced similar, except no changes in the nasal turbinates, but less severe effects. The differences in the responses between the W-B and N-O exposed animals were attributed to oral ingestion and continued inhalation exposure by the W-B exposed animals during post-exposure preening. In terms of modeling potential human exposure, N-O exposure served as a better model because the signal received by the W-B animals are irrelevant to human exposure. The NOEL for the W-B and N-O exposure was 23 and 138 mg/m³, respectively.
INHALATION TOXICITY AND FERTILITY STUDIES IN RATS WITH ACETONE CYANOHYDRIN AND ADIPONITRILE.
W.F. Heydens, L.D. Kier, M.V. Roloff, D.C.
Monsanto Company, St. Louis, MO

Toxicity and fertility studies were conducted in rats exposed to Acetone Cyanohydrin (ACY) or Adiponitrile (ADN). Mean analytical exposure concentrations were maintained near the following target levels: 10, 30, and 60 ppm (ACY); and 3, 7, and 23 ppm (ADN). For toxicity studies, animals were exposed 6 hrs/day, 5 days/wk for 13-16 weeks. After exposure to ACY, there were no apparent gross or microscopic lesions, or changes in body weights, organ weights, or hematological values. A dose-related decrease in serum glucose levels was observed, but this was not considered biologically significant. Dose-related increases in urine thiocyanate were noted, reflecting absorption and metabolism of ACY to cyanide. After ADN exposures, high level females had decreased hematocrit, hemoglobin, and red blood cell counts. No other changes or lesions were detected. Dose-related increases in urine thiocyanate were also noted in this study, but the values were much higher than following ACY exposure. In fertility studies, males and females were exposed (10 and 3 wks, respectively), and mated with untreated rats. Females were examined at mid-gestation. Mating efficiency, pregnancy rates, and nidation data were evaluated. No treatment-related effects on fertility were detected.

Departments of Pathology and Biosciences, University of Illinois, Urbana, IL

T-2 toxin has been implicated in chemical warfare as a component of "Yellow Rain." We have shown that T-2 toxin by intravenous (iv) or inhalation (inl) routes causes similar lesions in heart, pancreas, lymphoid tissue and gastrointestinal tract. Here we compare the effect of iv with inl exposure to T-2 toxin on pulmonary ultrastructure. Eight to 12 wk male castrated SPF-derived pigs were given iv T-2 toxin (0.2.4 mg/kg) or nebulized T-2 toxin (0 or 10 mg/kg; 20-30% retention). Pigs were killed 1/2 to 8 hrs later and lung was prepared for electron microscopy. With iv dosing diffuse changes were seen at 8 hrs. Capillaries were dilated and contained many neutrophils. Iv macrophages filled much of the lumen and contained phagocytosed cellular debris. With inl widely scattered focal changes were present. Capillaries also contained many neutrophils, and iv macrophages were prominent. Macrophages and neutrophils infiltrated the interstitium and alveolar spaces. Pulmonary changes following iv dosing are secondary to T-2 induced extrapulmonary cellular necrosis while changes following inl are presumably a direct effect of T-2 toxin on the lung.

PRODUCTION OF HYPERTERMIA AND RESPIRATORY RESPONSE TO INHALATION OF ENDOTOXIN IN GUINEA PIGS. P.S. Thorne, C.P. Yeske, and M.H. Karol.
Dept. Industrial Environmental Health Sciences, Univ. of Pittsburgh, Pittsburgh, PA

Production of endotoxin fever was observed in guinea pigs exposed to aerosols of 10-50 μg/m3 Escherichia coli endotoxin for 6 hr. Pulmonary function measurements included continuous monitoring of respiratory rate, tidal volume and breathing pattern for 30 hr beginning 4 hr before exposure and flow-volume loops at 0, 6, and 24 hr time points. These measurements utilized plethysmography techniques and microcomputer data acquisition. Intrapleural temperature was monitored continuously by radiotelemetry using Mini-Mitter temperature-controlled oscillators, AM receivers, and an IBM AT operating software of our own design. Baseline temperature monitoring of animals over many days indicated a normal variation of ± 0.5°C about the mean. Endotoxin inhalation induced an increase in core temperature that reached a peak 1.6°C change at 4.8 hr. Temperature returned to normal 1.5 hr post-exposure. Respiratory rate followed the same pattern, peaking at 4.2 hr with a mean increase of 52%. Tidal volume remained unchanged during the exposure. Lung lavage 24 hr post-exposure revealed a neutrophilia (65% PMN vs. 1% in controls) and a 4-fold increase in WBC. The role of mediator release in the etiology of this hyperthermia and respiratory response will be discussed. Supported by NIHES ES01932.

Lovalence Inhalation Toxicology Research Institute, Albuquerque, NM, and NIHS/ NIEHS, Research Triangle Park, NC

Occupational exposure to nickel subsulfide (NiS₂) may occur during roasting of nickel. This study evaluated the subchronic inhalation toxicity of NiS₂. F344/N rats and B6C3F1 mice were exposed to aerosols of NiS₂ 6 hr/day, 5 days/week for 13 weeks. Concentrations were 2.5, 1.2, 0.6, 0.3, 0.15 and 0 mg/m³. The aerosol had a mass median aerodynamic diameter of 2.4 µm. Exposure related mortality was not observed. Biochemical and cytological endpoints evaluated in bronchoalveolar lavage fluid indicated an inflammatory response in the lungs of both species exposed to either 2.5 or 0.6 mg/m³. Dose related histopathologic lesions were present in the nose, lung, lung associated lymph nodes, forestomach and thymus of both species. The major findings were inflammation of the lung andatrophy of the olfactory epithelium. The lowest exposure concentrations with lung lesions were 0.6 mg/m³ for mice and 0.15 mg/m³ for rats. Results indicate that inhalation exposure of rats and mice at concentrations near the current TLV for nickel produces significant lesions in the respiratory tract. (Research conducted under IAA Y01-ES-30108 between U.S. DOH Contract No. DE-AC04-76EV01013 and NIHES/ NTP).
Studies have been conducted to determine the toxicity of nickel oxide (N1O), nickel sulfate (NiSO4), and nickel sulfide (Ni3S2) after inhalation exposure of F344/N rats and B6C3F1 mice for two weeks. Exposure concentrations used (as mg/m³ of nickel) were 0.9 = 23.6 for NiSO4, 0.8 = 13.3 for NiSO4 and 0.4 = 7.3 for Ni3S2. NiSO4 was the most toxic compound with exposure-related mortality seen at levels of 6.7 mg/m³ and above in rats and 1.6 mg/m³ and above in mice. For Ni3S2, mortality was seen in mice (but not in rats) at the highest exposure level (7.3 mg/m³). No mortality was seen after N1O exposure. Extensive lesions of the lung and nasal cavity were seen in both rats and mice after exposure to NiSO4 and Ni3S2 at the highest four exposure levels. Lesions of the lung and nasal cavity were only seen at the highest exposure level after N1O exposure. The amount of nickel in the lungs at the end of exposure varied in relation to the water solubility of the compounds. Based on these two-week studies, the toxicity ranking was NiSO4 > Ni3S2 >> N1O. Further work is in progress to assess the relative toxicities of these three nickel compounds after 90-day exposures.

BIOCHEMICAL AND HISTOLOGICAL PULMONARY EFFECTS OF GALLIUM ARSENIDE COMPARED TO SILICA AFTER A SINGLE INTRATRACHEAL DOSE. B. J. Snider, J. Zhang and D. E. Carter, Dept. of Pharmacology and Toxicology, Univ. of Arizona, Tucson, AZ

The pulmonary changes induced in male Sprague-Dawley rats by single intratracheal doses of 30 and 100 mg/kg gallium arsenide (GaAs) were compared with those from 200 mg/kg silica, with a normal saline control. GaAs particles were prepared by sieving crushed crystals through a 10 μm sieve to yield a particle fraction that was about 3 μm as determined from EM. Compounds were suspended in saline immediately before dosing. At 1, 2 and 4 weeks, and 3 and 6 months, tissues were collected and analyzed for total arsenic in blood, urine, feces, lung, liver and kidneys; lung protein, DNA and 4-hydroxyproline; lung wet weight vs. dry weight; and lung lipids. Lungs were also examined for histological analyses using H&E, Masson's trichrome, reticulum stains, TEM and IF at 1 and 4 weeks and 6 months. Increases over control in silica and GaAs treatment groups were observed for lung wet weight, lipids and macromolecules. The silica group showed the greatest responses through 6 months followed closely by the 100 mg/kg GaAs at early time points. The 30 mg/kg GaAs values were statistically higher than control at the early times but less than the 100 mg/kg dose. The biochemical and histological data indicated that collagen deposition had occurred in the rats treated with GaAs at the early time points, but that lung changes were returning to normal by 3 months. (Supported by NIOSH Grant 82076.)
LUNG RETENTION AND METABOLIC ACTIVATION OF $^{14}$C-BENZO(A)PYRENE ADSORBED ON CARBON BLACK PARTICLES J.D. Sun, J.A. Bond, S.M. Hao, M.E. Carlini, and R.K. Wolff. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Increased retention of inhaled benzo(a)pyrene (BaP) in lungs when adsorbed on insoluble particles was previously shown. The potential biological consequences of this observation were investigated. Male F344 rats were intratracheally instilled with $^{14}$C-BaP adsorbed onto carbon black particles (CBP) (0.2%, 2.0% or 20% BaP by mass) or pure $^{14}$C-BaP. Retention of $^{14}$C in lungs was biphasic. The fraction of $^{14}$C in the long-term component of clearance increased as the percent adsorbed decreased, but did not change with differing amounts of CBP having the same BaP coating. Early (<1 h) metabolic events were assessed by similar instillations into an isolated perfused-ventilated rat lung (IPVL) system. HPLC analysis of perfusate showed significant amounts of BaP metabolites. $^{14}$C covalently bound to lung macromolecules in the IPVL was greater for pure BaP than BaP on CBP at 1h, but was greater for BaP on CBP at 1 to 30 days after in vivo instillations. These data suggest that adsorption of BaP to CBP may initially reduce its bioavailability for metabolism, but longer retention and slow leaching from particles eventually allows a larger fraction of the BaP to be metabolically activated and bind to target macromolecules. (Research funded under U.S. DOE Contract No. DE-AC04-76EV01013.)

PULMONARY AND PATHOLOGICAL RESPONSES OF RATS TO ACUTE INHALATION OF SYNTHETIC GRAPHITE. S.A. Thomson, D.C. Burnett, R.S. Anderson*, R.J. Hilaski. Chemical Research, Development and Engineering Center, APS, MD and *Chesapeake Biol. Lab., Univ. MD, Solomons, MD.

Synthetic graphite is a crystalline form of carbon made from high temperature treatment of coal or petroleum products, contains less than 1% quartz, and is regarded as an inert dust. Occupational exposure can occur during manufacturing and application processes. Inhalation hazards of synthetic graphite (Asbury Micro 260) were evaluated in Fischer 344 rats exposed to 4 acute concentrations (1, 10, 100, 500 mg/m³) for 4 hrs. Atelvaco macrophage (AM), bronchoalveolar lavage (BAL) physiological and pathological indices of damage were assessed in graphite and air exposed rats at 24 hrs, 14 da, 3 mos post-exposure (PE). Results indicate graphite is nontoxic to AM and is readily phagocytized. At 24 hrs PE, physiological changes and an influx of neutrophils in BAL fluid occurred at 500 mg/m³ but by 14 da PE these changes were reversed. No significant BAL or histopathological changes occurred at any of the lower exposure concentrations. Based on comparable studies with quartz and metal dusts, synthetic graphite is inert and fits the criteria of a nuisance dust.

RETENTION AND BINDING OF INHALED $^{14}$C-BENZO(A)-PYRENE ADSORBED ON CARBON BLACK PARTICLES R.K. Wolff, J.D. Sun, E.B. Barr, and S.J. Rothenberg. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

F344/N rats were exposed nose-only for 2 hrs to aerosols of benzo(a)pyrene (BaP) adsorbed on carbon black (CB) and pure BaP aerosols to determine if association with carbonaceous particles altered biological fate of inhaled polycyclic aromatic hydrocarbons. CB particles were coated with 0.2% and 2% $^{14}$C-BaP by mass and generated at total mass concentrations of 100 mg/m³. Lung retention of $^{14}$C was biphasic. The fraction of $^{14}$C in the long-term retention component was inversely proportional to the percent adsorbed on the particles (0.12, 0.012, and 0.001 for the 0.2% and 2% coatings, and pure BaP, respectively). $^{14}$C remaining bound to carbon black particles decreased with time. $^{14}$C covalently bound to lung macromolecules was much greater for BaP-CB than for pure BaP after 1 day. Covalently bound $^{14}$C levels increased progressively with time after exposure to BaP-CB. At 30 days, they represented 67% of the total $^{14}$C present and fractions of 0.039 and 0.002 of $^{14}$C initially deposited as 0.2% and 2% $^{14}$C-BaP-CB, respectively. These data suggest that slow leaching of BaP from carbon black particles increases the levels of reactive metabolites bound to potential target sites compared to pure BaP. (Research supported by U.S. DOE Contract No. DE-AC04-76EV01013.)


Silica increases the levels of surfactant phospholipids in the lungs of rats. These increases could result from either alterations in the numbers of alveolar Type II cells and/or in the biosynthetic capacity of those cells. We tested these hypotheses by examining the response of the Type II cell population in the lungs to intratracheally instilled silica (10mg/rat) and the ability of lung slices to incorporate 14C-choline and 3H-palmitate into surfactant phospholipids. At 7, 14, and 28 days following silica treatment, Type II cells had increased 1.3, 1.7, and 2.3-fold, respectively, compared to controls. Type II cell hypertrophy was also observed, with cell volumes increasing as much as 2-fold. Incorporation of 14C-choline and 3H-palmitate into surfactant phospholipids increased 2.7 and 2.3-fold, respectively, at these same time points. These data indicate that surfactant levels in the lungs of silica-treated rats are elevated through mechanisms involving both increases in the Type II cell populations and in their surfactant phospholipid biosynthetic capacity.

*Supported in part by ES 07126.
A murine model to assess the inflammatory and fibrotic potential of synthetic industrial fibers. LR Pustelnik and AK Hubbard. Sponsor: AJ Gandolfi, Dept. of Pharmacology/Toxicology, University of AZ, Tucson, AZ.

Several parameters of lung injury were measured in this murine model to ascertain the inflammatory potential of any of a number of industrial fibers. For example, the pulmonary inflammatory potential of man-made ceramic fibers of a specific size was compared to a known inflammatory particulate, silica. Male C57BL/6 mice (20 g) were intratracheally instilled with particulates (0.5, 1.5 or 4 mg) or saline. After 1 week the lungs were lavaged and the fluid analyzed for total/differential cells, protein and inflammatory mediators [angiotensin converting enzyme (ACE) and plasminogen activator (PA)]. The inflammatory cells (4.1 x 10^6/ml) in the lavage of the saline injected animals were 95-99% macrophages (PAM). Silica caused a dose-dependent increase in total cell number (13.6 x 10^6/ml) which could be attributed to neutrophils and PAM. Silica also caused a dose-dependent increase in lavage protein (915 ug/ml), ACE levels (17 units/mg), and PA levels (14%). Man-made ceramic fibers (JM; 2-3 um diam) did not elevate pulmonary cell number or protein levels whereas ACE rose to 7 units/ml (0.5 mg) and PA to 12.5% (1.5 mg). Ceramic fibers of this specific size appeared to have minimal inflammatory stimulus. (NIH HL 35744 and AZ Lung Association)

Chronology of a pulmonary inflammatory response induced by exposure to glass fibers in a murine model of pneumoconiosis. MS Stefaniak and AK Hubbard. Sponsor: AJ Gandolfi, Dept Pharm-Tox, University of AZ, Tucson, AZ.

Glass insulation fibers (GF; JM-100) have been shown to induce only a mild inflammatory response in mice 7 days post intratracheal instillation. To determine if a more intense response occurred earlier, the inflammatory response to GF was examined at day 3 and 5 in addition to day 7. Male C57BL/6 mice (20 g) were intratracheally instilled with GF, silica particles (0.5 mg) or saline. On days 3, 5, or 7, the lungs were lavaged and the fluid analyzed for total/differential cells, protein and inflammatory mediators [angiotensin converting enzyme (ACE) and plasminogen activator (PA)]. The cells in the lavage of the control animals (2.8-5.2 x 10^6/ml) were 95-99% macrophages (PAM). GF evoked a peak inflammatory response on day 5 in total cell number (15.1 x 10^6/ml) attributed primarily to PAM and in part to neutrophils (PMN). Peak levels of lavage protein (387-342 ug/ml) and ACE (15-16 units/ml) occurred on days 3 and 5; no significant levels of PA were detected. Instillation of GF appears to induce an early inflammatory response which may activate PAM and chemotactic factors for PMN and PAM. (NIH HL 35754 and AZ Lung Association)


A number of man-made fibers are now available and are promoted as replacements for asbestos. The purpose of this study was to evaluate the in vitro cytotoxicity of two aramid fibers, an aramid fiber (Kevlar®) by DuPont and a calcium sodium metaphosphate fiber (CSMP) by Monsanto on primary rat alveolar macrophages (AMs) and a mouse macrophage-like cell line (J774A.1). Following 24- and 48-hour incubations, the cells were observed under phase contrast microscopy for changes in cell morphology and cell viability was assessed using trypan blue exclusion. From these data, a viability index was calculated to reflect changes in both cell number and cell viability. Both fibers produced a dose-dependent reduction in viability index. In addition, lactate dehydrogenase (LDH) activity was measured as an indicator of cell membrane integrity. Both fibers produced a dose-dependent release of LDH in the primary AMs. Finally, the effect of these materials on AM chemotaxis was assessed using a modification of the Boyden method. No chemotaxis was observed in response to either fiber alone. However, both fibers stimulated measurable dose-dependent chemotaxis when incubated with AMs. These results suggest that both Kevlar and CSMP are cytotoxic to AMs in vitro in the form and at the concentrations (2.5, 25, 250 ug/ml) used in this study.

Deposition & clearance of inhaled kevlar® aramid fibers in rats. D.P. Kelly, K.F. Lee and G.L. Kennedy, Jr. E.I. du Pont de Nemours and Co., Haskel Laboratory for Toxicology and Industrial Medicine, Newark, DE

The deposition and clearance of lung-deposited Kevlar® aramid fibers have been investigated as part of a subchronic and chronic inhalation toxicity testing program. Lung-recovered Kevlar® fibers were microscopically counted and measured in Sprague-Dawley rats exposed to airborne fibers which were ~12 um median length (ML) and <0.5 um diameter. In each of 3 studies lung-recovered fibers were progressively shorter with time. Twenty-eight days after a 6-hour exposure at 400 f/cc lengths of recovered fibers were decreased to ~3 um ML. Eighteen months after a 3-week exposure to 25 or 100 f/cc fibers reached ~3 um ML. After 2 years of continuous exposure at 2.5, 25, or 100 f/cc or 1 year exposure-1 year recovery at 400 f/cc, fiber lengths approached 4 um ML. In the 2-year study, the lung-fiber accumulation rate/exposure concentration was similar for the 3 highest concentrations and was about 3K's greater than that seen at 2.5 f/cc indicating that concentrations ~>25 f/cc may overwhelm clearance mechanisms. The recovery from the lung of shorter fibers (<5 um) with time suggests that Kevlar® fibers may be less biologically active than other more durable fibers such as asbestos.
Elucidation of pathophysiologic processes underlying work performance degradations in rats following the inhalation of toxic substances requires the surgical placement of arterial and venous catheters. Usually, exercise performance studies are initiated within 24 hrs after such catheterizations. We have found, however, that as of this time the animals present signs of altered associated energy metabolism and extensive tissue necrosis, e.g., hyperglycemia, increased blood lactic acid (LA), lactate dehydrogenase (LDH), and serum glutamic-oxaloacetic transaminase (SGOT). These changes are accompanied by significant electrolyte imbalances. In order to overcome these disturbances, we have developed a postsurgical hyperalimentation strategy and catheter infusion system that (1) provides nutritional support to rats, (2) maintains the patency of indwelling catheters in order to extend recovery times, and (3) minimizes animal stress during recovery. With these approaches, post-catheterization blood glucose, LA, and LDH return to control levels within 72 hrs and SGOT is substantially reduced by this time. This work was supported by the DoD and conducted under the auspices of the DOE.

The toxicity of single and multiple fire gases is being studied to determine whether the toxic effects of the combustion products from materials can be explained by the biological interactions of the primary fire gases or if minor, more obscure gases need to be considered. LC50 values for Fischer 344 rats have been determined for carbon monoxide (CO) and hydrogen cyanide (HCN) (as individual gases) for 1, 2, 5, 10, 20, 30, and 60 minute exposures plus relevant post-exposure periods using the NBS Toxicity Test Method apparatus. The HCN LC50 (based on within plus 24 hour post-exposure deaths) concentration-time products were constant; however, the LC50 concentration-time products for CO decreased for exposures up to 5 minutes and increased for the exposures of greater duration. Combined CO and HCN experiments indicated that these toxicants act in an additive manner for all the time periods tested (5-60 minutes). Synergistic effects were found when the animals were exposed to certain combinations of CO and carbon dioxide (CO2) in tests ranging from 5-60 minutes. A mathematical model of the combined toxicological effects of CO, CO2, and HCN predicted the observed toxicity of the combustion products in 17 out of 24 cases of material decomposition.

A flexible polyurethane foam with FR and without a fire retardant (NFR) and a cotton fabric, common components of upholstered furniture, were thermally decomposed in large-scale (LS) room burns and small-scale (SS) toxicity tests. Sets of Fischer 344 rats were exposed sequentially to either smoldering or flaming smoke from the burn room or a room 40 feet away. SS testing of the individual components was conducted by the NBS toxicity test method under both flaming and non-flaming conditions. The LS smoldering and SS non-flaming tests produced essentially no within exposure deaths. Post-exposure deaths occurred following both the SS non-flaming foam or cotton tests, but not the LS tests. SS and LS flaming FR and LS flaming NFR products produced deaths within and post-exposure; whereas, SS flaming FR foam or cotton produced no deaths. The combined amounts of CO, CO2, and HCN were sufficient to account for the BS flaming deaths. In both the LS and SS tests, the production of HCN increased greatly when the foams were heated to high temperatures or burned following prior exposure to non-flaming conditions. Strictly flaming or non-flaming chair or foam tests produced little HCN. Little toxicological difference was noted between smoke from the fire room and 40 feet away.

The toxicity of single and multiple fire gases is being studied to determine whether the toxic effects of the combustion products from materials can be explained by the biological interactions of the primary fire gases or if minor, more obscure gases need to be considered. LC50 values for Fischer 344 rats have been determined for carbon monoxide (CO) and hydrogen cyanide (HCN) (as individual gases) for 1, 2, 5, 10, 20, 30, and 60 minute exposures plus relevant post-exposure periods using the NBS Toxicity Test Method apparatus. The HCN LC50 (based on within plus 24 hour post-exposure deaths) concentration-time products were constant; however, the LC50 concentration-time products for CO decreased for exposures up to 5 minutes and increased for the exposures of greater duration. Combined CO and HCN experiments indicated that these toxicants act in an additive manner for all the time periods tested (5-60 minutes). Synergistic effects were found when the animals were exposed to certain combinations of CO and carbon dioxide (CO2) in tests ranging from 5-60 minutes. A mathematical model of the combined toxicological effects of CO, CO2, and HCN predicted the observed toxicity of the combustion products in 17 out of 24 cases of material decomposition.

It was recently reported that trimethylolpropane-phosphoester (TMP-P), a neurotoxic bicyclic ester, is formed during high temperature pyrolysis of synthetic lubricants (Military Specification L-23699). To further evaluate this finding, GC/MS analysis and biological assay (by i.p. injection of rats and mice) were performed with pyrolised lubricants to detect BCPE formation. Formation of TMP-P from these oils was confirmed following sealed-tube pyrolysis at 500°-600°C for 30 mins. Six other toxic BCPE homologues, were not identified by GC/MS in the pyrolysed lubricants. Production of the neurotoxicity was limited to lubricants with a trimethylolpropane-ester base stock. Oils containing a pentaerythritol-ester base did not cause neurotoxicity in mice or rats following pyrolysis. L-23698 lubricants, which have been employed in aircraft engines under rigorous test conditions, produced no neurotoxicity when administered to rats. Thus, operational use of L-23699 lubricants would not produce acute neurotoxicity if exposure to these lubricants occurred. Evaluation of the relative inhalation hazard of neurotoxicants produced during open-to-air pyrolysis is pending.
The effects of three exposure levels of cigarette smoke (6, 9 & 12 exposures/day of 10% cigarette smoke, 5 days/week for 13 weeks) on the respiratory tract of female F344 rats were examined. Smoke exposures were alternated with exposure to air alone to limit smoke-related deaths. Body weights increased in sham-treated, 6, 9 & 12-exposure smoke-treated groups were 18, 17, 14 and 11% of starting weights, respectively, after 13 weeks of exposure. Exposure-related elevations in total free lung cells, total macrophages, total polymorphonuclear leukocytes (PMNs) and % PMNs in lavage cells were observed. Of the enzymes examined in lavage fluids, gammaglutamyltranspeptidase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase exhibited exposure-related increases. Acid phosphatase activity was increased in all smoke-treated groups, but not related to the number of exposures/day. The incidence and/or severity of squamous metaplasia and mucosal atrophy of the nasal turbinates and dorsal meatus, squamous metaplasia of the larynx, and hypertrophic or hyperplastic alterations in tracheal epithelium changed with the number of exposures. Thus, the extent of the overall pulmonary response appears to be related to total daily exposure of smoke.

The purpose was to understand the mechanism of thiol utilization in cigarette smoke exposed lungs. Lungs from rats and rabbits were initially perfused for 10 min. At every 30 sec one puff of freshly drawn cigarette smoke (6 ml for rat and 45 ml for rabbit, each time) was introduced in the trachea. Lungs were inflated for 10 sec with smoke followed by 20 sec of smokeless period, before introducing the next puff. GSH significantly decreased in both rabbit and rat lungs, without increase in GSSG. Protein-SH decreased significantly in rabbit lungs with a slight increase in protein-GSH mixed disulfides. Smoke inhibited GSH peroxidase activity. GSH S-transferase and GSSG reductase were decreased but not significantly in smoke challenged lungs. N-Acetylcysteine but not GSH protected rabbit lung from smoke-induced GSH depletion. Smoke condensate (100 mg/100 ml medium) also caused GSH depletion in rabbit lung, and GSH added to the medium protected the lung. These results indicate that the decreased GSH could be due to conjugation of substrates present in the smoke. These acute smoking effects could chronically lead to inefficient thiol-defensive mechanisms and other cumulative adverse effects. (Supported by Miss Lung Assoc. and HL-20622.)

In a previous study (Toxicologist, 6, 52, 1986), a 15-minute exposure to HCl (500, 5000, 10,000 ppm nominal concentrations) produced a concentration-dependent increase in respiratory rate and minute volume, and severe hypoxemia in 5000 and 10,000 ppm exposed baboons. This study was undertaken to compare the effects of PVC smoke containing HCl with those of pure HCl. Two groups of three baboons were exposed for 15 minutes in a head box to smoke generated by flaming or non-flaming combustion of PVC pellets. The PVC was combusted at 3 watts/cm² (nonflaming) and 6 watts/cm² (flaming) to achieve a nominal concentration of 5000 ppm HCl. Respiratory parameters (f, Vt, MV) were measured by inductive plethysmography. Pulmonary function tests (PFTs) and CO₂ challenge response tests were conducted at 3 days before and after exposure. Respiratory response to CO₂ smoke was similar under nonflaming and flaming combustion and was characterized by a breathing pattern of high f and low Vt (rapid, shallow breathing). In contrast to the effects of pure HCl, only two of the six animals exposed to PVC smoke developed hypoxemia. As with pure HCl, PFTs and CO₂ challenge responses at three days were not significantly different from preexposure values. (Sponsored by The Vinyl Institute.)

2, Ethylhexyl nitrate (EBN) is an additive used to increase the cetane number of diesel fuel. Groups of 10 male Crl:CD(SD) rats were exposed nose-only to EBN at vapor concentrations of 0, 14, 42 and 150 ppm 6 hrs/day, 5 days/week, for 2 weeks. After the 10th exposure, blood and urine specimens were collected from 5 rats per group for chemical analyses and were then killed for histopathological examination. Remaining rats were subjected to the same analyses after a 2 week recovery period. After the 10th exposure, rats from the 150 ppm group had increased hemoglobin and hematocrit values and increased erythrocyte and platelet counts. Exposure related histopathologic changes included eosinophilic cytoplasmic inclusions in cells of the renal proximal tubules and lipid-like cytoplasmic hepatoacellular vacuolation at 14, 42 and 150 ppm. A second subchronic inhalation study was conducted at exposure concentrations of 0, 4.3, 42 and 420 ppm to investigate the hepatic and renal injury. Histopathologic changes included hepatoacellular vacuolation in fasted, unexposed controls and at 4.3, 42 and 420 ppm; cytoplasmic inclusions in cells of the proximal tubules of hepatoacellular kidneys were seen only at 420 ppm. Since hepatoacellular vacuolation was not observed in a fasted control group, the hepatic effects were not considered to be exposure related. Under the conditions of this study, a NOEL was 42 ppm EBN.
Base stocks refined from crude oil serve as major components in a variety of lubricants. The possibility of occupational exposure to aerosols containing these base stocks signaled the need for evaluation of their inhalation toxicity. As part of a broad program to assess the toxicity of lubricant base stocks, a solvent-refined oil (SR0) and acid-refined white oil (WTO) were tested. Sprague-Dawley rats were exposed 6 hr/day, 5 days/wk for 4 weeks to respirable aerosols of SR0 or WTO at 0, 50, 220, or 1000 mg/cubic meter. Neither oil elicited signs of systemic toxicity or changes in body weight gain. At sacrifice on the day after the last exposure, no treatment-related changes were seen in clinical chemistry or hematologic parameters. Major organs were weighed and examined microscopically. Statistically significant increases were noted both in wet and dry lung weights in animals exposed to 1000 mg/cubic meter of either oil. Histologically, increased numbers of macrophages were noted in alveoli, particularly near alveolar ducts. Many of these macrophages were foamy in appearance due to the presence of numerous vacuoles. Under electron microscopy, the vacuoles appeared to be membrane-bound.

DIMETHYSULFOXIDE (DMSO) DOES NOT PROTECT AGAINST PULMONARY FIBROSIS. W.M. Haschek, K.E. Baer, and J.E. Rutherford. Department of Veterinary Pathobiology, University of Illinois, Urbana, IL

DMSO, a putative anti-inflammatory agent and free radical scavenger, was shown to protect against acute bleomycin-induced pulmonary fibrosis in the rat (Popin and Langner, Biochen. Pharmacol. 34:2386, 1985). We examined the effect of ip DMSO on pulmonary toxicity induced by 1) it bleomycin (BL) in mice (10-12 wk female Swiss inbred) and rats (200-300 gm male virus-free SD), or 2) ip butylated hydroxytoluene (BHT) in mice (10-12 wk male Swiss inbred). Bleomycin-induced mortality in mice (20% at 0.1 IU BL) and rats (50% at 1 IU BL) was increased to 100% by daily DMSO (5g/kg in 50% saline). Similar DMSO treatment after nonlethal doses of bleomycin (1 IU BL in rats and 0.075 or 0.05U in mice) increased lung hydroxyproline content (ug/lung + SEM) in the rat (BL + saline: 262±24 and BL + DMSO: 282±57) but had no effect in the mouse. Lung hydroxyproline content in mice 14 days after 400mg/kg BHT in corn oil (35±16) was also slightly increased by daily DMSO at 5g/kg (41±17). DMSO at 1 or 2g/kg had no effect. Daily DMSO (5g/kg) did not alter cellular proliferation ([H] thymidine incorporation into pulmonary DNA) in the lung at 2 or 5 days after BHT. Thus DMSO potentiated the lethality of BL, and potentiated or had no effect on BL- or BHT- induced fibrosis.

A NOVEL INHALATION CHAMBER FOR EXPOSURE OF RATS TO OXIDANT GASES. J.S. Wilson, D.M. Stavert, R.F. Achouleta, and B.E. Lehnert. Los Alamos National Laboratory, Los Alamos, NM

An important feature of an inhalation exposure system for toxicologic studies involving reactive gases and small animals is to deliver the agent of interest to the animals without the generation of additional reaction products and/or the loss of the physicochemical integrity of the material of interest. We have developed an inhalation exposure system designed to deliver oxidant gas-containing atmospheres to rats maintained within non-avoidance inhalation exposure tubes. Characteristics of this exposure system include: 1) the elimination of internal reactive surfaces by using quartz glass as the fabrication material, 2) a low internal gas volume, 3) short gas residency time, 4) the incorporation of short exposure tubes that eliminate animal avoidance from exposure atmospheres, 5) the simultaneous delivery of an oxidant gas-containing atmosphere to 12 rats, and 6) exposure tubes that serve as partial body flow plethysmographs for assessing ventilatory patterns during exposure. In addition, the exposure atmospheres are isolated from the exhaust flow, which prevents rebreathing, dilution, and/or modification of the chemical form of the inhaled gas. Steady state concentrations of nitrogen dioxide, for example, are achieved in the system within 15-30 sec and deviations from nominal gas concentrations are less than 5%.
INEXPENSIVE CONVERSION OF UPRIGHT AND INVERTED MICROSCOPES FOR POLARIZED LIGHT MICROSCOPY.
P.S. Thorne and W.G. Jones, Dept. of Industrial Environmental Health Sciences, University of Pittsburgh, Pittsburgh, PA. Sponsor: M.H. Karol

A simple adaptor was developed which allows one to utilize an upright or inverted light microscope as a full-featured, polarized light microscope (PLM). The adaptor can be built in less than 1 hr from readily available materials at a nominal cost. Components of the adaptor include a stage rotation device, a polarizer, an analyzer, a retardation plate and central stop. Conversion allows one to study morphology, pleochroism, birefringence, extinction characteristics, isotropy/anisotropy, sign of elongation and dispersion staining colors. Microscopes converted with this device are in use for commercial and classroom identification of asbestos in bulk insulation samples. They also have general utility for examination of various crystalline and noncrystalline materials. Use of the PLM adaptor for study of the effects of asbestos and other mineral dusts on cells in culture has been demonstrated using a Zeiss IM 35 in conjunction with phase contrast and epifluorescence microscopy. The adaptor has the potential to make PLM more accessible to toxicologists.

APPLICATION OF ELECTRON PROBE X-RAY ANALYSIS AS A DIAGNOSTIC ADJUNCT FOR NATURALLY-OCcurring AND EXPERIMENTAL VETERINARY PNEUMOCONIOSES.

Foreign-body pneumonia is not uncommon in animals. Light microscopy (LM) does not always permit definitive identification of foreign material in histologic sections. We have successfully used electron probe X-ray analysis (EXA) coupled with scanning electron microscopy for conclusive multi-element identification of foreign material in the lungs of several animal species with natural or experimental foreign body pneumonia. With EXA we have confirmed the presence of barium sulfate in the lungs of dogs, kaolinite aspiration in murine airways, and pulmonary silicosis in birds. In some cases, color dot mapping (CDM) permits greater appreciation for the diffuse distribution of a foreign material than LM. CDM also helps determine if a foreign material was inhaled as particulates or aspirated as a bolus. In conclusion, we have found EXA to be a useful diagnostic adjunct for definitive in situ identification of foreign material in the airways of animals. (Supported by Center for Environmental Toxicology, Michigan State University.)

INTEGRATED PRECONDITIONING PROCEDURES FOR IMPROVING THE QUALITY OF FERRETS SELECTED FOR BIOLOGICAL RESEARCH.
D. McLain, S. Mcgrain, Y. Greener, and E. Youkillys. Travenol Laboratories, Inc., Round Lake, IL.

While the heterogeneity of ferrets complements their usefulness for biological research with relevance to humans, a large inherent variance in several clinical pathology parameters may obscure or preclude the detection of pre-existing disease states. To optimize our test animal selection process and reduce the probability of assigning abnormal ferrets to research investigations, we have integrated various standard procedures during the preconditioning period. Establishment of these guidelines resulted in a more uniform population of test animals; prevented or caused regression of the estrus cycle, thereby eliminating or minimizing its chronic effects; and enhanced the detectability of the clinical manifestations of such disease states as proliferative colitis. Conscientious breeding efforts were identified with the potential to improve the quality of ferrets produced for biological research investigations.

THE EFFECTS OF SOLVENTS ON THE IN VITRO TOXICITY OF A BONE REPLACEMENT MATERIAL.

A bone replacement material consisting of a copolymer and a bone inductive agent is being developed. Current synthesis involves dissolving the copolymer in chloroform (CHL) or methylene chloride (MC). Although no toxicity has been observed, a new solvent is desired since CHL and MC are known or suspected carcinogens. The in vitro toxicity of the copolymer was tested with CHL, MC, acetone (ACT) or tetrahydrofuran (THF) using a cell growth inhibition assay, agar overlay test (solid and extracts) and a Cr-51 release assay using nylon disks as negative controls. Ethylene oxide (ETO) sterilization was used except for the Cr-51 test where half of the test disks were not sterilized to test the effects of ETO on toxicity. Growth inhibition test results at 24 hr showed zones of no cell growth around THF disks but no zones around other solvent disks. Agar overlay test results showed large zones of no stain around THF disks, but small or no zones with the other solvents. Cr-51 release results showed that without ETO, CHL disks had release rates above nylon by 19% at 4 hr and 101% at 24 hr. The other solvents were < 2% of the control. Results using ETO differed, with all solvents above the nylon control by 3-29% at 4 hr and 22-56% at 24 hr. THF had the largest release and ACT had the least. Acetone may be an acceptable alternative solvent, but ETO methods must be changed.

Hudson and Foster (Biosciences Review, 1964) previously reported that single subcutaneous doses of pyridostigmine bromide (PB) induced irreversible morphological changes in the neuromuscular junctions (NMJ) of diaphragm muscle in Sprague Dawley rats. In the present study, single oral or i.v. doses of PB equivalent to 0.1xLD50, were administered to Sprague Dawley Rats. Drug-related morphological changes were noted in the NMJ of diaphragm muscle of orally dosed rats, but not in the NMJ of i.v. dosed animals. The lesion, which was characterized as a marked dilatation of post synaptic organelles, including mitochondria and endoplasmic reticulum, in the vicinity of the motor endplate, was present one hour after dosing, but was no longer apparent at 28 or 56 days after dosing. Moderate muscle activity, induced during swim activity, caused an apparent exacerbation of this PB-induced lesion, but did not affect the reversibility of this morphological change.


Exposure effects to diesel exhaust (DE), carbon black (CB), ozone (O3), or nitrogen dioxide (NO2) on the metabolism of 1-(1-14C)palmitoyl-L-lysophosphatidylcholine(14C-LL) in freshly isolated pulmonary type II cells and macrophages were investigated. Phosphatidylcholine (PC) and palmitate (PA) were two major metabolites found in the metabolism of LL. Exposure length for DE and O3 was 4 weeks, while that of CB and NO2 was 6 weeks. O3 and NO2 exposure concentration was 0.5 ppm and 15 ppm respectively, while DE and CB had a particulate concentration of 6000 µg/m3. Type II cells exposed to DE or CB and O3 showed a 1.8- and 2.0-fold increase in the rate of incorporation of 14C-LL into PC. Macrophages showed no difference in the rate of incorporation when exposed similarly, except for a decline in O3. There was no change in the rate of incorporation of 14C-LL into PA in type II cells after exposure to DE, CB or O3. Macrophages exposed similarly showed a declining rate of incorporation. The results suggest that elevated and accumulated phospholipidosis in broncho-pulmonary lavaged fluid and macrophages are due to increases in the number of type II cells (previous results) and in production of surfactant by individual cells and decreases in rate of metabolism by macrophages.


Pyridostigmine bromide (PB) is currently being evaluated as a potential prophylactic for use in nerve agent poisoning. During preclinical studies in beagle dogs, the interaction between PB and red blood cell acetylcholinesterase (RBC AChE) was examined both in vivo and in vitro. Maximum inhibition was recorded at 2 to 3 hours after oral dosing and ranged from 10% (0.05 mg/kg) to 85% (5 mg/kg). Doses of 2 or 5 mg of PB/kg were administered every eight hours for 28 days. The level of enzyme inhibition remained constant during the 28 day treatment period with no indication of cumulative effects of or tolerance to PB-induced RBC AChE inhibition. Enzyme levels recovered to 80-90% of control activity within 72 hours after completion of the 28 day dose period. In vitro studies showed that the PB-RBC AChE complex was formed slowly with maximum inhibition recorded at one hour after addition of PB to untreated dog blood. This complex once formed, was relatively stable. Cell wash studies showed that the complex apparently hydrolyzed slowly, in vitro.

COMPARATIVE ACUTE TOXICITY OF CHLOROCITRATE AND FLUOROCITRATE IN DOGS. T. Bosakowski and A.A. Lavin, Dept. of Toxicology and Pathology, Research Center, Hoffmann-La Roche Inc., Nutley, NJ. (Sponsor: R.M. Mccullin)

The high dose effects of chlorocitrate [C-threo-chlorocitric acid] (CC) were compared in vivo to another halogenated citrate analog, and a well known inhibitor of the tricarboxylic acid (TCA) cycle, fluorocitrate (FC). Various biochemical changes that prevail during FC intoxication were monitored in an attempt to explain the collapse/recovery /or lethality (with no histopathologic change) observed in earlier subchronic studies with CC (Hayes et al., Toxicologist 3, 1983). The compounds were given to two dogs/group and a control group received an amount of citric acid equivalent to the dose of CC. Blood samples were obtained hourly up to 4 hr and liver and heart biopsies were excised with a rapid-freeze apparatus. EKG, rectal temperature, and bladder urine were taken just prior to surgical biopsies. CC (100 mg/kg) and FC (8 mg/kg) showed TCA cycle inhibition, evidenced by depletion of ATP (23%) and 33%) and accumulation of citrate in the liver (6-8 fold). CO was a much weaker inhibitor as shown by a significantly lower accumulation of serum citrate (2 fold vs 3 fold with FC) despite a much higher dose. Both compounds produced a similar diabetes-like syndrome (mild hypoglycaemia and glycosuria) mediated by a significant hyperuricamemia and slight hypouricaemia. CC was a more potent inducer of hypoglycaemia and a much greater build-up of plasma lactate ensued (18 fold vs 4 fold with FC), enough to explain lethality observed in earlier CC studies. In contrast, FC produced a severe life-threatening hypocalcaemia (~30%), and hypercalciumia was observed. This effect on calcium distribution was only minimal with CC. Both compounds had a similar depressive effect on circulation as evidenced by hypothermia, bradycardia, and elongation of the QT interval. These changes were found not to result from heart ATP depletion, but rather were considered the result of tacid acidosis and the ongoing ion imbalance. In conclusion, these results indicate that CC acts like FC in that they both inhibit citrate utilization in vivo. However, the differential mode of lethality observed indicates that major differences may exist in their relative potencies, disposition, or metabolism.
INTERRELATIONSHIPS BETWEEN ENERGY AND FAT METABOLISM AND HYPOPHAGIA IN RATS TREATED WITH PERFLUORODECANOIC ACID (PFDA). M.J. Van Ralghem, C.W. Noren, L.A. Menahan, and R.E. Peterson. School of Pharmacy, University of Wisconsin, Madison, WI.

Effects of PFDA (20, 40 or 80 mg/kg, ip) on energy metabolism and body composition were studied in male rats, 7 days after dosing. Cumulative feed intake and body weight were decreased in a dose-dependent fashion. Body weight loss was greater in PFDA-treated rats than in their pair-fed partners, even though feed intake in these two treatment groups was similar at each dose of PFDA examined. Energy expenditure, determined indirectly by measuring oxygen consumption and carbon dioxide production, decreased in a dose-dependent fashion, and no significant difference was detected between PFDA-treated rats and their pair-fed groups at a given dose. A dose-dependent decrease in carcass water and protein content was seen, while total amount of ash remained unchanged in both PFDA-treated and pair-fed rats. Observations were consistent with a reduction in caloric intake. In spite of their lower body weight, PFDA-treated rats found to have a higher carcass fat content than their pair-fed partners. Thus, with the same caloric intake, rats receiving PFDA lose more weight yet maintain a greater body fat content than pair-fed rats. (AFOSR 85-0207)

PERFLUORODECANOIC ACID (PFDA) AND LIPID METABOLISM IN THE RAT. M.J. Van Ralghem, J.P. Vanden Heuvel, C.W. Noren, L.A. Menahan, and R.E. Peterson. School of Pharmacy, University of Wisconsin, Madison, WI.

Male rats were studied 7 days after single ip doses (20, 40 or 80 mg/kg) of PFDA. PFDA treatment resulted in a dose-dependent decrease in carcass triacylglycerol (TG) due to hypophagia, yet at every dose examined, PFDA-treated rats had a greater carcass TG content than their pair-fed partners. Relative to total carcass fat content, carcass TG was significantly greater in PFDA-treated than in pair-fed rats. Reduced feed intake was responsible for dose-dependent decreases in carcass lipid phosphorus, total cholesterol and free cholesterol in PFDA-treated and pair-fed rats. While free fatty acid load presented to the liver was similar in PFDA-treated rats and their pair-fed partners, hepatic TG was increased in a dose-dependent fashion in PFDA-treated rats, but decreased in their pair-fed counterparts. Despite the elevated hepatic TG in PFDA-treated rats, no concomitant increase in plasma TG levels was seen. PFDA-treated animals showed a dose-related increase in liver total cholesterol, which was attributed solely to an increase in the esterified component. Liver lipid phosphorus was reduced to the same extent in PFDA-treated and pair-fed rats due to hypophagia. These findings indicate PFDA exerts profound effects on hepatic lipid metabolism. (AFOSR 85-0207)


Lipid metabolism is known to be influenced by numerous factors; diet, strain, season, chemicals, etc. The present investigation was undertaken to assess the role of diet in affecting the hypolipidemic effects of 2 phthalate esters: di-2-ethylhexyl phthalate (DEHP) and diisononyl phthalate (DINP). Male F-344 rats were fed 2.0% DEHP and 2.0% DINP for six days and sacrificed on day seven following an 18 hour fast. Blood was obtained on day six (ad libitum) and day 7 (fasting) of the animals. Both phthalates produced a significant depression in serum triglycerides and serum cholesterol under ad libitum conditions compared to untreated controls. However, this hypolipidemic effect was diminished or disappeared in the fasted rats. These findings indicate that (1) ad libitum rather than fasting is a more optimal condition to assess the hypolipidemic effects of phthalates and other chemicals, and (2) disreputant or incompatible dietary findings of various chemicals may be explained by differences in dietary status.
EFFECT OF VOMITOXIN (DEOXYNIVALENOL) ON INFANT CYNOBOLUS MONKEYS. F. Iversen, J. Trueove, E. Lok and E.A. Mera. Toxicology Research Division, Food Directorate, Health Protection Branch, Ottawa, Canada.

Vomitoxin, a tricothecene mycotoxin, has been detected in Canadian wheat. Previous studies at the Health Protection Branch have shown that vomitoxin inhibited protein synthesis, was immunotoxic and fetotoxic at elevated dose levels. Toxicity varied with the age, species and sex of the experimental system.

A pilot study was conducted using nursery reared 15 day old cynomolgus monkeys, 2 males and 2 females at each dose level of 0, 1, 2 and 5 mg/kg administered p.o. daily from 15 to 200 days of age. Both male high dose animals died within 7 days but all others appeared clinically unaffected. Histopathology on high dose males revealed thymus, spleen and lymph node alterations. Clinical chemistry, hematology, biochemistry (DNA, protein) and hepatic microsomal enzymes were normal at all dose levels. In vitro 3H thymidine incorporation was increased in the esophageal epithelium of both males and females.

The dose and sex related responses observed in monkeys are similar to those seen in the mouse. The narrow dose range between no apparent effect and death is seen in all species studied.

SUBCHRONIC ORAL TOXICITY STUDY OF 1-ETHYNYL-1-CYCLOHEXENE IN RATS. N.R. Siddiqui and E.J. Hobbs. Dow Corning Corporation, Midland, MI

A 28-day subchronic oral toxicity study was conducted with 1-ethynyl-1-cyclohexene (ECH) in laboratory rats. One control and three treatment groups of ten male and ten female Sprague-Dawley rats were given the test material by gavage at dose levels of 0, 100, 250, and 500 mg/kg/day for 28 days. One satellite group of male and female rats was also treated with 500 mg/kg/day of ECH. Signs of toxicity exhibited by rats include slight to mild salivation, lethargy and slight to mild ataxia in rats receiving the highest dose level. No significant treatment-related effects of toxicological significance were observed in the 100 mg/kg/day dose group. Significant and dose-related increases in cholesterol were observed in female rats. The other compound-related effects seen in animals of two high dose groups include decreased red blood cells, hemoglobin and hematocrit values in males and increased absolute and relative liver weights in females. Male rats given a 500 mg/kg dose of ECH also showed reduction in body weight gains and increased reticulocytes and platelets. No chemical-related gross or microscopic changes were observed in any of the organs or tissues of male and female rats. Most of the affected blood values at the highest dose level were returned to normal during a two-week recovery period.


The subchronic oral toxicologic profile of a novel anticholinergic bronchodilator, 3-(2-ethylamino) propyl)-1,2,3,4-tetrahydro-5H-[1] benzopyrano[3,4-C]pyridin-5-one (CI-923), was evaluated in dogs and rodents for a 13 week period. CI-923, when administered orally in gelatin capsules to Beagle dogs at daily dose levels of 15, 10 and 5 mg/kg resulted in sporadic emesis at all dose levels, and tachycardia at 15 and 10 mg/kg. CI-923 was well tolerated by Wistar albino rats when given as a dietary admixture at daily dose levels ranging from 200 to 500 mg/kg, and did not elicit clinical signs of drug toxicity. Rats dosed at 400 and 500 mg/kg had significantly reduced body weight gains over the study period. Gross pathologic evaluations revealed cecal distension which was seen at all dose levels in the rat study. There were no correlating histopathologic findings in the gastrointestinal tracts of these animals and the cecal distension was attributed to the pharmacologic effect of the drug.

SUBACUTE INTRAVENOUS TOXICITY OF CI-937, AN ANTIRAPYRAZOLE ANTIHECHT CANDIDATE, IN BEAGLE DOGS. D.G. Pegg and J.R. Watkins, Warner-Lambert/Parke-Davis Pharm. Res., Ann Arbor, MI

CI-937 is an intercalating agent which selectively inhibits DNA synthesis. Groups of beagle dogs, two per sex, were administered five consecutive daily doses of 0.32, 0.16, 0.05 or 0.016 mg/kg. One animal per sex was sacrificed after eight days and the remaining animals after 65 days. Clinical signs in these groups included emesis, dehydration, mucoid stool and diarrhea. Dose related leukopenia, thrombocytopenia and anemia were observed in animals administered doses of 0.05 mg/kg and greater. Changes in hematological parameters were reversible in the 0.02 mg/kg group animals. Bone marrow hypopcellularity was marked and in animals administered 0.16 mg/kg, only mature granulocytic series cells remained after eight days. Pathological lesions included atrophy or maturation arrest of germininal epithelium of testes, lymphoid depletion and necrosis, and primarily in the 0.32 mg/kg group, intestinal lesions which included erosions and necrosis of epithelium. The 0.016 mg/kg dose group was essentially asymptomatic. Though extremely dose potent in dogs, manifestations of CI-937 toxicity were consistent with the cytotoxic actions of the compound.
Recently, a new class of macrocyclic trichothecone mycotoxins has been isolated from a strain of *Myrothecium roridum*, which was associated with a pathogen on Texas tomatoes (Jarvis et al., *Tetrahedron Lett.* 26, 4859, 1985). This class of macrocyclic trichothecones, which has been termed mycotoxin A and B, is of particular interest because of its marked cytotoxicity against *in vitro* cell systems. In this study, mycotoxin B was compared to the most potent simple (T-2 mycotoxin), and macrocyclic trichothecones (verrucarin A). When tested in a mouse lethality assay, the LD<sub>50</sub> for mycotoxin B was 81 ± 15 μg/kg which makes it 12x more potent than verrucarin A and 100x more potent than T-2 mycotoxin. The minimum effective dose that caused skin irritation for mycotoxin B was 4.07 ± 0.59 ng/cm<sup>2</sup>, which means it is 3x more potent than verrucarin A and 16x more potent than T-2 mycotoxin. Thus, mycotoxin B appears to have the highest toxicity of the trichothecone mycotoxins which have been evaluated to date.

**TOXIC EFFECTS OF N-PHENYL-2-NAPHTHYLAMINE (PBN) IN F344/N RATS AND B6CF<sub>1</sub> MICE GIVEN THE COMPOUND IN THE FEED FOR UP TO 13 WEEKS.**

K.M. Abdo and C.A. Montgomery, NTP, NIHS, RTP, NC; A.C. Peters, Battelle Columbus Laboratories, Columbus, OH. Sponsor: R.S. Yang

Toxicity of PBN (an antioxidant used in the manufacture of rubber and plastics) was studied in F344/N rats and B6CF<sub>1</sub> mice. Groups of 10 males and 10 females of each sex and species were fed diets with 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm of PBN for up to 13 weeks. Compound-related mortalities occurred in rats and mice at 40,000 ppm. Mean body weight gains for rats >5,000 ppm and mice at >5,000 ppm were lower (P<0.5) than controls. There was a significant (P<0.05) increase in relative liver weight ratios in mice and male rats at >10,000 ppm and female rats at >5,000 ppm. Compound-related lesions observed in rats were: nephropathy in males at 20,000 or 40,000 ppm and in females at >10,000 ppm; kidney epithelial degeneration and hyperplasia in males and females at >500 ppm; testicular degeneration in males and females at >10,000 ppm; hematopoietic hypoplasia and atrophy of the femoral bone marrow in males at 40,000 ppm and females at 20,000 and 40,000 ppm; testicular hypospermato genesis, lymphoid depletion of the thymus and of the spleen in males and females at >40,000 ppm. In mice, multifocal kidney nephropathy was observed in males at 20,000 and 40,000 ppm and females at >5,000 ppm. The incidence and severity of the kidney lesions observed in rats and mice were dose-related.

**ASSESSMENT OF THE SUBCHRONIC INTRAVENOUS TOXICITY AND DISPOSITION OF (14C)-ACROLEIN IN THE RAT AND THE ACUTE AND SUBCHRONIC TOXICITY IN FERRETS.**

D. McLain, M. McCarty, S. Giovanetto, L. Martin, Y. Greener, and E. Youkilis, Travalen Labs, Inc., Round Lake, IL.

The potential toxicity and disposition of (14C)-acrolein in the rat was studied following intravenous (IV) administration for 14 days. Toxicity was also evaluated in ferrets subjected to acute (LD50) and subchronic IV exposure for 90 days (thrice weekly). No significant toxicity was elicited in rats treated with 0.06-6.0 μg acrolein/kg, and the peak concentration of (14C)-acrolein (sp. activity = 0.12 mCi/mole) in blood samples of rats treated with 6 μg/kg was not significantly different from background levels. No detectable levels of 14C were found in the major organs evaluated, and urinary excretion was the major route of elimination. In ferrets treated with 60-600 μg acrolein/kg, histopathologic changes were evident in lungs and injection site. These changes were typical of a local inflammatory response consisting of an alveolar macrophage accumulation (lungs) and perivascular and intimal changes (injection site). The pulmonary effects were considered to be reversible; several of the injection site lesions were irreversible. The acute IV LD50 of acrolein in the ferret was calculated to be 20 mg/kg.

The test material, a nonionic surfactant, is produced by reacting tridecyl alcohol (branched) with 4-5 moles of ethylene oxide. The test material was administered by the oral (gavage) route to rats for 7 days/week for 13 weeks at 0, 0.1, 0.5 or 1.0 g/kg/day. The incidence of in-life clinical observations was minimal; however, none were treatment related. Decreases in food consumption and body weights, and treatment related effects on organ weight parameters (liver, kidney, adrenal, brain and testes) were observed at doses ≥ 0.5 g/kg/day. No significant increase in the incidence of any non-neoplastic or neoplastic microscopic lesions was observed in selected tissues from the high dose animals compared to control animals. The results of this study support a low degree of systemic toxicity associated with subchronic oral exposure to tridecyl alcohol ethoxylate.

THIRTEEN WEEK SUBCHRONIC TOXICITY STUDY OF BUTYRALDEHYDE (BA) IN F344 RATS AND B6C3F1 MICE. G.W. Wolfe, M. Rodwin, J.E. French*, and C.A. Parker. Hazleton Laboratories America, Inc., Vienna, VA *NTP, NIEMS, RESEARCH TRIANGLE PARK, NC.

BA (CH3CH2CH2CHO) is one of a series of aliphatic aldehydes. BA was administered to 13 weeks via gavage in corn oil to F344 rats and B6C3F1 mice (10/sex/group) at dose levels of 0, 0.075, 0.15, 0.3, 0.6, and 1.2 g/kg. A dose-related increase in mortality was observed in the rats of both sexes (no survivors at 1.2 g/kg) while compound-related mortality was only observed at 1.2 g/kg in mice (2/10 males and 1/10 females). Decreased rate of body weight gain was observed in the 1.2 g/kg groups of both species and sexes. No compound-related gross necropsy findings were observed for the males and females of both species and sexes. Microscopic lesions were observed in the nasal cavity and stomach of both species and sexes. Nasal cavity lesions consisting of inflammation or necrosis were observed in the mice as low as 0.3 g/kg and in the rats as low as 0.075 g/kg. Stomach lesions (glandular) in the mice consisted of inflammation at 1.2 g/kg (1/10 males and 3/10 females). Stomach lesions (fore-stomach and glandular) in the rats included inflammation, erosion/ulceration, necrosis, hemorrhage, and epithelial hyperplasia in rats at 1.2 g/kg (5/10 males and 3/10 females) and 0.6 g/kg (5/10 males and 9/10 females).

THIRTEEN WEEK SUBCHRONIC TOXICITY STUDY OF CROTONALDEHYDE (CA) IN F344 RATS AND B6C3F1 MICE. G.W. Wolfe, M. Rodwin, J.E. French*, and C.A. Parker. Hazleton Laboratories America, Inc., Vienna, VA *NTP, NIEMS, RTP, NC.

CA (CH3CH=CHCHO) is representative of an alkyl-substituted vinyl aldehyde. CA was administered for 13 weeks via gavage in corn oil to F344 rats and B6C3F1 mice (10/sex/group) at dose levels of 0, 2.5, 5, 10, 20, and 40 mg/kg. Compound-related mortality was observed in rats of both sexes at 5, 10, 20, and 40 mg/kg while all mice survived to termination. Mean body weights were significantly decreased for the 40 mg/kg male rats at termination. Compound-related gross necropsy lesions observed in male and female rats at 20 and 40 mg/kg were thickened forestomach or nodules. No compound-related gross necropsy lesions were noted for the mice. Microscopic lesions were observed in the stomach of the mice and rats and in the nasal cavity of the rats. Stomach lesions in the mice included hyperplasia of the epithelial lining of the forestomach in most of the 40 mg/kg males and females and chronic active inflammation of the forestomach in two 40 mg/kg males. Stomach lesions in the rats included hyperplasia of the forestomach epithelium in the males and females at 10, 20, and 40 mg/kg and forestomach hyperkeratosis, ulcers, moderate necrosis, and acute inflammation at 40 mg/kg. Nasal lesions in the rats consisted of acute inflammation in the males as low as 20 mg/kg and females as low as 5 mg/kg.


Under an interagency agreement between the Agency for Toxic Substances and Disease Registry and the National Toxicology Program (NTP), the NTP is conducting toxicology studies related to the Superfund Act. As part of this effort, toxicologic studies were initiated on chemical mixtures of environmental concern, specifically ground water contaminants derived from hazardous waste disposal. The first protocol, centered on the subchronic health effects of drinking water contaminants, is at the contract review stage. Initial chemistry developmental work revealed that chemical interactions created limitations on the number and concentrations of chemicals in the mixture. Twenty-five chemicals, selected from over 1000 known contaminants in and around hazardous wastesites, are to be given to rats and mice in drinking water for up to 3 or 6 months. Five doses, in addition to the control group, are set at the average concentrations for individual component chemicals detected in ground water (i.e. baseline level; 1X) and 0.1X, 0.5X, 1X, 10X, and 100X of the baseline level. Toxicologic endpoints to be measured/observed include clinical signs, mortality, water and food consumption, body and organ weights, clinical and anatomic pathology, neurobehavioral tests, SMVCE, and cytogenetics.
A maximum contaminant level goal of 7.1 million fibers per liter in drinking water was proposed. Under the Safe Drinking Water Act, this must be a level that would not result in any known or anticipated adverse health effect. Very few studies are available to assess the contribution of asbestos in air from drinking water during showering and other non-consumptive uses.

Specific epidemiological studies conducted in several areas of the United States found no association between asbestos in water and cancer mortality. If any significant inhalation exposure of asbestos had occurred due to airborne asbestos (released from water), some evidence of bronchus, trachea, or lung cancers associated with asbestos inhalation exposure might have been expected but were not detected.

Results of drum-type humidifier usage, and measurement of air samples taken from showers (for asbestos) from asbestos contaminated water within Woodstock, N.Y. homes demonstrated that transfer of asbestos from water into air was negligible.

There is a paucity of toxicity data on a number of industrial chemicals currently in use in Canada. Nine such chemicals were administered individually by oral gavage to male and female rats at dose levels ranging from 1 to 100 mg/kg bw/day for 14 days. Cyclohexanone oxime caused increased spleen weights (females only), changes in hemostatic parameters, induction of mixed function oxidases (MFO's) and some renal and renal morphological changes at 100 mg/kg. At 100 mg/kg 2-butyne-4 diol depressed body weight gain in males, decreased liver and spleen weights in both sexes, altered serum biochemistry, induced MFO's and caused mild histological changes in liver and thyroid. p-Toluene sulfonhydrazide caused decreased body and organ weights (liver, spleen, heart) and produced significant changes in serum biochemistry, hematology and induced MFO activity in both sexes at 100 mg/kg. Males and females dosed with 100 mg/kg triallyl-5,6,7,8-tetrahydrothrene showed decreased body weight gain, decreased spleen weight and histopathological changes in kidney with some associated changes in serum biochemistry. No toxic effects were noted with the other chemicals tested.

Acute toxicity testing programs generally include the evaluation of response to exaggerated doses to determine such endpoints as lethal levels. To enhance the applicability of this data to man, a second (or third) species may be included in the program. This paper compares the literature data (from EEC, 1986) for all chemicals (544) for which both an LD50 in the rat and rabbit were reported, in order to evaluate the value of the data obtained from the rabbit. Chemicals were defined by acute toxicities as either extreme (LD50 ≤ 5 mg/kg), high (5.1-50), moderate (51-500), slight (501-5000), or very low (>5000). With approximately 70% of the chemicals (369/544), the classification category was the same for the rat and rabbit. In only 10 cases were the toxicity categories not adjacent (i.e., extreme vs. moderate). Of the 175 cases where the toxicity classification was different, 108 chemicals (61%) were more toxic to the rabbit. These data suggest that there is very little practical difference in the acute toxicity between the 2 species. Therefore, the inclusion of the rabbit in acute oral testing programs does not appear to add any substantial information to the toxicity data base in most cases.

An increasingly important aspect of toxicology is the use of screening tests for detecting the presence or absence of a single endpoint of effect such as mutagenicity or neurobehavioral effects. Such screens have a common set of operating characteristics which are not widely appreciated and which make traditional approaches to statistical analysis inappropriate, insensitive and inefficient. The characteristics of screens are presented and reviewed, along with several sets of actual data from observational screen (neurobehavioral) and mutagenicity studies. Two new alternative approaches to statistical analysis (a control chart approach and a graphical/exploratory data analysis approach) are presented along with a review of traditional contingency table and T-test/ANOVA methods. The performance of these methods in analyzing the presented screen data is then compared in terms of power and sensitivity. Both alternative approaches are shown to be superior to traditional approaches in performance towards the objectives of screens.
REQUIRED SAMPLE SIZES FOR INCREASING THE
STATISTICAL POWER OF HEMATOLOGIC-BIOCHEMICAL
EVALUATIONS IN A HETEROGENEOUS POPULATION OF
FERRETS. D. McLain, L. Lin, Y. Greener, and E.
Youkilis. Travenol Labs., Inc., Round Lake, II.

Hematologic and biochemical data were collected
from several hundred ferrets during the
preconditioning period. These data profiles
were statistically evaluated and compared with
data profile of beagle dogs. The animal to
animal variations for many parameters were
greater among ferrets than among beagle dogs.
To achieve a statistical power equal to that
routinely obtained with the more homogeneous
beagles, multivariate and/or univariate models
were applied for the prediction of sample sizes
required for ferrets. Comparative costs and
benefits of using ferrets vs. the beagle were
also evaluated.

THE USE OF BACKGROUND DATA IN THE
INTERPRETATION OF TOXICOLOGY STUDIES.
Huntingdon Research Centre, Huntingdon,
England. Sponsor: P.A. Johns

To interpret data from a particular
toxicology study, recourse is often
made to "background data," control
data from other studies carried out
at about the same time at the same
facility and with the same species
and strain of animal. Using examples
from the HRC data base, we illustrate
some aspects of this process not
usually considered:
(i) taking account of between and
within study variation;
(ii) using data from animals of
different ages, when insufficient data
are available at the required age;
(iii) using data from related
parameters simultaneously.

ANOTHER APPROACH TO TOXIC HAZARD RATINGS.
R. V. Lu, S. H. Kubner, J. T. Emsminger,
Oak Ridge National Laboratory*, Oak Ridge, TN.
and R. A. Yearly, Chemlawn, Columbus, OH.

Toxic hazard rating is one of the data require-
ments of Material Safety Data Sheets (MSDS)
under the OSHA Hazard Communication Standard
(Right-to-Know Law). Toxic hazard ratings not
only describe the first approximation of
poisoning potential, but also provide essential
information needed for the labeling of chemi-
cals. Presently, a number of rating systems are
available to classify toxic/hazardous
substances based on extrapolation of animal
toxicity data. Discrepancies occur among the
available systems -- especially for those
substances that are low in acute oral toxicity,
but are positive or potential carcinogens in
animal experiments. A rating system has been
developed for hazard identification by incor-
porating the carcinogenic potential, threshold
limit values, and acute toxicity values in
animals. To date, 2500 generic chemicals and
trade name products have been rated.

*Operated by Martin Marietta Energy Systems,
Inc., under contract No. DE-AC05-84OR21400 for
the U.S. Department of Energy.

HUMAN EXPOSURE ASSESSMENTS FOR LAUNDRY
PRODUCTS. P. J. Hakkinen and J. Yam.
The Procter & Gamble Company, Packaged
Soap and Detergent Product Development
Division, Cincinnati, OH. Sponsor:
J. E. Griffith

Human safety assessment of a laundry pro-
duct requires careful evaluation of 1) the
potential toxicity of the product and
its ingredients, and 2) the types and
extent of worker and consumer exposures.
Generally, the types of exposures (in-
halation, ingestion or dermal contact)
are predictable from a knowledge of the
manufacturing process, and of how con-
sumers use and misuse a particular type
of product. To assess the extent of
exposures to a product and its ingre-
dients, conservative yet realistic guide-
lines for assessing worker and consumer
exposure to laundry products and their
ingredients have been developed. Key
data for the proposed product include
laundry task frequency and duration, plant
dust level; and for new ingredients,
environmental levels, fabric deposition
and transfer to skin data, and percutan-
eous flux rate data.
A potential anti-inflammatory agent was administered orally to a single group of male and female rats as a suspension in 3% cornstarch at successively increasing doses. Controls received equivalent volumes of vehicle. Doses of 5 or 20 mg/kg were given for 4 consecutive days each, followed by doses of 40 mg/kg for 10 consecutive days. Animals were placed on a 3-day recovery period prior to each dose increase. Plasma levels of unchanged drug were obtained from similarly treated rats at 2, 5, and 24 hours after the fourth daily dose at each dose level. The compound was well tolerated at doses as high as 20 mg/kg; however, compound-related gastrointestinal toxicity and secondary clinical changes were noted at 40 mg/kg. Plasma levels indicated that exposure to the compound was dose-related from 5 to 40 mg/kg and the compound appeared to be slowly absorbed and/or slowly eliminated from plasma, with an estimated half-life on the order of 19 hours. Thus, the RDT study provided preliminary data for planning subsequent toxicology and metabolism studies in a manner which conformed animals and other resources.

A rapid and specific clean-up procedure for the gas chromatographic (GC) analysis of nicotine and cotinine in small volumes of plasma. E.L. White, L.E. Bates, and J.D. deBethizy. RJ Reynolds Tobacco Company, Bowman Gray Technical Center, Winston-Salem, NC

Reverse phase (C18) silica clean-up columns were chosen to remove selectively nicotine and cotinine from plasma prior to GC analysis. To determine the recovery of nicotine and cotinine from these columns, [14C] (+/−)nicotine and cotinine were applied to the column in phosphate buffered saline (pH 7.4) and eluted with methanol. Cotinine was easily eluted from the column, but nicotine apparently adsorbed to free silanol groups in the packing. Nicotine recovery was increased to that of cotinine (86+/−5%) by eluting with 1% (v/v) triethylamine in methanol. The elution profiles of the nicotine internal standard, N-ethylnornicotine, and the cotinine internal standard, N-ethylnorcotinine, from the C18 column were similar to the elution profiles of nicotine and cotinine, respectively. Standards of unlabeled nicotine and cotinine were prepared in fetal bovine serum, cleaned-up, and analyzed using a DB-5 capillary column and nitrogen-phosphorus detection. The detection was linear for concentrations of nicotine ranging from 10 ng/ml to 200 ng/ml (R2 = 0.99) and cotinine ranging from 20 ng/ml to 800 ng/ml (R2 = 0.98). The method was more rapid than extraction methods and required only 0.1 ml of plasma to quantify nicotine and cotinine.

Determination of pyrrolizidine alkaloid metabolites from mouse liver microsomes using tandem mass spectrometry. C.K. Winter, A.D. Jones, and H.J. Segall. Veterinary Medicine Pharmacology and Toxicology and Facility for Advanced Instrumentation, University of California, Davis, CA

The major pathways for the hepatic metabolism of the pyrrolizidine alkaloids (PA’s) (N-oxidation, hydroxylation and oxidation to pyrrolic compounds) have been established but detection of these processes has generally relied upon nonselective colorimetric assays. Efforts to identify specific PA metabolites using conventional gas and liquid chromatography have been complicated by the low volatility, thermal stability, and lack of appropriate uv chromophores of several of the metabolites. A novel approach for specific metabolite identification has been developed using tandem mass spectrometry with a VG ZAB-2F double-focusing mass spectrometer. Qualitative determination of the major PA’s senecionine and monocrotaline was accomplished using fast atom bombardment (FAB) and chemical ionization (CI) mass spectrometry in combination with collisionally activated decomposition/mass-analyzed ion kinetic energy spectrometry (CAD/MKES). Metabolites were quantified using selected ion and selected reacting monitoring. This non-chromatographic method is rapid and sensitive, requires minimal sample preparation, and should be applicable for the identification of metabolites of a wide variety of toxicants in several biological matrices.

Determination of cantharidin using capillary GC/MS in specimens from horses poisoned by ingestion of blister beetles (Euphoria sep.). A.G. Ray, M.J. Murphy, J.C. Reagor. Texas Veterinary Medical Diagnostic Laboratory (TVMDL), Texas A&M University, College Station, TX

Equine cantharidiasis is a recurring problem encompassing much of the U.S. Animals ingest alfalfa hay which has been contaminated with blister beetles during baffle-striped beetles (Euphoria tenax and occidentalis) are frequently involved, but other species (E. atrivittata, pardinis, albida, and pennsylvania) have been incriminated. Horses are very sensitive to the toxic principle cantharidin (lethal dose 2 mg/kg), which is an irritant. Analysis of adult beetles for cantharidin using HPLC showed wide intra- (0.89-5.40% dry wt) and interspecies (0.61-5.40% dry wt) variation. Capillary GC/MS has been adapted to confirm blister beetle toxicosis. After acidification of urine and dilute NaOH extracts of gastric content, cantharidin is partitioned into CHCl3, followed by cleanup using silica cartridges. Analysis is performed using a splitless injection system (port = 250°) and a 12 m methyl silicone column, beginning at 40° and programmed at 15°/min with a 4 min solvent delay (Rt = 8.5 min). Ions monitored are 70, 96 and 128 m/z. In the past year, 38 cases of equine cantharidiasis have been confirmed at TVMDL. Cantharidin concentrations in urine and gastric content from poisoned animals have ranged from 1 μg/kg to 2 mg/kg.

32P-postlabeling of DNA adducts is proving to be useful in detecting formation and following the persistence or disappearance of covalent complexes of deoxyribonucleotides and metabolites of known or potential mutagens and carcinogens. In order to improve reproducibility, increase the accuracy of quantitation and decrease the analysis time, we have adapted the 32P-postlabeling technique to HPLC. In our procedure, hepatic DNA is isolated by solvent extractions and enzyme digestions, then enzymatically hydrolyzed to deoxyribonucleotide-3'-5'-P's. Extraction with butanol produces a relative enrichment of adducted nucleotides. At 32P is used to produce labeled deoxyribonucleotide-3',5'-d3P's which are analyzed by reversed phase HPLC. Using this method we have been able to detect DNA-2-aminofluorene (AF) adducts formed in vitro and in vivo. We have measured the time course of formation of adducts in mouse liver following a single i.p. dose of AF and also detected adducts in rat liver following a chronic feeding study. The use of HPLC in conjunction with 32P-postlabeling provides a rapid and reliable approach to quantitation of DNA adducts produced by AF and potentially many other mutagens.

(Partially supported by PHS Grant CA 39018.)

CELL GROWTH STUDIED BY FOURIER TRANSFORM INFRARED SPECTROSCOPY. M.J.W. Chang, T.B. Huston, J.T. Keller and D.J. Long Rattelle Columbus Division, Columbus, OH. Sponsor: J.C. Page.

Fourier transform infrared spectroscopy (FT-IR) provides the sensitivity, selectivity, and variety of absorptions required for studies of molecular structure in biological systems. Our efforts are aimed at using FT-IR as a probe for cell biology and cellular biochemistry. Initial attempts found that Chinese hamster ovary (CHO) cells would grow on the surface of the optical crystal. Repeated IR scans from 900 cm⁻¹ to 2000 cm⁻¹ were made for a period of about two cell-cycles. Kinetic plots at different wave numbers were made. 3H-thymidine and 3H-leucine incorporation into DNA and protein were carried out in parallel cultures. Correlations were attempted. To further substantiate any correlation, hydroxyurea, an inhibitor for DNA synthesis and puromycin, an inhibitor for protein synthesis were applied to the culture and the effects on various kinetic plots were studied. Several positive correlations were identified. It is concluded that (1) in situ monitoring of cell growth by FT-IR is possible and (2) response of cells to toxicants can be observed. (Supported by NIH Grant RR-01367.)


Inhibition of cell-cell communication may be a mechanism of tumor promotion. Dieldrin and 2,2',4,4',5,5'-hexabromobiphenyl (HBB) are tumor promoters in vivo and block cell-cell communication in vitro. The scrape-loading/dye transfer (SL/DT) assay was used to quantify this in vitro effect at varying levels of each toxicant. SL/DT is based on intracellular loading of a fluorescent dye, Lucifer yellow (LY), and monitoring its transfer into adjacent cells via patent gap junctions. Dye transfer was measured with anchored cell culture and sorting (Meridian Instruments). Confluent F344-B cells (rat epithelial) cells were exposed to various nontoxic concentrations of HBB or dieldrin. The results indicate an inverse correlation between the degree of fluorescence in secondary recipient cells and the treatment concentration. The described methods provide quantitative analysis of dye transfer in measuring the dose-response of modulation of gap junctional permeability in cultured cells by environmental toxicants.


The effect of OOS-trimethyl phosphorothioate (OOS-TMP) (10 and 40 mg/kg, po) on hepatic MFO and heme pool in male rats was investigated. OOS-TMP decreased P-450 content and the activities of 7-ethoxycoumarin 0-deethylase, p-nitroanisole O-demethylase and aryl hydrocarbon hydroxylase (AHH) 24 and 72 hr after dosing relative to pair weighed or freely fed animals. AHH activity was decreased extensively in a dose-dependent manner. 6-Aminolevulinic acid synthase was somewhat decreased in treated animals while heme saturation of tryptophan pyrrolase was increased dramatically. Since this profile is similar to endotoxin shock related MFO decreases, we investigated the role of immune response in the decrease of MFO activities. It was found that OOS-TMP dramatically stimulates interleukin production in splenocytes; 196% of the control value at 40 mg/kg 72 hr after dosing. However, we could not detect any increased activity of interferons in serum. It was also proven that a factor(s) for depressing AHH activity in animals dosed with OOS-TMP was transferrable via serum. (Supported by U.S. PHS AG04419)

Sexually dimorphic hepatic metabolism of androgens in the rat does not fully develop until after puberty and is thought to depend upon neonatal hormonal status. Adult males hydroxylate testosterone (T) primarily at the 2 and 16 positions. Females reduce T at the 5a position. Alteration of the neonatal hormonal milieu may affect development of adult hepatic metabolism. The effect of DEX on neonatal T levels and hepatic T metabolism was examined. DEX (10 mg/kg) was administered ip to 1 day old Sprague-Dawley rats. At puberty (35 days) female 5α-reductase activities were decreased to 57% of controls (ps < 0.005) as measured by HPLC resolution of microsomal metabolites of 4α-T. At 70 days male 16α and 2α hydroxylation was reduced to 60% and 66% of controls (ps < 0.05). Plasma T levels measured by RIA were not significantly altered by DEX 24-96 hr after treatment. However, in both sexes hepatic 5α-reductase activities were reduced to 51% of controls (ps < 0.005) 48 hr after treatment while 6β, 16α and 2α hydroxylation activities were increased. This suggests that DEX decreases the conversion of T to dihydrotestosterone in the neonate. Decreased concentrations of this biologically inactive metabolite may be involved in the altered hepatic sexual dimorphism observed in the adult.

EFFECT OF VARIOUS PRETREATMENTS ON THE METABOLISM OF ACYLXONITRILE (ACN) IN RATS. L. Roulisse and S. Chakraborti. M.Sc. trav. & hyg. mil., Fac. méd., Univ. de Montréal, Montréal, Quebec, Canada

Because of involvement of both nonenzymatic and enzymatic pathways, the in vivo metabolism of ACN has been reexamined using various pretreatments. Male Fischer 344 rats were pretreated with either phenobarbital (PB) (50 mg/kg, ip, 5 days), or 3-methylcholanganthrene (3-MC) (15 mg/kg, ip, 3 days) or Arochlor 1254 (100 mg/kg, ip, 3 days) or pi-peronyl butoxide (PBO) (300 mg/kg, ip, 2 h) or diethyldimethane (DEM) (400 mg/kg, ip, 90 min) prior to an ip administration of ACN (0, 35 and 50 mg/kg) in corn oil. At 35 mg ACN, urinary excretion of chioconate was increased due to PB or 3-MC and decreased by PBO, whereas at 50 mg ACN, this excretion was increased by only PB and decreased by both PBO and Arochlor. DEM apparently reduced such excretion. At 35 mg ACN, the excretion of N-acetyl-S-(2-hydroxyethyl)-L-cysteine was decreased by PB and Arochlor, while PB and 3-MC had no effect. At 50 mg ACN, such excretion was reduced by PBO and Arochlor. DEM apparently reduced such excretion. PB, 3-MC, Arochlor or PBO had no further effect on the urinary excretion of N-acetyl-S-(2-cyanoethyl)-L-cysteine at 35 mg ACN, but at 50 mg ACN, such excretion was decreased by PB or 3-MC and decreased by Arochlor. DEM apparently reduced such excretion. These data indicate the complexity of various pathways for ACN metabolism. (Supported by IRSST, Quebec)

INFLUENCES OF DIETARY RESTRICTION AND AGE ON LIVER ENZYME ACTIVITIES AND LIPID PEROXIDATION IN MICE. A. Kozumi, R. Weindrup* and R. L. Walford*, Sponsored by T. Inimmure, Univ. of California, Riverside, CA *Univ. of California, Los Angeles, CA

Dietary restriction extends maximal lifespan in rodents by unknown mechanisms. We compared livers from 12- and 24-month-old mice fed control (C, ~95 kcal/μk) or restricted (R, ~55 kcal/μk) amounts of diet since 3 weeks of age. We hypothesized that R might alter the activity levels of enzymes with possible relevance to aging processes. The enzymes included several xenobiotic metabolizers, radical scavengers (catalase, superoxide dismutase, glutathione peroxidase), superoxide sources (xanthine oxidase, peroxisomal β-oxidation of palmitoyl-CoA), and glucose 6-phosphatase. Lipid peroxidation (LP) was also measured. Comparing 12- and 24-month-old mice, the strongest diet or age effect was an increased catalase activity for group R (42% higher at 12 mo, 64% at 24 mo). HP was clearly lower in group R at 12 mo (a 30% decrease) and somewhat lower (13%) at 24 mo than in group C. Similarly, in 12-month-old C and R mice injected with either the P-450 inducer β-naphthoflavone (3-μM in corn oil) or with corn oil alone, R mice showed higher catalase activity (40-44%) and lower LP (45-46%) in both β-μ-M-injected and vehicle-injected groups. These data suggest that in part CR can decrease oxidative tone by a selective increase in catalase activity. (Supported by U.S. PHS AG-06419 and by Life Extension Foundation)


Phencyclidine (PCP) and analogs containing the five (PCPy) and seven (PCHBMI) membered heterocyclic ring were examined for their inhibition of P450 dependent reactions in incubations with hepatic microsomes from PB induced rabbits. After a 30 minute, 10μM incubation at 300, the decrease in CO binding to reduced P450 (as percent of control) was 76, 72, and 94 for PCPy, PCT, and PCHBMI respectively. For each substrate (S), the rate of inhibition of benzphetamine metabolism was first order and plots of ln(ki) vs 1/(S) were linear, indicating active-site-directed inactivation. The saturation t1/2 (min) (and apparent Kd, μM) were 19.7 (49), 17.3 (19) and 110 (78) for PCPy, PCPy, and PCHBMI, respectively. The inhibition was NADPH dependent for each substrate. The observed trends are those expected from the equilibrium between the ring-closed, alpha-hydroxylated intermediate and the open chain aldehyde, since PCPy and PCT are expected to be more stable than closed ring structures which are proximal to the actual inhibitory intermediate. (Supported by USPHS DA02411).

The 4-azasteroid N,N-diethyl-4-methyl-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide (I) produces concentration- and time-dependent decreases in the viability of freshly isolated P-344 rat hepatocytes, exhibiting an LC50 of 0.52 mM at 3 hr. Testosterone and other steroids potentiated the cytotoxicity of I in a concentration-dependent manner (LC50 = 0.24 mM), while having no effect upon the toxicity of other chemical agents. The cytochrome P-450 inhibitor octylamine potentiated the cytotoxicity of I while the effect of metyrapone was not statistically significant. Induction of cytochrome P-450 isozymes by phenobarbital and β-naphthoflavone pretreatment protected the cells against azasteroid cytotoxicity, while acetone pretreatment had no effect. Phenobarbital pretreatment doubled the extent of metabolism of I. Galactosamine and sulfate-free media had no effect upon the cytotoxicity of I. These results suggest that cytochrome P-450 is involved in the detoxication of 4-azasteroids by rat hepatocytes while conjugative metabolism does not play a significant role.


Benzo(a)pyrene [B(a)P] has been demonstrated to produce cancer in lung and other organs of rodents and phenolic antioxidants such as BHA retard the incidence of B(a)P-induced neoplasia in mouse lung. In the present investigation we have examined the role of glutathione S-transferase (GST) in the detoxification of B(a)P and the mechanism of this antineoplastic activity of antioxidants. All the six isozymes of GST present in mouse lung (GST 9.8; GST 8.7; GST 7.9; GST 6.4; GST 5.7; and GST 4.9) were found to catalyze the conjugation of B(a)P-4,5-oxide and B(a)P-7,8-oxide with glutathione. However, GST 8.7 and GST 7.9 had relatively high catalytic activity towards these two epoxides. Also, these two isozymes (GST 8.7 and GST 7.9) non-catalytically bind B(a)P metabolites. GST activity as well as GST protein is increased by about 75% in the lung of BHA treated mice and the isoenzymes GST 8.7 and GST 7.9 are preferentially induced. These results suggest that the GST 8.7 and GST 7.9 isoenzymes play an important role in the detoxication of B(a)P metabolites and the antineoplastic activity of BHA against the B(a)P induced neoplasia in mouse lung may be due to their induction. (Supported in part by NIH grants CA 27967; GM 32304; EO 04396).

A NEW METHOD FOR THE MEASUREMENT OF HEPATIC APS KINASE ACTIVITY. T.J. Maziaaz and J.J. Hjelle. Dept. of Comparative Biosciences and the Environmental Toxicology Center, Univ. of Wisconsin, Madison, WI.

The sulfation of endogenous and xenobiotic compounds requires the high energy molecule adenosine 3′-phosphate 5′-phosphosulfate (PAPS). PAPS is synthesized from adenosine 5′-phosphosulfate (APS) and ATP by APS kinase. A major obstacle in the study of APS kinase has been the lack of a sensitive and convenient method to measure its activity. The present work describes a new procedure to measure APS kinase activity (a modification of the procedure of Robbins, Methods of Enzymology, 5, 964-977, 1962) that quantitates PAPS formation in vitro using a simple and sensitive method (Harrington et al., Drug Metab. Dispos., 13, 30-34, 1985). The assay was validated using cytosolic fraction prepared from liver homogenates and found to be linear with respect to time (0-10 min.) and tissue (0.78-3.13 mg liver tissue) and found to be optimal at Mn+ or Mg2+ concentrations of 2mM. PAPS, the product of the reaction, was found to be stable under the incubation conditions. Using this method, rat liver APS kinase activity was found to be 660±38 nmoles PAPS formed/min/g tissue and the specific activity was 2.63±.44 nmoles PAPS formed/min/mg protein. These data illustrate that rat liver has a high capacity to synthesize PAPS. (Supported by an MAF grant).

CARBON MONOXIDE-INDUCED INHIBITION OF AMINOPYRINE METABOLISM IN THE ISOLATED PERFUSED RABBIT LUNG. B.A. Trela, K.M. Johnson, P.R. Mayer and G.P. Carlson. Dept. of Pharmacology & Toxicology, Sch. of Pharmacy, Purdue University, West Lafayette, IN.

Although the inhibitory activity of carbon monoxide (CO) in drug metabolism has been established in vitro, its effect in intact organ systems has been less extensively studied. The aim of this investigation was to examine the effects of CO on xenobiotic metabolism in the intact lung since this organ, as opposed to the liver, is directly exposed to CO via the airways. Aminopyrine was used to evaluate xenobiotic metabolism in the isolated perfused rabbit lung. Aminopyrine and its metabolites, primarily 4-monomethylaminoantipyrine, were analyzed by HPLC. The lungs were exposed to air (control) or 7%, 1%, or 0.1% CO for 2.5 hours. When an artificial perfusion medium consisting of 4.5% bovine serum albumin and Krebs-Henseleit buffer containing 54 mg glucose/100 ml was utilized, aminopyrine metabolism was significantly inhibited only at the 7% CO level. When whole blood was used as the perfusate, the hemoglobin did not appear to protect the pulmonary drug xenobiotic metabolizing system from inhibition by binding the CO since aminopyrine metabolism was again inhibited at the 7% CO level. (Supported by NIH 30739 and ST3Z5E707039).
The large number of cytochrome P-450 isozymes present in rat liver microsomes presents a challenge to studying the effects of potential enzyme inactivators on individual cytochromes P-450. One approach is to monitor residual enzyme activity with a substrate which is metabolized by multiple isozymes, each at a specific site. For this purpose, we have used androstenedione since several laboratories have shown that hydroxylation of this substrate in each of four positions (7-alpha, 6-beta, 16-alpha, and 16-beta) can largely be attributed to a single cytochrome P-450 isozyme. The antibiotic chloramphenicol causes different rates of NADPH-dependent enzyme inactivation among the four hydroxylases (6-beta>16-beta>16-alpha>7-alpha).

Results with chloramphenicol analogs suggest that their selectivity as cytochrome P-450 inactivators is a function of at least three structural features: 1) the number of halogen atoms, 2) the presence of a para nitro group on the phenyl ring, and 3) substitutions on the ethyl side chain. For example, the compound N-(2-phenethyl) dichloroacetamide reversibly inhibits but does not inactivate the 6-beta hydroxylase, whereas N-(1,2-diphenethyl) dichloroacetamide rapidly inactivates the 6-beta hydroxylase. (Supported by NIH Grant ES 00151.)

A simple and sensitive method for the separation of carbon-14-labeled 4-hydroxycacetanilide, 3-hydroxyacetanilide, 2-hydroxyacetanilide and acetanilide was developed using thin layer chromatography. This separation is the basis for the assay of acetanilide hydroxylase activity. Microsomes from C57Bl/6NCrl and DBA/2NCrl male mice induced with isosafrole were incubated with [14C]acetanilide, acidified, extracted with ethyl acetate and applied to silica gel plates. The plates were developed with a hexane: isopropanol:ammonium hydroxide solvent system. The radioactive hydroxylated products and the parent compound were detected using a Berthold Automatic TLC-Linear Analyzer. Although the 4-hydroxylated compound was the primary product detected, this method can be used to detect other hydroxylated products. This method proved to be more sensitive than the colorimetric method of Mitoma and Udenfriend (1962) and more rapid than the HPLC method of Quentner et al. (1979).

The effects of safrole, isosafrole and dihydro-safrole on hepatic microsomal proteins of C57Bl/6 and DBA/2 mice were examined in DBA/2NCrl and C57Bl/6NCrl male mice after ip administration of 200 mg/kg/day for 3 days. Using gradient SDS-polyacrylamide gel electrophoresis, the microsomal proteins from the MDP-treated mice showed significant induction of a protein band with a molecular weight of 56,000 daltons which corresponds to a known cytochrome P-450 isozyme and an unknown protein with molecular weight of 27,000 daltons. The MDP pretreatments also induced total hepatic microsomal cytochrome P-450 and ethoxyresorufin-0-deethylase, acetanilide hydroxylase and ethylmorphine N-desethylase activities. However, benzo(a)pyrene hydroxylase activity was not induced by the MDP pretreatments. A type III metabolite-cytochrome P-450 complex was observed in all MDP pretreated microsomes. This type III spectrum can be displaced by incubating with butanol which increased some of the enzyme activities. Our results also demonstrated that the pattern of MDP induction was similar in C57Bl/6 and DBA/2 mice, indicating that the Ah receptor might not be involved in the mechanism of MDP induction of hepatic cytochrome P-450 in mice.


The organophosphate fenitrothion, a substrate for the cytochrome P-450 monooxygenase system, yields fenitrooxon by oxidative desulfuration (an activation reaction) and 4-nitro-m-cesol by oxidative dearylation (a detoxification reaction). Four constitutive cyt. P-450 isozymes and the major isozymes induced by phenobarbital and 3-NC were incubated with 14C-fenitrothion in reconstituted monoxygenase systems. Both metabolites were formed with all isozymes, but the ratio of products was considerably different with the different isozymes. Cyt. P-450 B2 and PB formed the highest percentage of oxon, 75% and 82%, respectively; while P-450 A1, B3, and 3-NC formed only 55% to 60% oxon. By contrast, P-450 A2 was primarily a cresol producer, with 62% of the product being 4-nitro m-cresol. Pretreatment of mice with fenitrothion is known to inhibit the subsequent microsomal metabolism of fenitrothion in vitro. Presumably, inhibition is due to destruction of cyt. P-450 caused by release of active sulfen occurring during desulfuration. Oxon formation is inhibited more than cresol formation suggesting preferential inhibition of P-450 isozymes which are high oxon producers. Fenitrothion bioactivation is therefore inhibited more than detoxication.
Cocaine-induced hepatotoxicity was examined in vivo in a dose response manner in C57BL, DBA, C3H and Balb mice. Cocaine (20-100 mg/Kg) was given ip and serum glutamic-pyruvic transaminase (SGPT) activities were determined 24 hours post injection. Significant elevations (100-150 fold) in SGPT were observed in male mice receiving cocaine. ED50 values for this response in males ranged from 40 mg/Kg for DBA to 80 mg/Kg for Balb. Female mice displayed significant resistance to cocaine-induced hepatotoxicity as indicated by only 2-10 fold elevations in SGPT values. The ED50 values (40-50 mg/Kg) for SGPT elevations in female mice were very similar among the four inbred strains. Phenobarbital pretreatment did not significantly alter SGPT elevations in male mice but increased female SGPT activities to values comparable with males. This suggests that the cytochrome P-450 isozyme responsible for activation of cocaine to a toxic metabolite is differentially active among inbred strains of male mice. Further, the data suggest that phenobarbital pretreatment induces the activity of this isozyme in female, but not in male mice. (Grant support: NIH AM49141)

Mechanisms of Toxicity of Cocaine in Hepatocytes Isolated from Mouse and Rat. D. Ross, A.J. Sadler, D.A. Donnelly, C.S. Boyer and D.R. Petersen. Molecular and Environmental Toxicology Program, School of Pharmacy, University of Colorado, Boulder, CO

Cocaine is hepatotoxic to both animals and man. The mechanism underlying cocaine-induced hepatotoxicity is unclear and no evidence exists regarding the cellular toxicity of cocaine. We have examined cocaine-induced biochemical changes and toxicity in hepatocytes isolated from both mouse (C3H) and rat (Sprague-Dawley). Cocaine induced depletion of reduced glutathione (GSH) in cells isolated from both mouse and rat and this could be accounted for in part by generation of oxidized glutathione (GSSG). Changes in cellular thiol redox status occurred at the lowest dose of cocaine tested (0.1 mg/ml). These results do not preclude the possible involvement of alkylation reactions in cocaine-induced thiol depletion and toxicity. Cocaine-induced cytotoxicity was accompanied by small amounts of lipid peroxidation (LPO) as measured by malondialdehyde production. Complete inhibition of LPO utilizing antioxidants, however, had no effect on cocaine-induced cytotoxicity. These data suggest that at the cellular level oxidative mechanisms may play a role in cocaine-induced cytotoxicity, but that LPO is not a critical determinant of toxicity.


We have observed that rat hepatic, cytosolic, cationic and anionic proteins with GSH S-transferase (GT) activity also catalyze defluorination [1], in disagreement with mice studies [2,3]. Therefore, hepatic cytosolic proteins from male CFW Swiss mice were separated on DEAE Sephadex A-50 and GSH-affinity columns and fractions were analyzed for GT activity and "defluorinase" (defl) activity measured with methoxyflurane (M0F) and fluoracetate (FAc). The majority of defl activities were catalyzed by anionic proteins that also catalyzed GT activity. Minor defl activity coeluted with cationic GTs. GT activity of anionic proteins was not separated from defl activities by affinity column. 17% and 36% of FAc and M0F defl activities, respectively, were not bound. Rates of defl were similar to those seen previously [1,2]. As with rats [1], the majority of defl activities were associated with anionic proteins exhibiting GT activity and adsorbed to the affinity column. Differences in total protein and in concentrations used could explain the discrepancy between our work and other mouse studies [2,3]. Thus, GTs appear to catalyze defluorinations. (Supported NIH GMS 22746 and VA)


Dose Differentiated Induction of Drug Metabolizing Enzymes by Clofiramozole. J. K. Ritter and M. B. Franklin. Dept. Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT

The dose dependence of the high magnitude induction of hepatic cytochrome P-450 (up to 4 nmoi/mg microsomal protein) and other drug metabolizing enzyme activities by the N-substituted imidazole, clomirazole, was examined. Clomirazole was administered (i.g.) in three daily doses ranging from 15 to 90 mg/kg to male rats. Whereas cytochrome P-450 increased only 50% with doses up to 45 mg/kg, small increments in dose above this resulted in up to 350% induction. The range and extent of oxidative and conjugative enzyme activities induced differed between low dose (15-45 mg/kg) and high dose (45-50 mg/kg). The low dose range response was characterized by increased p-nitroanisole demethylase (350%) and aniline p-hydroxylase (175%) activities. In contrast, the high dose range response was characterized by elevations in ethylmorphine (400%) and erythromycin (100%) demethylase activities and the extent of metabolic intermediate complex formation from troleandomycin (110%). The high dose range response was also associated with little or no additional increase in p-nitroanisole O-demethylase activity and a decline of aniline p-hydroxylase activity toward control values. UDP-Glucuronosyltransferase activity towards morphine was induced only by lower doses of clomirazole in a similar manner to p-nitroanisole demethylase activity. Glucuronidation rates of other aglycones, 1-naphthol, testosterone, and estrone were not induced by clomirazole.

The selectivity of induction of hepatic microsomal drug metabolizing enzymes, as demonstrated here with cytochrome P-450, may differ depending on the dose.

217

Benzene is a hemopoietic toxin which, on chronic exposure, causes aplastic anemia. Benzene is also a carcinogen associated with acute myelogenous leukemia and some of its variants in humans and lymphomas and certain solid tumors in rodents. Bioactivation of benzene is required for toxicity. We have shown that rat liver mitochondria, stripped of outer membrane to avoid microsomal contamination (mitoplasts), metabolize benzene in an NADPH-dependent reaction to phenol and secondary metabolites capable of covalent binding to mtDNA. We report here on the purification of a cytochrome P-450 which converts benzene to phenol. The activity has been solublized with 0.4% sodium cholate and purified 23-fold by polyethylene glycol fractionation. The production of phenol from benzene by the 5-15% polyethylene glycol fraction, as demonstrated by HPLC, requires NADPH and is completely dependent on bovine adrenodoxin/adrenodoxin reductase. Adrenodoxin does not support microsomal benzene metabolism, thus providing evidence that the benzene hydroxylase is indeed of mitochondrial origin. (Supported by NIHES grant ES02931 and a fellowship from the Percival E. and Ethyl Brown Fordegerer Foundation).


A C18 reversed-phase HPLC assay has been developed which quantitatively separates the known oxidative metabolites of aflatoxin B1 (AFB1, AFB2, AFD1, AFD2, AFB, aflatoxicol) and AFB-GSH. The ternary solvent system used consists of 0.05 M ammonium acetate (A), pH 3.4, 4% methanol : 66% THF (B) and water (C). Column temperature is maintained at 40°C C and a flow rate of 1.5 ml/min is used. Baseline resolution of all peaks and AFD (internal standard) is accomplished with a gradient program over 20 minutes. Production of hydroxylated metabolites is linear over 10 minutes and with increasing amounts of microsomal protein. To measure GST activity toward AFB, it is necessary to generate excess epoxide substrate in vitro. This was accomplished by using microsome obtained from mice pretreated with 500 mg/kg/day BHA incorporated in the diet. By appropriate dilution of cytost, AFB-GSH formation could be measured such that production was linear over 10 minutes, and with added cytost protein until the production of AFB-GSH 60 exceeded production of residual dihydrodiol. GST activity toward AFB-epoxide could be readily measured as low as 0.5 ml of mouse cytost. Time course studies of microsome formation of a new polar hydroxylated metabolite of AFB. Mass spectral analysis yielded a mass of 314, consistent with an O-demethylated and hydroxylated metabolite. Remethylation of the phenolic hydroxyl group with diazomethane resulted in a metabolite identical to AFD1. Time course studies demonstrated that this metabolite is produced by hydroxylation of AFB. This demethyl AFB1 metabolite is excreted as a glucuronide conjugate in bile of rats, and this is formed in vivo. (Supported by USPHS Grant ES-03415).

IN VITRO INACTIVATION BY CHLORAMPHENICOL OF THE MAJOR PHENOBARBITAL-INDUCIBLE ISOZYME OF DOG LIVER CYTOCHROME P-450. L.J. Ciaccio, D.B. Duigan, and J.R. Helperi, Dept. of Pharmacology and Toxicology, University of Arizona, Tucson, AZ

Chloramphenicol (CAP) is a potent and efficient mechanism-based inactivator of the major phenobarbital (PB)-inducible isozyme of dog liver cytochrome P-450 (PB-2). In a reconstituted system containing PB-2, CAP causes a time- and NADPH-dependent irreversible loss of 7-ethoxycoumarin deethylase activity, with no loss of spectrally detectable cytochrome P-450. Inactivation is enhanced by cytochrome b5, and in the presence of b5 the concentration of CAP at which the rate of inactivation is half maximal (Kb) and the maximal rate of inactivation (kb) are 5 μM and 1.2 min⁻¹, respectively. CAP binds covalently to PB-2 with a stoichiometry of 1 nmol (14C) CAP bound/nmol P-450 inactivated. CAP is a possible selective inactivator of PB-2. In intact microsomes from PB-treated dogs, CAP irreversibly inhibits androstenedione 16-alpha and 16-beta but not 6-beta hydroxylase. (14C) CAP covalent binding to dog liver microsomes is increased 5.5 times by PB induction. This increase correlates well with the increased levels of immunocohemically determined PB-2 (5.8 fold) and 16-alpha and 16-beta hydroxylase of androstenedione (3.7 and 3.8 fold) in PB versus control microsomes. In addition, anti-PB-2 IgG inhibits 90% the covalent binding of (14C) CAP to control and PB microsomes. (Supported by ES00151 and ES03619).


Aflatoxin B1 (AFB1), the major fungal metabolite of Aspergillus flavus and A. parasiticus, is mutagenic, teratogenic and carcinogenic. Chlorine gas was used to determine its effectiveness in destroying AFB1. Time course study of this treatment (100% AFB1 with 4 ml chlorine gas at standard temperature and pressure) showed that about 60 to 75% of AFB1 was destroyed within 10 min of exposure. During the treatment, at least three new fluorescent reaction products were produced and two of them were identified as 2,3-dichloro AFB1 and 2,3-dihydroxy AFB1 (dih). The use of radio-labeled AFB1 further confirmed the 5% destruction of parent AFB1 during 10 min. Chlorine-dose related study at 10 min exposure also indicated that even the treatment of 100 μg of AFB1 with 2 ml Cl2 caused about 75% destruction. Preliminary mutagenicity study using the Ames/ Salmonella assay indicated that the mutagenic activity of the treated sample in the presence of rat liver S-9 mix can be reduced to about 10% of the untreated control sample after 10 min. The results indicated that chlorine gas could be an effective agent in reducing aflatoxin toxicity.
OLTIPRAZ PROTECTS AGAINST THE HEPATOTOXICITY OF AFLATOXIN B1 (AFB1) IN THE RAT. Y.-L. Liu, B.D. Roebeck, J.D. Yager, J.D. Groopman, and T.H. Kensler. Dartmouth Medical School, Hanover, NH, Boston University School of Public Health, Boston, MA, Johns Hopkins University, Baltimore, MD.

Oltipraz, a 1,2-dithiol-3-thione, protects against the acute and chronic toxicity of AFB1. Male F344 rats were fed a purified diet with or without 0.075% oltipraz for 1 week prior to and during AFB1 treatment. Following single doses of AFB1 ranging from 25 μg to 400 μg per 100 g rat, acute toxicity was evaluated by presence of an hepatic enzyme in the serum and light microscopy. In subchronic studies, rats were subjected to 2/3 hepatectomy and the DNA prelabeled with 3H-thymidine during the subsequent hepatic regeneration. Two weeks later, control and oltipraz fed rats were gavaged with a tumorigenic regimen of 25 μg AFB1/100 g rat for 5 days/week for 2 weeks. Toxicity was assessed by determination of the rate of growth, liver weight, and loss of prelabeled DNA. In acute studies, oltipraz significantly reduced serum sorbitol dehydrogenase, the extent of hemorrhagic necrosis and bile duct proliferation. In the subchronic experiment, oltipraz dramatically prevented loss of body and liver weight associated with AFB1 treatment. Oltipraz protected against loss of prelabeled cells by greater than 75%. Taken together, oltipraz appears to offer a means to ameliorate the hepatotoxic effects of AFB1. Supported by NCI CA 39416, Biotrust, and AICR.

ROLE OF GLUTATHIONE (GSH) IN OLTIPRAZ (OTP)-INDUCED PROTECTION IN ACETAMINOPHEN (AAP) OR ALLYL ALCOHOL (AOH) HEPATOTOXICITY (HT) IN THE MALE HAMSTER. M.H. Davies, G.J. Schamber and R.C. Schnell, Deps. Pharmaceutical and Veterinary Science, North Dakota State University, Fargo, ND

GSH conjugation is involved in the disposition of both AOH and AAP; OTP (5-2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione markedly increases GSH content. GSH involvement in OTP-induced prevention of AOH- or AAP-induced HT was assessed in a 2x3 design. OTP (2.0 mmole/kg, po), buthionine sulfoximine (BSO, 9.5 mmole/kg, ip), AOH (1.0 mmole/kg, ip) AAP (2.6 mmole/kg, ip) or appropriate vehicle (VEH) were given at 0, 42 and 49 hr, respectively. HT was evaluated at 72 hr. BSO, a GSH synthesis inhibitor, lowered GSH levels (VEH 61% OTP 48%). Marked liver necrosis and elevated plasma enzymes were noted in groups dosed with VEH-VEH-AOH/AAP, while lethality (80%) resulted in animals receiving VEH-BSO-AOH/AAP. OTP-VEH-AOH/AAP afforded protection from HT. HT was potentiated by OTP-BSO-AOH, while HT in hamsters receiving OTP-BSO-AAP was ameliorated. OTP appears to be vital for prevention of AOH-induced HT, but OTP-induced protection in AAP-induced HT may be a function of other factors in AAP disposition (Supported by Sandoz Institute Fellowship (M40) and Burroughs-Wellcome Toxicology Scholar Award (RCS)).

AFLOTOXIN B1 HYDROXYLATION BY THE PCN-INDUCIBLE FORM OF RAT LIVER MICROSONAL CYTOCHROME P-450. M.R. Malvorson, S.H. Safe, A. Parkinson* and T.D. Phillips. Deps. of Veterinary Public Health and Physiology & Pharmacology, Texas A&M University, College Station, TX and *Kansas University Medical Center, Kansas City, KS.

The in vitro effects of various compounds which induce the pregnenolone-16α-carbonitrile (PCN)-inducible form of cytochrome P-450 (P-450) on the hepatic microsomal metabolism of aflatoxin B1 were investigated. Treatment of immature male rats with PCN resulted in a 6 fold increase in the formation of aflatoxin Q1 (AFQ1). Treatment of mature female rats with PCN resulted in a 16 fold increase in the formation of AFQ1. The age-dependent decline in constitutive cytochrome P-450 levels in female but not male rats resulted in a marked sex difference in the formation of AFQ1 (male/female=3.2) in liver microsomes from untreated rats. The formation of AFQ1 from AFBI was similar using microsomes from corn oil control or triacetyloleandomycin (TAO)-treated rats. However, the formation of AFQ1 from AFBI was selectively stimulated when liver microsomes from TAO-treated rats were treated with potassium ferricyanide, which dissociates the complex between cytochrome P-450 and TAO. Treatment of immature male rats with dexamethasone also increased the formation of AFQ1 (7 fold). The present results indicated that cytochrome P-450 selectively catalyzes the reaction of AFBI to AFQ1. (USDA Proj. 84CRS-2-2434.)

OTP-INDUCED PROTECTION IN ACETAMINOPHEN- OR ALLYL ALCOHOL-INDUCED HEPATOTOXICITY IN MALE HAMSTERS. M.H. Davies, G.J. Schamber and R.C. Schnell, Deps. Pharmaceutical and Veterinary Science, North Dakota State University, Fargo, ND

The ability of OTP (5-2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione to lessen acetaaminophen (AAP, 2.6 mmole/kg, ip) or allyl alcohol (AOH, 1.0 mmole/kg, ip)-induced hepatotoxicity (HT) was evaluated. OTP (2.0 mmole/kg, po) was gavaged either 48 hr prior to or 30 min following the toxin; HT expression was assessed 24 hr later. OTP pretreatment prevented marked elevations in plasma sorbitol dehydrogenase (SDH) and transaminases (ALT and AST), compared to animals receiving only AAP or AOH. The distribution and degree of hepatocellular necrosis agreed with plasma enzyme data. OTP, in an antiodotal role, did not alleviate AAP- or AOH-induced HT. However, OTP gavage after AAP administration did moderate AAP-induced HT. Biotransformation, via different pathways, is essential in the HT of both AAP and AOH, thus OTP may have multiple chemoprotective actions. (Supported by a Sandoz Institute Fellowship (M40) and a Burroughs-Wellcome Toxicology Scholar Award (RCS)).
EFFECT OF HEPATIC GLUTATHIONE (GSH) DEPLETION ON ACTIVATION OF INORGANIC SULFATE AND SULFATE ESTER FORMATION IN RATS. E. Gresus, C.A. White, S.R. Howell and C.D. Klagesm. Univ. of Kansas Medical Center, Kansas City, KS

Sulfation of organic compounds requires activation of inorganic sulfate via formation of 3'-phosphoadenosine 5'-phosphosulfate (PAPS). Inorganic sulfate can be formed by sulfoxidation of cysteine, which can be derived from GSH. Thus, a decrease in hepatic GSH may impair the synthesis of PAPS and the sulfation of chemicals. This hypothesis was tested by investigating the effect of GSH depletion on the levels of cysteine, inorganic sulfate and PAPS in liver or serum and the capacity to sulfate a xenobiotic. Phorone (2 mmol/kg, ip) decreased GSH (97%) and cysteine (83%) in liver, sulfate (63%) in serum and PAPS (48%) in liver. Both diethyl maleate and vinylidene chloride (6 mmol/kg each, ip) were less effective than phorone in decreasing GSH in liver and inorganic sulfate in serum, and did not alter hepatic PAPS levels. Three hr after phorone treatment, the nadir of hepatic PAPS concentration, harmol was injected in order to assess sulfation. Less harmol-sulfate and more harmol-glucuronide were found in the serum of phorone-treated rats than in controls. Phorone also reduced the biliary excretion of harmol sulfate while increasing the excretion of harmol glucuronide. These results indicate that severe GSH depletion decreases PAPS formation and sulfation of chemicals. However, an increase in glucuronidation may compensate for the impaired sulfation. (Supported by USPHS Grants ES-03192 and ES-07079)

INFLUENCE OF GSH DEPLETION ON THE UPTAKE OF ACRYLONITRILE (AN) VAPORS AND RESULTING THIOCYANATE (SCN) URINARY EXCRETION. D. Pilon and D.E. Rickert. CITI. RTP. NC

GSH appears to be an important factor in AN metabolism. We investigated the effect of GSH depletion on the uptake of AN vapor and the excretion of its urinary metabolite SCN after oral and inhalation administration. GSH was depleted in male Fisher-344 rats by combined Phorone/buthionine sulfoximine (PH/BSO) treatment. Rats were exposed to AN vapor at concentrations of 0, 25, 50, 100, 200. 500 and 750 ppm. AN uptake followed two distinct phases: a rapid and a slow phase. GSH depletion increased the uptake during the rapid phase for AN levels lower than 500 ppm. At both 500 and 750 ppm, rates were decreased compared to controls. During the slow phase, GSH depletion increased the rate of uptake at all AN concentrations. In a second set of experiments, rats received AN orally (4mg/kg) or by inhalation (95 ppm/27 min) with or without a PH/BSO treatment. SCN excretion in urine (24h) accounted for 27% of the total dose of inhaled AN but only for 17% after an oral dose. When GSH was depleted, the total amount of SCN excreted was increased 3-fold after administration of AN by either route and there was no longer a difference in urine SCN excretion by either route. This suggests that the difference in AN metabolism between routes of administration is dependent on the presence of GSH and that the formation of cyanide is greatly increased by GSH depletion. Partly supported by IRSSSTQ.

A SENSITIVE NONINVASIVE ASSAY TO DETERMINE ALTERED XENOBIOTIC METABOLISM BY GI MICROFLORA.

Chronic ingestion of environmental chemicals can lead to a selectively altered population of GI microflora. Chemical-microbial interactions are particularly important in young animals due to rapidly changing gut flora. To determine the magnitude and extent of chemical-microbial interaction as well as the ontogeny of microfloral enzyme activity in the developing animal, freshly voided feces are incubated with various substrates. Samples are collected by gently squeezing the rectal area of the animal and voiding the feces directly into sterilized flasks which have been evacuated and filled with CO2 three times. After addition of an anaerobic salt buffer, pH 7.0, evacuation and refilling the flask with CO2, substrate is introduced with a syringe through the rubber sleeve that seals the flask. Metabolites of substrates Y-hexachlorocyclohexane, p,p'-DDT, p-nitrophenyl-β-D-glucuronide, p-nitrophenyl sulfate, and 3,4-dichloronitrobenzene are analyzed by GC gas chromatography and are used to assess dechlorinase, dehydrochlorinase, β-glucuronidase, aryl sulfatase, and nitroreductase activity. The sensitivity of the analysis permits use of short incubation times, low substrate concentrations and small fecal samples. This is an abstract for a presentation and does not necessarily reflect EPA policy.


The AC/DC of benzidine (BZD) in vitro by hepatocytes and S9 liver preparations from rats and humans (organ donors) was studied to develop systems for predicting human metabolism of toxic xenobiotics. As observed in vivo, BZD was readily metabolized without added acetyl CoA (AcCoA), to monosacetyl- (MBZD) and diacetyl-benzidine by rat hepatocytes and to MBZD only with human hepatocytes during 4 hr incubations. The Km and Vmax for MBZD formation by human liver cells (N=3) were 10.4 µM and 36±20 pmol/min/10^6 cells. In contrast, essentially no metabolism of BZD was observed in human liver S9 without AcCoA. With AcCoA, BZD was very rapidly converted to MBZD, formation of which peaked at 9 min, followed by rapid DC and complete recovery of parent by 60 min. With other AC substrates studied, dapsone (DDS) behaved like BZD in human S9 incubations + AcCoA, but sulfamethazine was primarily acetylated with ≤ 1% DC in 2 hr. The relative rate of AC/DC of BZD and DDS could be altered by changing the S9 protein content or AcCoA concentration of the incubations. These results indicate that changes in the S9 incubation conditions can result in alterations in the balance between competing reactions whereas in isolated hepatocytes the relationships that exist in vivo are more likely to be maintained. (Supported by NIHES contract ES-55109).
Species differences in trichloroethylene (TCY) metabolism are being studied in human and rat hepatocyte suspensions. Hepatocytes were isolated by collagenase perfusion and incubated at 37°C for 4 hr in air-tight flasks with a center-well for TCY. TCY partitioned in the flasks with ratios of 3:1 and 12:1 (media:air) to produce noncytotoxic media concentrations of 0.2 mM and 1.7 mM. TCY and metabolites were analyzed by GC. TCY was metabolized by hepatocytes from 2 humans to 4 μM and 5.4 μM chloride (Cl) at 2 hr and about 5 μM at 4 hr, for both TCY concentrations, suggesting that this metabolic pathway is at steady state. Hepatocytes from one human formed 4 μM trichloroethanol (TCE) at 2 hr and 8 μM at 4 hr at both TCY concentrations, suggesting saturation of this pathway. In this liver these metabolites occurred in the same relative proportion. Hepatocytes from the other human produced 7 μM and 13 μM TCE at 2 hr from 0.2 mM and 1.7 mM TCY. Rat hepatocytes were more active, and neither Cl or TCE formation appeared saturated. They produced 8 mM Cl and 12 mM TCY from 0.2 mM TCY and 22 μM Cl and 28 μM TCE from 1.7 mM TCY. Glucuronidation of TCE accounted for only about 4.8% and 3.7% of the TCE in human hepatocytes, but 58% and 18% of the respective TCY concentrations in rat hepatocytes. (Supported by NIH contract ES-55109).

Investigating studies with primary hepatocytes use different incubation conditions. To optimize our research with human hepatocytes and compare results with those obtained by others, we studied benzo(a)pyrene (BAP) metabolism in rat hepatocytes in both suspension and attached to culture dishes. Hepatocytes were isolated by collagenase perfusion, and either added to glass reaction flasks, at 1 x 10^6 cells/ml or plated at 2.5 x 10^4 cells/60 mm dish. BAP (10 μM and 25 μM) or 50 μM was added immediately to suspensions or after a 2.5 hr or 24 hr attachment period. Incubations were at 37°C for 4 hr. Media samples were periodically taken for analysis of BAP metabolism and cytotoxicity. No significant differences in the rate of BAP metabolism or in cytotoxicity assessed by LDH release were found when results are calculated/10^6 viable cells but slight differences appear to exist/mg protein. Expressing results as % metabolism at 4 hr/10^6 viable cells, rat (N=3) hepatocytes metabolized 15% BAP at 10 μM, 13% at 25 μM, and 11% at 50 μM. In contrast, hepatocytes from 3 humans metabolized 1.8 ± 0.3% at both 10 μM and 25 μM. Hepatocytes of another human metabolized 8% of 10 μM and 7% of 25 μM HPLC metabolite profiles are being generated from these cultures. (Supported by NIH contract ES-55109).

To determine the activity of cytochrome P-450, glycuroxyl- and sulphotransferase in rat and human liver slices, 7EC, or its metabolite, 7HC, was incubated with slices of liver obtained from male Fischer 344 rats or patients undergoing liver resection. Storage of 7EC in Sacks' buffer at 3°C for 6 hr was investigated to determine whether the pathways of drug metabolism are functionally preserved. 25 μM and 100 μM doses of 7EC and 7HC were incubated in dynamic organ culture for up to 6 hr. Metabolite production was quantified using fluorescence spectrophotometry and conjugate formation identified using specific hydrolytic enzymes. The results indicated that rat and human liver slices, all three routes of metabolism (i.e., oxidation, glucuronidation and sulphation) were functional and comparable over the experimental time periods, and that the conjugation pathways possess more activity than the P-450-linked oxidations. Furthermore, the drug metabolizing activities were maintained when liver slices were stored in Sacks' buffer before tissue preparation. This study has demonstrated that the usefulness of this system to investigate cellular drug metabolism and to compare rates and routes of metabolism between species. (Supported by NCI-ES-55112.)
The demethylation of NDMA (2 mM), as measured by the production of formaldehyde (HCHO), was assayed in microsomes from untreated pigs and pigs given 1% ethanol, ad libitum, in the drinking water. All pigs were about 10 weeks old (sexually immature) and were numbered as follows: #1—untreated, female, 58 lb; #2—untreated, male, 54 lb; #3—female, 58 lb, administered ethanol for 1 week; #4—male, 53 lb, administered ethanol for 3 weeks. Microsomes from animals #1 and #2 catalyzed the production of 0.42 and 0.37 nmol HCHO/µg/min, respectively, a rate similar to microsomes from untreated rats (0.59 nmol HCHO/µg/min), but only half as much as microsomes from hamster livers (1.10 nmol HCHO/µg/min). The rate of demethylation of NDMA by liver microsomes from pig #3 (0.93 nmol HCHO/µg/min) was about twice that found with control microsomes. Demethylation by microsomes from pig #4 was 0.75 nmole HCHO/µg/min, suggesting that longer exposure to ethanol (3 weeks) did not result in any more enzyme induction.

Production of chloride (Cl) as a microsomal metabolite of chloropropanes was measured with a Cl-selective electrode. Sensitivity of measurement depended on minimizing endogenous Cl in the microsomes. Washed microsomes were prepared from phenobarbital-induced male F344 rats (170-195 g) using 50 mM Hepes/15 mM K2SO4. Incubation mixtures prepared with these microsomes, Mg2+, and an NADPH generator contained 2.5-3.5 mM Cl. These incubation mixtures catalyzed N-demethylation of benzphetamine (5.3 ± 0.21 mmol/min/mg) and dechlorination of 1,1-dichloroethane (7.6 mmol/min/mg) in agreement with literature values. Dechlorination of 50 mM 2-chloropropane and 1,2-dichloropropane in incubation mixtures containing 5 mg protein/ml was monitored continuously with the Cl electrode. Rates of Cl production were: 2-chloropropane, 1.9 and 1,2-dichloropropane, 2.0 mmol/min/mg. Concomitant with these oxidative dechlorinations is the formation of carbonyl carbon. In the case of 2-chloropropane, the carbonyl metabolite produced is acetone. Production of acetone was measured by formation of its dansyl hydrazide derivative followed by liquid chromatography with fluorescence detection. Rate of acetone production was similar to rate of Cl production, validating this technique for measurement of metabolically-produced chloride. (Supported by NIH ES 03911.)
ROLK OF QUINONE REDUCTASE (QR) IN IN VIVO ETHANOL (EtOH) METABOLISM. J.H. Chung and R.J. Rubin, Johns Hopkins University, Balt., MD

QR, in the presence of a suitable substrate, results in the generation of NAD+ from NADH. To test the hypothesis that QR can play a role in EtOH metabolism and toxicity, we have studied the effect of several quinones as well as of reduced levels of QR on EtOH administered in vivo to male rats. Butylated hydroxyanisole (BHA) is known to both induce QR and to be metabolized to t-butyl quinone (TBQ). Dietary BHA (0.75% for 10 days) , followed by oral EtOH (4 g/kg, by gavage), increased the rate of EtOH disappearance (ROD), and decreased both area under the curve (AUC) for blood EtOH and 24-hr hepatic triglyceride accumulation (HTA). TBQ (5 mg/kg, ip), although not significantly increasing ROD, led to decreased AUC and HTA. TBQ in BHA-fed rats resulted in greater effects on ROD and AUC than with TBQ or BHA alone. Another quinone substrate, menadione bisulfite (MEN-80 mg/kg sub Q), also resulted in a reduced AUC without a significant effect on ROD. However, when EtOH was administered iv, MEN treatment caused a significantly increased ROD (30%). In no case presented here did treatment result in a significant change in volume of distribution of EtOH. Thus, these results support the hypothesis that induction of QR and/or administration of quinones lead to enhanced in vivo metabolism of EtOH and decreased hepatotoxicity. Supported by NIH # RO1-AA06049.


Polyamines are important for stabilization of nucleic acid structure, cell growth, and proliferation. Some workers have shown the utility of polyamines to prevent chemical toxicity. Rat lung slices are capable of uptake of exogenously supplied polyamines. Studies on the uptake of polyamines by isolated, ventilated perfused rat lung are scanty. The objective of the present studies was to characterize the uptake and metabolism of polyamines in isolated, perfused ventilated lung preparations. Lungs were perfused using a constituted medium in which putrescine, spermidine and spermine were added at an initial concentration of 0.1, 0.5 or 5 mM. Perfusate samples were withdrawn at various time points and the lungs after 1 hr. perfusion were analysed. The uptake of polyamines by the isolated ventilated, perfused lung was linear (r = 0.99) for the three concentrations studied. The uptake was greater for putrescine followed by spermidine and spermine. The lung-to-perfusate ratio gave a linear plot indicating a greater tissue affinity for polyamines. Metabolic inhibitors like halamine (0.4 mM) and ouabain (0.2 mM) did not significantly affect the lung uptake of all three polyamines. (Supported by Miss. Lung Assoc.and HL-20622.)


The selective destruction of nigrostriatal dopaminergic neurons by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is dependent upon its oxidation to 1-methyl-4-phenylpyridine (MPP+) which is catalyzed by monoamine oxidase (MAO). A methylated analog of MPTP, 1-methyl-4-phenyl-2' (methylphénylamino) (2'CH3-MPTP) is a more potent nigrostriatal neurotoxin than MPTP in mice. HPLC analysis of brain extracts from C57 Bl mice treated with a single ip injection of [3H]MPTP or [3H]2'CH3-MPTP (20 mg free base/kg) indicates that 2'CH3-MPTP is converted to its corresponding pyridinium ion to a greater extent than is MPTP. The t1/2 for elimination is 160 min for 2'CH3-MPTP and 55 min for MPP+. Both the faster formation and slower clearance of 2'CH3-MPTP compared to MPP+ may contribute to the greater potency of 2'CH3-MPTP compared to MPTP. Nearly complete inhibition of MPP+ and 2'CH3-MPTP formation was achieved only by a combination of selective MAO-B and MAO-A inhibitors. Selective doses of MAO-B inhibitors protect against MPTP-induced neurotoxicity but fail to prevent 2'CH3-MPTP toxicity in mice. With 2'CH3-MPTP, but not with MPTP, it appears that the amount of MAO-A-mediated pyridinium formation is sufficient to produce neurotoxicity.

PHOSPHOROTHIONATE INSECTICIDE ACTIVATION BY RAT BRAIN MONOXYGENASES. C.S. Forsther and J.E. Chambers, Dept. of Biological Sciences, Mississippi State University, Mississippi State, MS.

Phosphorothionate insecticides are metabolized to their phosphate (oxon) analogs by microsomal monooxygenases. This activation produces a considerably more potent anticholinesterase; for example, the 150's for parathion and paraoxon for rat brain acetylcholinesterase (ACHE) are 4.4 x 10-5 M and 2.8 x 10-8 M, respectively. Since the brain's respiratory control center is an important target for organophosphate ACHE inhibitors, the activation of parathion and EPN was tested in microsomal preparations isolated from male and female brain regions (cerebral cortex, cerebellum, corpus striatum and medulla oblongata) and liver. Microsomes were incubated with the phosphorothionate and an NADPH-generating system; the resultant oxon was quantitated by its ability to inhibit exogenous ACHE. No difference in activation of parathion was observed between male and female brain microsomes, or among brain regions; microsomes from male liver were about 2.7 times more active than those from female liver. Liver, thus, demonstrated 6.6 and 2.9 times more activity than brain in males and females, respectively. Similar results were observed for the activation of EPN, although liver of both sexes activated less EPN than parathion. Even though brain monooxygenase activity is relatively low, it may be sufficient to contribute to symptoms of organophosphate poisoning.
A COMPARISON BETWEEN LIVER AND BRAIN PIG MICROSONES. M. Farage-Ellawar, B.H. Francis and E.H. Jeffery. Dept. of Veterinary Biosciences and Inst. for Environmental Studies, University of Illinois, Urbana, IL.

Levels of cytochrome P-450, cytochrome b5, NADPH-cytochrome c reductase and p-nitrophenol 0-deethylase (pNP) were evaluated in liver and brain of 3 to 5 month-old male pigs. Fifty mM Cysteine was added before homogenization of the brains, and spectral interference due to the high contamination of brain microsomes by mitochondrial cytochrome c oxidase was abolished by addition of 10 mM sodium succinate, 15 minutes prior to spectral determination. The amount of brain microsomal cytochrome b5 (nmole/mg microsomal protein) was 20% of the hepatic microsomal cytochrome b5 content. Similarly, NADPH-cytochrome c reductase/cytochrome P-450 ratio was four-fold larger in brain than in liver microsomes, and the ratio of cytochrome b5/cytochrome P-450 was twice as high in brain as in liver. Our results showed that, whereas brain contains much less cytochrome P-450 than the liver, the specific activity for pNP 0-deethylase was greater in brain [4.34 nmole/min/nmole cytochrome P-450] than in liver [2.8 nmole/min/nmole cytochrome P-450].

THE EFFECT OF ACUTE PHOSGENE EXPOSURE ON THE INFLUENZA PULMONARY CYTOTOXIC TLYMPHOCYTE RESPONSE. J.P. Ehrlich1 and G.R. Burleson.2

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Sponsor: J.A. Graham

The target organ of phosgene (carbonyl chloride) toxicity is the lung. There have been no previous studies on the effects of inhalation exposure to this toxic gas on local pulmonary cell-mediated immunity. This study was designed to investigate the effect of acute phosgene exposure on the pulmonary influenza virus-specific cytotoxic T lymphocyte (CTL) response in male Fischer 344 rats. Animals were infected with influenza/Port Chalmers/1/3H (H3N2) virus via intranasal administration 24 hr following acute exposure to 1.0 ppm phosgene or to clean filtered air for 4 hr. The influenza virus-specific CTL response was measured in whole-lung homogenate. The whole-lung homogenate was prepared as a single cell suspension by finely mincing lung tissue followed by collagenase digestion. The influenza virus-specific pulmonary CTL response is present 5 days after infection with a peak response occurring 7-10 days after infection. Acute exposure to phosgene resulted in a significant enhancement of the pulmonary CTL response 7 days post infection compared to air-exposed influenza infected rats. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

EFFECTS OF CYCLOSPORIN A (CYA) ON SUSCEPTIBILITY TO MURINE CYTOMEGALOVIRUS (MCMV) AND RELATED IMMUNE FUNCTION TESTS. M.J. Daniels,1 L.D. Lauer,2 J.H. Dean,3 and M.J.K. Selgrade.4 U.S. Environmental Protection Agency, Research Triangle Park, N.C. and Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

We examined the effects of CYA on susceptibility to MCMV, MCMV-augmented natural killer cell (NK) activity, assayed 5 days post infection (p.i.), and virus specific cytotoxic T cell-like (CTL) activity, assayed 9 days p.i. B6C3F1 mice were dosed with 50 mg CYA/kg/day i.p. for 5 consecutive days at various times relative to infection. Enhanced, virus-induced mortality (87% vs. 7%) and depressed NK (8% vs 46%) cytoly- sis and CTL (7% vs 12%) cytolyis) were seen in mice that received CYA on days 1-5 p.i. Mortality was also enhanced in mice treated with CYA 5-9 days p.i. (46% vs 29%), but there was no enhancement of mortality nor depression of NK in mice receiving CYA for 5 days immediately preceding infection. The study shows that CYA given 1-5 or 5-9 days p.i. affected susceptibility to MCMV and that CYA given 1-5 days p.i. also depressed virus-augmented NK and CTL responses. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

EFFECTS OF BENZ(α)PYRENE ON HOST RESISTANCE TO PLASMODIUM YOELII AND P. BERGHELI IN B6C3F1 MICE. M.M. Fount and S.G. Bradley. Dept. of Microbiology and Immunology, Medical College of VA/ Virginia Commonwealth University, Richmond, VA.

Two murine malaria parasites, Plasmodium berghei (PB) and P. yoelii 17XL (PY), are useful infectious models for assessing host resistance in immunotoxicologic studies. In the B6C3F1 mouse, the designated strain of the National Toxicology Program, PB produces a lethal infection and PY causes a self-limiting infection. The states of the humoral response and hemotopological system are important factors in host resistance. Mice that have recovered from a primary PY infection are resistant to rechallenge with PY or to challenge with PB. Subchronic exposure to 20 mg/kg and 40 mg/kg benzo(α)pyrene (BAP) has been shown to suppress the humoral response. Exposure to BAP also resulted in a prolonged, severe parasitemia with subsequent recovery in PY infected mice. The anemia was also more protracted in the BAP treated mice. Exposure to BAP did not alter the lethality of PB infection in mice, although parasitemia was severe in the highest dose group. Mice that were challenged with PY one month after the last BAP exposure had a severe, protracted parasitemia and anemia. Their eventual recovery, despite impaired humoral response, indicates that several factors are involved in host resistance to PY infections. (Supported by NIH ES55094)
MODULATION OF HOST RESISTANCE FOLLOWING INTRATRACHEAL INSTILLATION OF GALLIUM ARSENIDE (GaAs). H.H. Lysy, J.A. McCay, N.K. Snyder and W.L. White, Jr., Medical Coll. of VA/VCU, Richmond, VA.

GaAs is utilized in the production of semiconductor microcircuitry. Workplace exposure can result from inhalation of particulates during the manufacturing process. The objective of these studies was to determine if GaAs exposure altered host resistance to infectious organisms or tumors. GaAs was administered intratracheally at doses of 50, 100 or 200 mg/kg. The vehicle control group received an equal volume (100 µl) of 0.9% Tween 80 in saline. Fifteen days after a single exposure mice received a pathogen or tumor challenge. B6C3F1 female mice treated with the two highest doses of GaAs were less susceptible to Listeria monocytogenes (LM) infection than vehicle treated animals. A challenge with 5 x 10^5 CFU of LM produced 67% mortality among vehicle treated mice compared to 38%, 8%, and 5% mortality for GaAs treated mice. Susceptibility to Streptococcus pneumoniae was unaltered by GaAs exposure. GaAs mice challenged with 1 x 10^7 B16F10 tumor cells had a dose dependent increase in the number of tumor nodules. Clearance studies using radiolabeled B16F10 tumor cells demonstrated no differences among groups in the lung clearance of tumor cells on either 3 or 15 days after GaAs instillation. Preliminary studies suggest that GaAs exposure also decreases host resistance to the PYB6 fibrosarcoma tumor. (Supported by NIH ES 55094.)

IMMUNOTOXICOLOGICAL EVALUATION OF THE ANTIOXIDANT 4,4'-THIOBIS(2-N-Butylaminobenzenesulfonic Acid) (TBBC). K.L. White, Jr., D.L. Musgrove, J.A. McCay, M.D. Hollingsworth, Medical College of Virginia/VCU, Richmond, VA.

TBBC is a sulfur-containing antioxidant utilized in the plastic and rubber industries. As a result of its widespread use, there exists potential exposure occupationally and to the general population. Subchronic exposure for 14 days, by daily oral gavage at doses of 10, 100 or 200 mg/kg, produced significant alterations in immune function and host resistance in female B6C3F1 mice. TBBC decreased the IgM (44%) and IgG (48%) antibody forming cell response to SRBC, but had no effect on the delayed hypersensitivity response to KLH. The lymphoproliferative response to Con A, PHA and LPS was unaffected, while the MLR (44%) was significantly decreased. Increases in innate immunity included elevated complement CH50 (54%), NK activity (110%) and absolute number of neutrophils (177%). Bone marrow macrophage progenitors (CFU-M/M) were increased by 28%, while the increase in granulocyte-monocyte progenitors (CFU-GM/M) were (20%) did not reach significance. In host resistance assays, TBBC decreased resistance to challenge with Streptococcus pneumoniae and B16F10 melanoma, while decreasing resistance to the PYB6 fibrosarcoma. Host resistance to Listeria monocytogenes and herpes simplex virus (HSV-2) was not altered. (Supported by NIH ES 55094.)

EFFECT OF OZONE ON PULMONARY NATURAL KILLER CELL ACTIVITY. L.B. Fuller and D.R. Burleson, Northrop Services, Inc., Environmental Sciences, Sherburne, NC. Sponsor: J.A. Graham

Ozone is an oxidant gas and an ubiquitous air pollutant that causes an increased susceptibility to infectious diseases. There have been no previous studies of the effects of ozone exposure on local pulmonary cell-mediated immunity. Natural killer (NK) cell activity is an important first line of defense against viral and neoplastic disease. This study was designed to investigate the effect of acute ozone exposure on pulmonary NK cell activity. Pulmonary NK cell activity was measured using a single cell suspension of whole-lung homogenate prepared byincising lung tissue followed by collagenase digestion. Effector:target cell ratios of 100:1, 50:1, 25:1, 10:1, and 5:1 were used in a standard 4 h 51-chromium release assay. The YAC-1 cell line was labeled with 51-chromium and used as target cells. Fischer 344 male rats were exposed to 1.0 ppm ozone for 4 hr or to clean filtered air daily in Rochester exposure chambers. Pulmonary NK cell activity was not affected after ozone exposure for 1 or 3 days. However, NK activity in the lung was suppressed after exposure to ozone for 5 and 7 days. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

SPECIES COMPARISON OF IMMUNE FUNCTION ASSAYS COMMONLY INCLUDED IN IMMUNOTOXICOLOGY ASSESSMENTS. W.J. Murray, P.A. Melanson. The Froeter & Cemble Co., Miami Valley Labs, Cincinnati, OH

To compare mouse and rat models for use in immunotoxicity assessment, species variability in selected immune function assays was evaluated. Splenocytes from adult female B6C3F1 mice and F-344 rats were tested in parallel in cellular immune function assays measuring T and B cell mitogen stimulation, the mixed leukocyte response (MLR), and natural killer (NK) cell activity and also in the antibody plaque (AP) assay as a measurement of humoral immune function. Mitogen concentration vs. response curves were similar for mouse and rat splenocytes. The mouse splenic MLR was significantly higher than the rat MLR, while rat splenic NK activity was consistently higher than mouse NK activity. The peak IgM AP response to sheep erythrocytes occurred on day 4 in both species. The AP assay was also used to compare effects of the immunosuppres- sants, dexamethasone (Dx), in mice and rats receiving Dx (0.1-100 ug/day/mouse; 0.1-10 ug/day/rat) i.p. for 10 days. Numbers of AP/spleen decreased dose-dependently, up to 87% in mice and 96% in rats. Rats were more sensitive than mice to Dx-induced immunosuppression. These data suggest that while species differences exist in magnitude of immune responses and sensitivity to chemicals, either the mouse or rat model may be acceptable for identifying potential immunotoxicants.

Ochratoxin A (OA), a naturally occurring mycotoxin, has recently been shown to cause renal and hepatic carcinomas in mice. In the present studies, the effects of OA on immune mechanisms associated with tumor resistance were examined in mice using dose levels similar to those that cause neoplasia. OA was shown to specifically inhibit natural killer (NK) cell activity and increase the growth of transplantable tumor cells without altering T cell or macrophage-mediated anti-tumor activity. In contrast, ochratoxin B, a much less toxic ochratoxin, did not influence immune function. Poly I:C induced interferon was markedly reduced in mice following exposure to OA although total serum protein levels were slightly increased. Injection of poly I:C enhanced NK activity in the presence of OA, although the level of enhancement was slightly lower than produced by the agent in the absence of OA. Thus, ochratoxin appears to suppress NK cell activity by inhibiting production of basal interferon. Additionally, these findings suggest a possible role for altered NK cell function in the development of mycotoxin-induced carcinogenesis.

TARTRAZINE SPECIFIC REAGINIC ANTIBODY PRODUCTION: A DOSE RESPONSE STUDY. S. Nicklin, A.P. Hutchinson and K. Miller, BIBRA, Carshalton, UK. Sponsor G.G. Hard

We have previously demonstrated using UVG strain rats that the parenteral administration of a tartrazine/protein conjugate in association with carrageenan initiates transient tartrazine specific reaginic antibody production. In the present study UVG and BN rats received graded doses of ovalbumin-tartrazine conjugate plus carrageenan ip on d0 and d28. Serum was obtained weekly; total anti-tartrazine activity was determined by ELISA, reaginic antibody activity was assessed by passive cutaneous anaphylaxis (PCA). Both strains of rat initiated dose dependent anti-tartrazine antibody production. The reaginic response was inversely dose related and strain specific; the BN rats presented a strong PCA response which could be boosted whereas the equivalent UVG reaction was weak, transient and non-responsive to secondary challenge. These results provide some insight into the conditions governing reaginic antibody production and are clearly important for future development of animal models to examine the nature of adverse reactions reported to occur in susceptible individuals following the ingestion of food colours. (Supported by the UK Ministry of Agriculture Fisheries and Food.)

THE IN VITRO EFFECTS OF TRICHOThECENES ON THE IMMUNE SYSTEM. H.A.C. Atkinson and K. Miller, BIBRA, Carshalton, UK. Sponsor G.G. Hard

Trichothecenes, fungal toxins produced by Fusarium spp., have been implicated in a number of diseases in farm animals and man. The present study was undertaken in order to investigate the effects of low concentrations of deoxynivalenol, 3-acetyldeoxynivalenol, nivalenol and Fusarium-X on rat lymphocyte proliferation in vitro. Enhanced proliferation of PHA-activated lymphocytes was observed when cells were cultured in the presence of the trichothecenes at concentrations below 0.5 ng/ml. Incubation of macrophages with these compounds increased production levels of interleukin 1-like activity in the culture medium as demonstrated by a standard thymocyte co-stimulator assay. Similar results were obtained using the protein-synthesis inhibitor cycloheximide (CHX) which is known to interfere with the high-turnover-rate proteins that limit the translation of mRNA for interleukin. These results provide an explanation for the increased proliferative response seen in rat lymphocyte cultures, and suggest that in vivo effects of trichothecenes on the immune system may vary according to the level of exposure. (Supported by the UK Ministry of Agriculture Fisheries and Food.)

EARLY THYMIC EFFECTS OF DIOCYLTLIN DICHLORIDE (DOTC) AT A CELLULAR LEVEL. S. Volsen and K. Miller, BIBRA, Carshalton, UK. Sponsor G.G. Hard

Previous studies by us have shown that oral administration of diocyltin dichloride (DOTC) to rats resulted in suppression of thymocyte proliferation in vitro shortly after treatment. We now demonstrate, using discontinuous gradient separation, that thymocytes with the highest rate of DNA synthesis are found in the bands of intermediate density, and that one specific antigen bearing population of thymocytes is affected by DOTC treatment. The basic design of the experiments was to separate thymocytes from DOTC treated and control rats over Percoll gradients and to characterise the cells in each band. Reduced DNA synthesis and depleted numbers of mitotic figures correlated with a significant reduction in the number of thymocytes which normally express the Class I MHC antigen defined by the monoclonal antibody MRK 0X18. MRK 0X18 predominantly stains medullary thymocytes, suggesting that these cells may be required to sustain proliferation of other thymocytes. These functional and phenotypic alterations were detected as early as 48 hours post treatment at a time when no histopathological changes were observed. (Supported by the UK Ministry of Agriculture Fisheries and Food.)
ACETAMINOPHEN-INDUCED IMMUNOSUPPRESSION OF THE MURINE IGM ANTI SHEEP ERYTHROCYTE (SRBC) RESPONSE IN VITRO. Ali A. Abdelrahman, Stephen D. Cohen and Richard A. DiPaolo, Section of Pharmacology and Toxicology, School of Pharmacy, University of Connecticut, Storrs, CT.

This study determined the effect of acetaminophen (APAP) on the murine immune system. Primary anti SRBC (i gm) response was generated by C57Bl/6 splenocytes (4x10^7) cultured for 5 days (Mishell-Dutton) in 20% fetal calf serum in media with 2x10^5 SRBC. The numbers of plaque forming cells and IgM titers were determined by Jerne Plaque and Complement Fixation Assays. APAP (1.5-6x10^-5M) caused immunosuppression (24-98%) in a dose-dependent fashion as measured by both assays. There was no difference in cell viability or yield between APAP treatments. Immunosuppression occurred within 24 hrs, independent of the time of APAP dosing (i.e. day 1-4). APAP @ 6, 3, and 1x10^-5M resulted in 100% immunosuppression and a 20% decrease in cell viability and yield. Thus, short-term exposure of APAP is immunosuppressive for the murine IgM anti SRBC response in vitro.

Supported in part by NIHES Grant ES02524.

SYSTEMIC IMMUNE MODULATION PRODUCED BY INTRATRACHEAL INSTILLATION OF GALLIUM ARSENIDE (GaAs). J. A. McCoy, M.P. Holsapple S.G. Bradley and A.E. Manson. Medical College of Virginia/VCU, Richmond, VA.

GaAs was administered to B6C3F1 female mice by intratracheal instillation at doses between 50 and 200 mg/kg in a 100 uL volume. A time course study over a 25 day experimental period was conducted. Body, liver, spleen, thymus, kidney and brain weights were not affected by 200 mg/kg GaAs. Lung weights were increased by as much as 200% and did not decrease over the 25 day observation period. There were no changes in selected hematological and serum chemistry parameters. 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 response to SRBC was suppressed dose dependently with i.v. immunization, but no effect on the lung associated lymph node IgM response occurred with i.t. immunization. The DTH response to KLH was suppressed dose dependently to a level of 20% of control. MLR was suppressed dose dependently to a level of 46% of control, suggesting the effect may be at the level of proliferation; however, the response to T and B cell mitogens was not suppressed by GaAs exposure. Spleen B cell number was increased in GaAs exposed mice. These results indicate that the proliferative response is intact and that the action of GaAs is prior to proliferation. 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 NIH ES 55094.)
The effects of carbon tetrachloride (CCl₄) on murine antibody responses, body and organ weights, splenic proliferative responses to mitogens, and serum parameters were determined following a 7 day ip dosing regimen of 500, 1000 and 1500 mg/kg. In vivo sensitization of CCl₄ treated mice resulted in a dose dependent suppression of the T-dependent antibody response to SRBC at all doses, 36%, 48% and 55%, respectively. The T-independent antibody response to DNP-Ficoll was suppressive only at 1000 and 1500 mg/kg (27% and 32%). This dosing regimen resulted in increased liver weights and reduced thymus weights with no effect on spleen, kidney, lung and body weights. The serum chemistry profile indicated a dose dependent increase in serum SGPT levels (3300, 4500 and 4900-fold) and a non-dose dependent increase in bilirubin. Serum glucose, total protein and albumin levels were unaffected. Preliminary mitogen results indicated no effect on any concentration of LPS or the optimal concentration of Con A and a dose-related increase in sub-optimal concentration of Con A. Splenocytes from mice treated with 1500 mg/kg and sensitized in vivo demonstrated a comparable suppressed antibody response to SRBC and DNP-Ficoll as observed in vivo, 66% and 28%, respectively, and a control response to LPS and TNP-LPS. (Supported by NIH ES02564).

The effect of CsA on HgCl₂-induced autoreactivity was examined on ASW 2-month-old male mice. Each mouse received HgCl₂ in mg/kg sc. in the following schedule: 0.5 on days 1 and 2; 1.0 on day 3; 2.0 on day 6. In the first study 10 mice received only HgCl₂, and three groups (10 mice each) received CsA 5 mg/kg ip. daily for 8 weeks started 1 day prior to (group 2), concurrent with (group 3), or 1 week after HgCl₂ (group 4). In a second study 3 groups of 10 mice received the above HgCl₂ treatment. Group 5 received only HgCl₂ and groups 6 and 7 were treated with CsA 10 mg/kg as were groups 2 and 4, respectively. Mice were sacrificed 1 and 8 weeks after the end of CsA treatment in the first, and 4 weeks after the end of CsA in the second study. In all mice treated with HgCl₂, ANA developed and persisted. The GIC incidence per group of surviving mice was (1) 4/8, (2) 6/7, (3) 7/8, (4) 3/7, (5) 2/10, (6) 6/7, (7) 6/6 giving a total incidence in HgCl₂ only groups of 8/18 and in CsA treated groups, 31/34 (p=0.001). The results indicate that CsA did not inhibit the development of ANA and CsA increased the incidence of GIC deposition.

Administration of CY at low doses before antigen exposure results in an enhanced immune response. CY requires metabolic activation to the reactive phosphoramide mustard (PAM) and acrolein (AC) in order to produce its effects. The objective of this study was to determine which metabolite of CY mediates its immunoenhancing effects. Mice were administered acrolein (10, 30 and 100 umol/kg, i.v.) or CY (20 mg/kg, i.p.) and were sensitized with SRBC (i.v.) one day after drug exposure. The delayed-type hypersensitivity and the IgM antibody forming cell (AFC) response were augmented (150% of control) with AC (100 umol/kg) and CY. Exposure of naive splenocyte cultures to AC or 4-hydroperoxy-CY (4-HC; breaks down to the activated form of CY) for 1 hr prior to addition of SRBC to the cultures resulted in enhanced (145-160% of control) IgM AFC response (3x10⁶ and 10⁷ M), whereas PAM only produced an suppressive effect at higher doses (10⁷ M). These results suggest that the immunoenhancing effects of CY are mediated by acrolein. (Supported by ES55094 and ES05317).
MURINE POLYMORPHONUCLEAR NEUTROPHILS AS IMMUNOTOXICOLOGICAL TARGETS. M.F. Ackermann, K.R. Lamm, and M.I. Luster. Systemic Toxicology Branch, NTP, NIEHS, Research Triangle Park, NC.

Polymorphonuclear neutrophils (PMN) constitute the main cellular defense system of higher organisms against bacterial infections and have been implicated in mediator function and tumor cell destruction. Due to the lack of appropriate assay methodology, possible adverse effects of xenobiotics on PMN function have largely been ignored. We have devised a system which allows the recovery of large numbers of murine peritoneal PMN with purity between 85-90 percent. The PMN are resuspended in 0.5% bovine serum albumin (BSA) and injected intraperitoneal into mice. The recovered PMN are activated by low degranulation and superoxide production as well as virtually absent cytotoxic and cytostatic activity. However, they can readily be activated in vitro by various agents, thereby allowing studies assessing effects on PMN activation in vitro. This model could be helpful in the continuing effort to obtain a complete understanding of the effects of xenobiotics on the immune system.

ROLE OF METABOLISM IN DMN-INDUCED IMMUNOSUPPRESSION. D.H. Kim, K.N. Yang, K.W. Johnson, and M.P. Holsapple. KAIST, Seoul, Korea, and Medical College of Virginia/VCU, Richmond, VA.

In vitro immunosuppression by dimethylnitrosamine (DMN) was investigated by using a co-culture system consisting of primary hepatocytes (from B6C3F1 mice) as a metabolic activation system and splenocytes (from C57BL/6 mice) as target cells. DMN was not immunosuppressive when added to splenocytes alone (no hepatocytes), verifying the requirement for metabolic activation. The suppression by DMN was not produced when splenocytes were not allowed to contact with hepatocytes by 0.5% agar (<1 mm thick), indicating that cell to cell contact was required for the suppression by DMN. Radioactivity by 14C-DMN in TCA-ppt material in splenocytes was increased when co-cultured with hepatocytes. Aminocetonitrile (AAN) reversed the suppression by low concentrations of DMN (0.1 and 1.0 mM), but not by higher concentrations, in correlation with the degree of the inhibition of DMN demethylase activity by AAN. AAN also inhibited the binding of 14C-DMN to both hepatocytes and splenocytes. These results suggest that the suppression by DMN is mediated by the reactive intermediates released from hepatocytes. (Supported by NIH ES03564 and a Korea Science and Engineering Foundation Research Grant).


A concern in the development of new pharmaceuticals derived using recombinant DNA technology is the potential of the contaminant product or minor peptide contaminants to be antigenic under therapeutic conditions. Theoretically, immune responses to these antigens could interfere with the pharmacologic activity of the molecule or may contribute to autoimmune processes. In order to compare the antigenic potential of human growth hormone (hGH) preparations, groups of rhesus monkeys were treated with natural sequence biosynthetic human growth hormone (bio-hGH): 0.125, 0.375, 1.25 mg/kg daily for 37 days, 6 monkeys/group; methionyl human growth hormone (met-hGH), 0.045, 0.45 mg/kg, 3 times/wk for 91 days, 8 monkeys/group or pituitary-derived human growth hormone (pit-hGH), 0.1 IU, 1.0 IU/kg, 3 times/wk for 105 days, 8 monkeys/group. Serum samples were assayed for antibody to hGH and E. coli polypeptides and for immune complexes. Six of eight monkeys that received met-hGH and 4 of 8 monkeys that received pit-hGH demonstrated significant antibody responses by day 35 of treatment. None of the monkeys that received bio-hGH or control vehicle produced antibody to hGH. None of the animals produced antibody to E. coli polypeptides or developed immune complexes.

These data suggest that natural sequence bio-hGH may be less antigenic than met-hGH in the monkey. Confirmation in humans awaits clinical trial.

HUMORAL IMMUNOSUPPRESSION BY N-NITROSO COMPOUNDS: STRUCTURE ACTIVITY RELATIONSHIPS. N.E. Kaminski, S.D. Jordan and M.P. Holsapple. Department of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA.

Comparisons between changes in chemical structure of diethyl N-nitroso compounds and their ability to alter the day 4 IgM antibody response to sRBC, body weights, and organ weights in mice were investigated. Variations of the structurally simplest nitrosamine, Dimethylnitrosamine (DMN), by symmetrically lengthening the aliphatic chains resulted in a marked decrease in toxicity based on a mg/kg dose over a 7 day ip exposure regimen. Comparisons were made between DMN, Diethyl nitrosamine (DEN), Dipropynitrosamine (DPN) and Diisobutylnitrosamine (DBN). IC50 doses (i.e., the dose required to produce a 50% suppression of the control antibody response) for humoral suppression were 62 mM/kg DMN, 276 mM/kg DEN, 467 mM/kg DPN and 1557 mM/kg DBN. Dose dependant decreases in body weights, spleen weights and liver weights were observed with DEN, DPN and DBN. In contrast, DMN-treated animals demonstrated a marked increase in body weight due to ascites and increased liver and spleen weights, suggesting a different mechanism of toxicity from DEN, DPN and DBN. The non-symmetrical diethyl N-nitrosamine, Methylbutylnitrosamine (MBN), was the most toxic nitrosamine tested, with an IC50 of 47 mg/kg, and markedly decreased liver, spleen and body weights. (Supported by NIH ES03564).

The immunosuppressive capacity of benzo(a)pyrene (BaP) was examined as a function of aromatic hydrocarbon hydroxylase (AHH) inducibility and related to the ability of BaP to form covalent adducts with constituents of splenocyte surfaces. For the first objective, splenocytes from C57BL/6 (Ah') or DBA/2 (Ah') mice which express inducible or non-inducible AHH activity, respectively, were exposed to BaP at the initiation of culture with sheep red blood cells (SRBC). Five days later, direct anti-SRBC plaque forming cells (PFC) were enumerated and expressed as PFC/10^5 recovered cells. In a dose-dependent manner BaP (10^3 M to 10^3 M) suppressed (25-95%) the responses of C57BL/6 splenocytes, but not those of DBA/2 splenocytes. For detection of BaP adducts on cell surfaces, a monoclonal antibody (MAb; 3D1-G8) which reacts with BaP-diol epoxide derivatized proteins was produced and used in a flow cytometric analysis of C57BL/6 or DBA/2 splenocytes exposed for 48h to BaP (10^3 M) with or without the mitogen Con A. The 3D1-G8 MAb reacted with 20% of Con A/BaP treated C57BL/6 cells, but with less than 5% of similarly treated DBA/2 cells. Con A, BaP, benzo(a)pyrene, or vehicle treatment alone resulted in no 3D1-G8 binding above background. It is hypothesized that BaP adducts on Ah' splenocyte surfaces may be related to BaP-induced immunosuppression. (Supported by NIEHS Grant 04020).

920 HYDROQUINONE-INDUCED ALTERATION OF STROMAL CELL DEPENDENT B-LYMPHOPOIESIS. A.G. King, K.S. Landreth, and D. Wierda, Dept. of Pharm. & Tox. and Dept. of Microbiology, West Virginia University Medical Center, Morgantown, WV.

Benzone immunotoxicity occurs as a result of in vivo metabolism to polyhydroxy metabolites such as Hydroquinone (HQ). Previous investigations have shown that HQ inhibits B cell production within bone marrow cell cultures by preventing the maturation of pre-B cells into B cells (Sig^M). The present studies were initiated to determine whether the HQ-induced block in bone marrow pre-B cell maturation in vitro was mediated by toxicity to pre-B cells or to stromal cells which regulate B cell production. Pre-B cells were depleted of stromal cells by passage through Sephadex G-10 columns. Stromal cells were isolated by 24 hour adherence to culture dishes. Pre-B or stromal cells were then pulsed with HQ (10^-7 M) for one hour. HQ treated stroma could not support B cell production from untreated pre-B cells (25% of control). Untreated stroma supported pre-B cell maturation into Sig^M B cells (165% of control) even when pre-B cells were HQ treated. Our studies indicate that bone marrow stromal cells, rather than pre-B cells are selectively targeted by HQ. Our data also suggest that HQ may alter the production of stromal cell regulatory factors necessary for B-lymphopoiesis. Supported by NIOSH grant OH01542 and NIH AI23950.

921 THE EFFECT OF PARAOXON ON MURINE T AND B CELL PROLIFERATION IN VITRO. Steven Wood, Steven D. Cohen and R. A. DiCapua, Section of Pharmacology and Toxicology, University of Connecticut, Storrs, CT.

The organophosphate pesticide Paraaxon (Px) has been shown to suppress the murine in vivo anti-sheep red blood cell response. The in vitro response can also be suppressed in a dose dependent manner. The effect of Px on C57B1/6 splenocyte proliferation was measured with 10 ng/ml of lipopolysaccharide (LPS), a B cell stimulator or 1 mg/ml of concanavalin A (CONA), a T cell stimulator. Px was dissolved in culture media (RPMI 1640) and either LPS or CONA and cells added at time 0. The cells were incubated (37°C) for 48 hours, pulsed with 3H (luciferin) and harvested at 72 hours. B cell proliferation was inhibited only at 1x10^-4 M. Px. T cell proliferation was inhibited in a dose dependent range from 1x10^-4 to 1x10^-6 M. Px. The effect of Px on the mixed lymphocyte reaction (MLR) was also measured. C57B1/6 cells (H-2b) were mixed with immobilized (2000x) DBA cells (H-2d) at a 1:2 ratio and cultured as described above. Px suppressed the MLR in a dose dependent range from 1x10^-3 to 1x10^-6 M.

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Halothane induced immune response in a guinea pig model of halothane hepatitis. M. Stadat-Pajouh, T.P. Roth, A. Hubbard, A.J. Gandolfi, Dep. of Microbiology/Immunology and Anesthesiology, University of Arizona, Tucson, AZ.

A guinea pig model of halothane hepatitis explored the humoral immune response induced by halothane exposures and the potential role this response might play in contributing to liver damage. Guinea pigs were exposed to 1% halothane (21% or 80% O_2) every two wk for 4 wk. Blood samples were taken post exposure and evaluated for antibodies-cross reactive with trifluoroacetylated-guinea pig serum albumin (TFA-GPA) in an ELISA. The guinea pigs demonstrated on day 3 post exposure a halothane induced antibody that was cross-reactive with TFA-GPA. An increase in O_2 tension from 21% to 80% resulted in a 6 fold increase in halothane-induced antibody titers. In experiments to identify any halothane induced antigens, both halothane induced antibodies and specific anti-TFA-GPA detected a 13 k and 16 k antigen in liver protein from a halothane exposed guinea pig. Thus, multiple halothane exposures under a high O_2 tension induces an antibody in guinea pigs cross reactive with TFA-GPA. The generation of this antibody may be in response to the expression of halothane induced antigens. (NIH GM 34788)


The specificity of antibodies produced in halothane hepatitis patients (Ab_h) was studied using model haptens (reactive halothane metabolites) covalently linked by acetylation to the lysine residues of rabbit serum albumin (RSA). Quantitation was by ELISA and immunoblot techniques. Ab_h bound 5 times the quantity of trifluoroacetylated(TFA)-RSA than acetyl(AC)-RSA, indicating a preference for the trifluoroacetylated moiety. Use of a spacer arm, aminocaproic acid (ACA), in preparing the antigen increased the recognition of the haptens by Ab_h. The level of acetylation of the hapten to RSA was an important factor for recognition by Ab_h. Ab_h optimally recognize an antigen with 40% acetylation by TFA. The spacer arm allows for controlled acetylation of specific haptens and increased recognition of a specific hapten. The use of these synthetic antigens may aid in more closely mimicking the site and structure of the natural antigen responsible for the Ab_h response. (NIH GM 34788).
**CHRONOLOGY OF HALOTHANE-INDUCED ANTIGEN EXPRESSION IN HALOTHANE EXPOSED RABBITS.** TP Roth, O Khidir, S Schuman, AK Hubbard, AJ Gandolfi. Dept. of Anesthesiology, University of AZ, Tucson, AZ.

Multiple halothane exposures of rabbits induce an antibody (Ab) cross-reactive with trifluoroacetylated proteins. The generation of this antibody may be in response to expression of halothane-induced antigens (Ag). Male NZW rabbits were exposed to 1% halothane (80% O2) for 2 hr either once or 5 times at 2 wk intervals. Rabbits exposed once were sacrificed daily and their livers examined for immunogenic proteins by PAGE-Western analysis and detection with anti-TFA Ab. The peak Ag concentration appeared on the first day following exposure. Ag expression was highest after exposure 2 or 3. In multiple exposed animals Ag expression remained elevated for 4 days, but decreased by the 7th day post exposure. Five immunogenic proteins (85,3 k, 58.2, 53.2, 32.8 and 24.2 k) were consistently detected regardless of the number of exposures or day of sacrifice. These studies suggest that a similar series of Ags are produced by a single halothane exposure with peak levels on the 1st day or by multiple exposures with expression of the Ag for at least 4 days. (NIH GM 34788)

**CYTOFLUOROMETRIC ANALYSIS OF THYMIC AND SPLENIC LYMPHOCYTE POPULATIONS FOLLOWING ACUTE ADMINISTRATION OF O,O,S-TRIMETHYL PHOSPHOROTHIOATE.** K.E. Rodgers, D.D. Ellefson and C.F. Ware. Livingston Reproductive Biology Institute, USC Medical School, Los Angeles, CA. and Division of Biomedical Sciences, University of California, Riverside, CA.

O,O,S-trimethyl phosphorothioate has been previously shown to cause thymic atrophy in rats and mice. Therefore, the effect of acute administration of OOS-TMP on lymphocyte subpopulations in the thymus was examined. Three days following acute administration of 30 and 40 mg/kg OOS-TMP, there was a dramatic decrease in thymic lymphocyte number and in the percentage of remaining lymphocytes expressing T lymphocytes cell surface markers, such as Th1,2, L3T4 and Lyt2.2. In addition, the level of peanut agglutinin (a marker for immature thymocytes) binding on thymocytes was decreased. The basal and lymphokine-induced levels of proliferation of thymocytes were modulated following acute administration of OOS-TMP. The effect of OOS-TMP on splenic cell surface markers was also examined. At day 3 following treatment, the percentage of splenocytes expressing T cell markers was increased or decreased at a dose of 20 or 40 mg/kg OOS-TMP, respectively. In addition, at day 3 following administration of greater than 20 mg/kg OOS-TMP, the percentage of splenocytes expressing surface immunoglobulin was decreased.


Perioperative administration of nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and tolmetin, reduced postsurgical adhesion formation through mechanisms not yet understood. Since leukocytes are well known mediators of postsurgical repair in the peritoneal cavity, the effects of tolmetin on leukocyte function were determined. NSAIDs are known to inhibit the metabolism of arachidonic acid to prostaglandins which modulate a variety of macrophage functions. Studies presented here examine the effects of tolmetin administration on peritoneal resident and postsurgical leukocyte functions involved in the clearance of infection, such as phagocytosis (Pg), superoxide anion (O2-) release and tumoricidal (TC) activity. In nonsurgical controls, there was a elevation in O2- release and TC activity 24 hours after tolmetin administration. O2- release was suppressed at days 5 and 7 after treatment, but returned to control levels by day 14. Following surgery, administration of tolmetin significantly elevated O2- release at days 3 and 5, Pg at days 7 and 14 and TC activity at day 3. These studies indicate that compounds which suppress prostaglandin synthesis can modulate resident and postsurgical peritoneal cell function (HD 19002)

**WATER-SOLUBLE BENZENE METABOLITES IN F344/N RATS AND B6C3F1 MICE DURING AND FOLLOWING 1H-BENZENE INHALATION.** F.J. Sabourin, W. Bechtold, L. Birnbaum, G. Lucier and P. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM: NIEHS/NTP, Research Triangle Park, NC.

Benzene is a potent hematoxotoxin and is carcinogenic in both rodents and humans. Bone marrow, blood and urine concentrations of water-soluble benze metabolites were determined during and following inhalation exposure to 50 ppm for 6 h or 150 ppm for 2 h of 1H-benzene, F344/N rats and B6C3F1 mice. Water-soluble metabolites accounted for greater than 98% of the total metabolites. The area under the curve (AUC) of concentration vs. time was determined for each metabolite. In all cases, phenyl catechol sulfate were the major metabolites. Both species contained smaller amounts of muconic acid in the blood and urine. An unknown metabolite was present in appreciable amounts in rat but not mouse urine. Hydroquinone glucuronidation was high in the blood and bone marrow of mouse but not rat. Hydroquinone glucuronide and muconic acid were found in bone marrow of B6C3F1 mice, but not in rats. Results indicate species differences in metabolite profiles and that the rate of delivery of the "dose" affects the metabolites produced. (Research supported by NIEHS through IAA under U.S. DOE Contract No. DE-AC04-76EV01013.)
A COMPARATIVE IN VITRO EVALUATION OF FORMALDEHYDE METABOLISM IN THE EYE AND LIVER OF THE LONG-EVANS RAT. T. B. Moore and E. W. Lee, Biomedical Science Dept., General Motors Research Labs, Warren, MI.

The toxicity of methanol (M) is related to its metabolism to formate. The metabolism of formaldehyde (HCHO), an intermediate metabolite, was studied in the eye and liver of the Long-Evans rat. NAD-dependent HCHO oxidation, measured as the disappearance of HCHO, was analyzed by enzyme kinetics in respect to both total activity and the contribution from GSH-dependent formaldehyde dehydrogenase (FDH). Fresh homogenates of the two tissues in phosphate buffer, pH 7.4, were used to characterize the enzymes involved in HCHO oxidation. FDH activity had K_m values of 11.7 um (eye) and 12.7 um (liver). V_max values were 3.9 umoles/hr/g tissue (eye) and 25.3 umoles/hr/g tissue (liver). Km values for total HCHO metabolism were 306 um (eye) and 481 um (liver). V_max values were 28.2 umoles/hr/g tissue (eye) and 535 umoles/hr/g tissue (liver). HCHO metabolic capacity (tissue wt x V_max) for FDH was 0.6 umoles/hr (eye) and 352 umoles/hr (liver). The total metabolic capacity was 3.9 umoles/hr (eye) and 7.4 umoles/hr (liver). FDH was the enzyme which would most likely metabolize HCHO formed in either tissue due to its lower Km value. HCHO formed in the eye as well as in the liver after M exposure would likely be metabolized more rapidly than the oxidation of M to HCHO.

A COMPARATIVE IN VITRO EVALUATION OF METHANOL OXIDATION TO FORMALDEHYDE IN THE EYE AND LIVER OF THE LONG-EVANS RAT. T. B. Moore and E. W. Lee, Biomedical Science Dept., General Motors Research Labs, Warren, MI.

The toxicity of methanol (M) is related to its oxidation to formate. In conjunction with the development of a rodent model for M toxicity, the metabolism of M was studied in the eye and liver of the Long-Evans rat. Fresh homogenates of the two tissues in phosphate buffer, pH 7.4, were used to characterize the enzymes involved in the oxidation of M to formaldehyde (HCHO). No alcohol dehydrogenase-related metabolism of M was observed in either the eye or the liver. M was metabolized by both azide-sensitive catalase (A') and an azide insensitive alcohol oxidizing enzyme (A) in the liver and by the A' pathway in the eye. No A' metabolism was identifiable in the eye up to a 120 um M concentration. The A' activity had K_m values of 366 mm in the eye and 1.06 mm in the liver. The V_max values for the eye and the liver were 18.9 and 13.3 umoles/hr/g of tissue respectively. Tissue metabolic capacity of M (tissue wt x V_max) for the A' pathway was 2.7 umoles/hr in the eye and 255 umoles/hr in the liver. The A' activity in the liver had a Km value of 147 mm and a V_max of 30.8 umoles/hr/g of tissue with a metabolic capacity of 425 umoles/hr. The data show that A' is the only system active in the metabolism of M in the rat eye and that liver enzymes, A' and/or A, may contribute significantly to M oxidation to HCHO.


Human exposure to methanol occurs largely by inhalation of concentrations below those required to produce acute toxic effects. To characterize the pharmacokinetics of inhaled methanol and its metabolite, formate, three male rhesus monkeys were individually exposed to 0, 50, 200, 1200, and 2000 ppm methanol for 6 hr. These data were then compared to those previously collected from F-344 rats (The Toxicologist 6:229). The primate peak blood methanol concentrations were 7.7±3.5, 39.3±7.8, and 65.1±10.1 μg/ml following 200, 1200, and 2000 ppm, respectively, and were directly proportional to the vapor concentration. In contrast, the same exposure conditions in rats resulted in peak blood methanol concentrations that were not linearly proportional to the exposure concentration. Methanol was eliminated monoexponentially in both species. The primate half-life was 1200 and 2000 ppm were 3.5±0.6 hr and 3.5±0.5 hr, respectively, and were similar to the rat values. The 200 ppm exposure resulted in a shorter half-life, 2.4±0.3 hr. In both species, the exposures did not cause an elevation in blood formate concentrations that could be attributed to methanol metabolism. These results suggest the monkey and rat have similar capabilities for handling MeOH following low level inhalation exposure.

PHARMACOKINETICS AND BIOAVAILABILITY OF VITAMIN K IN DOGS. D.F. Gerken, R.A. Sams, G. Kociba, S. Ashcraft, and D. Brunseel. Ohio State University, Columbus, OH. Sponsor: V.L. Carter, Jr.

Brodifacoum is a "second generation" anticoagulant rodenticide which is more slowly eliminated than warfarin. Recommended therapy for treatment of brodifacoum toxicity in dogs consists of parenteral vitamin K followed by oral vitamin K. Results of human studies suggest incomplete (about 50%) bioavailability of oral vitamin K. Presently, there is very little information about the bioavailability of oral vitamin K in normal dogs or brodifacoum intoxicated dogs.

The objectives of this study were to determine the bioavailability of oral vitamin K in normal dogs and in fed and fasted brodifacoum intoxicated dogs. Six, normal, female dogs were administered intravenous and oral doses (3 mg/kg) of vitamin K. The dogs were then administered two oral doses of brodifacoum separated by 3 days. When OBT exceeded 40 seconds (within 2 days of the second brodifacoum dose), oral vitamin K was administered to fasted dogs and, after 24 hours to the same dogs with food. Blood samples collected after each vitamin K dose were analyzed for vitamin K and bioavailability was determined. Plasma vitamin K concentrations were determined by capillary gas chromatography with electron capture detection.

Little is known of the pharmacokinetics of drugs given to rodents via the diet despite the wide use of this dosing technique. Groups of rats were fed treated diets for 14 days which provided average daily calcium fenoprofen (F) doses of 38, 75, 134, or 245 mg/kg. The rats were killed (n=5/group) at 3-4 hr. intervals during the last 24 hours to determine feeding pattern and plasma concentrations of F. Plasma levels of F in each dose group were relatively constant during most if not the entire 24-hr. period; this correlated with the observation that food consumption occurred during most of the time intervals examined with nadirs around midnight and midday. Despite the linear relationship between dietary concentration and computed dose, the F peak plasma levels and AUC, appeared to reach a plateau at 134 mg/kg indicating that the systemic dose did not increase materially beyond this point. The plasma T1/2 for F at the end of 14 days on diet ranged from 6.8 to 9.0 hr. In a paired-dosing study, rats were given the equivalent low dose by single daily gavage for 14 days. By gavage, the peak plasma level of F on day 14 was ca 40% higher than that in rats given F in the diet; however, the AUC via the dietary route was ca 30% higher.

ABSORPTION OF BUTACHLOR IN RHESUS MONKEYS FOLLOWING TOPICAL ADMINISTRATION OF MACHETE® HERBICIDE. J.M. Kronenberg, and T.W. Fuhrenmann, Monsanto Company, P.O. Box 18110, St. Louis, MO 63164-1100, J.E. Johnson, International Research and Development Corp., Mattawan, MI.

The systemic dose of pesticides received by agricultural workers can be estimated using external skin deposition and/or biological monitoring techniques. These require knowledge of the dermal penetration rate or pharmacokinetics of the compound of interest. Rhesus monkeys are generally considered to be a good surrogate for man and were therefore used to assess the absorption and excretion profile of butachlor following topical administration of MACHETE herbicide. In a preliminary study, an average of 58% and 37% of the administered dose was recovered in the urine and feces, respectively, following intravenous administration of 14C-butachlor to rhesus monkeys. Granular and emulsifiable concentrate (EC) formulations of 14C-butachlor were then applied to the shaved abdomen for a 6 hour period. Urine and feces were collected for up to 20 days and analyzed for radioactivity. By comparing the amounts of radiolabel recovered in the excrata following intravenous and topical administrations, it was estimated that an average of 0.02% and 5% of the butachlor in the topically applied granular and EC formulations, respectively, were systemically absorbed.

PHARMACOKINETICS OF PROPETAMPHOS IN RATS. L.S. Silver AND W.C. Dauterman. Toxicology Program, N.C. State Univ., Raleigh, NC

Propetamphos, (E)-O-2-isopropoxy-carbonyl-1-methylvinyl O-methyl ethyl-phosphorodithioate is the active ingredient in the insecticide, Safrotime. Propetamphos is a cholinesterase inhibitor with a LD50 of 120 mg/kg (rat, oral). Practical and safe usage of this insecticide depends upon a knowledge of its pharmacokinetics and metabolism in animals.

The pharmacokinetics of propetamphos was investigated in vivo in the unanesthetized rat following rapid intravenous injection of a 12 mg/kg dose. A three compartmental pharmacokinetic model fit the experimental data for the time course of propetamphos concentration in plasma and was fitted to the C=Fe^-t + Ae^-t + Be^-t equation. Curve fitting was initially performed with a program C-STRIP, and was refined by the computer program NONLIN with a weighting procedure (1/C). The in vivo metabolism of propetamphos will be described in relationship to the pharmacokinetic model.

PHARMACOKINETICS OF RECOMBINANT HUMAN INTERFERON-\(\gamma\) (rIFN-\(\gamma\)) IDENTICAL IN SEQUENCE TO ENDOGENOUS IFN-\(\gamma\) EXCEPT FOR AN N-Terminal METHIONINE RESIDUE USED IN RHESUS MONKEYS TO SUPPORT ITS TOXICOLOGICAL EVALUATION IN THIS SPECIES. Intravenous (iv), intramuscular (im) and subcutaneous (sc) routes of administration were used in a crossover design. After 0.1 mg/kg iv, 6/11 animals showed a monoeponential decline of the serum levels: the half-life was 13.4±2.8 min. 5/11 animals showed biexponential decline; the half-lives were 12.3±2.3 and 130±66 min. Clearance (CL) was 18.7±9.0 ml/min/kg and the volume of distribution (Vd) was 510±465 ml/kg. The peak concentrations after 0.25 mg/kg rIFN-\(\gamma\) im and sc were 50.7±17.2 and 52.3±35.8 mg/ml, respectively. The observed concentration peak was at 480 min for the im and sc routes. The im and sc bioavailability were 92% and 90%, respectively. Administration of 0.25 mg/kg rIFN-\(\gamma\) iv caused no change in CL, half-life or Vd, suggesting that the kinetics of rIFN-\(\gamma\) are linear over this range of doses. Nearly all animals showed elevated antibodies to rIFN-\(\gamma\) after 3 doses at 2 week intervals (1 dose by each route), but the presence of non-neutralizing antibodies had no discernable effect on the calculated pharmacokinetic parameters.
PHARMACOKINETICS AND BIOAVAILABILITY OF GOSSYPOL IN MALE RATS. Mohamed A. Othman and Mohamed B. Abou-Dania. Duke University Medical Center, Durham, N.C.

The pharmacokinetics of (±)-gossypol were determined in male Sprague-Dawley rats following a single or 14 daily oral doses of 10 mg/kg in corn oil. Mean (± SE) oral bioavailability in rats was 11.7 ± 1.2%. The change in plasma (±)-gossypol concentration after a single or multiple dosing showed a biphasic pattern. A single oral dose of (±)-gossypol, however, was eliminated five times faster than the daily administered chemical. Thus, a single oral dose of (±)-gossypol was eliminated at the rapid rate of 0.042 hr⁻¹, corresponding to a half-life of 16.6 hr. The total plasma clearance (Cl), volume of distribution (Vd), and AUCplasma following a single oral dose of (±)-gossypol were 0.14 L/hr/kg, 3.3 L/kg, and 73.01 mg hr/L, respectively. On the other hand, 14 daily consecutive oral doses of (±)-gossypol were eliminated at a slower rate of 0.0087 hr⁻¹, corresponding to a half-life of 80.1 hr. Also, multiple dosing had the following pharmacokinetic parameters: Cl, 0.07 L/hr/kg; Vd, 7.9 L/kg; and AUCSS, 147.4 mg hr/L. The rapid elimination and short half-life of a single oral dose of (±)-gossypol compared to daily dosing are attributed to faster clearance rate and smaller volume of distribution following a single administration. Supported in part by World Health Organization Project 84040.

THE DIFFERENTIAL ELIMINATION OF 2,4,5,2',4',5'-HEXACHLOROBIPHENYL (6-Cl) FROM LACTATING MICE. L.A. Gallemburg and M.J. Vodticnik. Medical College of Wisconsin, Milwaukee, WI.

It has been suggested that highly lipophilic chemicals may be mobilized and eliminated relative to their time of sequestration. This was examined during the massive lipid mobilization associated with lactation. Female ICR mice received both unlabeled and 14C-6-Cl (2 mg/kg) i.v. The labeled dose was administered either to neonates (I) or to d15 pregnant animals (II) five weeks later. Thus, 14C identified the mobilization/elimination of either the first or second dose (I=14C, cold; II=cold, 14C). Disposition of II as a function of total dose was not different from that of I as determined from maternal tissue concentrations on d18 of gestation (e.g., parametrial fat: I=8.8±1.3%, II=11.4±1.1%). Litters were culled to 4 at delivery. The percentage of total dose II eliminated from maternal animals was significantly greater than dose I on d3 (I=18.7±2.9%; II=31.9±3.4%) and d5 (I=23.4±1.9%; II=30.9±2.2%) postpartum. The t1/2 of elimination for II was less than I from maternal parametral fat (I=18.8d; II=5.9d) and mammary gland (I=10.3d; II=5.5d) from d1-5 of lactation. The mobilization of the second dose of 6-Cl from maternal lipid depots and its elimination to suckling offspring was more rapid than that of the first 6-Cl dose. (Supported by ES 03493)

DISTRIBUTION AND ELIMINATION OF 4-ISOPROPYLBIPHENYL FROM THE RAT AND MOUSE. S.A. Carle, M.R. Roby, and D.E. Carter, Dept. of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

Oral administration of 14C-methyl 4-isopropylbiphenyl (4-IPB) to male Fischer 344 rats at doses ranging from 820 to 0.85 mg/kg (0.1 to 0.0001 LD50) resulted in dose-dependent distribution and excretion of radioactivity. The highest dose resulted in ulceration of the stomach and kidney congestion, with about 70% of the dose being eliminated in 72 hr. Decreasing dose levels exhibited increasing rates of both urinary and fecal elimination. Approximately 80% or more of the radioactivity was eliminated in 48 hr at the lower dose levels. An intravenous dose of 8.2 mg/kg 4-IPB was administered and tissues and excreta were examined for total radioactivity at 0.5, 1, 2, 4, 12, 24, and 48 hr (n=3). As was found for the orally-dosed animals, tissue levels of total radioactivity were highest in fat, muscle, skin and liver. Parent concentration was found to be highest in brain tissue at the initial time point (59 nmoles/g) and then in fat tissue at all subsequent time points. The percent of dose eliminated in the urine and feces was approximately 70% for the animals orally dosed at 8.2 mg/kg, suggesting the oral dose was well absorbed. An intravenous dose of 8.2 mg/kg 4-IPB was administered to male B6C3F1 mice. Elimination in mice was more rapid than rat with 70% of the dose found in the excreta of mice in 12 hr. The amount of dose in the feces plus intestinal contents at 12 hr was 22% for the mouse compared to 40% for the rat. Urine values were 56% in mouse and 26% in rats. (Supported by Electric Power Res. Inst. No. RP231004)

COMPARATIVE DISPOSITION OF ORALLY ADMINISTERED 4-VINYLCYCLOHEXENE (VCH) IN RATS AND MICE. B.J. Smith, M.R. Roby, and L.G. Sipes, Dept. of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

VCH is the major component in the gases discharged during rubber curing in the tire industry. When VCH was given chronically by gavage ovarian tumors developed in B6C3F1 mice but not F-344 rats. Disposition of VCH in female B6C3F1 mice and F-344 rats was investigated to determine if differences existed which might explain its species and target organ specificity. Animals were given a single dose of 400 mg/kg 14C-VCH in corn oil by gavage. Tissue distribution and elimination of 14C was determined at 1, 4, 8, 24, and 48 hr after administration. 14C-equivalents were excreted mainly in the urine and as expired organics in both species. No evidence for 14CO2 was obtained. Within 48 hr, rats and mice excreted 42% and 60% of total 14C in the urine, respectively. Both species exhaled a maximum of 30% of the dose by 8 hr. Rats and mice reached similar peak concentrations (1.2 nmo/l 14C/mg tissue) in the ovary 4 hr after dosing, however, after 4 hr, there was a more rapid decline in total 14C in the ovaries of mice. The data suggest distribution of VCH to the ovary does not explain the species difference in susceptibility. Studies are underway to determine the nature of the 14C that is retained by ovarian tissue. (Supported by ES-3-5031.)
PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR VINYLIDINE CHLORIDE. R.W. D'Souza and H.E. Andersen, Miami Valley Laboratories, Procter & Gamble Co., Cincinnati, OH and #AAMRL/TH, Wright Patterson AFB, OH; Sponsor: G.P. Daston.

A physiologically-based pharmacokinetic (PB-PK) model has been developed for vinylidine chloride (VDC). Simulations generated by the model offer insight into the behavior of VDC that would not be detected with compartmental modeling. Because of its low blood-air partition coefficient and saturable metabolism, the fraction of VDC dose that is metabolized or exhaled unchanged is dependent both on exposure dose and rate of absorption. Intravenous bolus studies cannot be used to elucidate the pharmacokinetics/metabolism of VDC as a major portion of VDC dose will be exhaled unchanged within a few minutes. Blood VDC half-life is not representative of metabolism, but is due to redistribution from fat to blood. A glutathione (GSH) depletion model is written as part of the PB-PK description and can be used to estimate the amount of intermediate epoxide that conjugates with GSH or binds to macromolecules. Simulations generated by the PB-PK model are consistent with literature data and have also been validated with appropriate experimental observations.

PHARMACOKINETIC MODELS FOR INDIRECT CARCINOGENS. R.H. Reitz, R.B. Conolly and M.E. Andersen. The Dow Chemical Company, Midland, MI; Northrop Services Inc., Dayton, OH; AAMRL/TH, Wright Patterson AFB, OH.

A number of "animal carcinogens" apparently act through indirect (nongenotoxic) mechanisms. Chemically-induced cell death followed by compensatory cellular regeneration (CCR) may be involved in the activity of these agents. To better understand this process, we have combined a two-stage carcinogenesis model (Moolgavkar and Knudson, JNCI 66:1037, [1981]) with a physiologically-based pharmacokinetic (PB-PK) model for chemical disposition (Ramsey and Andersen, TAP 73:159 [1984]). The model describes the uptake, distribution, and metabolic activation of chemical(s). Depletion of a hepatic macromolecule (MM) is correlated with bioactivation, and depletion of MM increases the probability of cell death. The population of liver cells is described dynamically, with spontaneous and chemically-induced rates of cell death and CCR. Simulation indicates that the population of cells with two mutations increases disproporionately with increasing chemical exposure and time. This work suggests that PB-PK models may be useful in exploring the relationship between chemical exposure, cytotoxicity, and tumor development.
The absorption, tissue distribution and excretion of \(^{14}C\)-4-NP in nulliparous rabbits were studied after a single oral dose of 10 mg/kg. After 72 hours, urinary excretion accounted for 82% of the dose and fecal excretion accounted for 5%. Major organs and carcass contained less than 1% and 2%, respectively. A similar pattern was seen in rats (Couch and Smith, 1986, Toxicologist 6, 282).

A comparative study of the pharmacokinetics of urinary elimination of \(^{14}C\)-4-NP equivalents was also undertaken. The elimination t1/2 was approximately 11 hours for nulliparous rats and rabbits and pregnant rats. Metabolites of \(^{14}C\)-4-NP in rats and rabbits were resolved as 12 radioactive peaks via reverse phase HPLC. The patterns for rats and rabbits were qualitatively similar but quantitatively different. Co-chromatography with nonradioabeled 4-NP showed essentially complete metabolism. \(^{14}C\)-4-NP is readily metabolized and excreted as water soluble metabolites in the urine of both rats and rabbits.

CITRAL: MODEL FOR TERPENE DISPOSITION IN RATS. J.J. Diliberto, G. Usha and L.S. Birnbaum. NIEHS, Research Triangle Park, NC

Citral (3,7-dimethyl-2,6-octadienal), an oxygenated monoterpene, occurs naturally in lemon and orange oils and is in many foods and beverages. Because of the high potential for human exposure and lack of knowledge concerning its toxicity, citral was nominated for toxicity testing. We have investigated the absorption, tissue distribution, and elimination of \(^{14}C\)-citral and P344 male rats. Animals were treated po with a single dose of 5,50 or 500 mg/kg and held in individual metabolism cages for 3 days. The major route of excretion was in the urine (54% of administered dose), but it was also eliminated to a significant extent in the feces (16%), via the lungs as \(^{14}C\)-CO\(_2\) (13%) and as unmetabolized citral (1%). At the end of 72 hrs, 4% remained in the carcass. Excretion of radioactivity after a single oral dose was rapid with no evidence of long-term storage in the body. Within 72 hours 84% of the oral low dose was absorbed and eliminated. The body burden was linear with increasing dose. These studies indicate the citral is readily absorbed, metabolized and excreted in the urine as water soluble metabolites. Fecal excretion suggests that the biliary route is involved and the rapid formation of \(^{14}C\)-CO\(_2\) implies that the aldehyde function is rapidly oxidized and decarboxylated. The rapid elimination suggests that significant bioaccumulation of citral would not occur.


PEA is widely used in fragrances. It is found in natural products including foods, spices, tobacco, wine and roses. In a study of the dermal absorption, distribution, metabolism, excretion and the influence of repeated dosing on these parameters, CD female and Long Evans male rats were dosed with 2-[\(^{14}C\)-]PEA by topical application. A dose (0.14 ml/kg) was applied to a 9cm\(^2\) area on the shaved back under occlusion for the duration of the experiment. A single dose was absorbed and rapidly excreted mainly in the urine (77.2%; 24 hrs). The amount excreted in the feces was 1.3% and 0.3% remained in the carcass. After 5 repeated daily applications, 63.4% of the cumulative dose was excreted in the 24 hr urine. Peak plasma concentrations occurred at 2 hrs. (mean value of 180 ug equiv./ml) indicating the applied dose was largely absorbed during this time. Concentrations declined rapidly (1.48 ug equiv./ml at 12 hrs). The initial half life was about 1 hr. Highest concentrations in all tissues of Long Evans rats occurred at 3 hrs. after a 6 hr. patch application. Kidneys, liver and fat contained the highest levels (87.5, 16.9 and 10.2 ug equiv./g resp.).
The disposition of three pyridine/phenyl ether (SC-1075, SC-1083, SC-1084) and one diphenyl ether (SC-1058) herbicide was determined in male CD rats. Each radiolabeled compound was administered to groups of 15 rats as a single, oral 50 mg/kg dose. Three rats from each group were sacrificed at 6, 24, 96, 240 and 360 hours. Plasma, whole blood, urine, feces, and various tissues were collected and analyzed for radioactive content. While the absorption of each compound was rapid, differences in excretion and disposition were noted. SC-1084 (t1/2-6h) was rapidly eliminated, primarily via the urine. At 15 days post-dosing, only 2% of the dose of SC-1084 had not been eliminated (total body burden). In contrast, SC-1075 (t1/2-12h) and SC-1083 (t1/2-12hours) were eliminated more slowly, with the majority of radioactivity being eliminated via the feces. Total body burden at 15 days for these 2 compounds were 19 & 18%, respectively. The diphenyl ether herbicide SC-1058 was eliminated most slowly (t1/2-255h). This compound was eliminated primarily via the feces and the total body burden at 15 days was 38%. These results suggest that variations in chemical structure can significantly alter the rate and route of elimination and total body burden.

The present study was designed to further investigate the unique sensitivity of the male rat kidney to d-limonene (DL) by determining both the NOEL and the temporal threshold for the compound's recognized nephrotoxic effects. Groups of male Fischer-344 rats were exposed to DL in a corn oil vehicle at 0, 2.5, 10, 30, or 75 mg/kg BW by single daily gavage (5 d/wk) for 91 days. Rats from selected dose groups were submitted to interim necropsy from 8-29 days while all groups were examined at the end of the study. Linear regression analyses indicated a dose-related trend for increased relative kidney and liver weights at 30 and 75 mg/kg. Histological examination of kidney tissue confirmed that DL induced a specific triad of events characterized by hyaline droplets, granular casts, and multiple cortical changes collectively classified as chronic nephrosis. The NOEL for these effects was 5 mg/kg while hyaline droplets formed as early as 8 days at the 75 mg/kg dose. These results indicate that even the lowest dose of DL administered in the recently completed NTP chronic bioassay (75 mg/kg) failed to meet the FDA's definition of an acceptable low dose level. Work from this laboratory on other compounds has questioned the relevancy of hyaline droplet formation for human risk assessment.

A novel enkephaline analog, SC-34871, was dissolved in sterile 3% dextrose at concentrations of 0.1, 0.5, and 5 mg free base/ml, and to assess irritancy, was injected once (1.0 ml) into the H. vastus lateralis of male New Zealand White rabbits (4/group). Venous blood samples were collected before dosing and 1,3,6,12,24,48, and 72 hours after dosing for determination of creatine phosphokinase (CPK) activity. Animals were sacrificed 72 hours after dosing and the muscles excised and grossly scored for irritation. Significant increases in CPK activity were seen 1,3, 5, 6, 12, and 24 hours following the 3 and 5 mg/ml doses, and 1, 6, and 12 hours following the 1 mg/ml dose. Peak CPK activity was seen 6 hours after the 5 mg/ml dose and 12 hours after the 1 and 3 mg/ml doses. Peak mean CPK activities were 941, 2998, 4283, and 6648 IU/ml for the 0.1, 0.5, and 5 mg/ml doses, respectively. At necropsy, the SC-34871 solutions were rated as marked irritants. In summary, SC-34871 (at 1 mg free base/ml and above) was an intramuscular irritant as evidenced by gross muscle necrosis and significant dose-related increases in CPK activity. This study demonstrates the marked irritancy of a derivative of an endogenous peptide and also illustrates the utility of CPK determinations in quantifying the muscular irritation of chemicals.


The acute oral toxicity of CI-944, a combination of CI-583 (meclonium) and CI-477 (codeine), and the individual components was assessed in mice and rats. The estimated median lethal dose (MLD) values in mice for CI-944, CI-583 and CI-477 were 320, 455, and 3155 mg/kg, respectively. Only gastrointestinal lesions following CI-583 administration were noted grossly. In rats, the estimated MLD values for CI-944, CI-583 and CI-477 were 560, 128, and 2230 mg/kg, respectively. Fibrinous peritonitis and/or intestinal ulcers were noted grossly at higher doses of CI-944 and CI-583. In a two-week study in rats, CI-944 was given orally at daily dose levels of 15, 30, 60, 125 and 250 mg/kg. Lethality was noted at 250 mg/kg and all animals given 125 mg/kg were sacrificed in extremis on Day 6. A decrease in red blood cell parameters was noted at 60 mg/kg and GI ulceration and visceral adhesions were noted at 125 and 250 mg/kg. A 13-week study in rats compared daily doses of 3, 10 and 35 mg/kg of CI-944 to 14.7 mg/kg of CI-583 and 8 mg/kg of CI-477. No drug-related effects were noted during the study.


Thrombocytopenia is a common adverse effect of antineoplastic drugs, but changes in mean platelet volume (MPV) and platelet distribution width (PDW) associated with thrombocytopenia in the rat are not well documented. Sprague-Dawley rats received single oral doses of busulfan (an alkyl sulfonate antineoplastic) at 0, 10, and 20 mg/kg (10 rats/sex/group). Venous blood was sampled 5 days before and 5, 12, and 19 days after dosing for platelet count, MCV, PDW, and other hematological parameters. Animals were necropsied, selected tissues examined histologically, and bone marrow smears prepared. Leukopenia was evident by day 5 and anemia was observed on days 12 and 19 in drug treated animals. Platelet counts decreased 22 and 91% in low and high-dose animals, respectively. Both MPV and PDW were increased 26-43% in high-dose animals and 10-21% in low-dose animals on day 12. Some recovery of platelet count and size was seen at both dosages by day 19. Histopathologic changes in drug-treated animals were consistent with exposure to a potent antineoplastic and the compensatory response to drug-induced thrombocytopenia in the rat is directed towards restoring platelet mass and function by increasing platelet size.

A 4-WEEK ORAL TOXICITY STUDY WITH ACETALDEHYDE AND FORMALDEHYDE IN RATS. H.P. TIL' R.A. WOUTERS, V.J. PERON and J.J. CLARY. TNO-CIVO Institutes, Zeist, The Netherlands and Celanese Corporation, New York, N.J.

A 4-week oral toxicity study with acetaldehyde and formaldehyde was carried out in rats. The aldehydes were administered via the drinking water; acetaldehyde at doses of 25, 125 and 675 mg/kg b.w./day and formaldehyde at doses of 5, 25 and 125 mg/kg b.w./day. Eight groups of 10 or 20 rats/sex/group were used. The controls received drinking water ad libitum. One of the groups was given an amount of drinking water equal to that of liquid consumed by the formaldehyde top-dose group. Food and liquid intake were decreased in the acetaldehyde and the formaldehyde top-dose group. Hyperkeratosis of the forestomach of top-dose rats was the only adverse effect of acetaldehyde. Changes attributable to formaldehyde were observed in the top-dose group only and included decreased protein and albumin levels in blood plasma, and thickening of the limiting ridge and hyperkeratosis in the forestomach. The number of variables in treated rats differed significantly from control variables but were comparable to those in rats receiving a restricted quantity of drinking water. It was concluded that the "no-toxic-effect levels" of acetaldehyde and formaldehyde in the present study were 125 and 25 mg/kg b.w./day, respectively.

The chronic toxicity of the new anti-convulsant zonisamide was evaluated in a detailed 52 week study in Beagle dogs at dose levels of 10, 30 and 75 mg/kg/day. The human therapeutic dose level is approximately 5-10 mg/kg. Zonisamide was well tolerated clinically. The only significant clinical laboratory change was a small decrease in plasma albumin concentration at the 75 mg/kg level. There were no significant histopathologic changes. However, the levels of high dose dogs were slightly enlarged and somewhat discoloured. Electron microscopic examination of livers from high dose male dogs revealed concentric lamellar bodies composed of smooth membranes which were not seen in controls. At the 10 and 30 mg/kg doses, plasma drug levels reached steady-state and were proportional to dose. At 75 mg/kg, plasma drug levels were disproportionately higher and never achieved steady-state, reaching concentrations approximately 10 times therapeutic levels after 52 weeks.


Phenylphrine HCl was incorporated into feed given to male and female F344/N rats and B6C3F1 mice in studies of 12 weeks and two years duration. In 12-week studies, body weight gains decreased with dose, and deaths of male rats and mice occurred at doses of 5,000 ppm and above, however, no organ-specific toxicity was evident. During two year studies, body weights of rats dosed at 0, 200 and 1,250 ppm and mice dosed at 1,250 and 2,500 ppm ranged from 97 to 85% of control. Survival of high dose male rats was signficantly greater than control (control, 30/50, low dose, 33/50, high dose, 42/50). Survival of other dose groups of rats and mice was similar to controls. Few nonneoplastic lesions were related to phenylphrine HCl dosing. Chronic focal inflammation of the liver, and inflammation of the prostrate were increased in dosed rats. No increases in neoplasms were observed in dosed rats or mice, and despite significantly greater survival of high dose male rats and only slightly lighter body weights than controls, mononuclear cell leukemia and adrenal pheochromocytomas occurred with significant negative trends in dosed male rats (leukemias; 24/25, 95/95, 5/50, historical control 27% ±9%; pheochromocytomas; 14/49, 11/50, 2/50, historical control 21% ±10%).


Geniant violet (hexamethyl-p-rosaniline) was fed ad libitum to Fischer 344 rats at dose levels of 0, 100, 300, and 600 ppm through three generations. The first generation was dosed for 80 days pre-mating, the second and third generation for 100-140 days. One male and one female from each of the second litters of each generation was used to perpetuate the next generation. A complete microscopic histopathology examination was done on two weanlings per sex per litter from the F3a generation. The geniant violet remained in the diet of each treatment group at all times. There were slight body weight and litter size reductions in the 600 ppm dose group. The stillborn animals and the deaths of pre-weanlings, while not consistent within an age group or in any of the generations, had higher incidences in the high dose group. The F3a, F2a, and F2b generations. There were no obvious effects or trends across doses or generations for fertility. The histopathology examination of the F3a weanlings showed no treatment related results. It is concluded that geniant violet administered in the diet continuously for three generations of rats had no markedly demonstrable effect except for a possible slight increase in mortality of pre-weanling pups.

MORPHOMETRIC ANALYSIS OF CULTURED HEPATOCYTES EXPOSED TO INDOMETHACIN. E.M.B. Sorensen. College of Pharmacy, Department of Pharmacology/Toxicology, University of Texas, Austin, TX.

Indomethacin, a nonsteroidal anti-inflammatory agent and potent inhibitor of cyclooxygenase, has been documented to cause liver dysfunction, especially in patients with hyperbilirubinemia secondary to rheumatoid arthritis or systemic lupus erythematosus. This study was undertaken to quantitate morphological changes in cultured liver cells exposed to indomethacin and to compare morphological assessments with standard assessments of functional integrity (i.e. enzyme release, urea levels, and dye exclusion). Primary cultures of rat hepatocytes were exposed for variable times to 0, 100, 500, or 1000 μM concentrations of indomethacin. After exposure, cells were preserved, embedded in plastic, sectioned, and subjected to double-blind morphometric analysis. This procedure systematically converts two-dimensional information into three-dimensional numerical data, which can be analyzed statistically. As the concentration of indomethacin was increased from 0 to 1000 μM, the relative percentage of Type I cells (indistinguishable from control cells) was reduced. For example, cultures exposed to 500 or 1000 μM indomethacin had significantly reduced percentages of Type I cells compared to controls exposed to lower concentrations of indomethacin. In contrast, the percentages of other progressively more damaged cell types (i.e. Types II, III, IV) increased as the concentration of indomethacin was increased. These morphometric results paralleled those obtained from functional assessments. Therefore, the use of morphometric techniques provided a good assessment of indomethacin-induced cytotoxicity and demonstrated the value of quantitative estimates in the evaluation of cellular injury.
AN IN VITRO MODEL FOR ASSESSING MUSCLE IRRITATION DUE TO PARENTERAL ANTIBIOTICS. P.D. Williams, R.C. Masters, L.D. Evans, D.A. Laska, and G.H. Hottendorf, Department of Experimental Toxicology, Bristol-Myers Company, Syracuse, NY. Sponsor: H. Madissac.

A rat skeletal muscle cell line (L6) was evaluated for its potential to discriminate the muscle irritating liability of several parenteral antibiotics. The cells were cultured in Medium 199 and exposed to clinical as well as diluted concentrations of cefoxitin, cephalothin, carbenicillin, tetracycline, erythromycin, ceforanide, ceftazolin and cephaloridine for one (1) hour. The cells were subsequently assayed for their content of the muscle-associated enzyme, creatine kinase (CK). Depletion of CK relative to control cultures was utilized as the index of cellular damage. The results of these analyses revealed the following ranking of antibiotic toxicity to L6 muscle cells: tetracycline, erythromycin, cefoxitin > cephalothin, carbenicillin > ceforanide, cefazolin > cephaloridine. The relative order of toxicity of these antibiotics to L6 cells is in good agreement with their reported muscle irritating liability in man. These results suggest that this in vitro model represents a useful adjunct to in vivo testing of parenteral antibiotics for muscle irritation liability.

IN VITRO NEUTRAL RED CYTOTOXICITY ASSAY WITH AN S-9 METABOLIC ACTIVATING SYSTEM. E. Borenfreund, Laboratory Animal Research Center, The Rockefeller University, NY, NY. Sponsor: D.M. Stark

Assays for in vitro cytotoxicity have not as yet been well explored. We developed a simple, semi-automated test, based on the binding of neutral red, a cationic supravital dye, to anionic sites in the lysosomal matrix of viable cells. Absorbance of extracted dye is proportional to the number of viable cells (Toxicology 39:121, 1986). To further simulate in vivo conditions, an aracotic-induced rat liver S-9 fraction was included into the assay procedure, to determine the effect of metabolic activation on the group of xenobiotics, such as pharmaceuticals, carcinogens and chemotherapeutic agents. 3T3 mouse fibroblasts seeded to 96-well microtiter plates were incubated for 18 hrs. with dilution of toxins in DMEM medium containing 2% FBS, with or without S-9 mix. Incubation was continued with fresh medium, containing neutral red. The lysosome bound dye was extracted and absorbance measured on a microtiter plate reader. Activated cytotoxic intermediates were found in one group of agents, a second showed reduced cytotoxicity due to detoxification and a third remained unaffected. The test is useful for screening of agents requiring metabolic conversion for activity and can aid in the design and modification of drugs and chemotherapeutic agents and in the selection of safe dosages for parenteral administration.

QUANTITATION OF EYE IRRITATION BY DRAIZE SCORE OR CORNEAL SWELLING AND CORRELATION WITH IN VITRO CYTOTOXICITY. M.E. Kennah II, D. Albulusce, S. Higmet and C.S. Barrow. RPS Industries, Inc. Environmental Sciences Center, Pittsburgh, PA.

For four decades, the Draize test has survived many criticisms to remain the accepted method for predicting eye irritation. The objectives of this study were (1) to determine if changes in corneal thickness would provide an alternative means of quantifying eye irritation and (2) develop an in vitro cytotoxicity assay and calibrate it with the in vivo eye irritation results. Corneal swelling and Draize scoring performed at 24 hours post-instillation of six surfactants (0.1 to 30%) and seven alcohols (1 to 100%). Eye irritation was dose-related and ranged from severe to inconsequential. Increases in corneal swelling were linearly correlated with the severity of eye irritation assessed by Draize scoring (r = 0.86). Cytotoxicity was determined from the decline in growth rate of BALB/3T3 cells. Linear regression of %growth rate inhibition versus log dose was used to calculate the volume-% of test chemical required to inhibit growth 50% (GI50). The in vitro GI50 values for surfactants ranged from 0.0013 to 0.578 volume-% while the alcohols were generally less cytotoxic with a range of 0.075 to 21.9 volume-%. These data were correlated with in vivo eye irritation. The correlations represent a promising experimental model for predicting in vivo eye irritation based upon in vitro cytotoxicity.
The skin is a primary site of chemical toxicity, yet only a limited characterization of the biochemical responses to dermatoxins has been achieved. Using the mouse model, we have found that topical treatment of skin with chemical toxins, such as dioxin and the phorbol ester tumor promoters, altered the synthesis of specific keratin proteins, reflecting disruptions in the normal tissue balance of growth and differentiation. Patterns of keratinization were analyzed by polyacrylamide gel electrophoresis after the pulse-labeling of excised skin fragments. The synthesis of proliferation-associated keratins (60, 54, and 52 kDa) was found to be increased in chemically treated epidermis, while keratins associated with differentiation (67 and 59 kDa) were decreased. The expression of these proteins provides a sensitive marker for the effects of chemicals on living epidermal cells. These markers may help to elucidate the various molecular mechanisms of toxicity induced by different chemicals.

We have previously demonstrated the enhancement of contact sensitization in mice fed a diet enriched in vitamin A acetate (VAA). Acrylic compounds and bisphosphonates are major causes of occupational allergic contact dermatitis, and this study compares the allergenic potential of a group of these substances in the 'VAA mouse model' and the guinea pig maximization test (GFT).

In the majority of cases results from both testing systems were equally efficient at predicting the sensitizing capacity of the chemicals, and corroborated well with clinical data. In both the mouse and the guinea pig tests the diacylates 1,6-hexanediyl diacylate (HDIA) and tetramethylene glycol diacylate (TGMA) gave very strong responses, whereas the dimethacrylate triethylene glycol dimethacrylate (TEGDMA) elicited a mild response. However, when 2-hydroxypropyl methacrylate (2-HPMA) and 2-hydroxyethyl acrylate (2-HEA) both monofunctional acrylates, as well as the biacrylate Grotan UK, were tested in the GFT erythematous skin reactions were produced in some control as well as test guinea pigs. This 'irritant' reaction was not evident in the 'VAA mouse model'. Sensitization can also occur due to impurities in commercial grade compounds, and three impurities isolated from triethylenetetramine as well as the parent compound were tested in the two systems. In the mouse, but not the guinea pig differences in the degree of response were evident.

(Sponsored by the U.K. Health and Safety Executive.


Despite adverse criticism the Draize test is of practical use in identifying eye irritants. In view of the potential of chemicals to cause a severe response in the eye it is important to approach chemical testing in a hierarchical, standard fashion. This should include the prior consideration of physical and chemical properties and results from dermal irritation and validated in-vitro tests. A review of approximately 1000 acute cutaneous studies revealed that pronounced primary irritation was observed in 24, 30, 13 and 33% of acute dermal systemic studies, rabbit skin, rat skin and rabbit eye irritation studies respectively, while severe responses were observed in only 11, 19, 6 and 7% of those studies respectively. When using the results of skin irritation tests to predict eye irritancy only 15% and 8% of rabbit and rat irritation studies respectively indicated comparable irritation, with 79 and 92% respectively of the studies under-predicting effects in the eye. As 6% of rabbit skin irritation studies over-predicted ocular effects, rat skin irritation data may be of greater value in predicting severe ocular effects.


Conventional single-exposure tests in animals have been of limited use in predicting the irritation potential of industrial petroleum-based products (cutting oils, base oils) following repeated dermal contact with human skin. The EGP Test (Vinson et al., unpub.) in which guinea pigs are wax-epilated 3 days prior to dosing, is a sensitive test for comparative mildness of cosmetic or household cleaning products at use levels; data correlate well with those of human arm immersion studies. In a modified EGP procedure, materials are applied via a Hill Top Chamber for 4-hrs/day for 4 days, with scoring of erythema at 24-hr intervals after each patch application. Reproducible responses to hydrophilic, lipophilic, and emulsified petroleum-related materials are obtained, with irritation increasing with concentration and the number of treatments. Hyperresponsiveness of the epilated animal coupled with a repeat exposure regimen enable fine discrimination among low-grade irritants. Data in hand agree well with human experience for similar materials. This procedure may be useful as a valid and sensitive predictor of the dermal effects of repeated contact with petroleum products in man.

242
DERMAL SENSITIZATION STUDY IN HAIRLESS GUINEA PIGS WITH DNCB AND BENZOCAINE. A. Chester, T. Terrell, L. Nave, L. DePass, Syntex Research, Palo Alto, CA.

This study was designed to investigate the use of adult female hairless guinea pigs, Charles River (CRL-1AFHAABR), as a model for dermal sensitization studies. These animals are euthymic and hairless except for continuous hair growth at the nose and feet. A modified Magnuson and Kilgman dermal sensitization procedure was followed. One group of Hartley and 7 group of hairless female guinea pigs were induced with 20% benzoic acid or 0.5% dinitrochlorobenzene (DNCB). Animals were first challenged with 10% benzoic acid or 0.5% DNCB. A second challenge was conducted with 5% benzoic acid and 0.1% DNCB. Compared to the Hartley guinea pigs, the hairless guinea pigs were more sensitive to the irritating effects of the compounds (supported histologically), had a greater incidence of sensitization to benzoic acid (90% vs. 50%; p < 0.05), and had a significantly stronger response to 10% but not 5% benzoic acid, corrected for baseline response. Advantages of the hairless guinea pigs included easier viewing of weak responses, fewer lesions due to the taping and shaving process, and fewer patches accidentally removed during the 24 hour exposure periods.

A NOVEL METHOD TO PREVENT A DERMAL DOSE FROM BECOMING AN ORAL DOSE IN RATS. M.C. Savides and J.P. Marciniszyn. Rica Inc., Palmersville OH

Information regarding dermal absorption is desired for many chemicals. In a typical dermal absorption study, the clipped back of a rodent is treated with test material and the material is left on the skin for a given length of time. After this time period, absorption through the skin is assessed. Although it is assumed that the only route of exposure is via the skin, oral exposure can occur due to the animal preening the dosed area. Although this undesired route of exposure has been documented to occur, many studies make no attempt to cover the treated area or to prevent the animal from preening this area. Mechanisms currently used to protect against oral exposure may not be of use for extended periods of time. One method involves the use of patches held in place with bandages, however, animals will often chew the bandages. Another protective mechanism involves the use of a "Queen Anne" collar, which may interfere with feeding when tunnel style feeders are used. A novel method is demonstrated in this poster which will prevent the animal from making contact with the dermally applied dose, yet not interfere with food consumption or the animal's comfort and mobility. The apparatus used consists of a screened template which is placed over the patched area of dosed skin. This template essentially lies flush with the rat's back.


Penetration of 14C-2,4,5,2',4',5'S-hexachloro-biphenyl (PCB) through skin of young (33 days) and adult (82 days) female Fischer 344 rats was determined in vivo and by two in vitro methods. In vivo dermal penetration at 120 hours was 45% in young and 63% in adults. As 72 hours in vivo dermal penetration was 35% in young and 25% in adults compared to 1.3% for young and 1.4% for adult as measured with a continuous flow in vitro system and 2.3% for young and 1.2% for adults as measured with a static in vitro system. Most of the absorbed PCB remained in the body as only 4.9% and 2.6% of this absorbed was excreted by young and adult rats, respectively, by 120 hours. The excretion of PCB into feces was approximately six times higher than into urine. A physiological pharmacokinetic model was fit to the organ and tissue distribution data. Parameters in the model determined from dermal dosing of female Fischer 344 rats were in reasonable agreement with those obtained from i.v. dosing of adult male Sprague Dawley rats. Differences in the model parameters between young and adult rats were found. The rate constant for dermal penetration was 0.83 x 10^-4 min^-1 for adults and 0.96 x 10^-4 min^-1 for young. The delay or lag time parameter for dermal penetration was 4.4 hr in adults and 1.1 hr in young.


Radiolabelled dinoeb was applied to previously clipped back skin of 33- and 82-day old male Fischer 344 rats. Radioactivity in the treated skin, tissues, urine, and feces was determined at 1, 6, 24, 48, and 120 hrs. In vivo dermal absorption was also measured in the same aged rats by static and flow-thru methods. In vivo dermal penetration in young and adult at 72 hrs was 65 and 90%, respectively. Static cells gave 75% for young and 55 or 71% for adults on two different runs. Flow cells gave lower results; 47% for young and 21 or 53% for adult on two different runs. Following in vivo application, adults excreted about 70% in urine, 16% in feces, and retained 17% in the body at 120 hrs. Results for young were about 85% of the adult values, which was the ratio of dermal penetration values for young to adult. Blood had the highest concentration of the tissue examined. Kidney to blood ratio averaged 0.60 in young and 0.41 in adult, while the liver and carcass to blood ratio averaged 0.18 in young and 0.11 in adult. Dermal absorption for young rats is slightly less than for adults, but the subsequent bodily retention and excretion appeared similar. In vitro dermal penetration of dinoeb was usually lower than in vivo absorption. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
Percutaneous fate of topical steroids: in vitro studies with mouse skin in organ culture. J. Kao, Dept. Drug Metabolism, SK&F Philadelphia, PA

With mouse skin maintained in a permeability chamber-organic culture system, the extent of metabolism during skin permeation of selected radionlabelled steroids was examined. Following in vitro topical application (10 μg/cm²), the rank ordered extent of permeation at 8 hr, as a percent of applied dose was testosterone (43.57%) > cortisol (11.48%) > estradiol (10.0%) > estrone (6.09%) > estradiol (1.52%). For estrone and testosterone, permeation was accompanied by extensive cutaneous first-pass metabolism with 77% and 41%, respectively, of the radioactivity recovered in the perfusion medium identified as metabolites. For estradiol and cortisol, limited first-pass metabolism was observed with only 20% being metabolites. In addition to water soluble metabolites, the principal metabolites identified were products of hydroxysteroid dehydrogenases and 5α-reductases. For estradiol, cutaneous metabolism was negligible. These observations support the contention that both cutaneous and metabolic processes are involved in the percutaneous fate of topical steroids and suggest that in future, studies concerned with topical drug absorption and bioavailability, an assessment of cutaneous first-pass metabolism should be included.

SKIN PENETRATION POTENTIAL OF 14C-POLYMERS JRA00 AND LR400 FOLLOWING A SINGLE APPLICATION TO FISCHER 344 RATS. S.W. Frantz, M.J. Tallant, C.M. Groose and B. Ballantyne, Busby Run Research Center/Union Carbide Corporation, Export, PA.

UCARES Polymers JRA040 and LR400 are quaternary nitrogen-containing cellulose ether polymers used as conditioning agents in human skin and hair care products, and were thus evaluated for their potential to penetrate skin. Separate material balances were determined using 14C samples of Polymers JRA040 and LR400 following a 24 hr exposure of the clipped dorsal skin of male and female Fischer 344 rats. For Polymer JRA040, no 14C was detected in blood at 1, 6 and 24 hr or in tissue samples, and < 1.5% of the dose was recovered in carcass and excreta. Following Polymer LR4000 exposure, no 14C was detected in either tissues or blood over several intervals up to 24 hr, and < 0.4% of the dose was recovered in carcass and excreta. In addition, autoradiographic analysis of dose site skin showed Polymer JRA040 was bound to the surface layers of the epidermis. The results of this study suggest that exposure of human skin to Polymers JRA040 or LR400 should not present a systemic risk to human health through topical application.

977

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SUBCHRONIC DERMAL TOXICITY STUDY OF BENZETHIONIUM CHLORIDE IN F344 RATS AND B6C3F1 MICE.
W. C. Eastin,* M. K. Placke, X. R. Heltman, M. J. Ryan and A. C. Peters. *National Toxico-
logy Program, RTP, NC and Battelle Columbus, OH.

A 13-week dermal toxicity study of benzethonium chloride (CAS No. 121-56-0) was conducted in F344
rats and B6C3F1 mice. Chemical was applied to clipped dorsal skin of each species (10/sex/
5) at dose levels of 25, 12.5, 6.25, 3.125, 1.563 or 0 mg/kg, 5 times per week for 13 weeks.
There was no evidence of systemic toxicity as a result of dosing except for a slight decrease
in body weight from high dose males of both
species. However, treatment resulted in clinically
and pathologically evident skin irritation
at the application site. The incidence,
severity, and time when lesions first appeared
in males differed between species (rats were
more sensitive than mice). Multiple
irregular epidermal crusts and thickened skin
occurred frequently in rats and mice dosed with
25 mg/kg or 12.5 mg/kg of the chemical with
multiple red foci observed in animals of both
dose levels. Microscopic lesions included
necrotizing (ulcerative) inflammation, chronic
dermal inflammation, acanthosis, and hyperker-
atinosis. Incidence and severity of dermal lesions
was dose-dependent and reached 100% in the
highest dose group of both species. (Supported
by Contract No. N01-ES-45066 from NTP).

ANTI-KERATIN MONOCLONAL ANTIBODY USED TO PROBE FOR PERTURBATION IN THE DIFFERENTIATIVE PROCESS INDUCED BY EXPOSURE OF RAT KERATINOCYTES TO BIS-(8-CHLOROETHYL) SULFIDE (BCES). B.J. Locey, and I.A. Bernstein. Toxicology Program, Dept. Env.
Ind. Health, Univ. of Michigan, Ann Arbor, MI.

BCES is a highly toxic alkylating vesicant
for human skin. The blister, formed at the der-
mal epidermal junction, is associated with the
 necrosis of the basal layer, which appears to be
selectively susceptible to BCES. Rat keratin-
ocytes grown in low Ca++ medium (1.1mM) form a
monolayer culture consisting primarily of a
proliferative population, but including cells
which exhibit characteristics of differentiated
cells.

Keratin polypeptides, the major constitut-
ents of the intermediate filament system of
keratinocytes, change as the cell differenti-
ates. Anti-keratin monoclonal antibodies can act
as specific and sensitive probes for normal
changes and abnormalities resulting from toxic
chemical exposure.

2D6, an anti-rat keratin monoclonal anti-
body developed in this laboratory, binds only
the basal layer in tissue cross-sections and a
54.5K peptide in immunoblot. In vitro exposure
in BCES between 1 and 10μM induces a dose-
dependent increase in 2D6 expression from 10 to
40 percent control, followed by a reduction at
higher doses. This suggests an altered cyto-
skeleton and a disruption of the normal process
of differentiation.

RESULTS OF A 90-DAY ORAL GAVAGE STUDY OF OXO
OCTYL ACETATE IN RATS. W.C. Daughtrey, M.
Eutemansor, S.W. Thompson*, and R.W. Biles.
Exxon Biomedical Sciences, Inc., East Millstone,
NJ. *Veterinary and Comparative Pathology, Sayre,
PA. Sponsor: G.F. Egan

Oxo octyl acetate is the acetic acid ester of an
isomeric mixture of branched aliphatic alcohols
having carbon numbers predominantly in the range
of C7 through C9 with C8 as the main component.
It is a solvent frequently used to release the
surface coating. The subchronic toxicity of oxo
octyl acetate was assessed following its admini-
stration to rats via oral gavage, 5 D/wk for 15
wks. Treated rats received undiluted oxo octyl
acetate at doses of 0.1, 0.5 or 1.0 g/kg. After
13 wks of dosing, all animals were sacrificed and
necropsied. Blood samples were obtained and
selected organs were weighed and prepared for sub-
sequent histological examination. Several treat-
ment-related effects were observed in the high
dose group (1.0 g/kg) animals. These effects
consisted of slight reductions in body weight and
food consumption, increased liver and kidney
weights, and evidence of "light hydrocarbon
nephropathy in high dose males only. With the
exception of increased liver weights in the mid
dose group, no other significant treatment-
related effects were observed in the mid or low
dose groups of animals. The results of this
study indicate that oxo octyl acetate possesses
an overall low degree of systemic toxicity when
administered orally to rats for 15 wks.

SUBCHRONIC TOXICITY STUDIES OF t-BUTYL ALCOHOL IN
RATS AND MICE. C. Lindenmoor III, D. R. Parnell,
J. D. Prejean, and R. R. Marcomot.* Southern
Research Institute, Birmingham, AL and *National
Toxicology Program, Research Triangle Park, NC.

The purpose of this study was to evaluate the
toxicity of t-butyl alcohol, an important commodi-
ity chemical, additive to unleaded gasoline and
contaminant of drinking water. Ninety-day tox-
icity studies were conducted in B6C3F1 mice and
F344 rats of both sexes using dosed water. Dose
levels were 0, 0.25, 0.5, 1, 2, and 4% (w/v). Lethality was observed at the 4% level of both
sexes and species. Weight-gain depression was
present in all dose levels of male rats; 4% fe-
male rats; 1, 2, and 4% male mice; and 2 and 4% female mice. Water consumption was increased at
lower dose levels in male rats and decreased in the
higher dose levels in rats of both sexes. Clinical
signs in rats were ataxia in both sexes and
hyperactivity in males. In rats, urine vol-
umes were reduced with putative uric acid crys-
tals observed in urine. Gross observations at
necropsy were normal. In mice, the urinary tract
had a shortening and thickening. No-effect levels for microscopic observations of hyperplasia of transitional
epithelia and inflammation in urinary bladder were
1% in male rats and mice and 2% in female mice
and rats. The results suggest that in rodents the
urinary tract is the target organ for t-butyl
alcohol toxicity and males are more sensitive to
t-butyl alcohol toxicity than females.

245

Male and female Sprague-Dawley rats received p- (50, 175, 450 or 600 mg/kg/day), m- (50, 150 or 450 mg/kg/day), or p-Cresol (50, 175, 450, or 600 mg/kg/day) or corn oil vehicle by gavage once daily for 13 weeks. Deficits in body weight and food consumption occurred at higher doses of the test materials during the first study week only. Convulsions and mortality occurred at high doses throughout the study, although the incidence was greatest during the first few weeks. The most prevalent clinical signs were dose-related in incidence and comprised three categories: 1) most groups affected, incidence increased or maintained throughout the study (salivation, myoclonus, tremors, urine wet abdomen); 2) all groups affected, greatest incidence during first few study weeks (hypertonia, rapid respiration); 3) only mid- and high-dose groups affected, incidence greatest during first study week (myoclonus, low body posture, labored respiration). Each animal was subjected to an observational battery of neurobehavioral tests at various times throughout the study. These tests revealed essentially no treatment-related deficits with regard to general appearance or neuromuscular, sensory or motor function for any of the three cresol isomers.


A subchronic toxicity study of ethylene glycol (EG) was conducted in male and female CD-1 rats. EG was administered in drinking water at concentrations of 0.5, 1.0, 2.0, and 4.0% for a 10-day study (both sexes). These dose levels were also used in a 90-day study for females, but doses for males in the 90-day study were 0.25, 0.5, 1.0, and 2.0%. At time of sacrifice gross necropsies were performed and tissues were submitted for evaluation. No mortality occurred in the 10-day study. In the 90-day study 8/10 females and 2/10 males in the high dose group died prior to sacrifice. Body weights were suppressed in a dose response fashion for both sexes. The most significant histological findings were kidney lesions. Lesions identified were corticomedullary mineralization, and tubular lesions such as dilatation, degeneration, crystals, necrosis and regeneration. These lesions were found to be predominantly in the males. (This abstract does not necessarily reflect EPA policy.)


In previous investigations high dermal doses of EGPE were found to induce a hemolytic anemia in rabbits. The present studies were designed to further investigate dermal exposure in rabbits, characterize the hemolytic response and evaluate differences in species susceptibility. Since potential human exposure is via skin, it is important to note that dermal application of up to 500 mg/kg/day of EGPE for 13 weeks to rabbits revealed no toxicologic effects. However, oral administration of EGPE to rabbits at 100 to 1000 mg/kg/day for 11 days resulted in a dose-related intravascular hemolytic anemia. The hemolytic anemia was characterized by decreased erythrocyte parameters, hemoglobinuria, splenic congestion, renal tubule damage and a regenerative erythroid response in bone marrow. Increases in erythrocyte fragility were observed without alterations in RBC glutathione or methemoglobin. Phenoxyacetic acid (PAA) was identified as the major blood metabolite of EGPE, but it was not directly responsible for erythrocyte hemolysis. Rats administered EGPE orally at 1250 or 2500 mg/kg/day for a maximum of 14 days did show overt signs of hemolysis.

1 Co-sponsored by Phenoxyethanol Producers Group.
p-XYLENE'S ALTERNATION OF THE RAT LUNG MICROSOMAL MEMBRANE. A. Roberta, J. Tegerio, R. Schatz, and D. Brown. Toxicology Program, Northeastern Univ., Boston, MA

Studies in our laboratory have shown that the organic solvent, p-xylene (1 g/kg, ip, 1h), inhibits pulmonary microsomal metabolism of benzo(a)pyrene (BaP) in the rat. The inhibition of BaP metabolism was found to be related to a p-xylene mediated reduction in microsomal P450. The current study describes the effects of p-xylene on the microsomal membrane. p-Xylene (1g/kg, ip, 1h) was found to decrease lipid peroxidation (LP), total phospholipid (PL) content, and phosphatidyicholine (PC) content of microsomal membranes 48%, 28% and 17%, respectively (p<0.05). In contrast, cholesterol (CL), sphingomyelin (SM), phosphatidylserine (PS), phosphatidylcholine (PL), and phosphatidylethanolamine (PE) levels were unchanged by p-xylene. The PL/CL ratio and the PC/PE ratio, indicators of membrane fluidity, were decreased 34% and 13%, respectively (p<0.05). Analysis by fluorescence polarization revealed that the fluidity of lung microsomal membranes from p-xylene treated rats (1g/kg, ip, 1h) was slightly less (5%) than the fluidity of control membranes (p<0.10). The decreases in LP, PL, PC, and fluidity are considered to contribute to changes in the microenvironment of microsomal P450 and to the inhibition BaP metabolism.

EFFECTS OF p-XYLENE ON CEREBRAL MEMBRANES. L.J. King, T. Aucoin and R.A. Schatz. Toxicology Program, Northeastern University, Boston, MA.

p-Xylene, an organic solvent, has been shown to cause changes in lung membranes in previous studies conducted in our laboratory. The fact that exposure to various organic solvents results in behavioral sequelae, presumably of CNS origin, led us to investigate the effects of p-xylene on brain membrane composition and function. In p-xylene-treated mice (2 g/kg i.p.), modest but significant decreases were seen in total PL levels in whole brain, 6% at 1h and 7% at 3h. Decreases in PC and PE at 3h were 30% and 47%, respectively. Total CL levels were also decreased by 25% at 1h and 16% at 3h. The ratios of PL/CL and PC/PE showed a significant increase, possibly corresponding to an increase in fluidity. Since an aldehyde is thought to be responsible for the observed membrane changes and since aldehydes may be detoxified by glutathione (GSH), we measured brain GSH levels in p-xylene-treated mice. Brain GSH levels were significantly decreased. Although we observed similar decreases in CNS phospholipids as observed in lung, comparison of ratios used to assess membrane fluidity (PL/CL, PC/PE) indicate that the physical state of membranes derived from brain and lung tissue may be different (decreased fluidity in lung and increased fluidity in brain). Partially supported by a grant from GE Plastics Division.
Experiments were conducted to determine the effects of toluene on immunocompetence and regional brain biogenic amines in CD-1 mice following four weeks of continuous oral exposure of toluene via drinking water ad libitum at the concentrations of 20, 100, and 500 ppm. Toluene produced a decrease in thymus weight. Lymphocyte proliferations to B and T cell mitogens were followed by a dose-related effect and significantly depressed in all groups. Mixed lymphocyte response showed a significant depression. The cytotoxic T-lymphocyte activity was only affected at the highest dose. Suppressions of antibody production were observed in treated mice after sensitization with sheep red blood cells. Oral ingestion of toluene induced biosynthesis of catecholamines and indoles. Significant increases of norepinephrine (NE) were found in the hypothalamus, midbrain and medulla oblongata. Levels of dopamine (DA) were increased in the corpus striatum and hypothalamus. Toluene also caused significant increases in serotonin (5-HT) levels in all dissected regions except cerebellum. Concomitantly, levels of the metabolites of these monoamines were increased in several brain regions. (Supported in part by a grant from U.S. Geological Survey.)

The peripheral nervous system is the target organ of a number of chemicals. Sensitivity to vibration has been used to evaluate the functional integrity of peripheral nerves. Vibration sensitivity of digits 3 and 5 was evaluated in normal volunteers selected for the absence of neurological disorders. Both fingers were tested on 3 occasions 1 week and 1 year apart. A computerized two-alternative forced-choice procedure. Temperature did not affect vibration threshold within the limits of this study. Vibration sensitivity thresholds were very stable over time (i.e. reliable).

Patients suffering from a compression of the median nerve (carpal tunnel syndrome) were also tested. There was a statistically significant difference between patients and controls when the difference between the sensitivity of digits 3 and 5 was compared. This study further demonstrates that vibration sensitivity assessment is a reliable and sensitive tool for characterizing the functional integrity of the peripheral nervous system in humans.

**Effect of 1,2-oxothiazolidine-4-carboxylic acid (OTCA) on the urinary excretion of mercurial acides in rats exposed to acetonyl-nitrile. J. Brodeur, L. Laliberté, D. Tardif. Dép. méd. trav. & hyg. m.l., Faculté de médecine, Université de Montréal, Montréal, Québec, Canada.**

N-Acetyl-S-(2-cyanoethyl)-L-cysteine (NAC) and N-Acetyl-S-(2-hydroxyethyl)-L-cysteine (NAHEC) are, with thiocyanate (TCN), important urinary metabolites of acetonitrile (ACN) in rats and good candidates for the biological monitoring of exposure to ACN. The effect of OTCA, a cysteine prodrug, on the excretion of NAC and NAHEC was studied in adult male rats given ACN i.p. at 190, 380 and 760 μmol/kg. Excretion of NAC increased linearly with dose of ACN, going from 55 ± 8 (mean ± S.E.M.), to 136 ± 23 and 346 ± 12 μmol/kg, respectively. Excretion of NAHEC remained low at 23 ± 3, 22 ± 1 and 20 ± 2 μmol/kg for the same doses respectively. The changes in excretion of TCN were moderate, going from 34 ± 3, to 26 ± 6 and 65 ± 9 as doses of ACN increased. Treatment with OTCA (6 mmol/kg, i.p.) increased markedly the ratio of excretion of liver GSH in rats given ACN (760 μmol/kg). It also increased significantly (2 x) the excretion of all 3 metabolites during the first 4-hr period following ACN administration. The results suggest that urinary NAC and NAHEC is a reliable monitor of exposure to ACN; in addition, OTCA provides a higher estimate of internal exposure to toxic reactive chemicals. (Supported by RBC grant MA-8943 and IRSSS, Québec).
994 EFFECTS OF PREVIOUS MATERNAL EXPOSURE TO 2,3,7,8-TCDD AND 1,2-DIMETHYLBENZENE ON THE BEHAVIORAL DEVELOPMENT OF APE-INFANTS


Adult female rhesus monkeys were fed diets containing 0, 3, or 25 p.p.m. TCDD for approximately 4 years. They were bred to unexposed males starting 10 months after TCDD exposure ended. Infants were born 6-11 months later, weaned at 4 months of age, and housed individually. Beginning when they were 8-10 months of age, they were socialized with monkeys of the same sex. Groups were matched nearly as possible for age, sex, and maternal TCDD exposure. When the infants were 18-24 months of age, they were shifted into groups of 3 monkeys each. The new groups were matched for age and sex, but each contained only monkeys from the same TCDD exposure condition (0, 3, or 25 p.p.m.). Throughout socialization, frequency and duration of 41 behaviors were observed daily using a focal animal scoring technique. When TCDD-exposed infants were regrouped with their similarly exposed peers, they showed a dose-dependent increase in self-directed behaviors. Infants in the 25 p.p.m. group showed an almost complete suppression of all interactive play behaviors for the 4 weeks immediately following the regrouping, and then gradually resumed play behavior. (Supported by EPA Grant R-811685.)


Cynomolgous monkeys (Macaca fascicularis) were dosed from birth with 100, 50, or 0 µg/kg/day of lead. This resulted in blood lead concentrations of 3-5, 5-9, or 0 µg/dl respectively before withdrawal of infant formula at 200 days of age, and declined thereafter to stable-state levels of 1, 1.5, or 0 µg/dl. At age 9 years of age the monkeys were tested on a spatial discrimination reversal task.

The monkey faced two pushbuttons, which could be backlit with a variety of forms and/or colors. The monkey's task was to respond on the right-most positioned button, irrespective of the stimuli presented on the button, in order to receive a juice reward. When the monkey learned the task to a criterion of at least 9 out of 10 correct, the other pushbutton became the correct one, for a total of 18 reversals. The set of three sets of increasingly complex sets of irrelevant stimuli were presented sequentially (for a total of 45 reversals). Treated monkeys performed more poorly than controls under some conditions, although deficits were not as severe as non-spatial discrimination reversal tasks or a delayed alternation task (which tests spatial memory) examined previously.


A total of 52 monkeys (Macaca fascicularis) were dosed orally from birth with 1500 µg/kg/day of lead on one of four dosing regimens (13 monkeys/group): 1) vehicle only, 2) lead from birth onward, 3) lead from 600 days of age and vehicle thereafter, or 4) vehicle from birth until 300 days of age and lead thereafter. This dosing regimen allowed evaluation of differential infant vulnerability as well as reversibility of the behavioral toxicity of lead. Blood lead concentrations averaged 3-6 µg/dl when monkeys were not being exposed to lead, and 32-35 µg/dl during lead exposure. When monkeys were 3-3.5 years old, testing began on an intermittent schedule of reinforcement, the multiple fixed-interval fixed ratio. All groups exposed to elevated performance different from controls, as manifested by differences in rate of response and increased variability of performance in the treated groups.


Delayed matching to sample tasks with visual cues have been used to quantify memory in primates and birds. A delayed matching to position (DMP) task was developed to model memory in rats, using spatial location as the critical stimulus. Rats received food for pressing 1 of 2 retracted levers in the choice phase of a trial if the location of the lever matched that of a single lever presented in the prior sample phase of that trial. When delays of 0-20 sec were imposed between sample and choice, matching accuracy declined with increasing delay. Reducing the intertrial interval (ITI) steepened the slope but did not change the intercept of this retention gradient, showing that interference from previous trials impaired memory but not discrimination. Over sessions, rats learned to "rehearse" responses during delay, but rehearsal accuracy (contacts with retracted levers) was unrelated to choice accuracy. One week after injection, trimethyldiazine (TMZ 7 mg/kg iv) reduced accuracy at nonzero delays but not at 0 delay, and did not affect rehearsal in delay. The ITI effects suggest that the DMP task adequately assessed memory. This is supported by the disruption of DMP by TMZ, which damages limbic structures supporting spatial memory in rats.
EFFECTS OF VOLATILE INHALANTS ON FLUROTOLYL INDUCED CONVULSIONS AND CHANGES IN OPERANT PERFORMANCE. D.C. Rees, K.R. Iorio, L.E. Black, E.E. Sikorski, T.M. O'Hara, R.L. Balster, Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA. SPONSOR: J.F. Borzelleca

The ability of some volatile inhalants to antagonize the convulsant and response rate decreasing effects of the convulsive inhalant flurothyl was assessed. Mice were trained to respond under a fixed-ratio 20 schedule of reinforcement. Response rates were measured for 5 min following 20-min exposures to flurothyl (1200 ppm) of which the last 15 min was in combination with either toluene (750-8000 ppm), halothane (750-6000 ppm), 1,1,1-trichloroethane (TCE; 500-8000 ppm), chloroform (50-4000 ppm) or pentobarbital (PB; 10 mg/kg). All agents blocked the response rate decreasing effects except chloroform which enhanced them. Anticonvulsant effects were assessed using cumulative dosing procedures. Individual mice were exposed to increasing concentrations of flurothyl in the presence of various concentrations of TCE (2000-15000 ppm), toluene (500-6000 ppm), halothane (500-4800 ppm) chloroform (2000-8000 ppm) or PB (30 mg/kg). All agents antagonized the convulsant actions of flurothyl except TCE which had no effect. Differences are apparent in the depressant effects of volatile inhalants. (Supported by Grant DA-03117)


The time course for the behavioral effects of carbon monoxide (CO) was examined in mice trained to lever press under a fixed-ratio 100 schedule of water reinforcement. Thirty-min test sessions were conducted either immediately or 30 min following ip injections of air (100 ml/kg) or CO (7.5, 15, 30, 50 or 100 ml/kg). Carboxyhemoglobin (COHB) levels were also determined for these doses at 5, 15, 30, 60, 120 min and 24 hrs post-injection. CO produced a dose-dependent decrease in rates of responding which was exhibited earlier and lasted longer with increasing doses. Peak COHB levels were observed at 15 or 30 min and were 20%, 32%, 42%, 51% and 60% for 7.5, 15, 30, 50 and 100 ml/kg, respectively. COHB saturation alone was not always a good predictor of behavioral effects since both level and duration of exposure contributed to behavioral impairment. The results also show that the ip route can be used to study the toxicity of CO. (Supported by grants ES-03809 and ES-07087)

EFFECTS OF SUBLETHAL DOSES OF IONIZING RADIATION ON SCHEDULE-CONTROLLED PERFORMANCE IN RATS. P.C. Mele, C.G. Franz and J.R. Harrison. Armed Forces Radiobiology Research Institute, Bethesda, MD. SPONSOR: V. Bogo

Male albino rats responded under fixed-ratio (FR) 50 or fixed-interval (FI) 2 min schedules of milk reinforcement. Separate groups were exposed to 50, 150, 450 or 0 (FI only) rads (r) of Co60 gamma three times at 42 d intervals; dose rate was 250 r/min. Under FR, response rates (RR) were decreased and pause times after reinforcement (PI) increased after 450 r. Maximal effects occurred 24 hr post-irradiation followed by recovery of post-irradiation performance over the next 3-7 d. Repeated 450 r exposures produced only a slight, nonsignificant enhancement of these effects. Under FI, RR were reduced by 450 r after the second exposure only; PI and index of curvature were not altered. Fifty and 150 r did not consistently alter FR or PI performance. Lastly, 650 r given to all groups disrupted both FR and PI performance; these effects did not vary as a function of prior radiation dose received. Radiogenic disruption of performance was dose-dependent, reversible, and dependent upon the schedule of reinforcement in effect.

EFFECTS OF ACUTE EXPOSURES TO CHOLINERGIC AGENTS ON OPERANT BEHAVIOR IN RATS. J.E. Chambers and H.W. Chambers. Deps. of Biological Sciences and Entomology, Mississippi State University, Mississippi State, MS.

Acute high level exposures to paraaxon (P), an organophosphorus anticholinesterase, and 2 forms of atropine, antagonists of muscarinic receptors, were studied for their effects on the retention of fixed ratio performance by male rats. Food deprived rats were trained to an FR10 schedule of reinforcement. Following stabilization, they were injected i.p. with one of the following: vehicle; atropine sulfate (AS); atropine methyl bromide (AMB); AS + F; AMB + F; or P. Animals were re-tested 1 and 2 days after treatment. Performance was depressed to 50% of pre-treatment rates by AMB at 1 day and recovered to 60% by 2 days, but performance was unaffected by AS. Performance was depressed to 30% of pretreatment rates by AMB+P or by AS+P at 1 day and recovered to 65% by 2 days. A slight decrease in average performance was observed after exposure to P alone at 1 day with recovery by 2 days. The percent inhibition of brain acetylcholinesterase observed following treatment with these dose levels of P were about 85%, 55% and 45% at 30 min, 1 day and 2 days following treatment, respectively. Performance is usually decreased by toxicants causing both hyper- and hypo- cholinergic activity, even when brain AChE activity is still greatly inhibited by P. (Supported by EPA grant R-811295)

Ionophore compounds which facilitate membrane ion transport are useful neurotoxicological probes. Previous work in our laboratory has shown that the calcium ionophore A23187 effects such behavioral changes in the rat at hypotonicity, elevated shock threshold, altered avoidance behavior and hypothermia. To further characterize the behavioral effects of A23187, treated adult male rats were tested for operant performance (FR-15) and circadian activity. Challenge tests were also conducted to determine the interaction between A23187 and the drugs – amphetamine (A), scopolamine (SC) or chlorpromazine (CPZ). Brain levels of norepinephrine (NE), dopamine, serotonin and their metabolites were also determined in treated rats. Dose-related decrements in rates of lever pressing were noted at 0.25 and 0.5 mg/kg (29% and 56% decrease from baseline). This was significant only at the 0.5 mg/kg dose (p<0.01). A and SC both attenuated, while CPZ exaggerated, the hypotonicity induced by 0.5 mg A23187/kg. At this dose circadian motor activity was unaffected. Neurochemically, A23187 at 1 mg/kg induced NE increase (p<0.01) in the N2 metabolite 3-methoxy-4-hydroxyphenylglycol. The data suggest that low doses of A23187 induce a specific pattern of neurobehavioral dysfunction which may include some involvement of the central noradrenergic system.


The effects of the putative adrenergic-acting formamidine, chlordimeform (CDM), and the cholinomimetic carbamate, carbaryl (CAR), were compared using a functional observational battery (FOB). The FOB, a series of observations and measurements that can be rapidly administered to toxicant-treated rats, includes home-cage and open-field observations, neuromuscular and sensorimotor tests, and physiological measures. Evaluations were made according to US EPA testing guidelines so as to determine dose-, time- and sex-related toxicant effects. Long-Evans hooded rats of both sexes were dosed i.p. with either vehicle, CDM (1, 25, 56 mg/kg) or CAR (3, 10, 30 mg/kg). Both CDM and CAR decreased body weight and temperature, and altered general activity, equilibrium, and home-cage resting states. CDM enhanced responses to several stimuli and increased grip strengths, whereas CAR either had no effect or decreased these measures. CAR but not CDM produced cholinergic autonomic signs of intoxication. Thus, the profiles of effect produced by these two pesticides could be clearly differentiated using the FOB.

1004 PRELIMINARY STUDIES OF MOTOR ACTIVITY AND TRENOR IN INTRATHECALLY IMPLANTED RATS. D. W. Herr* and H. A. Tilson. National Institute of Environmental Health Sciences, Research Triangle Park, NC

Systemic injection of noradrenergic agents modifies tremor produced by DDT (Toxicologist, 6: 892, 1986). Intrathecal infusion of similar drugs will be used to examine spinal modulation of DDT-induced tremor. Rats were sham operated or intrathecally cannulated (PE 10 tubing). Baseline activity was quantified in open field activity chambers with infrared photocells. A Fourier transformation of analog output produced by activity on a freely moving platform attached to a load cell transducer produced a spectral profile of bodily movements. Spectral profiles of DDT-induced tremor were also recorded. Sham operated and implanted rats had reduced activity and a decreased spectral profile relative to non-operated controls. Implanted and sham operated groups showed only tremor and both surgical groups had a similar spectral profile as controls when given 75 mg/kg DDT (po). Gavage with 30, 45, or 60 mg/kg DDT caused a dose- and time-related tremor, which appeared between 2-5 hr and peaked about 8 hr after DDT exposure. Maximal response to 45 and 60 mg/kg DDT was similar, while 30 mg/kg DDT caused tremor in both groups. Data indicate that decreased activity was due to surgery rather than cannulation and that implanted rats showed relatively normal tremor when given DDT. *Toxicology Curriculum, UNC. Funded by ES 07126.

1005 CHRONIC NICOTINE ADMINISTRATION AND WITHDRAWAL: EFFECTS ON RADIAL ARM MAZE PERFORMANCE IN RATS. E.D. Levin, A. Reyes, and G.B. Ellis. Dept. of Psychology, University of California, Los Angeles, CA. Sponsor: A. Cho

Tobacco use in humans has been reported to improve cognitive performance while withdrawal has been reported to impair cognitive function. In rats, nicotine has been found to improve response accuracy on a variety of operant tests. The effects of chronic nicotine administration and withdrawal have not been as widely studied. This experiment was conducted to examine the effects of chronic nicotine administration and withdrawal on radial arm maze performance in rats. Nineteen female Sprague-Dawley rats were trained to run in an 8-arm maze for sugar-coated food rewards. Only the first entry into each arm was rewarded. Nicotine was chronically administered to 10 of the rats for 3 weeks by a subcutaneously-implanted glass and silastic pellet which delivered 3.4 mg of nicotine base/day. Nine rats implanted with placebo pellets were controls. On the first week the treated rats did not differ from their predrug level of choice accuracy. On the second week they showed a significant (F(1,17)=5.52, p<0.05) rise in the number of arms entered before an error. They maintained this level of performance during the third week of nicotine and after withdrawal. (Supported by a grant from the MacArthur Foundation.)

Trimethyllin (TMT) produces behavioral and cognitive deficits resulting in part from limbic system toxicity. To determine whether these effects result from learning deficits or accelerated short-term memory loss, the present experiment examined long delay flavor aversion conditioning (LD-FAC) in TMT treated rats. Saline treated Long-Evans rats receiving injections of lithium after consuming saccharin flavored water later avoided saccharin ingestion: the degree of avoidance varied inversely with the time (0.5, 3, or 6 hr) separating initial saccharin availability and lithium injection. TMT treated rats (20 mg/kg iv, 30 days prior) showed impaired conditioning at the lost but not the short delay condition, suggesting that the effects were mnemonic and not associative. The effects of TMT on delay conditioning were accompanied by reduced body weights, hippocampal pathology, and impaired passive avoidance conditioning. In summary, LD-FAC appears to be a simple, reliable model of cognitive function with sufficient controls for distinguishing between functional alterations due to deficits in memory processes from those due to altered sensory, motor, or associative processes.

1007 BEHAVIORAL EFFECTS OF FyDRIN AND AMBUSH IN MALE MICE. J. Mitchell, M. Wilson, and M.J. Kallman, Depts. of Pharmacology and Psychology, University of Mississippi, University, MS.

Male Swiss mice, 20-25 g, were utilized to assess the effects of oral and oral administration of the pyrethroid insecticides Fydrin [P (30% fenvalerate)] and Ambush [A (25.6% permethrin)]. Animals were subjected to a conditioned taste aversion procedure using a normally preferred 0.3% saccharin solution. Subjects were allowed 30 min access to a drinking source containing the saccharin solution, followed immediately by the administration of the pyrethroid or control solution. P (0.5, 3.0, and 30.0 mg/kg orally; 60.0, 600.0 and 1800.0 mg/kg dermally) and A (0.5, 5.0, and 50.0 mg/kg orally; 30.0 and 300.0 mg/kg dermally) produced significant (p<0.05) reductions in the percent saccharin consumed. Total fluid intake, however, was not altered by any of the treatments. The effect of the insecticides both grouped and individual locomotor activity was ascertained in similar subjects. Activity measurements were taken over the 4 hr period immediately following the administration of the pyrethroid or control solution. P (30.0 mg/kg orally; 600.0 and 1800.0 mg/kg dermally) and A (50.0 mg/kg orally; 300.0 mg/kg dermally) significantly increased locomotor activity as compared to the vehicle control in both grouped and individual activity measures. Lethality (96 hr) was determined for each of the treatments. (Supported in part by the Research Institute of Pharmaceutical Sciences)


The three isomers of xylene are produced in high volume, are found in many industrial and household fluids, and have potential for widespread public exposure. NIOSH has set a time-weighted average TLV for mixed xlenes of 100 ppm; nevertheless, their neurotoxic properties are poorly characterized. Conditioned flavor aversions (CFAs) are induced easily by many toxic compounds, but have rarely been studied in inhaled chemicals. Male Long-Evans rats (40 days) were placed on a restricted water schedule (30 min/day) 1 week after arrival in the laboratory. Ten days later, all rats received 0.1% saccharin in place of water, and were then immediately placed into atmospheres of air or p-xylene at 50, 100, 200, 400, 800 or 1600 ppm for 4 hours. The restricted water schedule remained in effect for the next 72 hours, at which time the rats were given a choice between saccharin and water. p-Xylene inhalation reduced preference for saccharin at all concentrations, with maximal aversion at 800 and 1600 ppm. Total fluid consumption was slightly reduced after 1600 ppm but unaffected after lower concentrations. Thus inhaled p-xylene at a concentration of 1% the TLV caused a significant change in rats' normal flavor preference without disrupting fluid consumption.
The effects of p-xylene and toluene on motor activity were determined in rats following s.c., i.p., and p.o. administration. Motor activity was measured in commercial photocell devices that recorded horizontally (HA) and vertically (VA) directed activity. In each experiment, adult male Long-Evans hooded rats were either treated with no change, vehicle or a dose of solvent. Testing generally began 75 min later and lasted for 25 min. When given s.c., neither solvent (125-4000 mg/kg) affected motor activity levels. Similarly, no effects were obtained when p-xylene (500 mg/kg) was given 37.5-300 min prior to testing. When given i.p., p-xylene (125-1000 mg/kg) produced bimodal effects on HA; small to intermediate dosages increased HA while the largest dosage decreased HA. VA was unaffected by dosages that increased HA, but was decreased when HA was also decreased. Similar qualitative effects were obtained with toluene (125-1000 mg/kg), although the magnitude of the increase in HA was larger. When given p.o., p-xylene (125-1000 mg/kg) produced small increases in HA while the largest dosage decreased VA; toluene (125-1000 mg/kg) produced similar effects although the increases in HA were greater. These results emphasize the importance of route considerations in evaluating the effects of solvents on behavior.

Benzonitrile (BZN), used industrially as a solvent and in the production of amino resin compounds, has been identified in air and water supplies. The effects of BZN on body weight (BW) and behavior (motor activity, grip strength, hot plate and acoustic startle response) were assessed during a 13-week gavage study. Mice were exposed to BZN at doses of 0, 37.5, 75, 150, 300, or 600 mg/kg BW, while rats received 0, 19, 37.5, 75, 150 or 300 mg/kg. Testing took place at mid-study and during week 13 of exposure. An increase in startle response latency (600 mg/kg) and BW (150 and 600 mg/kg) was observed in female mice only at the mid-study test time. For female rats, hindlimb grip strength and BW were depressed in the 300 mg/kg group at mid-study, while pawlick latency was enhanced in the 150 and 300 mg/kg groups. BW was also decreased in the 150 and 300 mg/kg female rat dose group at this test time. At study week 13, a decrease in motor activity was observed for the 19, 37.5, 75, 150, and 300 mg/kg females, while pawlick latency was increased and BW depressed in the 300 mg/kg male rat dose group. These results suggest that BZN can induce neurological dysfunction in rodents and that the rat is a more susceptible species than the mouse. Supported by Contract No. N01-ES-55113.

Male Sprague-Dawley rats weighing between 200-250 gms. were treated with the known demyelinating agent Acetyl Ethyl Tetramethyl Tetraclin (NETT) (100mg/kg p.o. t.i.w) for 5 weeks and allowed to recover. Behavioral and morphological evaluation was performed at 4 time intervals at the cessation of dosing, 2 weeks after dosing, 7 weeks after dosing and 19 weeks afterwards. Behavior was evaluated using a battery of tests which included: openfield running, balancing beam, dorsal hindlimb retraction and grasp, acoustic startle, and forelimb grasp. Light and electron microscopic evaluations were performed using semi-thin and thin sections. Results indicate that the behavioral deficits observed at the cessation of dosing reversed themselves, except for balance, in a time-dependent manner. This correlates with morphological changes observed in the spinal cord and medulla in which myelin sheath integrity and diameter were decreased versus controls.
TDF is an agriculturally important triazole fungicide. The present experiments were conducted to characterize the behavioral toxicity of TDF using a measure of motor activity (MA). Dosage–effect, time–effect, and the effects of repeated dosing (7 days) were determined following TDF exposure. Male Long Evans hooded rats, approximately 70 days old, received TDF po in 2.0 mL/kg corn oil. MA testing was conducted for 1 hr in figure-eight mazes. For the dosage–effect determination, TDF (50–400 mg/kg) was administered 1 hr prior to testing. In the time–course study, TDF (200 mg/kg) was administered either 0.5, 1, 2, 4, 8, or 24 hr prior to testing. In the repeated dosing experiment animals received TDF (100 mg/kg) daily for 7 days and were tested 24 hr after the last exposure. TDF produced significant hyperactivity in the 100 and 200 mg/kg groups. This hyperactivity was rapid in both onset and recovery, with significant effects occurring only at the 0.5, 1, 2, and 4 hr time points. Repeated dosing with 100 mg/kg/day revealed no cumulative effects. These results indicate that TDF produces a transient hyperactivity at dosages 17 to 33% of the reported LD50.


MSG is an excitatory amino acid that produces neuronal degeneration, with neonates being most vulnerable. GABAergic neurons are the most severely damaged in certain brain regions. The purpose of this study was to examine the effects of MSG on the ontogeny of motor activity (MA) and the auditory startle response (ASR), two well-defined behaviors which are modulated by GABA. On postnatal day (PND) 1, Long Evans hooded rats (Charles River) were randomly assigned to litters (4 males and 4 females). MSG (4 g/kg) or saline were given ip daily on PND 2–9. Rats were tested at various ages from 12–84 days, measuring both 30 min figure-eight maze activity and the ASR. MSG-treated rats were hyperactive from PND 17–21 and their MA returned to control level by PND 41. The amplitude of the ASR was elevated in MSG-treated rats from PND 20–40 and returned to control level by PND 84. The apparent predisposition of GABAergic neurons to damage from MSG and the involvement of GABA in modulating behavioral arousal suggest that damage to GABAergic neurons is involved in the observed hyperactivity and elevated ASR. The transient nature of these effects was not expected and will also require further investigation. (*Supported by a NRC Research Associateship.)


To further characterize the behavioral toxicity of ANZ, comparisons were made between the effects of ANZ on motor activity (MA), the acoustic startle response (ASR), body temperature, and body weight in male Long Evans rats. Acute dosage–effect and time–effect determinations were made for MA and the ASR. ANZ (25–200 mg/kg) was administered po in 1.0 mL/kg of the vehicle (5% Equophor, 5% ethanol, 90% saline). ANZ produced a dosage– and time–dependent decrease in MA that lasted from 4–96 hr post-dosing. The effects of ANZ on the ASR revealed an increased latency and decreased amplitude and sensitization; effects on the ASR were more transient (4–24 hr) than for MA. ANZ-exposed animals lost body weight during 48 hr post-dosing, returning to control levels by 96 hr post-dosing. ANZ produced a dosage–dependent, transient (4 hr) decrease in body temperature ranging from 37.2°C in controls to 34.8°C in the 200 mg/kg ANZ group. Although 200 mg/kg ANZ produced a 65% mortality (within 72 hr), significant behavioral effects were seen at dosages as low as 25–50 mg/kg. These data indicate that asymptomatic dosages of ANZ disrupt sensory–motor functioning in the rat.


The prime aim of this study was to evaluate age-related effects of pyrethroids on the ASR; preliminary data also indicated that lethality in preweanling rats was greater than in adults. Both Type I (cypermethrin (CSM) and permethrin (PRM)) and Type II (deltamethrin (DLT) and cypermethrin (CPM)) pyrethroids were given to Long Evans hooded rats, ages 11 to 70 days. For ASR testing, pyrethroids were administered to both males and females in corn oil (po, 1 mL/kg) 2 hrs before testing at 17 and 21 days of age. The treatment effects at both ages were qualitatively similar to the adult, the lowest dosages affecting the ASR amplitude were 25–100% of the adult value. Acute lethality determinations were performed on 11-, 21- and 70-day old males following a single po dose in 4 mL/kg corn oil. Preweanling rats showed age-related differences in lethality which differed for the two types of pyrethroids. Compared with adults, preweanling rats were more susceptible to the Type II compounds (20X at 11- and 7X at 21-days of age). In contrast, 11-day old rats were less sensitive (CSM) and 21-day olds 2X more sensitive (CSM and PRM) to Type I compounds. These data indicate age-related differences in the acute toxicity of pyrethroids which vary with the measures used to evaluate toxicity.

Clonidine (CLO), an antihypertensive agent, may be useful in the treatment of several disorders occurring during pregnancy. Any expanded therapeutic role for CLO in pregnant patients will require detailed reproductive and behavioral toxicological screening. This study presents the results of a behavioral screen for toxicological effects of CLO following administration during the neonatal period (30 or 120 mcg/kg, ac. PND 2-18).

No significant differences were found in any of the following measures: eye opening, tooth eruption, surface righting, negative geotaxis, descending climbing skills, hind limb support, body weights, and survival rates. No differences were seen in adult rats tested on a progressive fixed-ratio (FR5, 20, 40, or 80) schedule.

These data are in agreement with and extend earlier studies from this lab in which clonidine, administered during pregnancy, failed to produce behavioral teratology.


Rat pups were indirectly exposed to 100 ppm chlorine dioxide (ClO₂), via drinking water, prenatally and until weaned at 42 days post conception (PC). The development of locomotor activity and ClO₂ exposed litters was monitored from days 31 through 42 PC. Additionally, sera from both groups were analyzed at various days PC, for circulating levels of thyroid hormones (T₃, T₄, T₃ uptake, free T₃ and free T₄). At 36 days post conception, the ClO₂ exposed animals exhibited significantly depressed locomotor activity levels and also depressed T₃ uptake. By day 42 PC, the locomotor activity levels and T₃ uptake of the ClO₂ exposed animals had returned to control levels. (This work was supported in part by CR809618, but does not necessarily reflect USEPA policy).

NEUROBEHAVIORAL DEVELOPMENT IN RATS AFTER PRENATAL KETAMINE. G.L. West, T.J. Sobotka, R.E. Brodige & D.Y. Quander. Div. of Toxicology, CFSAF, Wash., D.C. Sponsor: T.F.X. Collins

Ketamine was evaluated for use as a general anesthetic in studies of developmental effects of C-section birth in rats. Saline (1 ml/kg) or ketamine (200 mg/kg) plus xylazine (5 mg/kg) was given to pregnant rats on day 20 of gestation, i.e., one day before normal birth. Offspring were weighed weekly for nine weeks and were evaluated for physical and behavioral development. Two pups (1 M, 1 F) per litter were assigned to each of four evaluation batteries: (1) physical and reflex development (auditory startle, incisor eruption, eye opening, surface righting), postnatal rotordrom performance and natal sleep time; (2) olfactory discrimination (neonatal homing); (3) response inhibition (prewean conflict, postwean passive avoidance); or (4) activity (pre- and post-wean photocell and open field) and ketamine asleep time. Treatment effects were significant only for body weight and auditory startle. Age and sex factors were significant for various measures. Thus, ketamine appears to be a relatively safe general anesthetic for use in C-section studies.

EFFECT OF LEAD ON SEIZURE SPREAD AND MORTALITY. B.D. Davis¹, H.J. Kupferberg², N. Dombroski¹, and T.C. Raines¹. Technical Resources, Inc., Rockville, MD; ²National Institutes of Health, Bethesda, MD; ³National Bureau of Standards, Gaithersburg, MD.

In this study we investigated the effects of lead on seizure spread induced by pentylenetetrazol (PTZ). Lead acetate was administered to pregnant CD-1 mice in drinking water at concentrations between 0.05% and 0.1% (0.028% and 0.056% lead) from day 8 of pregnancy through weaning. Control animals received 0.1% sodium acetate. Following weaning, the offspring continued to receive lead acetate or sodium acetate until they were 6 and 7 weeks of age, males and females, respectively, when they were challenged with PTZ administered subcutaneously. Lead in tissue was analyzed by atomic absorption spectrometry with electrothermal atomization; lead acetate solutions were determined by flame atomic absorption spectrometry. No significant differences in body weights were observed between animals in the treated groups and controls throughout the study. Animals exposed to 0.1% lead acetate exhibited a higher incidence of tonic extension and mortality compared to controls, while seizure spread was similar in control animals and animals in the low dose group. These data suggest that lead affects mortality and seizure spread produced by PTZ.

255
We have determined the intraperitoneal convulsive doses (CD50) of CNS stimulants in pertussis vaccinated and control mice. Male N:NIH(S) mice were used. Pertussis vaccine was given i.p. (2 p.u. in 0.25 ml per mouse) three days before challenge with graded doses of five CNS stimulants, Metrazol (Met), thiosemicarbazide (TSC), picrotoxin (Pt), bicuculline methiodide (BM), and methyl 6,7-dimethoxy-4-ethylcarboline-3-carboxylate (DMCM). Six to ten mice were used for each dose level. CD50 for controls treated with Met, TSC, Pt and DMCM were 32.5, 10, 1.95, and 2.5 mg/kg, respectively and did not differ significantly from CD50 for vaccinated mice (38.5, 8.8, 1.32, and 1.78 mg/kg). In contrast, the CD50 for BM was significantly higher (P < 0.05) for control mice (134 mg/kg) than for vaccinated mice (84 mg/kg). This finding may imply a selective effect of pertussis vaccine involving GABAergic transmission.

Lindane (LND) has been shown to produce seizure activity after high dose exposure. We have previously shown that 24 hours after LND, seizure activity due to picrotoxin (PTX) is decreased in mice. Mice were treated with LND and challenged with PTX or tested for hepatic microsomal aniline hydroxylase (AH) and p-nitroaniline-sole-o-demethylase (ND) activity at 1 or 24 hours or 3 or 7 days after administration. Convulsions were scored using a seizure rating scale. Enzyme activities were determined spectrophotometrically. The results show decreases (28 and 44% of control) in PTX-induced seizure activity 1 and 3 days after LND respectively, but not at 7 days. AH was increased (124% of control) 1 day after LND while ND was increased 1, 3 and 7 days after LND (126, 135 and 140% respectively). Since the decreased response to PTX does not correspond temporally to increases in metabolic activity, it is concluded that the decrease in PTX-induced seizures after LND may be due to a CNS-mediated mechanism.

Low doses of ionizing radiation induce hyperthermia and high doses produce hyperthermia in rats. The purpose of this study was to determine the effect of variable doses of ionizing radiation on the temperature of guinea pigs, to compare these results to those obtained for rats, and to elucidate the mechanisms involved in the temperature responses. Exposure of guinea pigs to 10000-100,000 rad of 60-Co irradiation induced only hyperthermia. Intracerebroventricular (i.c.v.) doses of 10-30 ug of mezpramine or cinetidine (histamine H1 and H2 receptor antagonist, respectively) antagonized the hyperthermia due to ionizing radiation (1000 rad). Administration of 1-10 mg/kg i.p. or 10-50 ug i.c.v. of disodium cromoglycate (a mast cell stabilizer) attenuated radiation hyperthermia. However pretreatment of an antisera to drug methysergide (10-30 ug i.c.v.) had no antagonistic effect on the radiation-induced hyperthermia. Systemic (50-300 mg/kg i.p.) administration of WR 2721 (a radioprotectant) did not antagonize radiation hyperthermia, whereas central administration (50-300 ug i.c.v.) of the same drug attenuated it. These results indicate that ionizing radiation induced only hyperthermia in guinea pigs and that this hyperthermia is mediated by histamine H1 and H2 receptors.

Toxicological studies using schedule-controlled performance typically involve within-subject experimental designs. Statistical methods for estimating dose-response parameters (e.g., the dose producing a 50% maximal effect) must account for between subject differences which contribute error to the estimates. We illustrate a procedure which involves first estimating the parameter of interest and corresponding standard error from each subject's data separately. These estimates are combined to obtain a group estimate and corresponding standard error which accounts for both within-subject and between-subject variation. We illustrate the method using data from an experiment in which mice were exposed to carbon monoxide (CO) and effects on responding under a fixed-ratio schedule were examined. Nonlinear regression methods were used to obtain within-animal ED50 estimates and standard errors. The method of maximum likelihood was then used to produce a group ED50 value and standard error, (Supported by grants ES-03809 and ES-07087)
A NEW STATISTICAL MODELING FOR LEARNING BEHAVIOR IN WATER-FILLED MULTIPLE T-MAZE TESTING.
K. Suzuki, N. Hasegawa, T. Kamei, K. Kikukawa and T. Imamichi. Department of Physiology, Nippon Veterinary and Zootechnical College, Musashino City, Tokyo, Japan. Sponsor: J. P. Lee

To distinguish subtle behavioral alterations, we applied a newly proposed statistical equation expressed as Yi = b12 ln Xi + b13 Xi + b14 + E to water-filled multiple T-maze learning (Biel type), where Yi is for cumulative sum of errors, Xi for times of trials and b1 for number of animals. A trial for straining rat on the 1st day and 3 trials per day at 1000, 1300 and 1600 for next 3 consecutive days were applied to the Wistar-Inamichi strain rats with following conditions: (1) 3-10 weeks of age, (2) eyelid ectomized and intact, and (3) maternally treated with various single doses of ethylenethiourea or cytosin arabinocid.

The coefficient b13 gave positive values in cases of juvenile, eyelid ectomized and animals maternally treated with the chemicals even though they did not show any gross malformations, while it gave negative values in mature inacts. Thus, the sign + or - of the coefficient b13, showing qualitative difference in learning procedure, is considered a sensitive parameter for learning behavior.

Usefulness of this statistical modeling in behavioral teratology, especially the significance of the coefficient b13 as a judging cue for abnormality will be discussed.

A DATABASE FOR COMPUTER ANALYSIS OF REPORTED NEUROTOXIC EFFECTS. M.I. Gogel, Neurotoxicology Div., NERL, USEPA, Research Triangle Park, NC.

Automatic methods are becoming essential for accurate and thorough analysis of the increasing amount of toxicological data. Database management systems, readily available for personal computers, can be used to codify and summarize existing data. A published table from a chapter entitled "Chemicals Affecting Behavior" (Anger and Johnson, 1985) was inserted into a relational database management system on a personal computer. Listed are uses and neurotoxic effects as reported in eight secondary sources for 764 chemical entries, chronicity of the exposure, occurrence of the effect in humans or other animals, and the report citation. As a database, this information was automatically queried for specific, summarized information not easily observable in the original printed form.

The following are examples of some data that were calculated. A total of 1128 differently described effects were reported. Weakness was cited most frequently for 156 substances followed by convulsions, tremor, vertigo, CNS depression, narcosis and ataxia. Each of these was observed with over 80 chemicals. Listed chemicals had 214 different uses, but the use for 104 of these was not specified. An advantage of inserting data when collected into a database management system is the ease with which large volumes of information may then be evaluated. Tables may be created of summarized data from the database for publication.

HUMAN AND RAT METABOLISM AND MUTAGENESIS OF 2-ACETYLMETHYLFLUORONE BY INTACT HEPATOCYTES: A SYSTEM FOR SHORT-TERM IN VITRO GENOTOXICITY TESTING USING HUMAN TISSUES. K. Rado1,2, W.C. Daumrman2, and Robert Langenbach1. 1Cellular and Genetic Toxicology Branch, NIEHS, RTP, NC, 2Toxicology Program, N.C. State Univ., Raleigh, NC.

A method has been developed to assess the metabolism and mutagenic activation of AAF in human liver in vitro utilizing a slicing technique to prepare hepatocytes from non-perfusible biopsy and resected surgical liver tissue. AAF metabolites produced by human and rat hepatocytes were similar and consisted primarily of AF, with ring hydroxylated products and N-hydroxy-AAF also produced as organosoluble products. Sulfate and glucuronide conjugates of ring hydroxylated metabolites and AF were detected from human and rat hepatocytes. Levels of metabolism and mutagenicity showed interindividual variation in humans but were generally higher when compared to rat levels. A correlation appeared to exist between increasing levels of AF production and increased AAF mutagenesis on a case by case basis by human hepatocytes. This may suggest a role for further metabolism of AF in the mutagenic activity of AAF. The usefulness of such human and rodent tissue data in the extrapolation of rodent carcinogenesis data to humans will be discussed.

POTENTIATION OF 2-ACETYLMETHYLFLUORONE-INDUCED UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES BY THE PEROXISOME PROLIFERATORS CLOFIBRATE AND DI(2-ETHYLHEXYL)PHthalATE. M.T.S. Hsia and C.M. Chang, University of Wisconsin, Environmental Toxicology Center and Department of Entomology, Madison, WI.

The hypolipidemic drug clofibrate and the plasticizer di(2-ethylhexyl)phthalate (DEHP) have been found to induce hepatic peroxisome proliferation and are carcinogenic in rodents. However, these chemicals do not appear to be mutagenic in either prokaryotic or eukaryotic test systems. This study was conducted to see whether peroxisome proliferators could enhance the genotoxicity of other chemical carcinogens. Monolayer cultures of hepatocytes isolated from adult male Sprague-Dawley rats were exposed to 2-acetylmethylefluorone (2-AAF) or methyl methanesulfonate (MMS) with or without the addition of clofibrate or DEHP. The unscheduled DNA synthesis (UDS) response was measured scintillometrically using the membrane retention method. Both clofibrate and DEHP were found to exhibit dose-dependent potentiation of 2-AAF-induced UDS response, while MMS-induced UDS response was not affected by these two peroxisome proliferators. This potentiation phenomenon may be unique for the peroxisome proliferators as other epigenetic carcinogenic compounds (polychlorinated biphenyls, DDT, and toxaphene) failed to produce any detectable enhancement of 2-AAF-induced UDS response under the same experimental conditions.
VALIDATION OF THE HUMAN HEPATOCYTE PRIMARY CULTURE (HPC)/DNA REPAIR TEST.
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American Health Foundation, Valhalla, NY

Ten years ago, autoradiographic detection of DNA repair in cultured rat hepatocytes was introduced as an in vitro system to screen for carcinogens. The rat HPC/DNA repair test has been extensively validated and is widely accepted. Hepatocytes from several species are now being utilized. Only a limited number of chemicals has been tested in human hepatocytes. A systematic evaluation of the response of human hepatocytes to carcinogens and structurally related noncarcinogens has been undertaken. Thus far, livers were obtained from two brain-dead donors. Monolayer cultures of hepatocytes were simultaneously exposed to the test chemical and 3H-thymidine. DNA repair was detected by autoradiography. A concentration-dependent positive test was observed with the carcinogens 2-aminofluorene, 2-acetylaminofluorene and aflatoxin B1 while related noncarcinogens were negative. Benzo(a)pyrene was positive in only one of the two preparations. Diethylnitrosamine, tested in one preparation, was positive while dimethylformamide was negative. However, although differences were noted, human hepatocytes were capable of both activating carcinogens to DNA damaging products and repairing damaged DNA. These findings demonstrate that human hepatocytes can be used to identify a variety of genotoxic chemicals.

DNA DOUBLE-STRAND DAMAGE AND REPAIR IN ISOLATED SPERMATOGENIC CELLS FOLLOWING GAMMA IRRADIATION.
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Various cell types in spermatogenesis exhibit differential sensitivity to radiation-induced DNA damage. The investigation of DNA radiosensitivity in vitro is complicated by the heterogeneous population of male germ cells (MGC) present in isolated single cell suspensions. In the present investigation, γ-irradiation-induced DNA double-strand damage (DSR) was assessed in spermatogonia (SG) and preleptotene spermatocytes (PL). DNA of SG and PL was labelled by treatment of Sprague-Dawley rats with [3H]-thymidine 24 hours prior to enzymatic isolation of spermatogenic cells. DSR and repair was assessed using the method of neutral elution. DNA from SG and PL was detected isotopically, whereas total DNA was quantitated using a fluorometric method. DSR was induced in a dose-dependent manner in each of the heterogeneous cell population, as well as in the SG and PL. SG and PL were more sensitive to γ-irradiation-induced DSR compared to the heterogeneous MGC population. Repair of DSR was rapid (maximal repair within 30 minutes) and incomplete (45%). These studies demonstrate 1) the enhanced sensitivity of SG and PL to γ-irradiation-induced DSR and 2) the limited capability of SG and PL to repair DSR in vitro.

ACTIVITY OF GERM CELL MUTAGENS AND NONMUTAGENS IN THE RAT SPERMATOGENIC CELL DNA REPAIR ASSAY
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The rat spermatogenic cell DNA repair assay was developed to detect chemically induced germ cell DNA damage. In the current study, eight chemicals of known germ cell mutagenicity were tested to better characterize the specificity of the assay for germ cell mutagens. After exposure of Fisher-344 rats to the test compounds, the testes were removed and enzymatically digested to produce a spermatogenic cell suspension. The cells were incubated 24 hours in medium containing 3H-thymidine, and DNA repair was assessed as unscheduled DNA synthesis by autoradiography. The number of net grains per nucleus (NG) was determined in the pachytene spermatocytes. Five compounds, methyl methanesulfonate, triethylenemelamine, cyclophosphamide, N-ethyl-N-nitrosourea, and N-methyl-N-nitrosourea, which cause specific loci mutations and/or dominant lethal mutations, all induced a dose-dependent increase in NG and in the percent of spermatocytes in repair (>4 NG). In contrast, N-methyl-N'-nitro-N-nitrosoguanidine, diethylnitrosamine, and dimethylnitrosamine, strong somatic cell mutagens which do not affect germ cell mutagenesis, were negative in the rat spermatogenic cell repair assay. These results indicate that the rat spermatogenic cell DNA repair assay is useful in determining the mutagenic potential of chemicals in male germ cells.

INDUCTION OF UNSCHEDULED DNA SYNTHESIS (UDS) IN XENOGRAFTS CONTAINING HUMAN BRONCHIAL EPITHELIAL CELLS.
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Species-specific differences in carcinogen metabolism and DNA repair complicate extrapolation of rodent genotoxicity data to humans. Thus a xenograft system using human bronchial epithelial (HBE) cells was established. HBE cells from human bronchus were grown in culture, inoculated into deepithelialized rat tracheas, and implanted subcutaneously into nude mice. Within ten weeks, HBE cells lined the lumen of the trachea with a differentiated mucociliary epithelium. In situ hybridization with human DNA probes confirmed the human origin of the epithelial lining. Xenografts were cut into 1 mm thick rings, incubated 4 or 18 hr in media containing 3H-thymidine (10 μCi/ml) and N-ethyl-N'-nitro-N-nitrosoguanidine (MNNG, 3-1000 μM), and then fixed, sectioned and processed for autoradiography. A time- and concentration-dependent increase in number of grains/nucleus was observed, indicating induction of UDS by MNNG. A maximum of 14.6±1.6 grains/nucleus was observed 18 hr after treatment with 300 μM MNNG (control was <1 grain/nucleus). This system provides a relevant model for studying genetic effects in a differentiated human epithelium.
1034 EVALUATION OF IN-VIVO MUTAGENICITY OF METHYLENE CHLORIDE FOLLOWING INHALATION EXPOSURE IN MICE BY DOMINANT LETHAL TEST. W. Basso, R. Baje and M. Greening. A & M Schwartz College of Pharmacy & Hlth. Sciences, Long Island University, Brooklyn, NY.

Methylene chloride, a widely used industrial solvent, has been shown to be mutagenic by the Ames Test. This study was undertaken to determine its in vivo mutagenicity. Groups of 20 S/W male mice, seven weeks old, sexually mature, were exposed in a glass chamber to airflow of 10 liters/minute carrying 100, 150 or 200 ppm of methylene chloride. The exposure was carried out 2 hours/day, 5 days/week for 6 weeks. The control group was exposed to air alone. Two days after the last exposure, each male mouse was mated with a S/W virgin, adult female. Presence of vaginal plug was taken as a sign of successful mating, and was designated as day zero of gestation. All females were weighed twice a week, and etherized on day 17. After opening the abdomen, both uterine horns were excised, the fetuses removed and processed by a standard procedure. Testes and brain of the male mice from each group were removed following the establishment of successful mating, preserved in 10% formalin and processed. No significant difference in any of the mutagenicity parameters was found between control and treated groups. No microscopic lesions were found in the testes or brain of the methylene chloride treated male mice.

1035 DIETARY FAT MODIFIES HEPATIC ACTIVATION OF COOKED-FOOD MUTAGENS IN THE RAT.


Cooked food mutagens (CPM's) are activated by hepatic mixed function oxidases, the activities of which are modified by dietary fat. The activation to mutagenic metabolites of the CPM's: IQ, MeIQ and MeIQx were 2-4 fold greater with hepatic S9 fractions from rats fed purified diets containing 35% w/w beef dripping, coconut oil, sunflower oil or olive oil than from rats fed a low (1% w/w) fat diet. The greatest increase was in S9 fractions from olive oil-fed rats. GLC analysis revealed olive oil to contain 0.28 mg polychlorinated biphenyl/kg equivalent to 4.1 ug Aroclor 1254/NJ available in the diet. This contrasted with sunflower oil (0.13 mg PCB/kg; 1.9 ug Aroclor 1254/NJ available in the diet), containing sunflower oil supplemented with Aroclor 1254 to mimic the level found in the olive oil had a reduced ability to activate the mutagens studied. Thus the enhanced activation capacity of liver homogenates from rats fed high fat diets cannot be explained by the PCB content of the fat. Supported by the UK Ministry of Agriculture Fisheries and Food.

1036 DIETARY QUERCETIN MODIFIES HEPATIC TRANSFORMATION OF COOKED-FOOD MUTAGENS TO BACTERICI GENOTOXINS IN VIVO AND IN VITRO.


Dietary quercetin induces hepatic mixed-function oxygenases. These enzymes are involved in the conversion of cooked-food mutagens (CPM's) to bacterial mutagens. Quercetin also inhibits conversion of CPM's to bacterial mutagens in vitro. Female BALB/c mice were fed purified diets containing 42 mmoles quercetin/kg diet, an equivalent amount of its glycoside rutin or a control diet for 5 weeks. They were either killed and hepatic microsomal fractions prepared or used in a host-mediated bacterial mutation assay. The indicator organism was S. typhimurium TA98. Numbers of revertants induced by the CPM Trp-P-2 in the host mediated assay were elevated in mice fed quercetin or rutin whereas MeIQ induced mutagenesis was only increased in mice fed quercetin. Microsomes from mice fed quercetin but not rutin showed increased capacity to activate Trp-P-2 and MeIQ to mutagens in vitro. Thus although quercetin inhibits in vitro conversion of CPM's to bacterial mutagens, in vivo it's inductive effect antagonises inhibition and enhances activation of CPM's. Supported by the UK Ministry of Agriculture Fisheries and Food.

1037 NUTRITIONAL TOXICOLOGY: MECHANISMS OF FORMATION OF POTENT CARCINOGENS DURING COOKING.

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A new area of nutritional toxicology is the formation of new powerful carcinogens during cooking. This review deals with the underlying mechanisms. A liquid-reflux system of ethylene glycol/5% water, and realistic frying and browning of lean-ground beef models, were used to produce mutagens/carcinogens of the 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and quinoxaline (IQx) class. Pyrazine or pyridine compounds which react directly with creatinine in reflux models to form IQ-type mutagens have yet to be identified, but we have found that the free amino acids, L-threonine (thr), or L-serine (ser), which self-cyclize to form various pyrazine compounds upon heating, produce high levels of IQ-type, or IQ-like, mutagens when refluxed with creatinine. However, related compounds (3-hydroxybutyric acid; 2-aminoobutyric acid) do not; thus, cyclization is seen here as requisite for mutagen formation. The formation of those mutagens can be completely inhibited by L-tryptophan. (NCI grants CA-24217 and CA-42381.)
It has been well established that an inducible DNA repair system (i.e., "SOS" repair) is present in many prokaryotic organisms such as E. coli. However, with the exception of Saccharomyces cerevisiae, an analogous DNA repair system has not been well established for eukaryotic systems. Calkins, 1967 (Int. J. Rad. Biol. 12(2):297-301) reported "reactivation" phenomena in the eukaryote, Tetrahymena pyrifoma, in which cells exposed to X-rays did not follow the predicted monotonic dose response. The phenomenon was interpreted to be an induction of DNA repair processes similar to "SOS" repair found in E. coli. The work presented here further expands on this hypothesis showing that other mutagens (such as EMS and 4-NNO) can also reactivate UV damage. In addition to chemically-induced reactivation, other analogues to the E. coli "SOS" repair system have also been made. Utilizing a tunable dye laser to generate monochromatic light (265 nm), we have demonstrated an analogous UV-induced reactivation of survival in the excision defective strain of E. coli, B5-1. This reactivation may be an important part in our understanding of the inducible repair process, as well as being an important consideration in the evaluation of mutagenic agents.

The mutagenicity of NG and NSG was assessed using the mouse lymphoma (ML) and the CHO sister chromatid exchange (SCE) assays both with and without metabolic activation (S-9). NG was tested at concentrations of 4 to 0.01 mg/ml with a 4-hour (ML) and a 24-hour (SCE) exposure period. NSG was tested using the same 4 and 24-hour exposure periods for each assay and at concentrations ranging from 1.5 to 0.01 mg/ml. In both cases, the higher concentrations approximated the limits of solubility for each compound in aqueous solution. NG with or without S-9 was moderately cytostatic at the highest dose but did not induce either an increase in the mutant or SCE frequency. NSG showed marked cytotoxicity (growth less than 60% of controls) in the ML assay for doses greater than 1.2 mg/ml. This toxicity was greatly increased with S-9. Significant (p<.05) mutagenic activity, with a correlated dose response, was observed both with and without S-9. A significant (p<.0001) rise in the SCE rate, with correlated dose response, was also produced by NG without S-9. Thus while NG was nonmutagenic, its potential environmental degradation product, NSG, was moderately mutagenic in both systems tested.
Studies were conducted to assess possible health effects of CP (monochloropropanone: MCP, 1,1 and 1,3 dichloropropanone: 1,1 DCP and 1,3 DCP). It was found that CP could react with glutathione (GSH) without metabolic activation. The CP-GSH reaction rate increased as pH increased from 6 to 8, had an almost of reactivity: 1,3 DCP > MCP > 1,1 DCP, and was stoichiometric to chlorine(s)/molecule. MCP and 1,1 DCP but not 1,3 DCP were substrates for GSH S-transferase from rat liver cytosol. Cytotoxicity and mutagenicity of CP were tested in rat hepatocyte suspensions and the Ames assay. All CP were cytotoxic in hepatocytes from 0.5 - 10 mM with a corresponding decrease in cellular GSH. Mutagenic potencies among the CP differed greatly. 1,3 DCP was mutagenic in the range, 1,1 DCP was weakly mutagenic in the same range and MCP was not mutagenic. Thus, despite the structural similarity of the CP studied, notable differences in cytotoxicity and mutagenicity were observed suggesting a highly selective action of 1,3 DCP in mutagenesis compared to nonspecific effects of CP resulting in cellular damage. (This abstract does not necessarily reflect EPA policy).

Effects of low-level exposures to inhaled diesel exhaust and coal dust, both alone and combined, on induction of sister chromatid exchanges (SCEs) and chromosome aberrations in lymphocytes of monkeys was investigated. Male cynomolgus monkeys were exposed 7 hours/day, 5 days/week for 24 months to filtered air (control), 2 mg/m3 respirable coal dust, 2 mg/m3 diesel exhaust, or 1 mg/m3 respirable coal dust plus 1 mg/m3 diesel particles. At termination of exposures whole blood was cultured, and 50 and 200 metaphases per monkey were evaluated for SCEs and chromatid/ chromosome-type aberrations. Frequencies of SCEs per metaphase in each treatment group were 8.0, 8.2, 8.4, and 8.4, respectively, for control, coal dust, diesel exhaust, and the mixture of diesel exhaust plus coal dust. These values were not statistically different nor were any statistically significant differences noted in the number of chromatid- and chromosome-type aberrations in exposed groups compared to controls.
Oximes are a class of organic compounds containing the functional group \[ \text{C}=\text{N}=\text{O} \cdot \text{H} \]. Uses for the oximes vary greatly, however many of them are used as chemical intermediates. Few data are available on the toxicology of oximes. We have examined six oximes for their mutagenic effects in Salmonella typhimurium and LS178Y TK+/− mouse lymphoma cells. The oximes examined were: Cyclohexanone oxime; 2-butanone oxime; butanal oxime; acetaldehyde oxide; glyoxanilide oxime; and Aldicarb oxime. The compounds were tested in the presence and absence of rat liver S-9 in the LS178Y mouse lymphoma assay and in the presence and absence of rat liver and hamster liver S-9 in the Salmonella assay. Five Salmonella strains were used. Glyoxanilide oxime produced a weak positive response in strain TA1535 in the absence of activation. The remaining oximes were negative in Salmonella under all activation conditions. Positive responses were noted for all six oximes in LS178Y cells in the absence of S-9 activation. S-9 was also produced a positive response in the presence of S-9 activation. These results are the first on mutation induction by oximes in mammalian cells. Research sponsored by NCI contracts No. NIH-CF-41004 and NIH-CF-41030.


Cigarette smoke contains carcinogens and mutagens and affects the health of smokers. Recently, increased research has proven the potentially protective effect of selenium (Se) against heavy metal toxicity, cancer, and other health disorders. Accordingly, we have proposed the fortification of tobacco with Se to develop safer cigarettes. As a start in evaluating any biological effects of added Se, we have determined the mutagenicity of fresh, mainstream (MS), or inhaled, cigarette smoke condensate (CSC), with or without Se, in the plate incorporation assay of the Ames test. Initially, it was shown that Se by itself was not mutagenic at 0–80 \( \mu \text{g} \) Se/plate with TA1538 and TA1978. Subsequently, the effects of different levels of Se, added to 100 or 150 \( \mu \text{g} \) CSC/plate, were examined with TA98, TA100, and TA1538. On addition of 10 \( \mu \text{g} \) Se to 100 \( \mu \text{g} \) CSC/plate produced mutagenicity reductions of about 50%. Higher levels of added Se yielded greater reductions. Fresh cigarette sidestream (SS) smoke, collected between puffs, was also tested. Again, 5–50 \( \mu \text{g} \) of Se added to 100 or 150 \( \mu \text{g} \) SS-CSC/plate gave similar reductions, indicating similarity in mutagenicities of MS and SS CSC.


In the toxicological assessment process described, we have taken into consideration that the occupational exposure to organic chemicals is more often multiple than single. We designed a study which deals with the prediction of potential combined effects of chemical exposures on multiple target sites. We chose 5 halosilanes widely used in industrial settings (chloroform, carbon tetrachloride, 1,1,1-trichloroethane, trichloroethylene and 1,2-dichloroethane) and studied their effects on 4 target sites (CNS, heart, kidney and liver). In Phase I, we used dose-response relationships, and attempted to predict the interactive capacities of these halosilanes for each target organ. In Phase II, we tested the validity of our predictions by preparing combinations of these reference halosilanes with six other chemicals. Our results indicate that few supraadditive interactions occurred (kidney and liver). Moreover, the overall degree of predictability was low. (Supported by NSERC and IERST, Québec).
The role of haloalkane dosage in the potentiation of halokane hepatotoxicity by ketones is not well understood. We investigated the effect of the dose of CCl₄ on the liver injury potentiated by 1,3-butanediol (BD), methyl n-buty1 ketone (MBK) or methyl isobutyl ketone (MIBK) in rats. Male Sprague-Dawley rats were orally treated with different concentrations of BD, MBK or MIBK (in corn oil) 24 h prior to a challenge of CCl₄ given i.p. (0.005, 0.01, 0.05, 0.10 or 0.50 ml/kg). ALT activity and bilirubin level were assayed in plasma 24 or 48 h after the challenge as indices of hepatic dysfunction. When the CCl₄ dose was increased 10-fold, the minimal effective dosage (MED) of the ketones required to potentiate CCl₄ hepatotoxicity decreased proportionately. The product of the dose of CCl₄ (T) by the corresponding MED (P) was constant for CCl₄ dosages ranging from 0.01 to 0.10 ml/kg and was equivalent for the 3 potentiators. For doses of CCl₄ lower than 0.01 ml/kg, no potentiation occurred even when toxic doses of the potentiators were administered. At the 0.50 ml/kg dose of CCl₄, potentiation occurred but at a higher than expected P X T products. This suggests that CCl₄ plays an active role in the ketone/potentiation phenomenon and that both components of the ketone/CCl₄ combination should be taken into account when predicting the severity of the resulting liver injury. Supported by MRC and IRSST Quebec.

Chloroenceone (CD) markedly potentiates CCl₄ hepatotoxicity. Based on hepatotoxicity and lethality assessments, recent studies showed a significant protection in animals stimulated for active hepatic cell regeneration, using normal diet (N) or CD treated (10 ppm in diet for 15 days) male S-D rats undergoing sham (SH) or partial hepatectomies (PH) on day 15 of the dietary protocol. Greatest "H-T incorporation in nuclear DNA occurs at 2 days post-PH which returns to basal levels by 7 days. CD treatment alone did not change the above phenomenon. CCl₄ (0.1 ml/kg, i.p.) was administered 2 and 7 days post-PH or SH in rats on N or CD diets. Hepatotoxicity was assessed at 6 or 24 h. CCl₄ induced serum enzyme alterations were less in 2 days post-PH rats than in SH rats or 7 days post-PH rats indicating that CCl₄ hepatotoxicity is significantly reduced when there is a greater regenerative activity. These results support the concept that suppressed hepatic cell regeneration is responsible for CD potentiated CCl₄ hepatotoxicity. (EPA grant R-811072 and ES-07045.)
PROTECTION OF CHLORDEcone POTENTIATED CCl4 HEPATOTOXICITY BY PARTIAL HEPATECTOMY. A.N. Bell, R.A. Young, V.G. Lockard and H.M. Mehen-dale. Dept. Pharmaco1 and Toxico1 and Dept. Path. Univ. MS Med. Ctr. Jackson, MS

Chlordcone(CD) markedly potentiates CCl4 hepatotoxicity. We have hypothesized that the combination of CD + CCl4 suppresses hepato-cellular regeneration following CCl4 intoxication. To test this hypothesis, CCl4 hepatotoxicity was evaluated in actively regenerating livers using CD treated partially hepatectomized (PH) rats as a model. Peak mitotic activity occurs 1 day post-PH with a return to baseline at 7 days post-PH. CCl4 (100 uL/kg, ip) was administered 1 and 7 days post PH or sham operation (SH). Hepatotoxicity was assessed 24 hrs. later by measuring serum SGPT, SGOT and ICD and by histomorphometric analysis. In the 1 day post-PH group, CCl4-induced serum enzyme elevations were less than in the SH group. This difference was not observed in the 7 day post-PH group. CCl4 lethality was decreased in the 1 day post-PH group, relative to the SH group, but not in the 7 day post-PH group. While histomorphometric analysis revealed the presence of balloon and necrotic cells in the 1 and 7 day post-PH groups, mitotic figures were observed only in the 1 day post-PH group. These findings indicate that suppression of hepato-cellular regeneration is involved in the CD + CCl4 interaction. (Supported by ES-07045 and EPA R-811072).

PROTECTION OF HEPATOTOXIC AND LETHAL EFFECTS OF CCl4 BY PARTIAL HEPATECTOMY K.S. Prasada Rao, U.M. Joshi, R.A. Young, A.N. Bell, and H.M. Mehen-dale, Dept. of Pharmaco1 and Toxicol1, Univ. Miss. Med. Ctr., Jackson, MS

Bell et al. (Toxicol. Lett. 31 (suppl.): 101, 1986) have reported protection of chlordcone potentiated CCl4 toxicity by partial hepatectomy. This may mean that the progressive phase of the end effects of CCl4 might be due to suppressed hepato-cellular regeneration. This hypothesis was tested in male S-D rats undergoing sham hepatectomy (SH), or 2/3rd hepatectomy (PH). Incorporation of H-thymidine (H-T) in hepato-cellular nuclear DNA, was used as an index of hepato-cellular regeneration. Peak regeneration occurs at 2 days post-PH and liver regeneration phases out by 7 days. SH and PH rats were challenged with a single ip dose of either corn oil vehicle, or CCl4 (0.1 mL/pO or 2.5 mL/kg). At 6 or 24 hr later, hepatotoxicity was assessed by serum enzymes (PT and OT). In another group of rats, 48 hr mortality was recorded. The high dose of CCl4 was significantly hepatotoxic and lethal in SH rats while in PH rats both hepatotoxic and lethal effects were significantly decreased. H-T incorporation highly stimulated by PH, was significantly decreased by high dose of CCl4. These findings are supportive of the concept that the progressive phase of CCl4 toxicity is due to suppressed hepato-cellular regeneration and tissue repair. (EPA R-811072 and ES-07045.)

INTERACTIVE HEPATOTOXICITY OF CCl4 AND TRICHLOROETHYLENE: ANY ROLE FOR ENHANCED LIPID PEROXIDATION? L.G. Spies and C.D. Eskelson, Dept's. of Pharmacology & Toxicology & Surgery, University of Arizona, Tucson, AZ

Halogenated hydrocarbons contaminate many drinking water supplies. Although any individual agent is usually present at non-toxic concentrations, the potential for synergistic interactions is of concern. When P344 or S/D rats (260-250 g) were administered simultaneously CCl4 (0.1 mL/Kg ip) and trichloroethylene (TCE, 0.25 to 1.0 mL/kg ip), a dramatic potentiation of CCl4 induced liver injury was observed. Similar potentiation was observed when TCE was administered prior to CCl4 (either ip or in the drinking water). To determine if this synergism resulted from enhanced lipid peroxidation, exhalations of ethane was quantitated in rats receiving ip. CCl4 (0.1 mL/Kg), TCE (1.0 mL/Kg) or both chemicals simultaneously. Ethane was trapped on tennax at 30 min intervals for 4 hr, desorbed, and determined by GC. Ethane exhalation was linear for 4 hr at which time 60, 130 or 160 nmoles of ethane were exhaled by the CCl4, TCE, or CCl4 and TCE groups, respectively. Incoporation of TCE and CCl4 reduced exhalation of CCl4, but enhanced the exhalation of CHCl3, a metabolite of CCl4. The mechanism by which TCE potentiates the hepatotoxicity of CCl4 remains unknown. It appears that CCl4 metabolism is enhanced, but not lipid peroxidation. Other indices of peroxidation must be measured to insure these solvents do not retard ethanol exhalation. Supported by ES-3-5531 and EPA No. CR-812557.

264

Inhalation of p-xylene for three days (6 h/d, 1600 ppm) increased cytochrome P-450 content of the liver in male F344 rats. Subsequent (next day) exposure to a single dose of carbon tetrachloride (0.075 ml/kg by gavage) resulted in a greater decrease of cytochrome P-450 in the liver when compared to rats treated with CCl4 alone. Changes in other parameters such as liver weight and liver-to-body weight ratio were also enhanced by the combined exposure. The three day exposure to p-xylene only did not reliably affect any other measured parameters, and cytochrome P-450 levels were not statistically different from controls by the second day after cessation of exposure. A single 6-hour exposure to p-xylene did not reliably affect cytochrome P-450 content of the liver. However, small but statistically significant changes were noted in body weight, liver weight and liver-to-body weight ratio. These results indicate that p-xylene is not a strong hepatotoxicant under these conditions, it produces a marked enhancement of the hepatic effects of carbon tetrachloride. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

EFFECT OF VEHICLE ON THE RELATIVE UPTAKE OF HALOALKANES ADMINISTERED BY GAVAGE. R.H. Granger, J.B. Coleman, L.W. Condit*, R.G. Lamb and J.F. Borzelleca. Deps. of Pharmacology/Toxicology and Internal Medicine, Medical College of VA, Richmond, VA and U.S.E.P.A., Cincinnati, OH.

The effect of vehicle (water, corn oil (CO), 5% emulphor in water (5%/E)) on the uptake of lipophilic chemicals, e.g. chloroform (CHCl3), carbon tetrachloride (CCl4), has been investigated. Rats with indwelling arterial cannulas were gavaged with equivalent doses of CHCl3 or CCl4 dissolved in different vehicles. The absorption, elimination and relative uptake of each chemical from the different vehicles was determined. The rate and amount of chemical absorbed are related to the degree of lipophilic interaction between the chemical and the vehicle. CCl4 in CO displayed a significant decrease in uptake, rate of uptake and peak blood concentrations when compared with the less lipophilic vehicles, water or 5%/E. The uptake and rate of absorption of CHCl3 (more water soluble than CCl4) was affected to a lesser extent. These results indicate that 5%/E, which is capable of solvating quantities of haloalkanes several orders of magnitude greater than water alone, will more closely simulate the absorption of haloalkanes from water. (Supported by EPA Cooperative Agreement 81255A.)

DICHLOROACETATE INCREASES CHLOROFORM TOXICITY IN FEMALE RATS. W.R. Davis, West Virginia Univ. Med. Ctr., Morgantown, WV

Dichloroacetate (DCA) and chloroform (CHCl3) are both formed during chlorination disinfection of drinking water. DCA has effects on intermediary metabolism to increase plasma concentrations of keto-acids, an effect associated with increased susceptibility to CHCl3 toxicity. The present studies were designed to determine if DCA enhances the toxicity of CHCl3. Male and female SD rats were given DCA (either 0.92 and 2.45 gmole/kg) by gavage (2 ml/kg) 3 times. The solutions were neutralized; controls received the equivalent Na load. CHCl3 was given (0.75 ml/kg, ip) 3 hrs after the last DCA dose. DCA decreased lactate concentrations in liver and plasma. CHCl3 decreased plasma lactate more markedly and the effect was not additive. BUN was not increased by CHCl3 alone (20 ± 3 vs 26 ± 5 mg/dl) in NaCl controls, but was elevated by DCA (72 ± 3 and 58 ± 14 for low and high DCA doses), the interaction was significant. SGPT was increased in NaCl controls (55 ± 2 vs 70 ± 6 U/ml). DCA treatment enhanced this (241 ± 57 and 125 ± 49). CHCl3 toxicity was not increased by DCA treatment in male rats. (Supported by US EPA Cooperative Agreement CR811906; this does not necessarily reflect official EPA policy.)


Although CCl4 is of concern as a drinking water contaminant, it has been necessary in oral toxicity studies to give CCl4 as a suspension/emulsion or in an oil vehicle, due to its limited water solubility. The objective of our study was to assess the influence of dosing vehicles on the acute hepatotoxicity of CCl4. Fasted 200 g male S-D. rats were given 0, 10, 25, 50, 100, 250, 500, 1000 or 2500 mg CCl4/kg by gavage in: corn oil; as an aqueous emulsion; as the undiluted chemical; and in the 10 and 25 mg/kg doses only in water. Blood and liver samples were taken 24 hr after dosing for measurement of serum and microsomal enzymes. Pathological examination of liver samples was also conducted. Dose-dependent increases in serum enzyme levels and pathological changes, and dose-dependent decreases in microsomal P-450 and glucose-6-phosphatase activity were observed in each vehicle group. CCl4 was less hepatotoxic at each dosage level when given in corn oil than when given as an emulsion or as the pure chemical. CCl4 in corn oil was also less toxic than CCl4 in water at the 10 and 25 mg/kg doses. Our findings demonstrate that dosing vehicles significantly influence the acute hepatotoxicity of CCl4 in rats. (Supported by U.S. EPA 812267)
EFFECT OF DOSING VEHICLES ON THE PHARMACOKINETICS OF ORALLY ADMINISTERED CARBON TETRACHLORIDE (CCl₄) IN RATS. J.J. Crock, H.J. Kim, C.E. Dallas, R. Ramathan, S. Muralidhara, and J.M. Gallo. Dept. Pharmacology & Toxicology, *Dept. Pharmaceutics, College of Pharmacy, Univ. of Georgia, Athens, GA.  

Because of the common use of oil vehicles as a dosage vehicle in oral toxicity experiments and their potential influence on the pharmacokinetics and toxicity of CCl₄, studies were undertaken to assess potential effects of different vehicles on the pharmacokinetics of CCl₄. Fasted 200 g male S-D rats with indwelling arterial cannulas received 25 mg/kg CCl₄ by gavage. In corn oil; as an aqueous emulsion; in water; and as pure undiluted chemical. A 25 mg/kg dose was also given iv for calculation of bioavailability. Serial blood samples were taken and analyzed for CCl₄. Peak concentrations of CCl₄ in the blood were reached within 8 min after dosing in the emulsion and saturated water groups. These peak levels were slightly higher than in the pure CCl₄ group and substantially higher than in the corn oil group. There was evidence of later secondary peaks of lesser magnitude in the corn oil group. The absolute bioavailability for the emulsion and saturated water groups was higher than for the corn oil and pure chemical groups, and comparable to the iv group. These results suggest that corn oil has a sufficient effect on the pharmacokinetics of orally-administered CCl₄ to require a reappraisal of its use in acute oral toxicity experiments with CCl₄. (EPA CR 812267)

EFFECTS OF 4-HYDROXY-2-NONENAL (4-HNE) ON SULFHYDRYL LEVELS AND GLUCOSAMONIDASE (G-6-Pase) ACTIVITY ON RAT LIVER. J. Renn, J.J. Kadis, H.E. Ferran, Jr., A. O’Connor. Dept. of Biod., La Salle University, Philadelphia, PA and Dept. of Pharmacol., Thos. Jefferson University, Philadelphia, PA. 

4-HNE, an unsaturated aldehyde formed by incubating CCl₄ with liver microsomes has been postulated to mediate the hepatoxicity of CCl₄ (Biochem. Biophys. Acta 620, 231, 1980). CCl₄ is known to reduce liver G-6-Pase activity, elevate serum glutamic-pyruvic transaminase (SGPT) and have essentially no effect on liver NPSH levels. To compare the effects of 4-HNE with those known to be produced by CCl₄ on the liver we studied the effects of intraperitoneally (ip) and intraperitoneally (ip) administered 4-HNE on G-6-Pase activity, SGPT as well as liver NPSH and protein-bound sulfhydryl (PSSH) levels in male Sprague-Dawley rats. 4-HNE synthesized in our laboratory had an LD50 ip of 35 mg/kg in rats. Intraperitoneal injection of 35 mg/kg 4-HNE reduced liver NPSH levels 27% and at 70 mg/kg, 51%. Neither PSSH nor G-6-Pase activity were reduced by either of these doses nor were SGPT levels elevated. The results of ip injection of 35 mg/kg 4-HNE 3 hr after treatment did not differ significantly from those seen after ip injection of 4-HNE. Liver microsomes incubated for 30 min with 4.4 mM 4-HNE, as with CCl₄, lost 55% of their cytochrome P-450 levels, however. In summary, most of the characteristic hepatotoxic effects of CCl₄ were not seen after ip or ip injection of lethal doses of 4-HNE to rats. To what extent 4-HNE generated within hepatocytes may simulate the hepatotoxic effects of CCl₄ still remains an open question. (Supported by a grant from La Salle University and a USPHS Toxicology Training Grant to TJJ)

ADENOSINE 3'-PHOSPHATE 5'-PHOSPHOSULFATE (PAPS) AND UDP-GLUCURONIC ACID REGULATION DURING CARBON TETRACHLORIDE-INDUCED HEPATIC FIBROSIS IN THE RAT. T.J. Mazliss, N.G. Shipp and J.J. Hjelle. University of Wisconsin, Madison, WI. 

The development of hepatic fibrosis is associated with changes in various hepatocellular functions including significant changes in the synthesis of extracellular matrix components (e.g., sulfated glycosaminoglycans). Given that glycosaminoglycan synthesis and drug biotransformation both require PAPS and UDPGA, it was of interest to determine whether hepatic fibrosis was associated with changes in the concentrations and synthesis of these co-substrates. Rats were treated with CCl₄ (0.5 ml/kg, sc., twice weekly) for 1, 3 and 6 weeks. Histopathologic examination revealed the development of fibrosis after 6 weeks. Although the activity of the PAPS synthetic enzymes, ATP sulfurylase and APS kinase, were decreased at timepoints (minimal values of 36 and 31% of control, respectively), hepatic PAPS concentrations were not altered. Serum sulfate concentrations, a PAPS precursor, were also unaffected. UDPGA concentrations were decreased (6 weeks, 73% of control) but the synthetic pathway (UDP-glucose and UDP-glucose dehydrogenase) was unaffected. These data indicate that the availability of hepatic co-substrates is not markedly altered during fibrogenesis caused by subchronic treatment by CCl₄. (Supported by a PMAF grant.)

ADENOSINE 3'-PHOSPHATE 5'-PHOSPHOSULFATE (PAPS) AND UDP-GLUCURONIC ACID (UDPGA) SYNTHESIS DURING LIVER NECROSIS AND REGENERATION. T.J. Mazliss, N.G. Shipp, W. Wickman and J.J. Hjelle. University of Wisconsin, Madison, WI.

In order to further assess the effects of acute liver injury on hepatic drug conjugation and phase II co-substrates, the concentrations and synthesis of PAPS and UDPGA were determined in rats treated with carbon tetrachloride (CCl₄, 1 ml/kg in corn oil, ip.) and killed 12, 24, 48 or 72 hrs later. Histopathologic examination and serum ALT and AST activities confirmed that maximal hepatic cellular necrosis occurred after 24 hrs and active regeneration occurred 48 hrs after CCl₄ treatment. UDPGA concentrations were decreased at 12 and 24 hrs with the minimum occurring at 24 hrs (65% of control). Changes in hepatic UDP-glucose concentration and UDP-glucose dehydrogenase activity were not observed. PAPS concentrations were decreased at all timepoints (minimum at 48 hrs, 55% of control). Activities of the PAPS synthetic enzymes, ATP sulfurylase and APS kinase were decreased at 24 and 48 hrs. Minimum activity was at 48 hrs in each case and represented 56 and 28% of control activity, respectively. Serum sulfate increased at 24 and 48 hrs to 130% of control. These data show that UDPGA and PAPS concentrations are decreased by acute liver injury and that the decline in PAPS concentration is associated with lower activities of the synthetic enzymes. (Supported by a PMAF grant.)
1066 THE COVALENT BINDING OF 14C-CCl4 METABOLITES WITH PHOSPHO-LIPID OF HEPATIC MICROSOMES FROM PHENOBARBITAL TREATED RATS. B.S. Kaphalia and C.A.S. Ansari, Division of Chemical Pathology, The University of Texas Medical Branch, Galveston, Texas.

Covalent binding of carbon tetrachloride (CCl4) metabolites to cell constituents may be a contributing factor in the acute hepatotoxicity of the compound. The present studies were conducted to examine the binding of CCl4 metabolites to microsomal lipids. The hepatic microsomal preparation of rats treated with phenobarbital was incubated with 14C-CCl4 in vitro in the presence of NADPH generating system. The total lipids were extracted with chloroform/methanol (2:1, v/v) and were further separated into neutral and phospholipid fractions. The phospholipid fraction contained seven-fold higher radioactivity than found in the neutral lipids. The phospholipid fraction was separated into major phospholipids using thin layer chromatography (TLC) on silica gel G and chloroform/methanol/ammonia (65:25:4, v/v) as the developing solvent followed by autoradiography. Two major radioactive bands associated with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions were obtained. The 14C metabolite binding was 42 and 48 mmol/mg (dry wt basis), in the PC and PE fractions, respectively. The TLC separated fractions were further purified by normal phase HPLC using a gradient of hexane: isopropanol (6.8:1, v/v) to hexane:isopropanol/water (6.8:1, v/v). Transesterification and phospholipase A2 cleavage studies were further carried out to identify the fatty acid moiety bound to 14C metabolites in the molecules. The structure elucidation of these adducts is in progress. (Supported by NIH Grant No. AM-27135).

1068 THE INFLUENCE OF CHLORINATED HYDROCARBONS ON HEPATOCYTE FUNCTION IN VIVO AND IN VITRO. R.G. Lamb, J.B. Coleman, H. Granger, T.W. Condie* and J.F. Borzelleca. Depts. of Pharmacology and Toxicology and Internal Medicine, Medical College of VA, Richmond, VA and W.U.S.P.A., Cincinnati, OH.

Intact rats (in vivo) and primary cultures of adult rat hepatocytes (in vitro) were exposed to carbon tetrachloride (CCl4), chloroform (CHCl3), trichloroethylene (TCE), perchloroethylene (PCE) and selected combinations of these agents. Chemical dependent changes in hepatocyte function were assessed by measuring alterations in the cell's capacity to: (1) reduce MTT (mitochondrial function); (2) incorporate 3H-choline into phosphatidylcholine (endoplasmic reticulum function); (3) release of GGT (AST) and/or GPT (ALT) (plasma membrane integrity). In both studies, CCl4 and CHCl3 altered hepatocyte functions more than TCE and PCE. CCl4-mediated changes in hepatocyte function were increased by CHCl3, PCE and TCE and reduced by inhibitors of metabolism (SKF 525A and metyrapone). These results suggest that CCl4, CHCl3, TCE and PCE produce similar alterations in cellular function of hepatocytes in vivo and in vitro. Therefore, cultured hepatocytes may be an appropriate system for determining the hepatotoxic potential of chemicals. (Supported by EPA Cooperative Agreement 812558 and NIH Grant AM31115.)


The role of carbon tetrachloride (CCl4) metabolism on the CCl4-dependent alterations in hepatocyte phospholipase C activity in intact rats and monolayers was assessed. CCl4 treatment (0.5 ml/kg, i.p.) for 4 hr produced a 2-fold increase in microsomal phospholipase C (PLC) activity, a major phospholipid degradative enzyme. Phenobarbital pretreatment increased: (1) 450- mediated CCl4 metabolism, as measured by a rise in the binding of 14C-CCl4 adducts to protein and lipids; (2) the CCl4-mediated increase in PLC activity (2-fold). A 6 hr incubation of cultured hepatocytes with 0.5 mM CCl4 produced a 2-3 fold increase in PLC activity that was blocked by metyrapone. Metyrapone was also able to inhibit the covalent binding of CCl4 to hepatocyte protein and lipids. In both systems, these increases in PLC activity preceded other cellular alterations such as decreases in mitochondrial and microsomal function and membrane permeability. These data suggest that the PLC-mediated degradation of membrane phospholipids may be an early event in CCl4-induced hepatocyte injury, and this event is preceded by covalent adduct formation. (Supported by NIH AM 31115, EPA Cooperative Agreement 812558, and NIH ES 07087.)

1069 COMPARISON OF BIOCHEMICAL ALTERATIONS PRODUCED BY CCL4 EXPOSURE IN RAT LIVER AND IN PRIMARY CULTURES OF RAT HEPATOCEYS. R.M. Lang and L. Moore. Dept. of Pharmacology, USUHS, Bethesda, MD.

In order to evaluate how well the development of CCl4 hepatotoxicity in vivo can be modeled in primary cultures of rat hepatocytes, biochemical alterations were determined in liver samples from rats given 1.5 ml/kg CCl4, and in liver cells cultured for 18 hours then exposed to 2 mM CCl4. Soluble thiol levels matched closely between tissue and hepatocytes (11 vs. 12 µg/mg protein). Comparable concentrations of CCl4 resulted in 6.3 mM at 30' and in the culture medium (0.5 mM at 5'). Simultaneous inhibition of ER Ca2+ pump and stimulation of phospholipase occurred at early times in vivo (30') and in vitro (5'). Glucose-6-phosphatase was inhibited next in liver (120') and in cells (20'). 5'-AMPase was not affected at all times examined in either system. Leakage of glutamic-pyruvic transaminase and depletion of glycogen were maximal at later times in vivo (6 hrs.) and in cells (30'). Calcium increased several-fold in liver (24 hrs.), but was not elevated in hepatocytes. Lack of calcium accumulation in cells likely resulted from impaired mitochondrial Ca2+ uptake. Thus, CCl4-induced changes followed nearly the same continuum in both models, although the progression was much more rapid in vitro than in vivo. (Supported by grant #PS03457 from NIH.)
A COMPARISON OF ARGINOSUCCINIC ACID LYASE TO OTHER INDICES OF CARBON TETRACHLORIDE (CCl₄)-INDUCED HEPATOTOXICITY. D.A. McMillan and I.G. Sipes, Dept. of Pharmacology and Toxicology, University of Arizona, Tucson, AZ

Arginosuccinic acid lyase (ASAL) is widely used as an index of toxicity in cultured hepatocyte systems but has only recently been used as an in vivo index of hepatotoxicity. The enzyme is stable for 4 hr at 40°C. By 10 hr 33% of the activity is lost and at 24 hr only 9% of the activity remains. At 0°C enzyme activity is reduced to 85% by 24 hr to 48 hr and 76% at 72 hr to 96 hr. An evaluation of ASAL was done in comparison with bilirubin, alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) on carbon tetrachloride (CCl₄)-induced hepatotoxicity (0.1 and 1.0 ml/kg, i.p.). Bilirubin levels increased over time for both CCl₄ groups from 8 to 24 hr. ALT activity was maximal from 12 hr to 24 hr for both CCl₄ treatment groups. SDH activity showed the greatest elevations over control levels at 24 hr, 33- and 150-fold respectively, for the low and high dose CCl₄ groups. ASAL, which had barely measurable control levels, also served as a sensitive marker of hepatic damage. At 4, 12 and 24 hr the activity of ASAL was increased 10-, 65- and 46-fold over controls at the low dose and 19-, 232- and 323-fold at the high dose, respectively. A major drawback in the use of ASAL may be its loss of activity over time. (Supported by EPA No. CR-812597.)


The effect of ethionine on the in vitro mitochondria and microsomal calcium uptake was evaluated. Ethionine is known to deplete the liver of ATP and thereby damages mitochondria. Such an effect is more pronounced in female rats. Female Sprague-Dawley rats were administered ethionine (400 mg/kg) in two divided doses and the animals sacrificed after three hours. Other groups of rats received ethionine+CCL₄ (0.5 ml/kg) phenobarbitol (75 mg/kg) for three days + CCL₄ or phenobarbital + Ethionine + CCL₄. Animals were sacrificed one hour after CCL₄ administration. Appropriate controls were maintained. Liver mitochondrial and microsomal 45Ca uptake was measured. Activities of mitochondrial succinic dehydrogenase and microsomal glucose-6-phosphatase were determined. Serum transaminases were also measured. Enhanced inhibition of mitochondrial and microsomal 45Ca uptake was clearly evident in ethionine + CCl₄ and phenobarbitol + ethionine + CCl₄ groups. Mitochondrial succinic dehydrogenase was significantly inhibited. The results indicated that mitochondrial activity associated with increased CCl₄ metabolism might be involved in progressive hepatocellular damage induced by CCl₄. (Supported by: PSC-CUNY 666258 and ES-04172)


Hepatotoxicity resulting from exposure to high CCl₄ doses in rats is reportedly preceded by marked inhibition of calcium uptake by isolated liver microsomal (ER) and plasma membrane (PM) subcellular fractions. However, the relationship between toxicity and calcium uptake by isolated ER and PM at low CCl₄ doses has not been well characterized. To determine this relationship, we administered CCl₄ to mice at doses of 0.005-2.0 ml/kg, measured serum alanine aminotransferase activity (an index of liver necrosis) 24 hrs post-dose, and measured ER and PM calcium uptake activities 2 hrs post-dose. Low yet hepatotoxic CCl₄ doses did not affect calcium uptake by plasma membrane vesicles, and only a modest inhibition of this activity was observed at the highest CCl₄ dose, 2.0 ml/kg. In contrast all hepatotoxic CCl₄ doses reduced microsomal calcium uptake activity to less than 40% of control. This inhibition of microsomal calcium uptake activity was not associated with reduced microsomal protein thiol content. We conclude that CCl₄ hepatotoxicity in mice is correlated with early inhibition of calcium uptake by microsomal, but not plasma membrane, subcellular fractions. Supported by NIH Grants ES-03373 and CA-36277.

TETRACHLOROETHYLENE METABOLISM AND HEPATOTOXICITY. M.H. Rosner and D.E. Carter, Univ. of Arizona, College of Pharmacy, Tucson, AZ

Tetrachloroethylene (PERC) has been shown to produce different hepatotoxicities in several mammalian species. Our study investigated whether differences in metabolic rate in the same species were related to the observed toxicity in the same species. Metabolism was quantified by measuring urinary metabolites and hepatotoxicity was assessed by serum glutamic oxalacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) which were analyzed as prescribed by Sigma Diagnostic Kit #505. Male Sprague-Dawley rats (275 ± 25 g) were gavaged 0.10-2.50 ml/kg PERC for a dose response effect and given 0.75 ml/kg PERC following pretreatment with phenobarbital, 3-methylcholanthrene, β-naphthoflavone and Aroclor-1254 to observe the effects of inducing agents. A group was also pretreated with the inhibitor, SKF 525A. Serum enzyme levels were not increased after increased levels of PERC; decreased enzyme levels were observed in some cases. Metabolism of the PERC was induced by β-naphthoflavone and Aroclor-1254 but without increased serum enzyme levels. SKF 525-A decreased urinary oxalate levels and showed increased SGPT levels, but this was caused by SKF 525-A alone. The rate of metabolism and liver toxicity do not appear to be directly related. (Supported by Electric Power Res. Inst. No. RP231004.)
RELATIVE UPTAKE AND ELIMINATION OF 1,1,1-TRICHLOROETHANE (TCE) IN INHALATION AND ORAL EXPOSURES IN RATS. C.E. Dallas, A. Amadament, S. Muralidhara, J.M. Gallo, and J. V. Bruckner. Dept. of Pharmacol. & Toxicol. and *Dept. Pharmaceutics, College of Pharmacy, Univ. Georgia, Athens, GA.

In order to assess the utility of route to route extrapolation of pharmacokinetic data for volatile organics, the uptake and elimination of TCE were contrasted in male S-D rats subjected to equivalent oral and inhalation exposures. Fifty or 500 ppm TCE was inhaled for 2 hr through a one-way valve by anesthetized rats of 325-375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently and analyzed for TCE in order to characterize systemic uptake and elimination profiles. To administer an equivalent dose by oral and inhalation exposures, an oral bolus dose equal to the total cumulative uptake measured during the 2-hr inhalation exposures was given to other rats and serial blood samples taken. Similar, rapid rises in blood TCE levels were seen during the initial min of the oral and inhalation exposures. Blood levels peaked 5-12 min after oral dosing and declined steadily thereafter. Blood levels in rats inhaling TCE approached steady-state and remained there for the duration of the 2-hr exposures. Bioavailability of inhaled TCE was significantly greater than that of orally administered TCE. (Supported by U.S. EPA CR 812267)

COMPARATIVE PHARMACOKINETICS OF INHALED AND INGESTED TRICHLOROETHYLENE (TCE) IN RATS. J.V. Bruckner, C.E. Dallas, S. Muralidhara, R. Emanathan, and J.M. Gallo. Dept. Pharmacology & Toxicology and *Dept. Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA.

The relative uptake, disposition, and elimination of equivalent inhaled and ingested doses of TCE were studied in male S-D rats of 325-375 g. Unanesthetized rats with an indwelling arterial cannula inhaled 50 or 500 ppm TCE for 2 hr through a 1-way valve. Samples of the inhaled and exhaled breath, as well as arterial blood, were collected concurrently and analyzed for TCE. Based on cumulative uptake at the end of the 2-hr inhalation exposure, an equivalent dose was given orally as a single oral bolus to unanesthetized rats and serial blood samples taken from an arterial cannula. Blood levels of TCE rose rapidly to a peak within 10 minutes of the oral bolus and were similar in magnitude to blood levels measured in the same time frame after initiation of corresponding inhalation exposures. There were substantially higher concentration of TCE in the blood during the steady-state and elimination phases of the inhalation exposures than during the same time-frame following oral dosing. Predictably, the bioavailability of ingested TCE was significantly less than that of inhaled TCE. (Supported by U.S. EPA CR 812267)


This study was designed to elucidate the biochemical events involved in trichloroethylene (TCE) nephrotoxicity. Male Wistar mice (30 g) were given i.p. 1 500 mg/kg 3-Bromolione-5-P-Clorokorimine (500) to deplete glutathione. Two hours after 500 pretreatment TCE was adminis- tered i.p. to mice in a dosage from 125 to 1 000 mg/kg in sesame oil. Lipid peroxidation in vivo was measured as exhalation of ethane over 2 h. Subsequently mice were killed and malondialdehyde (MDA) production was measured in liver and kidney tissue and renal accumulation of p-sinohippurate (PAH) and tetraethylammonium (TEA) was determined.

TCE administration induced a dose-dependent increase in ethane exhalation and a dose-dependent increase in MDA production in the kidney but not in the liver tissue. Renal accumulation of PAH and TEA decreased in a dose-dependent manner. This decrease correlated well with the increase in MDA generation in the renal cortex.

In conclusion, TCE could be bioactivated in the liver or/and in the kidney to a reactive radical which initiates the peroxidative damage of the membrane lipids in the kidney cells.

PERCHLOROETHYLENE METABOLISM BY THE GLUTATHIONE CONJUGATION PATHWAY. J. Odum and T. Green. Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: E.A. Lock.

Percchloroethylene (PER) has produced a low incidence of tumours in the F344 rat but not the B6C3F1 mouse kidney. Many halogenated alkenes are metabolised by glutathione (GSH) conjugation. These conjugates are degraded to nephrotoxic and sometimes mutagenic cytosine conjugates. In this study PER was conjugated with GSH in vitro in F344 rat liver S9. The GSH conjugate of PER (trichloroethyl glutathione) was found in bile after PER was given orally. The mercapturic acid was identified in urine from rats dosed orally with PER (1500mg/kg) and in urine from rats and B6C3F1 mice exposed by inhalation (400 ppm 6h/day). The urinary concentrations of the mercapturic acid were 10x lower in the mouse than in the rat. Trichloroethyl cytosine (a known mutagen) was a substrate for kidney 8-lyase. Km for mouse kidney cytosolic 8-lyase was 5x higher and Vmax 5x lower than that for the rat. The difference in amounts of PER metabolised by this pathway and the lower activity of 8-lyase in the mouse may account for the species difference in carcinogenicity.
90-DAY TOXICITY DATA FOR 1,2,3-TRICHLOROPROPANE. R.D. Lauter, M. Robinson, J.P. Berge, E.L. Long and L.W. Condie, Toxicology and Microbiology Division, Health Effects Research Laboratory, Cincinnati, Ohio. 1Pathology Associates, Ijamsville, Maryland.

Because 1,2,3-trichloropropane (TCP) is one of several short chained, chlorinated aliphatic contaminants in drinking water and because little was known about its toxicity, subacute, subchronic toxicity studies were conducted. The results of the 10 day study have been presented [The Pharmacologist 28:182 (1986)]. The results of the 90 day study are now available. The exposure groups were 0, 0.01, 0.05, 0.10 and 0.40 mmoles per kg of rat body weight (M.W. of TCP = 143.43). The doses used in this study did not result in food or water consumption differences. The results of the 90 day study corroborate and extend the findings of the 10 day study indicating a correlation between TCP exposure and myocardial inflammation, degeneration and necrosis. Detailed results of the histopathology findings, clinical chemistry and hematology parameters will be presented. (This abstract does not necessarily reflect EPA policy).

PERFLUORO-N-DECANIC ACID (PFDA)-INDUCED INCREASE IN HEPATIC PALMITATE OXIDATION IN VITRO IN MALE F344 RATS. G.D. Pilcher, M.E. George and M.F. Andersen, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH.

Perfluoro-n-decancic acid (PFDA) increases liver size, alters hepatic fatty acid composition, causes hepatic ultrastructural changes including disruption of mitochondria and the appearance of peroxisomes, and decreases fatty acid oxidation in vivo. In the present study, total and peroxisomal fatty acyl-CoA-oxidase was measured in liver homogenates from PFDA-dosed (50 mg/kg) and paired control rats 2 or 8 days after a single dose. PFDA increased total palmitate oxidation compared to paired control 2 and 8 days after dosing by 70% and 50%, respectively (p < 0.05). In the same treatment groups, peroxisomal (cyanide-insensitive) palmitoyl-CoA oxidase was increased in PFDA-dosed rats 2 and 8 days after dosing by 5- and 10-fold, respectively. The in vitro results indicate that PFDA increases the capacity for total fatty acid oxidation in liver due, in part, to enhanced peroxisomal fatty-acyl oxidase. These data are unexpected based on in vivo utilization of [14C] palmitate and may indicate increased biosynthetic incorporation of fatty acids or altered conditions in the in vitro versus in vivo oxidation assays. (Supported by APOSH).


Our objective was to evaluate pathologic changes caused by ingestion of the soil fumigant DCP. DCP was given in corn oil by gavage to male Sprague-Dawley rats in daily doses of 0.1, 0.25, 0.5 and 1.0 g/kg for 1, 5 and 10 days. Only the liver showed slight changes 24 hr after a single high dose. After 5 days, the 1.0 g/kg group and some lower dose rats exhibited hepatic centrilobular degeneration, necrosis, inflammation and early fibrosis. At 10 days, hepatic changes were not so severe as at 5 days. Nucleolar enlargement in hepatocytes was seen at all dose levels at both 5 and 10 days, as was hemolytic anemia which increased in severity with increasing dose.

In the subchronic study, rats were gavaged with 0.1, 0.25, 0.5 or 0.75 g/kg, 5 days per week, for up to 90 days. Livers of rats given 0.5 and 0.75 g/kg exhibited centrilobular hepatitis and fibrosis. Testicular degeneration and adrenal cortical lipidosis and medullary vacuolization also occurred in the 0.5 and 0.75 g/kg rats. Hemolytic anemia was manifest at all 4 dosage levels. Our findings indicate that the liver and red blood cells, and possibly the testicles and adrenals are target organs for DCP. (Supported by U.S. EPA 811213)

TOXICITY OF PERFLUOROALKYL ETHYL METHACRYLATE. G.L. Kennedy, Jr. E.I. duPont deNemours & Co. Newark, DE

Perfluoroalkyl ethyl methacrylate (FAEM) is a copolymer with a molecular weight of from 1500-3000 and is used for topical coatings. The acute oral toxicity is very low with doses of 5 to 25 g/kg tolerated by the rat. No signs of a response were seen in rabbits treated dermally with 2 g/cm2. In the rabbit, FAEM is a mild skin irritant and is a moderate eye irritant producing only transient changes. The material was not a skin sensitizer in the guinea pig. The approximate lethal dose following a 4-hour inhalation by rats of a respirable aerosol is 1,700 mg/m3 which classifies FAEM as moderately toxic by this route. Genetic screening studies including the Salmonella and the CHO/HGPRT gene mutation assays show FAEM to be inactive. The subchronic toxicity is also very low with rats given 28 daily oral doses of up to 1000 mg/kg showing no clinical signs, no hematologic, clinical pathologic, or tissue pathologic changes. FAEM is very low in toxicity following various routes of acute exposure and repeated oral doses did not produce any remarkable changes in rats.
SUBCHRONIC TOXICOLOGY STUDIES OF HEXACHLORO-1,3-BUTADIENE (HCBD) IN B6C3F1 MICE FOLLOWING DIETARY INCORPORATION. R.S.H. Yang, K.M. Abdo, and H.R. Elwell, NIHES/NTP, RTP, NC; A.C. Levy, Microbiological Associates Inc., Bethesda, MD; L.H. Brennecke, PAI, Indianapolis, IN.

Groups of 5 mice/sex were exposed to 0, 30, 100, 300, 1,000, or 3,000 ppm HCBD in the feed for 14 days. Toxic responses, primarily in the higher dose groups, included abnormal clinical signs, mortality (all mice in the top two doses died by day 7), body and organ weight depression, gross and histopathological changes. The most prevalent microscopic lesion, seen in all HCBD-treated mice of both sexes, was renal tubule necrosis and/or regeneration. Histopathological changes were also seen in liver, lymphoid tissues, and testes. Subchronic studies were conducted where groups of 10 mice/sex were exposed to 0, 1, 3, 10, 30, or 100 ppm HCBD in the feed for 13 weeks. No treatment-related clinical signs or mortality were observed. Body weight gain was reduced in the 30 and 100 ppm males (-49% and -56%, respectively), and the 100 ppm females (-47%). Significant reduction in kidney weights were seen in the 30 and 100 ppm males and 100 ppm females. A treatment-related increase in tubular regeneration in the renal cortex was confirmed. This lesion, characterized by a diffused increase in basophilia of the tubular epithelial cytoplasm and an increase in the number of nuclei, increased in severity with increased dose.

DNA-PROTEIN CROSS-LINKING IN RAT LIVER NUCLEI BY HALOGENATED ACETALDEHYDES. D. A. Keller, R. C. Brown, and H. d'A. Heck, Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

The carcinogenic formaldehyde and acetaldehyde form DNA-protein cross-links (DFX). The ability of chloral (trichloroacetaldehyde), a trichloro-ethylene metabolite, chloroacetaldehyde, a vinyl chloride metabolite, and bromal (tribromacetaldehyde) to form DFX was investigated in rat liver nuclei. The percentage of nonextractable (interfacial) DNA (IP-DNA), an index of DFX formation, was increased from 12 to 23% by 47 mM chloroacetaldehyde in 30 min at 37°C, and from 12.9 to 24.3% by 200 mM acetaldehyde in 30 min at 4°C. Yields of total DNA were decreased by 44% with chloroacetaldehyde and by 12% with acetaldehyde, perhaps due to extensive cross-linking. Chloral hydrate (100 mM) and bromal (173 mM) exposed nuclei had non-significant increases in IP-DNA, from 11.8 to 13.2% in 90 min at 37°C, and from 13.4 to 14.3% in 30 min at 37°C, respectively. Reactivity of the aldehydes with thiosemicarbazide was investigated. Chloroacetaldehyde reacted extensively, acetaldehyde reacted to a lesser extent, while chloral and bromal were unreactive. The failure of chloral and bromal to react may be due to the stability of their respective hydrates. This may also explain why these compounds apparently do not form DFX in vitro.


Glutathione (GSH) content in liver homogenate and p-450 content, aninopyrine demethylase (AmDM), aniline hydroxylase (AnHX), 7-ethoxyxocoumarine deethylase (EC0E) and 7-ethoxyresorufin deethylase (EroE) activities in liver microsomes were determined 3 hr or 18 hr after orally administering to rats halocacetonitriles (HAN), including monochlorocacetonitrile (MCN), dichlorocacetonitrile (DCN), trichlorocacetonitrile (TCN), dibromocacetonitrile (DBN) and bromochlorocacetonitrile (BCN). Liver weight to body weight ratio was increased at 10 hr in rats treated with 4 dihalocacetonitriles (DHAN). At 3 hr GSH was depleted by MCN and increased by TCN. By 18 hr GSH recovered to the control level in MCN treated rats and was significantly higher in DHAN treated rats. All DHAN decreased p-450 content and EroE activity both at 3 hr and 18 hr. They also inhibited AnHX and EroE activities at 18 hrs. Only DCN and TCN decreased AnHX activities at 3 hr. AmDM activity was inhibited by DCN, TCN and BCN at 18 hr. In general HAN inhibited species of p-450 involved in EroE and AnHX more than those involved in AmDM or EroE, with DCN and TCN the most reactive, followed by DBN and CN and, MCN the least reactive. This abstract does not necessarily reflect EPA policy.

ACETONE EFFECTS ON N-(3,5-DICHLOROPHENYL)-SUCINIMIDE-INDUCED NEPHROTOXICITY. G.O. Rankin, H.H. Lo, V.J. Teets, D.J. Yang, and F.I. Brown. Depts. of Pharmacology and Anatomy, Marshall University School of Medicine, Huntington, WV.

Acetone has been shown to potentiate the hepato- and/or nephrotoxic effects of numerous halogenated hydrocarbons which require bioactivation to a toxic species. Previous studies from our laboratory have demonstrated that N-(3,5-dichlorophenyl)-sucinimide (NDS) requires biotransformation to its ultimate nephrotoxicant. The purpose of this study was to determine if acetone pretreatment could alter NDS-induced nephrotoxicity. Male Fischer 344 rats received acetone (1, 5 or 10 mmol/kg) or corn oil (10 ml/kg) orally followed 18 hr later by a single intraperitoneal injection of NDS (0.2 or 0.4 mmol/kg) or sesame oil (2.5 ml/kg). Renal function was monitored at 24 and 48 hr. Acetone pretreatment did not potentiate the renal effects produced by NDS (0.2 mmol/kg) or reduce the nephrotoxicity produced by NDS (0.4 mmol/kg). These results suggest that the mechanism of activation of other halogenated hydrocarbons by acetone is different from the mechanism required for the activation of NDS. Supported by NTH grant DK 31210.
PCBs IN GOATS. HASTENING WITHDRAWAL USING MINERAL OIL. D. Polin, M. Underwood, E. Lehning, S. Burian, and P. Wiggers, Dept. of Animal Science, Michigan State University, East Lansing, MI.

Sixteen pigmy goats weighing 8 to 22 kg were fed 15 ppm PCBs (Aroclor 1254) for 14 days. Two were euthanized at zero time of withdrawal, along with 2 controls. At that time, four goats were fed clean diet, 5 were fed 5% mineral oil (MO), and 5 were fed 10% MO plus restricted in feed intake to 50% of ad libitum controls. Two goats remained on clean diet from the start of the experiment. On day 21 of withdrawal, all goats were euthanized and carcasses (minus head, lower legs, and digestive tract) with abdominal fat were ground into hamburger consistency. PCB analyses revealed that those at zero time withdrawal had 16 ppm, as compared to an average 12 ppm (100%) on day 21 of withdrawal. Those fed MO averaged 92 ppm (49%) whereas those fed 10% MO + 50% feed restriction averaged 7.84 ppm (65%). Mineral oil was effective in reducing PCBs over a 21 day period to about 58% of the concentrations in non-treated goats.

COMPARISON OF THE PHARMACOKINETICS OF TWO TOXICOLOGICALLY DIVERSE POLYCHLORINATED BIPHENYL ISOMERS IN MICE. M.A. Clevenger, R.D. Harbison, and R.C. James, Div. of Toxicol., Univ. Ark. For Medical Sciences, Little Rock, AR.

The chlorination pattern of the biphenyl molecule is important in both the toxicity and elimination kinetics of PCBs, however the relationship between pharmacokinetic behavior and toxic potency of PCB isomers has been only minimally explored. The steady-state pharmacokinetics of 2,2',5,5'- (2,5-TCB) and 3,3',4,4'-tetrachlorobiphenyl (3,4-TCB) were compared using a physiological model approach. Male ICR mice were gavaged with either 100 mg/kg of 2,5-TCB once daily for 8 doses or 8 mg/kg of 3,4-TCB every other day for 10 doses. The terminal elimination phase kinetics were followed in serum, liver, and adipose tissue. The elimination half-life from serum was 1.4 days for 2,5-TCB and 2.2 days for 3,4-TCB. Both isomers were cleared from liver and adipose at rates similar to clearance from serum. Adipose contained the highest concentrations of TCBs with levels being 200 and 450 times serum levels for 2,5-TCB and 3,4-TCB, respectively. 3,4-TCB also showed greater disposition for liver and thymus relative to 2,5-TCB. The two isomers studied displayed differences in both elimination kinetics and accumulation in target organs suggesting that PCB burdens in target tissues contribute significantly to the diverse toxic potencies of PCB isomers. (Supported in part by USPHS Grant ES02824).

SUBACUTE TOXICITY OF 2,2',3,4,4',6-HEXACHLORODIPHENYL ETHER IN THE RAT. D.C. Villeneuve, V.E. Secours, I. Chu, W. Eamon, M. Beadette and V.E. Valli, Health Protection Branch, Ottawa, Canada, and Biopath Analysts, Guelph, Ontario.

Chlorodiphenyl ethers are demonstrated environmental contaminants and only limited information is available on their toxicity. The purpose of the present study was to investigate the subacute oral toxicity of 2,2',3,4,4',6-hexachlorodiphenyl ether (CDE), one of the predominant isomers found in the environment. CDE was administered orally, by gavage to male and female rats daily at dose levels ranging from 0.04 to 40 mg/kg bw for 28 days. CDE caused increased body weight gain in females dosed 4.0 or 40 mg/kg bw and increased liver weights in both sexes receiving 40 mg/kg. Serum LDH, uric acid and potassium levels were increased in females at 4.0 and 40 mg/kg. Hepatic mixed function oxidase activity was not induced in either males and females and hematology profiles were unaffected by treatment. Mild to moderate histological changes occurred in the thyroid, liver and kidney of both sexes, and in general were more severe and prevalent in males than females. Residue analysis showed that CDE accumulated in a dose-dependent manner in both fat and liver with levels in fat 10-20 times higher than in liver. On an overall basis the no-effect level for this compound was judged to be 0.4 mg/kg bw/day.

CHRONIC EXPOSURE TO 3,4,5,3',4'-HEXACHLORODIPHENYL (HCB) ALTERS THE DYNAMICS OF VITAMIN A METABOLISM. P.A. Bank, M.E. Cullum, R.K. Jensen, and M.H. Zile, Deps. of Food Science/Human Nutrition and Pathology, Michigan State University, East Lansing, MI. Sponsor: Stuart Sleight.

3H-retinol complexed to retinol binding protein-transhyretin was given iv to female rats that had been fed 3.2 umol HCB/kg diet for 140 d. Radioactivity and/or vitamin A were determined in blood, tissues and excreta for 48 h following administration of 3H-vitamin A complex. HCB treatment increased (3-4 fold) the urinary and fecal excretion of 3H and increased serum turnover rate, while fractional turnover rate was not altered. Total liver 3H in HCB rats was decreased (50%) at 12-48 h time points; equilibration of 3H into the retinyl ester (RE) pool occurred 12 h later in treated rats but at 24 h sp act of these RE pools were similar. HCB rats did not recycle hepatic 3H-RE at 48 h as observed in control animals. In the kidney 3H in HCB rats was increased after 12 h, due primarily to equilibration of the dose into a RE pool. Other vitamin A target tissues did not show any significant difference in total 3H. Thus, HCB can alter kinetic parameters associated with recycling, mobilization and excretion of vitamin A. (NIH ES05347).
Evidence for 3,4,3',4'-tetrachlorobiphenyl-mediated oxidation of uroporphyrinogen by chick embryo liver supernatants. P. Sinclair, R. Lambrecht, & J. Sinclair, VA Medical Center, White River Jct., VT and Dartmouth Medical School, Hanover, NH Sponsor: E. Smith

Various PCB and PBB congeners cause accumulation of uroporphyrin (URO) in cultures of chick embryo hepatocytes. We have shown that this accumulation appears to be mediated by a form of cytochrome P450 that is inducible by methylcholanthrene (MC) as well as 3,4,4'-trichlorobiphenyl. Liver supernatants from MC-pretreated chick embryos catalyzed the oxidation of uroporphyrinogen I (UROgen) in the presence of both NADPH and 3,4,3',4'-tetrachlorobiphenyl. Liver supernatants from either untreated embryos or embryos treated with glutethimide, a phenobarbital-like inducer of P450, did not catalyze the oxidation of UROgen. This oxidation was inhibited by CO, piperonyl butoxide and by specific antisera against the MC-inducible P450 of chick embryo liver. The oxidation was not inhibited by catalase, superoxide dismutase or desferrioxamine. These results suggest that the primary action of PCB's to cause URO accumulation in the avian system is the induction of a specific P450 followed by oxidation of UROgen. Supported by the VA and NIH (CA 25012).

Role of glutathione(GSH) content in modulating cytoskeletal perturbation induced by 1-chloro-2,4-dinitrobenzene(CDNB). M.F. Leung and I.N. Chou. Dept. of Microbiology, Boston University School of Medicine, Boston, MA Sponsor: A.E. Rogers

We have shown that CDN-induced microtubule(MT) disassembly in mouse 3T3 cells and that taxol prevents CDN effects. Since CDN is an excellent substrate for glutathione-S-transferase, we have investigated the role of cellular GSH content in modulating CDN-induced perturbation to the organization of MT and microfilaments(MF) as visualized by fluorescence microscopy. Treatment of 3T3 cells with 2.75 uM CDN for 30 min resulted in depletion of total GSH by 75% and MT began to disassemble. At 3 hr, GSH content was undetectable and MT were almost completely disrupted. In contrast, MF bundles increased in density, especially in peripheral areas where MT distribution was deficient while MF were heavily packed. Interestingly, CDN-treated cells began to reaggregate at 5 hr as evidenced by partial restoration of GSH content to 10% of the control value and MT began to reassemble. At 9 hr, GSH content reached 20% of the control value and, under these conditions, MT reassembly was nearly complete and MF also returned to their normal content and distribution pattern. These results suggest that total cellular GSH content may be an important element in controlling the dynamics of MT state and MF distribution in the cytoplasm.

Comparison of uroporphyrinogen decarboxylase activities and uroporphyrin accumulation in mice, Japanese quail and cultured chick embryo hepatocytes treated with polyhalogenated aromatic compounds. R. Lambrecht, P. Sinclair, W.J. Bement, J. Sinclair, VA Medical Center, White River Jct., VT, and R. Carpenter, Oregon State University, Corvallis, OR. Sponsor: R. Smith

The purpose of this study was to compare the activity of uroporphyrinogen decarboxylase (UROD) in the hepatic porphyria caused by certain polyhalogenated aromatic compounds. Under these conditions, mice (C57BL/6 males) developed porphyria in weeks, quail in days and chick hepatocytes in hours. UROD activity was assayed in 10,000 X G supernatants using penta-carboxyl porphyrinogen III as substrate. Highly porphyrinically mice were found to have decreased UROD activities, ranging from 13% to 69% of the control values, depending on the degree of uroporphyrin accumulation. However, in neither Japanese quail nor chick hepatocytes was a statistically significant decrease in UROD activity observed for treated versus control groups, despite the high concentration of uroporphyrin in the treated groups. These results suggest that the decrease in UROD activity previously reported by many workers is not the primary cause of the accumulation caused by the halogenated compounds. Supported by funds from the VA and NIH (CA 25012).


Three synthetic oils were applied to the shaved backs of collared rats, 5 days/week, in separate 13-week studies. Toxic signs, weight gain, serum chemistry, hematometry, urinalysis, organ weights, and gross and microscopic changes were evaluated. A condensed, hydroxylated polyolefin (viscosity of 28.1 CSt at 40°C), a dibasic acid ester (26.4 CSt at 40°C), and a polyol ester of C5-C9 acids (5 CSt at 100°C), were tested at 0, 800, or 2000 mg/kg/day. A small (10%) time and dose-related reduction in mean body weight gain was seen in males, with lesser effects in females. Serum glucose levels were reduced up to 24% in treated males, and to a lesser extent in females. Slight desquamation with trace erythema was observed at the treatment sites at both dose levels, in both sexes, with all 3 materials. Other than mild protoporphyria and very slight ketonuria evident in rats dosed with the dibasic acid ester, no evidence of toxicity was found by any of the test parameters. These results should pose minimal risks to humans.
A COMPARATIVE STUDY ON THE EFFECT OF ANIMAL AGE ON THE PERCUTANEOUS ABSORPTION OF CAPTAFOLO.

The effect of animal age on the percutaneous absorption of Captafo was examined in young and mature male Sprague-Dawley rats (240-259 g, Group I; 457-516 g, Group II). A single 0.5 mg dose of 14C-Captato was labeled in the tetrachloroethyl moiety was applied topically in 0.05 ml of saline onto a 10 cm² site on the back. The rats were placed in Metrap® restraining metabolism chambers for 2, 8 or 24 hours, and urine, feces, CO₂ and volatiles were collected. At the end of each time interval, four animals from each group were sacrificed, and the skin from the application site, acetone and methanol/water skin rinses, blood, carcass and excreta were analyzed for 14C content. After the 24 hr exposure period, almost 99% of the 14C-Captato remained on the skin application site. 97% of the 14C-label found at the skin site could be removed by washing with acetone. Mean (range) total systemic absorption after the 24 hr exposure was 0.9% (0.5-1.3) in Group I and 1.2% (0.8-2.0) in Group II. These results indicate that Captato is poorly absorbed when applied dermally to rats and the age of the animal is not a determinant of the extent to which the test material is absorbed.

EFFECTS OF P. GINSENG ON ACUTE ALCOHOL INTOXICA-

To study the effects of P. ginseng on blood alcohol clearance, the blood alcohol level in human subjects were examined using each individual as his own control. Alcohol 1.11 g/kg b.w. or alcohol containing crude water extract of ginseng 50 mg/kg b.w. was consumed over a 45 min period. At 40 min after the last drink, blood samples were taken and alcohol content was determined. The blood alcohol level of the control subjects was 0.15% (g/100 ml), whereas that of the test subjects receiving ginseng extract along with alcohol was only 65% of the control level. When the elimination in men from ginseng-Dawley rats (200 g) of the final alcohol metabolite, CO₂, was determined by the measurement of 14CO₂ trapped in ethanalamine solution, cumulative counts of 14CO₂ revealed that in 5 hrs, about 60% of the alcohol injected was eliminated in control animals, while in test animals receiving ginseng extract 50 mg/kg b.w., over 90% was eliminated. These experimental results indicate that P. ginseng extract enhances the metabolism of ethyl alcohol, thereby lessening acute alcohol intoxication.

A RELEVANT VAGINAL IRRITATION/SUBACUTE TOXICITY MODEL IN THE RABBIT AND OVARIECTOMIZED RAT.

The use of the rabbit and ovariectomized rat to predict human vaginal irritation can be enhanced by the addition of a nonoxynol-9 control group. In two separate studies, three groups of 5 female rabbits were dosed with 1.0 ml of normal saline, experimental product extract or 2% nonoxynol-9. Similar groups of ten ovariectomized rats were dosed with 0.2 ml of the same agents. Ovariectomized rats were chosen to offer consistent, thin, non-cornified stratified squamous epithelium devoid of the epithelial variation associated with hormonal cycle. The rabbit was chosen for its history of sensitivity associated with its single layer of tall columnar epithelium easily stripped by mild irritants. Following ten days of dosing, the animals were sacrificed and selected tissues were preserved for microscopic examination. Microscopic vaginal irritation in nonoxynol-9 treated animals was comparable to or greater than that seen in the saline controls or experimental extract-treated animals. The use of this positive control material helps to bring the test material results into perspective, i.e., relatively low potential for vaginal irritation in humans.

ACUTE ORAL TOXICITY OF 2-THIOTRIAZONE IN RATS.
T.M. Tate, L.P. Ruhr, and W. Flory. Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA.

The acute toxicity of 2-thiazone (a new compound under investigation as a potential fertilizer), closely resembles the toxic effects of alpha-naphthylthiourica (ANTU), a rodenticide. This compound is highly toxic to adult male rats with an oral LD₅₀ of 4.6 mg/kg. There were no toxic effects observed in immature rats dosed up to 1000 mg/kg body weight. Where clinical signs occurred they included: dyspnea, prostration, cyanosis, convulsive activity and a white frothy discharge. Gross lesions included pulmonary edema and effusion. These signs developed within 3 hours after dosing. They reach a maximum in 2-4 hours and then either death or recovery ensues. The results of this study indicate that the lung is the target organ with gross and histologic alteration being apparent.

Human HL-60 promyelocytic leukemia cells are induced to differentiate into monocytes following treatment with phorbol esters, and into granulocytes following treatment with dimethylsulfoxide (DMSO) or retinoic acid. A change seen early in phorbol ester-induced differentiation was production of cellular chondroitin sulfate with a decreased sulfation content. In this respect 20 mM 12-O-tetradecanoylphorbol-13-acetate (TPA) induced an 80% reduction of the available free sulfate did not appear to be the cause of this decrease. Serum starvation also had no effect on sulfation, indicating the process was related to differentiation rather than concurrent cell growth arrest. Glycosaminoglycans have been implicated in regulation of cell growth and differentiation, therefore the ability of other differentiation inducers to produce this effect was examined. The granulocytic inducers DMSO (1.5%) and retinoic acid (1 μM) also inhibited the sulfation of chondroitin sulfate by 60 and 30% respectively. These findings therefore suggest that alterations in glycosaminoglycan sulfate content may play an important role in cell differentiation induced by chemical agents. Supported by Grant ES07073.

1100 OCCURRENCE OF THE MYCOTOXIN, CYCLOPIAZONIC ACID, IN MEAT AFTER ORAL ADMINISTRATION TO CHICKENS. William P. Norred, Joe W. Dorner and Richard J. Cole. Russell Research Center, ARS/USDA, Athens, GA and National Peanut Research Laboratory, ARS/USDA, Dawson, GA.

Cyclopiazonic acid (CPA) is a mycotoxin produced by several economically important fungi including Aspergillus flavus and Penicillium camemberti. The toxin has been identified in mold-contaminated corn and peanuts. We earlier demonstrated, in a distribution study with [14C]CPA administration to rats, that a significant percentage of either oral or parenteral doses of CPA distributed to the carcass. Consumption of CPA-contaminated feed by domestic animals might result in similar distribution in edible meat, with the result that human consumers may be exposed to the mycotoxin. We therefore developed a high pressure liquid chromatographic method for analysis of CPA in meat, and then administered 0, 0.5, 5.0 or 10.0 mg CPA/kg body weight to 4-week-old chickens by crop intubation. At 3, 24, 48 and 96 hr intervals, birds were killed and breast and thigh muscle removed. Analysis of the meat indicated a dose-response relationship, with some samples from birds administered 10 mg CPA/kg having as high as 4000 ppm of CPA. The levels of CPA recovered from the meat declined with time, so that by 96 hr after dosing, CPA was detectable only in the high dose group. There appeared to be at least 2 polar metabolites of CPA in the meat.


A group of carbamoylpyridine and nitroctylpyriderazine compounds, synthesized in this department, have exhibited in vitro activity to inhibit ADP-induced human platelet aggregation (1a). Some of these were tested for acute toxicity in 8 & 9 ICR mice to select those compounds which offer most promise as therapeutic agents for further in vivo evaluation, and to obtain in vivo toxicity data for refinement in targeting new molecular designs for future syntheses.

Biological activity was noted as CMS effects (sedation, respiratory depression), with some compounds inducing muscle tremors/convulsions at higher doses. A small amount of bloody exudate was noted around nostrils or eyes of some animals. Acute ip LD₅₀'s ranged from 85 to 529 mg/kg. No compound-related damage to the heart, liver, lungs or kidneys was noted by light microscopy.

The compound with the best LD₅₀/Aₙ₄ ratio (C73) was safety tested po (ig) in 5-0 rats at doses up to slightly > 4.5 times the mouse ip LD₅₀. These doses were not lethal, although sedation, respiratory depression and slight bloody discharge from nostrils or eyes of some rats was observed.

Supported by NIH Grant RO1- HL-22326.


Seventy-two, 3-month old pastel mink were fed 0, 33, 60, 108, 194, or 350 ppm supplemental fluoride (F), as sodium fluoride, for 382 days to assess its effects on growth, fur quality, reproduction, and survival. No differences were observed in body weight gains or fur quality between the controls and F-treated mink. Males exposed to 350 ppm F for 4 months showed weakened frontal and parietal bones. The F treatments had no adverse effects on breeding, gestation, whelping, or lactation, although only 14% of the kits whelped. Females fed 350 ppm F survived to 3 weeks of age. No differences were observed in hemostatic parameters or serum calcium concentrations between the controls and treated mink. Serum F levels and alkaline phosphatase activities were significantly elevated in mink fed 194 and 350 ppm F. Urinary and femoral concentrations were closely correlated with dietary F levels. Femoral ash content of the 194 and 350 ppm F-treated mink was significantly less than the controls. The survivability of the adult mink was adversely affected only at 350 ppm. Clinical signs of toxicity included skeletal and dental lesions, general unthriftness, and hyperexcitability.
Anatoxin-a, 2-acetyl-9-azabicyclo[4.2.1]non-2-ene, is a potent cholinergic alkaloid produced by a toxicogenic strain of the cyanobacterium blue-green algae (Anabaena flos-aquae, LD50 = 0.25 µg/kg, i.p., mouse). It has been responsible for the death of livestock, pets and wildlife when toxic blooms have occurred in lakes of temperate North America. Factors which contribute to and select for the onset of a toxic bloom remain unclear. It has been suggested that certain key nutrients, such as nitrate and phosphate play an important role in this process.

Recently, this laboratory has developed a rapid, sensitive GC-EC method for the detection of anatoxin-a which is three orders of magnitude more sensitive than the mouse bioassay, i.e. 25 ng/10 ml sample. This provides a means for the detection of sublethal, pretoxic levels of anatoxin-a. Using this method, concentration, carbon dioxide, light and pH are being assessed for their effect on growth and anatoxin-a production of a cultured strain of anatoxin-a producing Anabaena flos-aquae.

**Prophylaxis and Treatment Against the Toxicity of Organophosphate (OP) Compounds in Rat by Memantine and Atropine.** R.C. Gupta, M.J. McLean, and W.D. Dettbarn. Departments of Pharmacology and Neurology, School of Medicine, Vanderbilt University, Nashville, TN

Male Sprague-Dawley rats injected sc with a non-lethal acute dose of OP compound such as soman (100 µg/kg), tabun (200 µg/kg), sarin (110 µg/kg), VX (12 µg/kg) or DFP (1.5 mg/kg) had seizures within 15-20 min, maximal at 1 hr and lasting 4-6 hr. Larger doses of these compounds were lethal. A single dose of the novel anti-convulsant, memantine HCL (MEM, 18 mg/kg, sc), and atropine sulfate (AS, 16 mg/kg, sc) 60 and 15 min, respectively, before OP compound administration prevented seizures, muscle necrosis and lethality, and behavior remained normal. Neither MEM nor AS, alone or in combination, affected the activity of acetylcholinesterase, butyrylcholinesterase, or carboxylesterase in various brain regions (cortex, stem, striatum, and hippocampus) or skeletal muscle (soleus, extensor digitorum longus (EDL), and diaphragm). MEM + AS reduced OP-induced inhibition of these esterases by > 50-60%. These data suggest that, in addition to cholinolytic effects of AS, MEM may prevent OP toxicity by enhancing their degradation and/or esterase reactivation and by direct neuronal actions demonstrated in cell culture.

**Anatoxin-A(s), an Irreversible Anticholinesterase from Blue Green Anabaena Flos-Aquae NRC 525-17.** N.A. Mahmood and W.W. Carmichael, Wright State Univ., Dayton, OH. Sponsor: R.W. Gardier.

Anatoxin-a(s) [ANTX-A(S)] from cyanobacterium A. flos-aquae NRC 525-17 has been isolated, purified by HPLC and pharmacologically characterized. Results from acute toxicity tests in laboratory animals with ANTX-A(S) indicated an excessive stimulation of the cholinergic nervous system similar to anticholinesterase poisoning. Whole blood cholinesterase activities from dosed rats (0.1-1.0 mg/kg, i.p.) were inhibited. In vitro inhibition of eel acetylcholinesterase (ACHE, EC 3.1.1.7) and human erythrocyte AChE was found to be time and concentration dependent and follows first-order kinetics, indicative of irreversible inhibition. Inhibition of horse serum cholinesterase (EC 3.1.1.8) was less specific than AChE and observed to be non-linear. The irreversibility of AChE inhibition was confirmed by equilibrium dialysis and a plot of Vmax versus total enzyme [E].

**Nutrient Concentration and Anatoxin-A Production.** D.K. Stevens and R.I. Krieger. Pharmacology/Toxicology Program and Washington Animal Disease Diagnostic Laboratory, Universities of Washington State, Pullman WA and Idaho, Moscow ID.

Anatoxin-a, 2-acetyl-9-azabicyclo[4.2.1]non-2-ene, is a potent cholinergic alkaloid produced by a toxicogenic strain of the cyanobacterium blue-green algae (Anabaena flos-aquae, LD50 = 0.25 µg/kg, i.p., mouse). It has been responsible for the death of livestock, pets and wildlife when toxic blooms have occurred in lakes of temperate North America. Factors which contribute to and select for the onset of a toxic bloom remain unclear. It has been suggested that certain key nutrients, such as nitrate and phosphate play an important role in this process.

Recently, this laboratory has developed a rapid, sensitive GC-EC method for the detection of anatoxin-a which is three orders of magnitude more sensitive than the mouse bioassay, i.e. 25 ng/10 ml sample. This provides a means for the detection of sublethal, pretoxic levels of anatoxin-a. Using this method, concentration, carbon dioxide, light and pH are being assessed for their effect on growth and anatoxin-a production of a cultured strain of anatoxin-a producing Anabaena flos-aquae.
11-DAY CONTINUOUS EXPOSURE INHALATION TOXICITY STUDY/ETHYL CHLORIDE (ETC1):
13 WEEKS/60 RATS AND B6C3F1 MICE GIVEN THE COMPOUND IN THE FEED FOR UP TO
13-WEEK EXPOSURE TO TETRAFLUOROMETHYL /TOXICOLOGIC EFFECTS OF
13-WEEK EXPOSURE./ TOLERANCE TO TRIMELLITIC ANHYDRIDE IN RATS
13-WEEK NESE-OONLY-EXPOSURE STUDY IN FISCHER 344 RATS./CHLORPYRIFOS:
14-DAY EXPOSURE TO SUBLETAL CONCENTRATIONS OF CARDIOMEGALY IN RATS AFTER
14C-) ACROLEIN IN THE RAT AND THE ACUTE/OUS TOXICITY AND DISPOSATION OF
14C-BENZO(A)-PYRENE ADSORBED ON CARBON BLACK P/ION AND BINDING OF INHALED
14C-BENZO(A)-PYRENE ADSORBED ON CARBON BLACK P/AND METABOLIC ACTIVATION OF
14C-BROMOBENZENE METABOLITES TO LIVER SLICES IN VITRO/COVALENT BINDING OF
14C-LABELED DNA/CITY BY DIMETHYLNITROSAMINE - DISTRIBUTION STUDIES WITH
14C-NIPLURIDIN INTO MILK OF LACTATING RATS: A COMPARISON OF/EXCRETION OF
14C-PHENYLETHYL ALCOHOL (PEA)/TRIBUTION AND EXCRETION OF TOPICAL DOSES OF
14C-PHENYL1-3-PYRAZOLIDINE ADMINISTERED BY/G/ABOLIC FATE OF [U-
14C-POLYMERS JR400 AND LR400 FOLLOWING A SI/SKIN PENETRATION POTENTIAL OF
14C/14 METABOLITES WITH PHOSPHOLIPID OF HEPATIC M/TH/COVALENT BINDING OF
14C] (4-NPI) IN RATS AND RABBITS/F 4-NITRO-N-METHYLPYRZALIMIDE [CARBOXYL-
14C/ETHEL-3-ETHOXYPROPIONATE IN THE RAT/T METABOLISM AND DISPOSATION OF
14C/FORMALDEHYDE (HCHO) IN THE NASAL MUCOSA /SIS OF DNA-BOUND [3H]- AND
2CH3-MPPT IN MOUSE BRAIN/METABOLISM AND CLEARANCE OF MPT AND
2,5-HD AND DMH EXPOSED RATS/PLASMIC TRANSPORT IN SCIATIC NERVES OF ACR,
2,5-HD, DMHD AND IDPN UPON MICROTUBULLE CYTOSKELETAL NETW/OFFECTS OF ACR,
2,5-HD AND DMHD UPON THE MICROTUBULE: MITOSIS AND TUB/TOXIC ACTION OF ACR,
2-WEEK SUBCHRONIC INHALE STUDY ON 3,4-DICHLOROANILINE IN RATS/A
7-WEEK SUBCHRONIC INHALE STUDY ON PERACETIC ACID IN RATS/A
7-YEAR DIETARY CHRONIC TOXICITY /COVALENT GENICITY STUDY/6-METHOXYDUMONIC ACID:
30-DAY INHALATION EXPOSURES OF SPRAGUE-DAWLEY RATS TO AEROSOLIZED LUBRICA
31P-NUCLEAR MAGNETIC RESONANCE (NMR)./ RATE OF ABSORPTION AS MONITORED BY
32P-POSTLABELED DNA-2-AMINOFLUORENE ADDUCTS/.HPLC ANALYSIS OF
3H-BENZENE INHALATION IN F344/N RATS AND B6C3F1 MICE DURING AND FOLLOWING
3H/ESTRADIOL-17beta-D-GLUCURONIDE (E217G) AND 3H-TAUR/EASES THE UPTAKE OF
3H-ETHYL/ETHYLAZONIUM IN VITRO/ ISOLATED FEMALE RATS/TH-GLUCURONIDE (E217G) AND
3H]- AND [14C]FORMALDEHYDE IN THE NASAL MU/ANALYSIS O OF DNA-BOUND [3H]-
3H]-AMP BINDING IN VITRO BY MIREX (M/PK) ISOENZYME I (IE2) ACTIVITY AND [3H]-
2,3,7,8-TETRACHLOROBENZOFURAN (TCDF) - SYNTHESIS AND CHARACTERIZA/
373 CELLS FROM CADMIUM TOXICITY./ DIFFERENTIATION-INDUCING AGENTS PROTECT
373 FIBROBLASTS./D NICKEL REDUCE CADMIUM-INDUCED MORPHOLOGICAL CHANGES IN
4-NPI) IN RATS AND RABBITS/F 4-NITRO-N-METHYLPYRZALIMIDE [CARBOXYL-14C] (4-
4-WEEK ORAL TOXICITY STUDY WITH ACETALDEHYDE AND FORMALDEHYDE IN RATS/A
54MCG12/S ON DISTRIBUTION AND EXCRETION OF MANGANESE AFTER INHALATION TO
6MP) AND 6-ThIOGUANINE (6TG) COMBINED /IVE EFFECTS OF 6-MERCAPTOPURINE (6TG)
70-DAY ORAL GAVAGE STUDY OF OXOACTYL ACETATE IN RATS/RESULTS OF A
90-90-DAY TOXICITY DATA FOR 1,2,3-TRICHLOROPROPA-

A -

5
10

A23187 PRODUCE DIFFERENT SPECTRA OF EFF/MEMH), 2,5-HEXANE-2,4,6-DIONE (HD), AND
A23187./FURTHER NEUROBEHAVIORAL CHARACTERIZATION OF THE IGONPHORE
ABSORPTION AND DISPOSITION OF 1,2-DIHYDRO-2,4,6-TRIMETHYLPYRROLINA/DERMAL
ABSORPTION AS MONITORED BY 31P-NUCLEAR MA/ BY TPF AND TCFP AND THE RATE OF
ABSORPTION DISTRIBUTION AND EXCRETION OF TOPICAL DOSES OF 14C-PHENYLETHYL
ABSORPTION KINETICS OF ORGANOPHOSPHATES, STEROIDS, CAFFEINE./PERCUTANEOUS
ABSORPTION OF BENZO(a)PYRENE (BAP) FROM COMPLEX MIXTURES APP/PERCUITANEOUS
ABSORPTION OF BUTACHLOR IN RHEUS MONKEYS FOLLOWING TOPICAL ADMINISTRATION
ABSORPTION OF CAPTAFO/1/1D ON THE FACT OF ANIMAL AGE ON THE PERCUTANEOUS
ABSORPTION OF GLYPHOSATE IN THE MALE FISHER RAT
ABSORPTION STUDIES/USE OF EXCISED HUMAN SKIN FOR IN VITRO PERCUITANEOUS
ABSORPTION STUDY OF DIMAC WITH SILVER SULFADIAZINE AS COMPARED TO SILVADE
ACCEPTABLE DAILY INTAKE/EXCURSIONS ABOVE THE
ACUMULATION AND MORPHOLOGICAL CHANGES IN LIVERS OF ENVIRONMENTA/SELENIUM
ACUMULATION IN MAMMAL BRAIN TISSUE./BISNUTH
ACETALDEHYDE AND FORMALDEHYDE IN RATS./A 4-WEEK ORAL TOXICITY STUDY WITH
ACETALDEHYDES.-A-PROTEIN CROSS-LINKING IN RAP LIVER NUCLEI BY HAGELEN
ACETAMINOPHEN (AA) IN RATS PRETREATED WITH S/T'S OF ACUTE ADMINISTRATION OF
ACETAMINOPHEN (AA) HEPATOTOXICITY IN RATS BY ENDOCRINE STAT/MODULATION
ACETAMINOPHEN (AA) IN RATS./ACID (UDP-GA) DEPLETION ON THE DISPOSITION OF
ACETAMINOPHEN (AA) METABOLITES.:ATION IN BILIARY AND URINARY EXCRETION OF
ACETAMINOPHEN (AA): EFFECT OF CYTOCHROME P/INDEX FOR TOXIC ACTIVATION OF
ACETAMINOPHEN (APAP) OR ALLYL ALCOHOL (AAL)/6 (OTP)-INDUCED PROTECTION IN
ACETAMINOPHEN (APAP) EFFECT OF USE OF METHYL/DING AND DEFINED DIET ON
ACETAMINOPHEN (APAP) HEPATOTOXICITY/TUNED ELECTROPHILE PRODUCTION DURING
ACETAMINOPHEN (APAP)-INDUCED INHIBITION OF /ASE (MPO) ACTIVITY AND AGE ON

277
ANTIBODIES/N OF GAMMA-GLUTAMYL TRANSFERASE (GGT) BY ACYVINIC AND ANTI-GGT
ANTIBODY (ANA) AND GLOMERULAR IMMUNE COM/SA ON HgCl2-INDUCED ANTINUCLEAR
ANTIBODY PRODUCING ABILITY OF HUMAN LYM/IN ON MITOGEN RESPONSIVENESS AND
ANTIBODY PRODUCTION: A DOSE RESPONSE STUDY./TARTRAZINE SPECIFIC REAGIN
ANTIBODY RESPONSE IN VIVO AND MITOGEN RE/PECTS OF T-2 TOXIN ON THE MURINE
ANTIBODY TO SUBTILASIN A: CORRELATION WI/SSAY (ELISA) TO DETECT HUMAN IgE
ANTIBODY USED TO PROBE FOR PERUTURATION IN THE DI/ANTI-KERATIN MONOCLONAL
ANTICANCER AGENT, IN RATS AND RABBITS/TENTIAL OF DEZUAGINE (CI-908), AN
ANTICANCER DRUG CANDIDATE, IN BEAGLE DOGS/TY OF CI-937, AN ANTHRAPHYRAZOLE
ANTICOLINERGIC EFFECTS FROM BLUE GREEN ANABAENA F/TOXIN-A(S), AN IRREVERSIBLE
ANTICONVULSANT MEMBRANE FLUIDITY EFFECTS_/PRECONVULSANT UP
ANTICONVULSANT ZONISAMIDE IN BEAGLE DOGS/CHRONIC TOXICITY OF THE
ANTISTEROGENIC EFFECTS IN TCD TREATED GUIN/NPSERASE (UDPGL) ACTIVITY AND
ANTIGEN EXPRESSION IN HLOTHANE EXPOSED R/CHRONOLOGY OF HALOTHANE-INDUCED
ANTIGEN-PRESENTING CELLS (APC)./ROL OF CONTACT SENSITISATION BY CUTANEOUS
ANTIGENIC DETERMINANT IN THE ACETAMINOPHEN-ADDUCT/CHARACTERIZATION OF THE
ANTIGENS WHICH DETECT ANTIBODIES TO DIPH/IZATION OF ISOYCNATE-CONTAINING
ANTINOPLASTIC AGENT, SKF 10177, IN MALE /TRO HEPATOTOXICITY OF A NOVEL
ANTINUCLEAR ANTIBODY (ANA) AND GLOMERULAR /FORIN A (CSA) ON HgCl2-INDUCED
ANTIOXIDANT 4,4'-THIOBIS(6-E-BUTYL-CRESO)/OXICLOGICAL EVALUATION OF THE
ANTIOXIDANT DEPENSES AND CELL KINETICS IN /CtS OF HEPATOPATHY ON TUMOR CELL
ANTIOXIDANT ENZYMES AND TRACE ELEMENTS IN RAT KIDNEY/EFFECT OF CADMIUM ON
ANTIOXIDANT ENZYMES AND TRACE ELEMENTS IN RAT KIDNEY/EFFECT OF CADMIUM ON
ANTIOXIDANT ENZYMES AND TRACE METAL LEVELS/DIETARY COPPER AND SELENIUM ON
ANTIOXIDANT ENZYMES IN RATS FED VARIOUS LE/S OF CADMIUM ON LUNG CYTOSOLIC
ANTIOXIDANT ENZYMES IN THE HEPATOPANCREAS /MICROSOMAL OXYGEN REDUCTION AND
ANTIOXIDANT ENZYMES AND CYTIC AMP AGONISTS PREVENT THE INHIBITION OF MOUSE HEPA
ANTIOXIDANTS IN THE DETOXIFICATION OF BENH/NE- S-TRANSFERASES AND PHENOLIC
ANTIOXIDANTS/CINOMAS IN PARTIALLY-EXPECTOMIZED RATS INDUCED BY PHENOLIC
ANTIPATELET COMPOUNDS/AU TOXICITY STUDIES ON SOME NOVELS
ANTIPROTEASE ACTIVITY ASSOCIATED WITH PROTECTION FROM HEATPCELLULAR TOXI
ANTITUMOR DRUG, CIS-DIAMMINEDICHLOOR-PLAT/ING THE MAJOR DNA ADDUCT OF THE
ANTIOXIDANT./GEN PEROXIDE-MEDIATED CHROMOSOME DAMAGE IN VITRO BY PHENOLIC
AORTIC SMOOTH MUSCLE CELLS IN CULTURE/PHENOTYPIC MODULATION OF
APOPROTEIN E BY THE USE OF DIMETHYLATED ANALOG/DATIVE EFFECTS OF ACETAMINOPHEN
APAP) HEPTATOCITIC/NEURODEVELOPMENTAL EFFECTS OF ACETAMINOPHEN (APAP)
APAP) IN MICE/CE OF ASCORBIC ACID/STRESSORS ON METABOLISM OF ACETAMINOPHEN
APAP) IN MICE/CE OF ASCORBIC ACID/STRESSORS ON METABOLISM OF ACETAMINOPHEN
APAP)-INDUCED INHIBITION OF HEPATIC MITOCH/AND AND AGE ON ACETAMINOPHEN
APLASYA SILENT NEURONS./E (DFP) ON THE ELECTROPHYSIOLOGICAL PROPERTIES OF
APPLICATION OF ELECTRON PROBE X-RAY ANALYSIS AS A DIAGNOSTIC ADJUNCT FOR
APS KINEASE ACTIVITY./A NEW METHOD FOR THE MEASUREMENT OF HEPATIC
AQUATIC ECOSYSTEM./IMPACT OF A PAPER MILL WASTE DISCHARGE ON AN
AQUATIC POLLUTANTS TO BLUEGILL (BF-2) AND FAT/COMPARATIVE CYTOTOXICITY OF
ARCHICONDROPHIC ACID RELEASE BY MURINE PERITONEAL /OXY RADICAL PRODUCTION AND
ARAMID AND METAPHOSPHATE FIBERS ON MACROPHAGE VIABILITY AND/THE EFFECT OF
ARAMID FIBERS IN RATS/DEPOSITION & CLEARANCE OF INHALED KEROS
ARGININOSUCCINATE LYSASE): A SENSITIVE INDICATOR OF HEPATOTOX/NERUM ASAL
ARGININOSUCCINIC ACID LYSATE TO OTHER INDICATORS OF CARBON TE/A COMPARISON
AROCOLOR 1254 AS A 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ANTAGONIST IN CS7BL
AROCOLOR 1254./CITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN: ANTAGONISM BY
AROCOLOR 1254./ICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN: ANTAGONISM BY
ARSENIC AS DETERMINANTS OF CYTOTOXICITY /TION OF PENTAVALENT TO TRIVALENT
ARSENIC IN THE RABBIT /PANIMALS WITH ADMINISTRATIVE DISTRIBUTION OF ARSENIC
ARSENE COMPARISON TO SILICA AFTER A SING/ICAL PULMONARY EFFECTS OF GALLIUM
ARSENE EXPOSURE: ULTRA-STRUC/UAL AND /REAL STRUCTURE/UNCTION BY ACUTE
ARSENITE, AND METHYMERCURY IN CULTURED F/ROTOPHILE REASSEMBLY BY CADMIUM,
ARSENE./ TRANSFORMATION IN CSH/10G1/2 C18 B MEFROMBY CELLS BY SODIUM
ARSENITE AND GALLIUM ARSENIDE (GA): DEVELOPMENTAL TOXICITY STUDIES.
ARSENITE: ACUTE AND SUBCHRONIC INHALATION TOXICITY STUDIES, EXPERIMENTAL
ARSENITE: ALTERATIONS IN THE HEMATOPOIETIC SYSTEM OF RATS AND MICE.
ARSENITE: TOXICITY DATA FROM ACUTE, SUBCHRONIC AND SUBCHRONIC INHALATION EXPOSURES.
ARYL AMINES IN HUMAN AND RAT LIVER ACETYLATION/DEACETYLATION (AC/DC) OF
ARYL ALCOHOL ETHER PHENOX/ETHANOL ARE AFFECTED/EMBRYOTOXICITY OF THE
ARYL HYDROCARBON HYDROXYLASE (AHH) KIN/OF RAINBOW TROUT MARKEDLY ALTERED
ARYL HYDROCARBON HYDROXYLASE (AHH), ETHOXYRESORUFIN O-DEETHYLATION (EROD),
ASAL (ARGININOSUCCINATE LYSASE): A SENSITIVE INDICATOR OF HEPATOTOX/SERUM
ASBASTOS FROM CONTAMINATED DRINKING WATE/OF INGESTION STUDIES OF AIRBORNE
ASCORBATE ON NO2-INITIATED PEROXIDATION/EFFECT OF DHE-TOCOPHEROL AND
ASCORBIC ACID (AA) AND ASCORBYL PALMITATE (AP) ON 12-O-TETRADEC/EFECT OF
ASCORBIC ACID EFFECTS ON METABOLISM OF ACETAMINOPHEN (APAP) 1/FORINE OF
ASCORBIC PALMITATE (AP) ON 12-O-TETRADEC/EFFECT OF ASCORBIC ACID (AA) AND
ASPERMATOGENESIS INDUCED BY SAH 59-801 IN TH/ULAR TUBULE DEGENERATION AND
ASPIRIN IN DOGS. PROTECTION BY RO 22-132/ENT OF GASTRIC MUCOSAL DAMAGE BY
ASSAY (ELISA) TO DETECT HUMAN IgE ANTIBODY/AN ENZYME LINKED IMMUNOSORBENT
ASSAY (NDA)/CITY TEST FOR CULTURED HEPATOCYTES USING THE NEUTRAL RED DYE
ASSAY FOR FOUR HYDROXYLATION ENZYMES TOWARDS A FLAT/DEVELOPMENT OF AN HPLC
ASSAY TO DETERMINE ALTERED XENOBIOTIC METABOLISM /A SENSITIVE NONINVASIVE
ASSAY TO MEASURE INHIBITED CELL-CELL CO/F THE SCRAPE-LOADING/DYE TRANSFER
ASSAY TO MEASURE INHIBITION OF INTERCELLULAR CO/APPLICATION OF THE 'FRAP'
ASSAY WITH AN S-9 METABOLIC ACTIVATING /IN VITRO NEUTRAL RED CYTOTOXICITY
ASSAY A/AITILE AND NONVOLATILE CARCINOGENS ON THE V79 METABOLIC COOPERATION
ASSAYS COMMONLY INCLUDED IN IMMUNOTOXICITY/IES COMPARISON OF IMMUNE FUNCTION
ASYMPTOMIC/NUCLEAR ANTIBODY (ANA) AND GLOMERULAR COMPLEXES (GIC) IN
RECEPTOR AND IN VIVO EFFECTS. LORODIBENZO-P-DIOXINS AS LIGANDS FOR THE
Ah RECEPTOR IN HAMSTERS ADMINISTERED 3H-2,3,7,8/LONG-TERM KINETICS OF THE
Ah RECEPTOR IN HEXACHLOROBENZENE (HCB) PORPHYRIA: STUDIES/ROLE OF THE
Ah RECEPTOR IN THE DOWN REGULATION OF UTERINE AND HEPATIC EST/ROLE OF THE
Ah RECEPTOR/PHYSICOCHEMICAL PROPERTIES OF THE HUMAN AND RODENT
ATP PRODUCTION BY BRAIN MITOCHONDRIA EXPOSED TO NEUROFILAMENTS AXONOPAT
AXOPATHY PRODUCED BY BRAIN AXOPATHY TRANSPORT IN SCATIC NERVES OF ACR, 2,5-HD AND D
ATPases FROM VERTEBRATE AND INVERTEBRATE ACTION OF GOSSYPOL ON SOLUBLE F1
ATPases OF FISH AND RAT. NATIVE STUDIES ON THE EFFECTS OF MERCURY ON BRAIN
ATPase EFFECT OF CHLOROCON ON MATURING RAT BRAIN
ATROPHY MAY NOT BE A PRIMARY EFFECT OF 2,3,7,8-TETRAHCHLORODIBENZO-/THYMIC
ATROPHY/C57BL/6J AND DBA/2J MICE EFFECTS ON ENZYMES DESTRUCTION AND THYMIC
ATROPHIA AND DF3-INDUCED DELAYED NEUROPATHY IN THE CHICKEN
ATROPIQUE AND MIXED TOXICITY USING A VAGA/C RESPONSE OF RHESUS MACAQUES TO
Ay STUDY OF ORGANOPOPHOSPHATE (OP) COMPOUNDS IN RAT BY MEMERNTINE AND
Ay STUDY OF ORGANOPOPHOSPHATE (OP) COMPOUNDS IN RAT BY MEMERNTINE AND
Audiovisual Training Program in Toxicology
Auditory Startle Response (ASR)/F PYRETHROIDS: ACUTE LETHALITY VERSUS THE
Auditory Startle Response. AS NEONATES EXHIBIT HYPERACTIVITY AND ELEVATED
AVERSIONS INDUCED BY INHALED p-XYLENE/RATION-DEPENDENT CONDITIONED FLAVOR
AVIAN NEURAL ESTERASES TO ORGANOPHOSPHORUS COMPOUNDS IN VITRO SENSITIVITY
AXONAL DEGENERATION FOLLOWING REPEATED INTRACRANIAL TRANSPORT OF DOXORUBICIN (DOX)
AXONOPATHY INDUCED BY 1,3-DIMETHYLINDOLINILOVINPHOSPHATE (DB/CENTRAL DISTAL
AXONOPATHY PRODUCING NEUROTOXINS/MITOCOCHONDRIA EXPOSED TO NEUROFILAMENTOUS
AXONS IN 2,5-HEXADIONE (2,5-HD) EXPOS / T ALTERATIONS IN OPTIC TRACT (OT)
AXONS/ENTAL DISTRIBUTION IN RAT DORSAL ROOT GANGLION CELLS IN AND INJURED
AXOPATHY TRANSPORT IN SCATIC NERVES OF ACR, 2,5-HD AND D/ATP, CP, AND
AXOTOMY/DECREASED DE NOVO ACHE SYNTHESIS FOLLOWING
AXOTOMY/VINCA ALKALOIDS INHIBIT THE NEURON PERIKARYAL RESPONSE TO
AXOTOMY/VINCA ALKALOIDS INHIBIT THE NEURON PERIKARYAL RESPONSE TO
AZA-ATAXIA TOWARD RAT HEPATOCYTES: ROLE OF CYTOCHROME/CYTOTOXICITY OF 4-
AZO DYES DERIVED FROM BENZIDINE (B) /TRUCITION ACTIVITY RELATIONSHIPS OF

B

B CELL PROLIFERATION IN VITRO./THE EFFECT OF PARAOXON ON MURINE T AND
B CELL ACTIVATION AND DIFFERENTIATION/ABOMATIC HYDROCARBON ALTERATIONS IN
B3C3F1 MALE MICE./HEPATIC TUMOR PROMOTION BY DIETARY FAT IN
B3C3F1 MICE AND F344 RATS/EXPOSURE TO BENZONITRILE ON NEUROBEHAVIOR IN
B3C3F1 MICE DURING AND FOLLOWING 3H-BER/WE METABOLITES IN F344/N RATS AND
B3C3F1 MICE EXPOSED FOR 13 WEEKS//OF NICKEL SUBSULFIDE TO F344/N RATS AND
B3C3F1 MICE FOLLOWING DIETARY INCORPORATION/EXCHLORO-1,3-BUTADENE (HCB) IN
B3C3F1 MICE FOLLOWING HIGH FAT DIETARY AND PHOSPHOLIPIDE PRODUCTION AND ON
B3C3F1 MICE/MICRO-RNA COMPLEX IN P/YLAMINE (PBNA) IN F344/N RATS AND
B3C3F1 MICE/ACID 2-YEAR DIETARY CHRONIC TOXICITY-ONCOGENICITY STUDY IN
B3C3F1 MICE/SUBSTRIBUTION OF MANGANESE IN CHRONICALLY EXPOSED F344 RATS AND
B3C3F1 MICE/CHRONIC TUMOR STUDY OF BUTYRALDEHYDE (BA) IN F344 RATS AND
B3C3F1 MICE/ENE ON HOST RESISTANCE TO PLASMODIUM YOELII AND P. BERGHEI IN
B3C3F1 MICE/HRONIC TOXICITY STUDY OF CROTONALDEHYDE (CA) IN F344 RATS AND
B3C3F1 MICE/LUCEENCE OF VIRAL INFECTIONS ON TUMOR INCIDENCE AND SURVIVAL OF
B3C3F1 MICE/PHARYNCHITIS EXPERIMENT IN DIETHYLNOITOAMINE (DENA) INITIATED
B3C3F1 MICE/NEONATAL TOXICITY STUDY OF BENZETHONIUM CHLORIDE IN F344 RATS AND
B3C3F1 MICE/tc1)/11-DAY CONTINUOUS EXPOSURE INHALATION TOXICITY STUDY IN
B3C3F1 MOUSE./ THE KIRSTEN-RAS (KI-RAS) ONCOGENE IN THE LIVER OF THE MALE
B3C3F1 MOUSE./NIKEL SULFATE, AND NICKEL SUBSULFIDE IN THE F344/N RAT AND
B3C3F1 MICE (SKIN PAINT)/YDR/O-2,2,4-TRIMETHYL-MONOLINE IN F344 RATS AND
BACILLUS THURINGIENSIS STRAIN HDI-1 AND HD2-1 AGAINST UV/STABILIZATION OF
BACKGROUND DATA IN THE INTERPRETATION OF TOXICITY/JOY OF S. TOL. TOXICITY OF
BACTERIA AND MAMMALIAN CELLS IN VITRO/TOXICITY OF SIX OXME COMPOUNDS IN
BACTERIAL GENOTOXINS IN VIVO AND IN VITRO/TION OF COOKED-FOOD MUTAGENS TO
BAP FROM COMPLEX MIXTURES APPLIED TO /OUS ABSORPTION OF BENZO[a]PYREN (BAP)
BAP-INDUCED DNA DAMAGE AND REPAIR IN AEW/E (HCHO) - AND BENZO(A)PYRENE (BAP)
BARBITAL (Na.BB) ON URINARY BLADDER AND /LS OF PHENOBARBITAL (Na.PB) AND
BARRIER/TEM TO STUDY THE EFFECTS OF LOW LEVELS OF LEAD ON THE BLOOD-BRAIN
BASE LAMINA AND EXTRACELLULAR MATRIX OF THE FETAL MOUSE/TCD EXPOSED TO THE
BCNU ON ACETAMINOPHEN BIOTRANSFORMATION AND GLUTATHIONE-5-TETRA/EFECTS OF
BEEF (EPICAUTA spp.)./EMS FROM HORSES POISONED BY INGESTION OF BLISTER
BINDING(c) DUAL LABEL STUDY OF 1,2-DIBROMOBENZENE METABOLISM AND COVALENT
115 BIOASSAY FOR ASSESSING LUNG TOXICITY IN DEVELOPMENT OF A COMPREHENSIVE
119 BIOASSAY SYSTEM FOR DETECTION OF CARCINOGENS./VALIDATION OF RAPID
938 BIOAVAILABILITY OF OSSYPOLE IN MALE RATS/PHARMACOKINETICS AND
931 BIOAVAILABILITY OF TOLUENE IN ORALLY EXPOSED MALE /SOIL ADSORPTION ALTERS
933 BIOAVAILABILITY OF VITAMIN K IN DOGS/PHARMACOKINETICS AND
1069 BIOCHEMICAL ALTERATIONS PRODUCED BY CCL4 EXPOSURE IN RAT LIVER/COMPARISON OF
791 BIOCHEMICAL AND HISTOLOGICAL CHANGES IN LUNG LAVAGE FLUIDS FOLLOWING THORACIC/CELLULAR AND
315 BIOCHEMICAL INDICATORS OF NEUROTOXICITY./ING RAT ALTERS MORPHOLOGICAL AND
317 BIOCHEMICAL MECHANISMS OF ORGANOMETAL TOXIC/EXAMINATION OF THE POTENTIAL
318 BIOCHEMICAL PATTERNS SIMILAR TO RAT LIVER/TREATMENT IN MOUSE LIVER INDUCE
200 BIOCHEMICAL RESPONSES OF MICE TO ENDOXAN AND CANTHARIDIN ACID./HEPATIC
48 BIOCHEMISTRY AND HISTOPATHOLOGY/EFFECTS OF INHALED PHOSGENE ON PULMONARY
806 BIOCHEMISTRY FOLLOWING CIGARETTE SMOKE EXP/PATHOLOGY AND LAVAGE CYTOTOLOGY/
410 BIOCHEMISTRY OF ACUTE CYCLOX (O3) EXPOSURE ON HUMAN AND RAT LAVAGE FLUID
626 BIOGENIC AMINE ALTERATIONS IN DIFFERENT BRAIN REGIONS OF MICE EXPOSED TO
797 BIOSYNTHESIS IN THE LUNGS OF SILICA-TREATED RATS/SURFACTANT
465 BIOTRANSFORMATION AND GLUTATHIONE-S-TRANSFERASE/TS OF BCNU ON ACETAMINOPHEN
1067 BIOTRANSFORMATION IN THE ACTIVATION OF HEPATO/OLE OF CARBON TETRACHLORIDE
437 BIOTRANSFORMATION OF CYCLIC 1,3-DIKETONES/PEROXIDATIVE
601 BIOTRANSFORMATION OF ROBONONE/50-DEPENDENT MONOXYGENASES ON THE IN VITRO
1088 BIPHENYLISOMER IN MICE./S OF CHEMICALLY DIVERSE POLYCHLORINATED
395 BISPHENOL (PCBS) ON INITIATION OF HEPATOC/CTS OF TWO PURE POLYCHLORINATED
675 BIPYRIDYL HERBICIDES./CES IN STIMULATION OF MICROSOMAL NADPH OXIDATION BY
781 BIS-(2-METHOXYETHYL) ETHER IN THE ADULT MALE RAT /MENT ON THE METABOLISM OF
781 BIS-(2-METHOXYETHYL) ETHER PRETREATMENT ON THE /EFFECT ON PHENOBARBITAL OR
997 BIS-(B-CHLOROETHYL) SULFIDE (BCS)/D BY EXPOSURE OF RAT KERATINOCYTES TO
630 BISPHENOL-A (BPA) AEROSOL IN MICE AND RATS./IRRITATION RESPONSE TO
595 BIVALVE MOLLUSCOS/IDANT ENZYMES IN THE HEPATOPANCREAS OF TWO MID-ATLANTIC
411 BLADDER AND KIDNEY IN MALE P344 RATS INT/ AND BARBITAL (Na.BB) ON URINARY
670 BLASTOCYSTIC RESPONSE TO HERPES SIMPLEX VIRUS 2(HSV2) BY/INHIBITION OF THE
664 BLEOMYCIN COMPLEXES IN ISOLATED SPERMATOG/MDA FORMATION INDUCED BY IRON-
135 BLEOMYCIN-INDUCED DAMAGE OF MITOCHONDRIA DNA FROM MOUSE AND L1210 TUMOR M
849 BLISTER BEETLES (EPICAPNA SPP.)./ENS FROM HORSES POISONED BY INGESTION OF
530 BLOOD AND BLOOD REGIONS./ TOXICITY AND CHOLINESTERASE (ChE) INHIBITION IN
819 BLOOD CELL ACETHYLCHOLINESTERASE IN BEAG/ OF PYRIDOSTIGMINE BROMIDE ON RED
47 BLOOD CONCENTRATIONS OF CYANIDE AND CARBON MONOXIDE IN MICE FOLLOW/LETHAL
301 BLOOD LEAD LEVEL IN PLUMBERS USING PLASMA ATOMIC /DIRECT DETERMINATION OF
306 BLOOD LEAD/EYTHROCYTE MEMBRANE FLUIDITY AS RELATED TO
364 BLOOD PRESSURE AND HEART RATE IN RATS/SURE TO COMPLEX ORGANIC MIXTURES ON
65 BLOOD-BRAIN BARRIER/TU TO STUDY THE EFFECTS OF LOW LEVELS OF LEAD ON THE
44 BLOOTY MOUTHER AFTER 8 WEEKS OF 5.0 PPM N/RAY FUNCTION IN THE OUTBRED MALE
599 BLUEGILL (BF-2) AND FATHEAD MINNOW (FHM)/OXICITY OF AQUATIC POLLUTANTS TO
329 BONE FOLLOWING DIETARY AND ADMINISTRATION /CITY AND ALUMINUM CONCENTRATION IN
554 BONE IN FISCHER 344 RATS/ OF HETEROXYMOGENE ON CALCIUM HOMEOSTASIS AND
554 BONE LEAD IN INTERACT LEGS BY L X-RAY FLUO/NONE-INVASIVE DETECTION OF TIBIAL
509 BONE MARROW NEUTROPHILS/ON OF INCREASED OXIDANT GENERATION BY DBA/2 MOUSE
156 BONE MINERAL-/CADIUM AND OVARIECTOMY-INDUCED LOSS OF
817 BONE REPLACEMENT MATERIAL./ECTS OF SOLVENTS ON THE IN VITRO TOXICITY OF A
247 BOVINE HEPATOCYTE CULTURES - A TOOL FOR THE MEASURE OF XENOBIO TIC CYTOTOX
496 BP) BY THE RAT AND HAMSTER./ERENCES IN THE METABOLISM OF BENZO(a)PYRENE
193 BRAIN ACETYCHOLINESTERASE (AChE) BY OR/ION AND REACTIVATION OF MAMMALIAN
531 BRAIN ACETYCHOLINESTERASE (AChE) INHIB/BONOPHOSPHATE INDUCED PERSISTANT
609 BRAIN ACETYCHOLINESTERASE./REACTIVATION AND AGING OF RODENT AND FISH
327 BRAIN AND CORRELATION WITH BEHAVIOR./UM INDUCED LIPID PEROXIDATION IN
604 BRAIN ATPases OF FISH AND RAT./ATIVE STUDIES ON THE EFFECTS OF MERCURY ON
222 BRAIN ATPases.EFFECT OF CHLORODEONE ON MATURE RODER
65 BRAIN BARRIER/EM TO STUDY THE EFFECTS OF LOW LEVELS OF LEAD ON THE BLOOD-
198 BRAIN BIOGENIC AMINES BY TOXAPHEN:E A HIGH PERFORMANCE L/DELETION OF RAT
353 BRAIN CYTOSOL OF CHICKENS TREATED WITH /CALMODULIN KINASE II ACTIVITY IN
665 BRAIN LIPIDS./CYANIDE AND PEROXIDATION LEAD -
209 BRAIN MALFORMATION IN THE DEVELOPING RAT./ACUTE ETHANOL EXPOSURE AND
529 BRAIN MITOCHONDRIA EXPOSED TO NEUROFILAMENTOUS AXONOPAT/ATP PRODUCTION BY
893 BRAIN MONOXYGENASES./PHOSPHOROTHIONATE INSECTICIDE ACTIVATION BY RAT
894 BRAIN FIG MICROSOMES/A COMPARISON BETWEEN LIVER AND
626 BRAIN REGIONS OF MICE EXPOSED TO BENZOL/IC AMINE ALTERATIONS IN DIFFERENT
530 BRAIN REGIONS./ TOXICITY AND CHOLINESTERASE (ChE) INHIBITION IN BLOOD AND
630 BRAIN TISSUE./BISMUTH ACCUMULATION IN MAMMAL
629 BRAIN WITH MAGNETIC RESONANCE.-IMAGING MANGANESE IN THE PRIMATE
31A BRAIN-/CYSTEINE AND GLUTATHIONE ENZYME METHYLMERCUY UPTAKE BY THE
517 BRAIN/OXYGEN FREE RADICAL MEDIATED ISCHEMIA-REFPERFUSION DAMAGE TO GEBRIL
891 BRAIN/METABOLISM AND CLEARANCE OF MPTP AND 2'3'CH-2'MPTP IN MOUSE

284
BRAINSTEM INVOLVEMENT IN THE MODIFICATION OF THERMOREGULATORY PR/POSSIBLE
634 BREAST CANCER CELLS/GEN REGULATED FLASMINOGEN ACTIVATOR ACTIVITY OF MCP-7
586 BROMIDE IN THE MALE F-344 RAT/REPRODUCTIVE EFFECTS OF INHALED METHYL
578 BROMIDE, CHLORINATED STYRENES, BROMINATED /HEALTH EFFECTS REVIEWS: METHYL
577 BROMIDE./THE OLFACTORY EPITHELIUM FOLLOWING INHALATION EXPOSURE TO METHYL
539 BROMIDE/RACERIZATION OF THE REACTIVITY OF METALLOTHIONEIN TOWARDS METHYL
537 BROMINATED DIPHENYL ETHERS./REVIEWS: METHYL BROMIDE, CHLORINATED STYRENES,
522 BROMO-2,5-DIHYDROXY-THIOPHENOL/NEUROTOXICITY: STRUCTURAL ACTIVITY REQUIR/6-
523 BROMO-2,5-DIHYDROXY-THIOPHENOL/SYNTHESES AND NEUROTOXICITY OF 2-
239 BROMOBENZENE METABOLITES TO LIVER SLICES IN VITR/OVALENT BINDING OF 14C-
354 BROMOBENZENE ON ORGANIC ION UPTAKE BY RAT /ONJUGATES OF ACYLONITRILE AND
362 BROMOHYDROQUINONE (BHQ)-EQUIVALENTS TO PROTEI/WEEN COVALENT BINDING OF 2-
771 BRONCHIAL AND EARLY ALVEOLAR CLEARANCE IN/EMPORAL CHARACTERISTICS OF LATE
1033 BRONCHIAL EPITHELIAL CELLS./SYNTHESIS (UDS) IN XENOGRAFTS CONTAINING HUMAN
155 BRONCHOALVEOLAR LAVAGE/MIUM STIMULATES PULMONARY INFLAMMATION MEASURED BY
682 BRONCHODILATOR/SUBCONSCIOUS ORAL TOXICITY EVALUATION OF AN ANTI-CHOLINERIC
494 BROWN ADIPOSE TISSUE THERMOGENESIS /INDUCED BY 2,3,7,8-TETRACHL/CHANGES OF
426 BaP-DNA ADDUCTS IN MOUSE SKIN/INFLUENCE OF COMPLEX ORGANIC MIXTURES ON
286 BUDWORM/IN VITRO CUNTRATIVE PENETRATION OF FENVALERATE IN THE TOBACCO
704 BUILDER./CE OF TERATOGENIC RESPONSE IN RATS AND RABBITS GIVEN A DETERGENT
603 BULLHEAD CATFISH (ICTALURUS NEBULOSUS)/CITY OF ACETAMINOPHEN IN THE BROWN
596 BULLHEAD FROM A CONTAMINATED NEUSE RIVER/NCTION OXIDASE ACTIVITY IN BROWN
528 BUTACHLOR IN RHEUS MONKEYS FOLLOWING TOPICAL ADMINISTRATION/ABSORPTION OF
550 BUTADIENE (HCBD) IN B6C3F1 MICE FOLLOWING/LOG STUDIES OF HEXACHLORO-1,3-
450 BUTENAL OF THE RAT HEPATIC MICROSOMAL M/IHIBITION BY CROTONALDEHYDE (2-
250 BUTOXYETHANOL; BE) VIA ALCOHOL/ALDEHYDE DEH/NE GLYCOL MONOBUTYL ETHER (2-
427 BUTYL ACRYLATE IN MALE FISHER RATS/METABOLISM AND DISPOSITION OF N-
961 BUTYL ALCOHOL IN RATS AND MICE/SUBACUTE TOXICITY STUDIES OF t-
959 BUTYL BENZYL PHthalate and MODIFIED MATING TRIALS /SUBACUTE TOXICITY OF
769 BUTYLPHthalate (TRP)/SUBACUTE TOXICITY OF BPH/IN Trp/hydroxyanisolole in V/v/A
104 BUTYLATED HYDROXYANISOLE (HBA)/SPECIAL TOXICITY OF TERT-A
146 BUTYLATED HYDROXYTOLUENE (BHT)/OP RETINYL ACETATE (RA) HEPATOXICITY BY
264 BUTYLCYCLOXEGANE IN MALE F-344 RATS WITH HYALINE /METABOLISM OF TERTARY-
211 BUTYLDICHLOROVINYLPHOSPHATE (BDVCP)/L DISTAL AXONOPATHY INDUCED BY DI-n-
836 BUTYLALDEHYDE (BA) IN F344 RATS AND B6C3F1 /SUBACUTIC TOXICITY STUDY OF

- C -

C19-373, AN ANTHrapyrazOLE ANTICANCER DRUG/SUBACUTE INTRAVENOUS TOXICITY OF
1043 C102 WITH FATTY ACIDS UNDER AQUEOUS CONDITION/REACTIONS INVOLVING HOC1 OR
835 C36/101/2 C31 8 MOUSE EMBRYO CELLS BY SOD/PROMOTION OF TRANSFORMATION IN
618 C57BL/6 MICE BY PRETREATMENT WITH DIETHYLAMINE/3,6,7-TEATRAHYDROPYRIDINE (MTPP) IN
654 C57BL/6 AND DBA/2 MICE./ (MDP) COMPOUNDS ON HEPATIC MICROSOMAL PROTEINS OF
646 C57BL/6 MICE/OR IN HEXACHLOROBENZENE (HC) Porphyria: Studies in Congenic
639 C57BL/6 AND DBA/2J MICE - EFFECTS OF EM/RODIGENZO-p-DIOXIN ANTAGONIST IN
424 CA+2 STIMULATE PHOSPHORYLATION AND INHIBIT ACTIVITY OF PLASMA RIGGSOME-ACTIVATING
276 CADMIUM AND DEXAMETHASONE (DEX) IN MICE/ALLOTHIONEIN (MT) ISOFORMS BY ZINC,
290 CADMIUM AND MERCURY MOUTH WITH OUBAIN-RECEPT/MECHANISM OF INTERACTION OF
156 CADMIUM AND OVARIECTOMY-INDUCED LOSS OF BONE MINERAL.
283 CADMIUM CARCINOGENESIS IN THE WISTAR RAT BY ZINC TREATMENT/ANTAGONISM OF
275 CADMIUM CARDIOTOXICITY: INFLUENCE OF DIETARY COPPER AND SELENIUM ON ANTIHOT
275 CADMIUM EXPOSED RATS/ENHAL METAL AND METALLOTHIONEIN LEVELS IN CONTROL AND
273 CADMIUM IN AMBIENT AIR./APPROACHES TO NONCARCINOGENIC RISK ASSESSMENT FOR
274 CADMIUM INDUCTION OF METALLOTHIONEIN (MT) ISOFORMS IN CADMIUM RESISTANT AN
283 CADMIUM ON ANTIOXIDANT ENZYMES AND TRACE ELEMENTS IN RAT KIDNEY/EFFECT OF
287 CADMIUM ON ANTIOXIDANT ENZYMES AND TRACE ELEMENTS IN RAT KIDNEY/EFFECT OF
287 CADMIUM ON LUNG CYTOSOLIC ANTIOXIDANT ENZYMES IN RATS FED VARI/EFFECTS OF
274 CADMIUM RESISTANT AND SENSITIVE STRAINS / METALLOTHIONEIN (MT) ISOFORMS IN
155 CADMIUM STIMULATES PULMONARY INFLAMMATION M/CHRONIC EXPOSURE OF HUMANS TO
274 CADMIUM TOXICITY AND ENZYME INHIBITION BY DIMETHIOTHIETH/PROTECTION TO
777 CADMIUM TOXICITY DATA FOR EXTRAPOLATION FROM IN VIT/NASAL EPITHELIAL CELL
284 CADMIUM TOXICITY,/DIFFERENTIATION-INDUCING AGENTS PROTECT 3T3 CELLS FROM
280 CADMIUM TRANSPORT ACROSS BLOOD BARRIER MEMBRANE VESICLES FROM RAT SMALL IN
282 CADMIUM, ARSENITE, AND METHYL MERCURY IN /ION OF MICROTUBULE REASSEMBLY BY
628 CADMIUM, COPPER, ZINC, CALCIUM AND IRR/OF DIETARY SELENIUM ON SELENIUM,
627 CADMIUM, SELENIUM, COPPER, ZINC, CALCIUM AND/EFFECTS OF DIETARY COPPER ON
571 CADMIUM-INDUCED CHANGES IN SPERMATOZOAN CHOL/THIOCARBAMATE INHIBITION OF
322 CADMIUM-INDUCED MORPHOLOGICAL CHANGES IN 3T3 FIBRO/ZNIC AND NICKEL REDUCE
570 CADMIUM-INDUCED PATHOLOGICAL CHANGES IN THE RAT/LATRO AND IN VIVO MEASURES OF
777 CADMIUM-TREATED RATS.-/ENIUM, COPPER, ZINC, CALCIUM AND IRON IN THE EYE OF
286 CADMIUM-TREATED RATS/ADMIUM, COPPER, ZINC, CALCIUM AND IRON IN THE EYE OF
281 CADMIUM./METAL AND STRESS PRETREATMENT ON THE SUBCELLULAR DISTRIBUTION OF
7 CAFFEINE IN RATS./POTENTIAL REVERSIBILITY OF SKELLETAL EFFECTS OF

285
CAFFEINE, AND BENZOIC ACID IN THE ISOLATE/OF ORGANOPHOSPHATES, STEROIDS, 627
CALCIUM AND IRON IN THE EYE OF CADMIUM-T/CADMIUM, SELENIUM, COPPER, ZINC, 628
CALCIUM AND IRON IN THE EYE OF CADMIUM-T/SELENIUM, CADMIUM, COPPER, 629
CALCIUM HOMEOSTASIS AND BONE IN FISCHER 3/ EFFECTS OF HEXACHLOROBENZENE ON 554
CALCIUM HOMEOSTASIS IN FEMALE RATS./ND CCL4 INTERACTION ON HEPATOCYTE 1072
CALCIUM RELATIONSHIPS/INTRACELLULAR LEAD- 304
CALCIUM UPTAKE ACTIVITIES IN LIVER SUBC/RELATIONS WITH RAPID EFFECTS ON 1071
CALCINOSIS: [C] IN PUBLIC KIDNEY CELLS (LLC-PK1)-INDUCED INCREASES IN CYTOSOLIC 859
CALMODULIN IN RABBIT INJECTED EFFECTS OF CYCLOOXYGENASE 58
CALMODULIN KINASE II ACTIVITY IN BRAIN CYTOSOL OF PIGS TREATED/ALTERED 535
CAMP AND CGMP CHANGES IN DEVELOPING MICE/SECALONIC ACID D-INDUCED PATALAL 68
CAMP BINDING IN VITRO BY MIREX (MX)/K ISOENZYME (IE2) ACTIVITY AND [3H]- 68
CAMP-DEPENDENT PROTEIN KINASE (CAMP-PK) ISOENZYME (MODULATION OF HEPATIC 68
CAMP-PK) ISOENZYME (IE2) ACTIVITY AND [3] CAMP-DEPENDENT PROTEIN KINASE (634
CANCER CELLS/GEN REGULATED PLASMINOGEN ACTIVATOR ACTIVITY OF MCF-7 BREAST 41
CANCER IN THE RAP/CHANGES IN HEPATIC ENDOPLASMIC RETICULUM DURING MAMMARY 48
CANCER OF THE LUNG/O MODEL FOR THE INDUCTION OF CARCINOMAS AND SMALL CELL 74
CANCER RISK IN WORKERS EXPOSED TO OXADIAZON.-ASSESSMENT OF 55
CANTHARIDIC ACID./HEPATIC BIOCHEMICAL RESPONSES OF MICE TO ENDOThAL AND 200
CANTHARIDIN USING CAPILLARY GC/MS IN SPECIMENS FROM HORSE/DETERMINATION OF 849
CAPTAFOLE/DY ON THE EFFECT OF ANIMAL AGE ON THE PERCUTANEOUS ABSORPTION OF 1094
CAPTAN TO CD-1 MICE./OMIC ALTERATIONS FOLLOWING DIETARY ADMINISTRATION OF 189
CARBAMAZEPINE AND STEROIDS ON THEIR HEPATOTOXICITY/EFFECT OF INTERACTION BETWEEN 484
CARBARYL USING A FUNCTIONAL OBSERVATIONAL/CMPARISON OF COMPARATIVE TOXICITY AND 1003
CARBARYL-IMMUNOLOGICAL STUDIES ON THE COMPARATIVE TOXICITY AND METABOLISM OF 485
CARBENDAZIM EFFECTS ON THE HYPOTHALAMIC-PITUITARY REPRODUCTIVE AXIS. 558
CARBENDAZIM-INDUCED ALTERATIONS OF REPRODUCTIVE/ DOSE RESPONSE ANALYSIS OF 717
CARBOFURAN TECHNICAL./REPEATED DOSE DERMAL TOXICITY STUDY IN RABBITS WITH 174
CARBON BLACK PARTICLES/BINDING OF INHALED 14C-BENZO(A)-PYRENE ADSORBED ON 795
CARBON BLACK PARTICLES/BINDING OF INHALED 14C-BENZO(A)-PYRENE ADSORBED ON 794
CARBON BLACK PARTICLES/BINDING OF INHALED 14C-BENZO(A)-PYRENE ADSORBED ON 794
CARBON DIOXIDE (CO2) ASSESSMENT OF REPRODUCTIVE EFFECTS. 47
CARBON DIOXIDE IN MICE FOLLOWING INHAL/OOD CONCENTRATIONS OF CYANIDE AND 999
CARBON MONOXIDE ON FIXED-RATIO PERFORMANCE IN /EFFECTS OF INTRAPEPTONEAL 861
CARBON MONOXIDE-INDUCED HEMOLYSIS OF AMINOPYRINE METABOLISM IN THE ISOLA 752
CARBON MONOXIDE./ EFFECTS IN RATS EXPOSED TO SUB-LETHAL CONCENTRATIONS OF 753
CARBON MONOXIDE./ARYL AFTER 14-DAY EXPOSURE TO SUBLETHAL CONCENTRATIONS OF 751
CARBON MONOXIDE/KINETICS IN RATS EXPOSED TO SUB-LETHAL CONCENTRATIONS OF 754
CARBON MONOXIDE/HISTOPATHOLOGICAL CHANGES IN RATS EXPOSED TO 754
CARBON TETRACHLORIDE (CC14) IN RATS./Vehicles on acute hepatotoxicity of 1061
CARBON TETRACHLORIDE (CC14) IN RATS./MICROANATOMICAL ANALYSIS OF ORALLY ADMINISTERED 1062
CARBON TETRACHLORIDE (CC14) INDUCED HEP/IC ACID LYASE TO OTHER INDICES OF 1070
CARBON TETRACHLORIDE AND CHLOROFORM//INTERACTION STUDIES WITH 440
CARBON TETRACHLORIDE AND METALLOTHIONEIN INTERACTION/IN VITRO STUDY ON 1067
CARBON TETRACHLORIDE BIOTRANSFORMATION IN THE ACTIVATION OF HEPA/ROLE OF 520
CARBON TETRACHLORIDE HEPATOXICITY./VITAMIN A POTENTIATION OF 1058
CARBON TETRACHLORIDE ON THE LIVER/INDUCED HEPATOTOXICITY OF 1058
CARBON TETRACHLORIDE-INDUCED HEPATIC PI/GLUCURONIC ACID REGULATION DURING 1052
CARBON TETRACHLORIDE./INTERACTION BETWEEN 1,3-DICHLOROPROPANONE AND 138
CARBON TETRACHLORIDE/OXYTETRA CYCLES FOLLOWING IN VIVO EXPOSURE TO CHLOROGENE AND 910
CARBON TETRACHLORIDE/PRESSURE OF HUMORAL IMMUNITY BY 910
CARBON-14 (T/C) DUAL LABEL STUDY OF 1,2-DIBROMOBENZENE METABOLISM/THIOTHIOU/ 436
CARBONYL-14C) (4-NPI) IN RATS AND RABBITS/4-NITRO-N-METHYLFURFALDIME [ 946
CARBOSULFIN ON REPRODUCTION IN RATS/EFFECT OF 534
CARBOXYLIC ACID (OCA) ON THE URINARY EXC/EFFECT OF L-2-OXOTHIAZOLIDINE-4- 289
CARCINOCAGENESIS ON THE URINARY EXC/EFFECT OF L-2-OXOTHIAZOLIDINE-4- 289
CARCINOGEN AND TISSUE SPECIFIC ONCOCENE ACTIVATION. 926
CARCINOGENESIS IN THE WISTAR RAT BY ZINC TREATMENT/-ANTAGONISM OF CADIUM 283
CARCINOGENESIS RESEARCH INFORMATION SYSTEM./CRCIS: CHEMICAL 164
CARCINOGENESIS/TIME OF FEEDING ZINC DEFICIENT DIET IN ESOPHAGEAL 407
CARCINOGENESIS: DOSE EFFECTS AND NATURAL KIL/ - MAGNESIUM INTERACTIONS IN 160
CARCINOGENIC POTENTIAL OF DIBROMONYL PHTHA/ THE CHRONIC ORAL TOXICITY AND 54
CARCINOGENICITY OF METHYLAMINE IN MICE./N AND TESTOSTERONE ON THE RENAL 325
CARCINOGENICITY OF NICKEL DITHIOCARBAMATE. 325
CARCINOGENS AND TUMOR PROMOTERS./ODEL SYSTEM FOR THE STUDY OF IRRITANTS, 336
CARCINOGENS DURING COOKING/ TOXICOLOGY: MECHANISMS OF FORMATION OF POTENT 1037
CARCINOGENS ON THE V79 METABOLIC COOPERATI/ON OF VOLATILE AND NONVOLATILE 655
CARCINOGENS./PHARMACOKINETIC MODELS FOR INDIRECT 419
CARCINOGENS./VALIDATION OF RAPID BIODESTROY SYSTEM FOR DETECTION OF 74
CARCINOSIS AND SMALL CELLS: CANCER OF THE L/VIVO MODEL FOR THE INDUCTION OF 394
CARCINOMAS IN PARTIALLY-HEPATECTOMIZED RATS INDUCED BY PHENOL/FORESTOMACH 394
CARDIAC MYOCYTES/ERGIC AGONIST-INDUCED CYTOTOXICITY IN ISOLATED ADULT RAT
CARDIAC MYOBLASTS BY SODIUM NITRITE OR HYDROXYLAMINE/IN VIVO OXIDATION OF
CARDIOMEGALY IN RATS AFTER 14-DAY EXPOSURE TO SUBLETHAL CONCENTRATIONS OF
CAROTIDIC AGENTS./IAL MYOCYTE REAGGREGATE CULTURES (MMR) ON EXPOSURE TO
CAROTIDOCYTOTOXICITY: INFLUENCE OF DIETARY COPPER AND SODIUM ON ANTIO/CADMIUM
CAROTIDOCYTOTOXICITY OF N-DIETHYL-M-TOLUAMIDE (DEET)
CAROTIDOCYTOTOXICITY/IN VIVO AND IN VITRO MODELS FOR ASSESSING
CAROTIDOCYTOTOXICITY: IN VIVO EVIDENCE OF METABOLISM TO ACR/ALLYLAMINE
CARNITINE PALMITOYL TRANSFERASE I IN RAT LIVER MITOCHONDRIA/INHIBITION OF
CASTRATION AND TESTOSTERONE ON THE RENAL CARCINOGENICITY OF ME/EFFECTS OF
CATHEPSIN D/RELATIONSHIP BETWEEN TISSUE ELASTICITY AND CYTOTOXICITY OF THE COTTON DEFOLIANT DEF ON
CATIONIC AMPHIPHILIC DRUGS./THE IMMUNOTOXICITY OF
CATS./ITY OF NALIDIXIC ACID-INDUCED ACUTE ELECTRORENTINOGRAPHIC CHANGES IN
CBDP ON TISSUE CARBOXYSTERASE (CaE)/RESYNTHESIODIACYLPHOSPHORIN OXIDE (C
CBDP PRETREATMENT OF THE RAT ALTERS SO/RESYNTHESIODIACYLPHOSPHORIN OXIDE (C
CC14 BY PARTIAL HEPATECTOMY/OPTION OF HEPATOXICITY AND LETHAL EFFECTS OF
CC14 HEPATOXICITY IN MICE: CORRELATIONS WITH RAPID EFFECTS ON CALCIUM U
CC14 IN RATS/VEHICLES ON ACUTE HEPATOGENESIS IN ACID TETRACHLORIDE (C
CC14, MONOCHLOROBENZENE, AND LEAD ACETATE./TOXICITY OF MIXTURES OF
CC14 AND TRICHLOROETHYLENE: ANY ROLE FOR EN/INTERACTIVE HEPATOXICITY OF
CC14 DOSAGE ON THE POTENTIATION OF CC14 HEPATOGENESIS BY/INFLUENCE OF THE
CC14 EXPOSURE IN RAT LIVER AND IN PRIM/IOCHEMICAL ALTERATIONS PRODUCED BY
CC14 HEPATOXICITY BY KETONES/ OF THE CC14 DOSAGE ON THE POTENTIATION OF
CC14 HEPATOXICITY BY PARTIAL HEPATECTOMY/TECTION OF CHLORODONE POTENTIATED
CC14 HEPATOGENESIS IN ISOLATED HEPATOCYTES OBTAINED FROM CHLORODEONE(ICD)
CC14 HEPATOGENESIS IN PARTIAL HEPATOCYTE RADICALS CLICHORODEONE INJECTED
CC14 INTERACTION ON HEpatocallic CALCIUM HOMEOS/EFFECT OF ETHIONINE AND
CC14 ON THE JAPANESE MEDAKA EMBRYO (OR/E OF METABOLISM IN THE TOXICITY OF
CC14 IN RATS/MACROKINETICS OF ORALLY ADMINISTERED CARBON TETRACHLORIDE (C
CC14-INDUCED HEPATOGENESIS/E TO OTHER INDICES OF CARBON TETRACHLORIDE (C
CCRIS: CHEMICAL CARCINOGENESIS RESEARCH INFORMATION SYSTEM.
CD RAT./SUBCHRONIC TOXICITY STUDY OF 1,4-DITHIANE IN THE
CD RATS EXPOSED TO CYCLOHEXANE VIA /WO-GENERATION REPRODUCTION STUDY IN
CD RATS/DEVELOPMENTAL TOXICITY EVALUATION OF SOMAN IN MAN
CD RATS/DEVELOPMENTAL TOXICITY OF 1,1,1-TRICHLOROETHANE (TCEN) IN
CD-1 MICE./GISTIC ALTERATIONS FOLLOWING DIETARY ADMINISTRATION OF CAPTAN TO
CD-1 MICE./TERATOLOGIC EVALUATION OF NITROFURAZONE (HF) IN
CD-1 MICE/REPRODUCTIVE TOXICITY OF 3 PHTHALATE ESTERS IN
CD-1 MICE/UNOLOGICAL AND NEUROCHEMICAL EVALUATIONS OF TOXICITY TO TETRAETHYLPYRROLIDONE (C
CD-1 MICE/TERATOGENICITY OF ETHYLENE GLYCOL IN
CDN)./ CYTOPSKELETAL PERTURBATION INDUCED BY 1-CHLORO 2,4-DINITROBENZENE(.
CELL ACETYLCHOLINESTERASE IN BEAGLE DOYRIDOSTIGMINE BROMIDE ON RED BLOOD
CELL ACTIVITY BY OCRHOTAXIN A./DEPRESSION OF NATURAL KILLER
CELL ACTIVITY WITHOUT INDUCTION OF SUPPRESSOR C/INHIBITION OF CYTOTOXIC T
CELL ACTIVITY/EFFECT OF OZONE ON PULMONARY NATURAL KILLER
CELL ACTIVITY/EFFECT OF PHOSGENE ON LUNG NATURAL KILLER
CELL ANTIOXIDANT DEFENSES AND CELL KINETICS/EFFECTS OF HYPEROXIA ON TUMOR
CELL ANTIOXIDANT DEFENSES AND EFFECTS DATA FOR EXTRAPOLATION FROM IN VIT/HASAL EPITHELIAL
CELL CANCER OF THE LUNG/O ROLE OF THE INDUCTION OF PARATHYROID AND SMALL
CELL CULTURE AS AN IN VITRO TERATOGEN SCREEN./CHICK EMBRYO RETINA.
CELL CULTURES AND ITS RELATIONSHIP TO /3-DINITROBENZENE BY RAT TESTICULAR
CELL CULTURES FROM DRUG-INDUCED INJURY BY PLAVENTON/PROTECTION OF RAT LIVER
CELL CULTURES./ TOXIC EFFECTS OF MAMBA SNAKE VENOMS IN PRIMARY MYOCARDIAL
CELL CULTURES./ HYDROQUINONE INHIBIT MYELOPOIESIS IN FIBROBLASTOID STROMAL
CELL CULTURES./ TOXIC EFFECTS OF MAMBA SNAKE VENOMS IN PRIMARY ENDOTHELIAL
CELL DEPENDENT B-LYMPHOCYTES./HYDROQUINONE-INDUCED ALTERATION OF STROMAL
CELL DEPENDENT B-LYMPHOCYTES ALTERATION OF HUMAN PROMYELOCYTIC LEUKEMIA
CELL DNA REPAIR ASSAY./ MUTAGENS AND NONMUTAGENS IN THE RAT SPERMATOGENIC
CELL FUNCTION IN VITRO AFTER THE ADDIT/PECIFIC INDICES OF ALTERED SERTOLI
CELL FUNCTION./ TIN SODIUM DIHYDRATE ON NORMAL AND POSTSURGICAL PERITONEAL
CELL GROWTH STUDIED BY FOURIER TRANSFORM INFRARED SPECTROSCOPY
CELL INJURY./DIFFERENTIAL MECHANISMS OF CA2+/DEPENDENT
CELL KINETICS IN VIVO./HYPEROXIA ON TUMOR CELL ANTIOXIDANT DEFENSES AND
CELL LEUKEMIA AND PSEUDEOHYMOCYTOMA IN /CREASED INCIDENCES OF MONONUCLEAR
CELL LINKS./HYPEROXIA REDUCES EXPERIMENTAL LUNG METASTASIS FROM SENSITIVE
CELL LOSS AT NEUROTOXIC DOSES./EDIONE CAUSES INREVERSIBLE PESTICIDAL GERM
CELL MUTAGENS AND NONMUTAGENS IN THE RAT SPERMATOGENIC C/ACTIVITY OF GERM
CELL PROLIFERATION IN VITRO./THE EFFECT OF PARAXOXON ON MORINE T AND B
CELL TRANSFORMATION./T OF MAGNESIUM AGAINST NICKEL-INDUCED DNA DAMAGE AND
CELL-CELL COMMUNICATION IN WB CELLS BY PB/SCF RESISTANCE TO MEASURE INHIBITED
CELL-CELL COMMUNICATION: MECHANISM OF IMM/UMOR PROMOTER, FAILS TO INHIBIT
CELL-DERIVED rDNA HUMAN GM-CSF IN RHEUS MONKEYS/IMMUNOREACTIVITY OF CHO
CELLS (APC). / ROLE OF CONTACT SENSITISATION BY CUTANEOUS ANTIGEN-PRESENTING
640 CELLS (EC). / MEMBRANE FLUIDITY AND INSULIN RECEPTOR BINDING OF ENDOThelial
659 CELLS (LLC-PK1). / INDUCED INCREASES IN CYTOSOLIC CALCIUM ([Ca2+]i) IN PIG KIDNEY
678 CELLS (MGC). / PROMOTIONAL AGENTS/LATE CYCLASE (AC) IN ISOLATED MALE GERM
622 CELLS AND INJURED AXONS/ENTAL DISTRIBUTION IN RAT DORSAL ROOT GANGLION
640 CELLS BY HEPATIC TUMOR/PROLIFERATION IN RAT ISOLATED SPERMATOGENIC
659 CELLS BY N-[2-(2-OXO-1-IMIDAZOLIDINYL)]EUDUCTION IN STIMULATED PHAGOCYTIC
648 CELLS BY NAPHTALEN in THE ISOLATED PERFUSED M/SSELECTIVE DAMAGE TO CLARA
657 CELLS BY PBB AND DIELDRIN./EASURE INHIBITED CELL-CELL COMMUNICATION IN WB
676 CELLS BY SODIUM ARENETH./ TRANSFORMATION IN C3H/10T1/2 C1 8 MOUSE EMBRYO
567 CELLS CO-CULTURE DNA SYNTHESIS IN VITRO/ OF ME AND MA ON RAT SERTOLI-GERM
596 CELLS EXPOSED TO HYDROQUINONE INHIBIT MYELOPOIESIS/MACROPHAGE-LIKE STROMAL
1031 CELLS FOLLOWING GAMMA IRRADIATION/GE AND REPAIR IN ISOLATED SPERMATOGENIC
156 CELLS FOLLOWING IONIZING RADIATION/ WITHIN NEONATAL MOUSE CEREBELLAR
284 CELLS FROM CADMIUM TOXICITY-/DIFFERENTIATION-INDUCING AGENTS PROTECT 373
747 CELLS FROM NITROGEN DIOXIDE (NO2)-EXPOSED RA/SENSITIVITY OF ISOLATED LUNG
92 CELLS IN 3,4,5,3',4',5'-HEXACHLOROBENZOPHENONE/Y WITHOUT INDUCTION OF SUPPRESSOR
551 CELLS IN CULTURE./ DIOXIDE ON IODINE UPTAKE AND ORGANIZATION BY THYROID
676 CELLS IN CULTURE/PHENOTYPIC MODULATION OF AORTIC SMOOTH MUSCLE
5 CELLS IN PRIMARY CULTURE./PRODUCE DIFFERENT SPECTRA OF EFFECTS ON SERTOLI
568 CELLS IN VITRO/ALATE (MESH) ON THE HORMONAL RESPONSIVENESS OF RAT SERTOLI
1046 CELLS IN VITRO/OXIDATIVE STRESS OF SIX OXIME COMPOUNDS IN BACTERIA AND MAMMALIAN
327 CELLS INDUCED BY A PHORBOL DIESTER/ OF HUMAN PROMYELOCYTIC LEUKEMIA HL-60
160 CELLS INVOLVEMENT/IONS IN CARCINOGENESIS: DOSE EFFECTS AND NATURAL KILLER
6 CELLS TO CADMIUM/RENTAL RESPONSE OF PRIMARY RAT SERTOLI AND INTERSTITIAL
110 CELLS TO CEPAHARIDINE TOXICITY IN VITRO/IN VIVO ON THE KIDNEY AND LIVER
653 CELLS./ERICAERIAL COMMUNICATION BY 2,2',4',5',5'-HEXABROMOBIPHENYL IN WB
153 CELLS/GENOTOXICITY OF METHYLmercury IN MAMMALIAN
510 CELLS/IDENCE FOR REACTIVE OXYGEN SPECIES INDUCING MUTATIONS IN MAMMALIAN
326 CELLS/NIKEL TOXICITY IN HUMAN AND RAT ALVEOLAR EPITHELIAL
668 CELLS/INDUCED DNA DAMAGE IN HYPOXIC AND NORMOXIC KERATINOCYTE
328 CELLS/IONS IN DNA-PROTEIN INTERACTION FOLLOWING CHROMATE TREATMENT OF CHO
73 CELLS/S OF FORMICINS AND DILAZEP ON GROWTH ARREST AND RECOVERY OF LS178Y
566 CELLS/T OF SEVERAL POTENT TESTICULAR TOXINS ON CO-CULTURES OF TESTICULAR
1033 CELLS/ SYNTHESIS (UDS) IN XENOBOTS CONTAINING HUMAN BRONCHIAL EPITHELIAL
293 CELLS/ AFTER MERCURIC CHLORIDE EXPOSURE IN CULTURED RENAL PROXIMAL TUBULE
294 CELLS/ MERCURIC CHLORIDE INDUCED CYTOTOXICITY IN CULTURED RENAL PROXIMAL TUBULE
664 CELLS/ACTION INDUCED BY IRON-BLOOMYCIN COMPLEXES IN ISOLATED SPERMATOGENIC
210 CELLS/EFFECT OF DELTA-9-TETRAHYDROCANNABINOL ON RAT NEUROBLASTOMA
634 CELLS/GEN REGULATED PLASMINOGEN ACTIVATOR ACTIVITY OF MCP-7 BREAST CANCER
670 CELLS/METABOLISM OF METHOTREXATE IN HEPATIC
424 CELLS/OXYLATION OF DISTINCT RIBOSOME-ASSOCIATED PROTEINS IN GH3 PITUITARY
663 CELLS/PARACQUIRED CYTOSED RUNIC KININ IN CULTURED LUNG EPITHELIAL
51 CELLS AND BIOCHEMICAL CHANGES IN LUNG LAVAGE FLUIDS FOLLOWING THORACIC
667 CELLS DAMAGE CAUSED BY THE PYRROLOZIDINE ALKALOIDS LIPID PEROXIDATION AND
294 CELLS ENZYMATIC MEASUREMENT AS AN INDICATOR OF MERCURIC CHLORIDE INDUCED CYTO
684 CELLS/ LIVER INJURY/TRYPAN BLUE EXCLUSION: A POOR INDICATOR OF
905 CELULAR LEVEL./EARLY THYMIC EFFECTS OF DOXYCILYCHLORIDE (DOTC) AT A
159 CELULAR UPTAKE AND METABOLIC REDUCTION OF PENTAVEHONG TRIVALENT ARSEN
360 CEPAHARIDINE AND HEXACHLOROBUTADIENE IN RABBIT CORT/IN VITRO TOXICITY OF
110 CEPAHARIDINE TOXICITY IN VITRO/SCAPABILITY OF KIDNEY AND LIVER CELLS TO
70 CEPAHARIDINE NEPHROTOXICITY: EFFECTS ON RENAL MICROSONAL PROTEINS.
150 CEPAHARIDINE CELLS FOLLOWING IN VIVO EXPOSU/EXPRESSION WITHIN NEONATAL MOUSE
302 CEREBELLUM/DISTRIBUTION OF PE, CU, ZN, AND HG IN THE MOUSE
208 CEREBRAL CORTEX/EFFECT OF CHOL2 ON THE MORPHOLOGY OF THE DEVELOPING RAT
989 CEREBRAL MEMBRANES/EFFECTS OF P-XYLENE ON
621 CEREBRAL SULFUR CONTAINING COMPOUNDS./ OF L-METHIONINE-dl-SULFOXIMINE ON
755 CF-1 MICE./EFFECTS OF INHALED ETHYL CHLORIDE ON FETAL DEVELOPMENT IN
8 CHM CHANGES IN DEVELOPING MICE/SECALONIC ACID D-INDUCED PALATAL CAMP AND
224 CHAIN LENGTH/STIMULATION OF H2O2 GENERATION BY FATTY ACIDS: EFFECT OF
813 CHAMBER FOR EXPOSURE OF RATS TO OXIDANT GASES./A NOVEL INHALATION
316 CHELATION ON LEAD AND ZINC CONCENTRATIONS IN HUMANS: COMP/EFFECTS OF EDTA
312 CHELATION THERAPY./MOBILIZATION OF LEAD OVER THE COURSE OF DMSA
513 CHEMOTACTIC ACTIVITY./OLAR MACROPHAGES TO RELEASE NEUTROPHIL AND MONOCYTE
451 CHEMOTHERAPEUTIC ACTIVITY./ FOR PROCARBAZINE-INDUCED SPERMATOXOTOXICITY AND
1091 CHICK EMBRYO HEPATOCYTES TREATED WITH F/MICE, JAPANESE QUAIL AND CULTURED
1091 CHICK EMBRYO LIVER SUPERNATANTS./EDIATED OXIDATION OF UROFORPHONYRINOL BY
564 CHICK EMBRYO RETINA CELL REAGREGATION CU/ CHANGES IN SPONTANEOUSLY BEATING
1 CHICKEN EMBRYO TOXICITY OF PAPYRUS IN THE
216 CHICKENS DURING POSTEROPHOSPHATE-INDUCED/NIC (13-29) EVALUATION IN LAYING
480 CHICKEN.-METABOLISM OF THE PYRIDINE/PHENYL ETHER HERBICIDE SC-1084 IN THE
539 CHICKEN/ATROPINE AND DFP-INDUCED DELAYED NEUROTOXICITY IN THE
535 CHICKENS TREATED WITH TRI-O-CRESYL PHOSPHATE/II ACTIVITY IN BRAIN CYTOSOL OF
1100 CHICKENS./OTOXIN CYCLOPONIAZONIC ACID, IN MEAT AFTER ORAL ADMINISTRATION TO

CULTURES (MMR) ON EXPOSURE TO CARDIOTOXIC/K MYOCARDIAL MYOCYTE REAGGREGATE
CULTURES - A TOOL FOR THE MEASURE OF XENOBIOTIC CYTOTOXIC/BOVINE HEPATOCYTE
CULTURES AND ITS RELATIONSHIP TO TARGET /ROBENZENE BY RAT TESTICULAR CELL
CULTURES FROM DRUG-INDUCED INJURY BY FLAVONONE/PROTECTION OF RAT LIVER CELL
CULTURES OF HEPATOCYTES BY EPIDERMAL GROWTH OF DNA SYNTHESIS IN PRIMARY
CULTURES OF POSTNATAL RAT HEPATOCYTES./DINE AND DNA SYNTHESIS IN PRIMARY
CULTURES OF RAT HEPATOCYTES BY NONGENOTO/CHOLESTEROL SYNTHESIS IN PRIMARY
CULTURES OF RAT HEPATOCYTES BY PHOSPHATE/B (GTPase) EXPRESSION IN PRIMARY
CULTURES OF RAT HEPATOCYTES MAINTAINS CY/SENSE OF HEXOBARBITAL IN PRIMARY
CULTURES OF RHEating ON THE EFFECT OF /ROBENZENE EXPOSURE IN RAT HEPATOCYTE
CULTURES./TOXIC EFFECTS OF MAMBA SNAKE VENOMS IN PRIMARY MYOCARDIAL CELL
CULTURES./HYDROQUINONE INHIBIT MYELOPOIESIS IN FIBROBLASTOID STROMAL CELL
CULTURES./TOXIC EFFECTS OF MAMBA SNAKE VENOMS IN PRIMARY ENDOTHELIAL CELL
CUMENE/ONE-MONTH INHALATION STUDY WITH
CUTANEOUS ANTIGEN-PRESENTING CELLS (APC)./ROL OF CONTACT SENSITISATION BY
CUTANEOUS EPIDERMIS, IN VITRO/GENERATION OF A HUMAN
CUTANEOUS PENEQUINANCE IN THE TOBACCO /IN VIVO AND IN VITRO
CYANAMIDE/POTENTIATION OF ALCOHOL-ALCOHOL-INDUCED HEPATOTOXICITY BY
CYANIDE AND CARBON MONOXIDE IN MICE FOLLOW/LETHAL BLOOD CONCENTRATIONS OF
CYANIDE AND CYSTAMINE/M ISOLATED PERFUSED RAT HEART UPON REOXGENATION BY
CYANIDE AND PEROXIDATION OF BRAIN LIPIDS.
CYANIDE-INDUCED CYTOTOXICITY/MECHANISM OF
CYANOHYDRIN AND ADIPONITRILE/Y AND FERTILITY STUDIES IN RATS WITH ACETONE
CYCLASE (AC) IN ISOLATED MALE GERM CELLS/TION OF GTP-RESPONSIVE ADENYLALE
CYCLIC AMP AGONISTS PREVENT THE INHIBITION OF MOUSE HEPAT/ANTIOXIDANTS AND
CYCLIC PEPTIDE HEPATOTOXIN PRODUCED BY /P OF THE DEHYDROAMINO ACID FROM A
CYCLODIENE INSECTICIDE TOXICITY./NEUROPSYCHOLOGICAL EVALUATION OF
CYCLOHEXANONE VIA INHALATION./ON REPRODUCTION STUDY IN CD RATS EXPOSED TO
CYCLOHEXENE IN RATS/SUBCHRONIC ORAL TOXICITY STUDY OF 1-ETHYNYL-1-
CYCLOHEXIMIDE IN METHYL ISOBUTYL KETONE POTENTIATION OF TAUROLITHOCHOLIC
CYCLOPHOSPHAMIDE (CY-) INDUCED IMMUNOEHANCEMENT IS MEDIATED BY ITS METABO
CYCLOPIAZONIC ACID, IN MEAT AFTER ORAL ADMINISTRATION/OF THE MYCOTOXIN
CYCLOSPORIN A (CsA) ON HsCL2-INDUCED ANTINUCLEAR ANTIBODY (ANA)/EFFECT OF
CYCLOSPORIN A ON SUSCEPTIBILITY TO MURINE CYTOMEGALOVIRUS/EFFECTS OF
CYHEXATIN./A ONE-YEAR DIETARY TOXICITY STUDY IN BEAGLE DOGS ADMINISTERED
CYSTAMINE/M ISOLATED PERFUSED RAT HEART UPON REOXGENATION BY CYANIDE AND
CYSTEINE AND GLUTATHIONE ENHANCE METHYLMERCURO UPTAKE BY THE BRAIN.
CYSTEINE CONJUGATES OF ACYLONITRILE AND BROMOBENZENE ON ORGAN/EFFECTS OF
CYSTEINES/NIUM IONS IN THE FORMATION AND REACTIONS OF S-(2-HALOETHYL)-L-
CYTCHROME B245 IN RAT ALVEOLAR MACROPHAGES/OX INHALATION DECREASES
CYTOCHROME C REDUCTASE (CCR) BY ACRYLAMID/OAMIDE DEHYDROGENASE (LpDH) AND
CYTOCHROME P-450 (GP-450) IN THE GUINEA P/GRUPIN O-DEETHYLASE (EOD), AND
CYTOCHROME P-450 (P-450) CATALYZED METABOLISM OF 2,2,2-TRIFLUOROETHANOL A
CYTOCHROME P-450 AND ENHANCES THE EFFECT OF CARBON TETRA/P-XYLENE INDUCES
CYTOCHROME P-450 FROM AN INSECTICIDE RESIST/TERIZATION OF MULTIPLE FORMS OF
CYTOCHROME P-450 IN DETOXIFICATION/STEROIDS TOWARD RAT HEPATOCYTES: ROLE OF
CYTOCHROME P-450 INDUCERS AND INHIBITORS./F ACETAMINOPHEN (AA): EFFECT OF
CYTOCHROME P-450 INDUCTION AND GLUTATHIONE/INVESTIGATIONS INTO THE ROLE OF
CYTOCHROME P-450 ISOZYME RESPONSIBLE FOR /ACTORIZATION OF THE DOG HEPATIC
ISOZYME (P-450 ISOLATED FROM T/BRAIN OF C/MECHANISM OF ACTION OF ETHANOL-
CYTOCHROME P-450 ISOZYME FROM UNTREATED /CHARACTERIZATION OF TWO HEPATIC
CYTOCHROME P-450 ISOZYME./SULFURATION AND DEARYLATION OF FENITROTHION BY
CYTOCHROME P-450 LEVELS AND DRUG METABOL/ES OF RAT HEPATOCYTES MAINTAINS
CYTOCHROME P-450, 17-HYDROXYLASE AND 17,2/SUPPRESSED RAT TESTICULAR HEME,
CYTOCHROME P-450/./ THE MAJOR PHENOBARBITAL-INDUCIBLE ISOZYME OF DOG LIVER
CYTOCHROME P-450/D MECHANISMS OF ISOYCYANIDE BINDING TO PURIFIED RAT LIVER
CYTOCHROME P-450/LATION BY THE FCN-INDUCIBLE FORM OF RAT LIVER MICROSOMAL
CYTOCHROME P-450/LATION WITHOUT THE USE OF NON-DEGRADATION OF RAT LIVER MICROSOMAL
CYTOCHROME P-450p AND UDP-GLUCURONOSY-L/TR/ULATION OF RAT LIVER MICROSOMAL
CYTOCHROME P450 BY HETEROCYCLIC ANALOGS OF /MECHANISM-BASED INHIBITION OF
CYTOCHROME P450d1./INHIBITION OF 2-AMINOPHENOL MUTAGENESIS BY INDUCERS OF
CYTOCHROME P450p IN VIVO/INHIBITION OF RAT LIVER MICROSOMAL
CYTOSOME(S) P-450 (P-450P-B), AMINOPYRIN/NDEUCE PHENOBARBITAL-INDUCIBLE
CYTOSOME P-450 BY XENOBIOTICS/MECHANISM OF INDUCTION OF
CYTOSOMES p-450/OCARBON HYDROXYLASE (AHH) KINETICS BUT SLIGHTLY ALTERED
CYTOSPLASMA ETIC AND SPLENIC LYM-NANCY OF THE RESISTANCE TO GlUCOCORTICOSTERON
CYTOGENETIC EVALUATION OF MINERAL FIBER INDUCED RAT PLK/MORPHOLOGICAL AND
CYTOLOGY/BIOCHEMISTRY FOLLOWING CIGARETTE SMOKE/HISTOPATHOLOGY AND LAVAGE
CYTOMEGALOVIRUS (MCMV) AND RELATED IMMUNE FUNCTION ON SUSCEPTIBILITY TO MURINE
CYTOMETRIC INVESTIGATION OF THE EFFECT OF METALS ON NUCLEI/FLOW
CYTOMETRY & SPERM MICRO-INJECTION./NUCLEI ASSESSED ACROSS SPECIES BY FLOW
CYTOMETRY/DUCTION OF IL-2 RECEPTOR EXPRESSION BY DMSA DETECTED USING FLOW
CYTOKINAS ASSOCIATED WITH /BETA-LACTAM AN/E-MEDIATED HEMOLYTIC ANEMIA AND
CYTOSKELETAL INJURY IN CULTURED LUNG EPITHELIAL CELLS/PARAGANGI-ASSOCIATED
CYTOSKELETAL NETWORKS./CTS OF ACR, 2,5-HD, DMAE AND IDP ON MICROTBUBE

292
DATA ACQUISITION AND MANAGEMENT OF RADIONUCLIDE A COMPUTER-BASED SYSTEM FOR DATABASE FOR THE REVIEW OF ACUTE TOXICITY STUDIES/ THE DEVELOPMENT OF A DATABASE FOR COMPUTER ANALYSIS OF REPORTED NEUROTOXIC EFFECTS. DBA/2 MICE. (MDP) COMPOUNDS ON HEPATIC MICROSOMAL PROTEINS OF C57BL/6 AND DBA/2 MOUSE BONE MARROW NEUTROPHILS/ON OF INCREASED OXIDANT GENERATION BY DBA2 MICE - EFFECTS ON ENZYME INDUCED/DIOXIN ANTAGONIST IN C57BL/6J AND DEEP/XCITY AND TESTICULAR TOXICITY OF SELECTIVELY METHYLATED ANALOGS OF DECP/VINYL IN THE ADULT HEN/ DUCTED BY DI-N-Ethyl-2,2-DICHLOROVINYL-Phosphonate (DECP)/TPP)/GUTIONS AND CONCENTRATED IN RATS EXPOSED TO 1,2-DICHLOROETHANE (DCP)/ACUTE, SUBACUTE, AND SUBCHRONIC PATHOLOGY OF 1,2-DICHLOROPROPANE (6188 C57BL MICE P RETREATMENT WITH DIETHYLDITHIOCARBAMIC ACID (881 DEARYLATION OF FENITROTHERION BY CYTOCHROME /OXIDATIVE DESULFURIZATION AND DECALIN ON MALE RAT NEPHROPATHY/DY OF THE ACUTE EFFECTS OF d-LIMONENE AND DECANOIC ACID (PFDA)-INDUCED INCREASE IN HEPATIC PALMITATE O/PERFLUORO-N-DECARBOXYLASE (CODC) INDUCTION AND TUMOR PRO/UCED DNA SYNTHESIS, ORNITHINE DECARBOXYLASE ACTIVITIES AND UROPHORPHIN A/COMPARISON OF UROPHORPHYRINON DECARBONYLASE ACTIVITY (ODC) IN GASTRIC MUC/ AND NON-STIMULATED ORNITHINE DECARBONYLASE ACTIVITY: A SENSITIVE MARKER OF LUNG RESPONSE TO ORNITHINE DECHELORINATION OF CHLOROPROPANES./MICROSOMAL DECOMPOSITION PRODUCTS OF POLYMERS CONTAINiON OF THESE GASES OR THERMAL DECONTAMINATION STRATEGIES FOR HEPTACHLOR CONTAMINATED LIVESTOCK DECYLDODECYL BENZENES. A THERMAL STUDY OF A MIXTURE OF DEET/ANATROPOCUTANEOUS EFFECTS OF N,N-DIETHYL-M-TOLUAMIDE 643 DEHYDROXTAL (ERD), AND CYTOCHROME P-450 (/YLASE (AH), ETHOXYRESORUFIN O DEFICIENCY/IMMUNE DYSFUNCTION WITH SEVERE OR MARGINAL COPPER DEPLUMPING BY HEPATOLIGINE S TRANSFERASES. DEGENERATION OF NASAL EPITHELIUM IN RATS AND MICE CAUSED/INFLAMMATION AND DEHP AND PHENOBARBITAL ON GROWTH AND PROGRE/EVIDENCE FOR LACK OF EFFECT OF DEHP ON MILK COMPOSITION AND MAMMARY G/P? THROUGH RAT MILK AND EFFECTS OF DEHP AND MONO(2-ETHYLHEXYL) PHTHALATE /R OF DI(2-ETHYLHEXYL) PHTHALATE (DEHP) AND WP-14,643 (WT) IN RATS GENTS, DI(2-Ethylhexyl)Phthalate (DEHP) ER 344 RATS FOLLOWING MATERNAL EXPOSURE TO DIETHYLHEXYL PHTHALATE (271) DEHYDRATASE (ALAD) BY LEAD (Pb)/S INHIBITION OF DELTA-AMINOLEVULINIC ACID DEHYDROAMINOCIC FROM A CYCLIC PEPTIDE HE/E/TOXICITY RELATIONSHIP OF THE DEHYDROGENASE (ECH) mRNA BY WP-14,643 IN RA HYDRATASE : 3-HYDOXYACYL COA DEHYDROGENASE (LpDH) AND CYTOCHROME C REDUC/CYPF INHIBITION OF LIPOAMIDE DEHYDROGENASE. VANADIUM INHIBITION OF YEAST GLUCOSE-6-PHOSPHATE DEHYDROGENASE/DATION PRODUCTS BY PURIFIED RAT HEPATIC MICROSOMAL ALDEHYDE DEHYDROGENASES (ALDH)/ED RAT LIVER MICROSOMAL AND MITOCHONDRIAL ALDEHYDE DEHYDROGENASES/NONOBUTYL ETHYL (2-BUTOXYETHANOL; BE) VIA ALCOHOL/ALDEHYDE DEIODINATION BY IODINATED ISOMERS OF AMINO / OF HEPATIC AND RENAL THYROXINE DELAYED NEUROPATHY (OPIDN) IN ADULT HENS/S DURING ORGANOPHOSPHATE-INDUCED DELAYED NEUROPATHY (OPIDN) IN CHICKENS./PONENT OF ORGANOPHOSPHORUS-INDUCED DELAYED NEUROTOXICITY IN YOUNG CHICKENS/F ADMINISTRATION OF ORGANOPHOSPHATE DELTA-9-TETRAHYDRO-CANNABINOL/RESPONSE TO HERPES SIMPLEX VIRUS 2(HSV2) BY
DELTA-9-TETRAHYDROCANNABINOL ON RAT NEUROBLASTOMA CELLS/EFFECT OF
DEMETHYLASE ACTIVITY AND LIVER HYPERTROPHY/50 (P-450PB-B), AMINOPYRINE N-
DEMETHYLATION OF N-NITROSODIMETHYLAMINE (NDMA) CATALYZED BY PIG LIVER /
DEMETHYLATION: A CORRELATIVE NEUROBEHAVIORAL/RH OF THE NERVOUS SYSTEM FROM
DENMIN: INITIATION/ESSENTIAL HEPATOCYTE RESPONSE TO DIETHYLNITROSAMINE (1)
DENA INITIATED B6C3F1 MICE/ATIC TUMOR EXPRESSION IN DIETHYLNITROSAMINE (1)
DENMATHEMATICAL CHILDREN'S MUSCLE/MINE ON TETANIC CONTRACTURE IN INNERVATED VS.
DENMATHEMATICAL CHILDREN'S MUSCLE/MINE ON TETANIC CONTRACTURE IN INNERVATED VS.
DENMATHEMATICAL CHILDREN'S MUSCLE/MINE ON TETANIC CONTRACTURE IN INNERVATED VS.
DEPOSITION & CLEARANCE OF INHALED KEVLAR ARAMID FIBERS IN RATS
DEPOSITION AND METABOLISM OF PROPANOOL AND ACETONE VAPORS IN THE NASAL CAV
DESMAL ABSORPTION AND DISPOSITION OF 1,2-DIHYDRO-2,2,4-TRIMETHYLOXYLONINE
DESMAL DOSE FROM ACOMING ORAL DOSE IN RATS/A NOVEL METHOD TO PREVENT A
DESMAL EXPOSURE TO COMPLEX ORGANIC MIXTURES ON BLOOD PRESSURE A/EFFECT OF
DESMAL IRRITANT TESTS: PREDICTiveness OF IRRITATION POTENTIAL USING SMALL
DESMAL PENETRATION AND PHARMACOKINETICS OF 2,4,5,2'-IN VIVO AND IN VITRO
DESMAL SENSITIZATION STUDY IN HAIRLESS GUINEA PIGS WITH DNBC AND BENZOCAL
DESMAL SENSITIZATION STUDY IN HAIRLESS GUINEA PIGS WITH DNBC AND BENZOCAL
DESMAL TOXICITY OF METISFURON METHYL IN THE RABBIT/SUBCIRRHOSIC
DESMAL TOXICITY OF THREE SYNTHETIC OILS IN RATS/SUBCIRRHOSIC
DESMAL TOXICITY STUDIES OF ETHION TECHNICAL IN RABBIT/3/SUBCIRRHOSIC
DESMAL TOXICITY STUDY IN RABBITS WITH CARBOFURAN TECHNICAL./REPEATED DOSE
DESMAL TOXICITY STUDY OF BENZETHIONUM CHLORIDE IN P344 RATS AN/SUBCIRRHOSIC
DESMAL APPLIED CHEMICAL CLASS FRACTION/INTURES: TERATOGENICITY OF THEIR
DESMAL APPLIED CLARIFIED SLURRY OIL IN/AL: DEVELOPMENTAL TOXICITY OF 2-METHYLNAPHTHOL APPLIED
DESMAL TO OCLUDED AND NON-OCLUDED SI/CITY OF 2-METHYLNAPHTHOL APPLIED
DESMAL TO PREGNANT RATS/THE EFFECT OF PHENETHYL ALCOHOL APPLIED
DESMALOTOXINS..ERNS OF EPIDERMAL KERATIN EXPRESSION ARE INDUCED BY POTENT
DESMAL-N ACTIVATOR EXHIBIT PULMONARY SENS/MIMIZED BY INJECTION WITH HDI OR
DESMALIFICATION AND DEARYLATION OF FENITROTHION BY /COMPARISON OF OXIDATIVE
DESMAL DETERGENT BUILDER/CE OF TERATOGENIC RESPONSE IN RATS AND RABBITS GIVEN A
DESMAL DETERGENT/LIVER MICROSOMAL CYTOCHROME P-450b WITHOUT THE USE OF NON-IONIC
DESMAL TOXICITY TO PEPTIDES TOWARD RAT HEPATOCYTES: ROLE OF CYTOCHROME P-450 IN
DESMAL TOXICITY OF BENZO(A)PYRENE IN LUNG./ND PHENOLIC ANTIOXIDANTS IN THE
DESMAL TOXIFICATION-D/DT-DIAPHOREAS AND QUINONE
DESMAL TREATED 3-METHYLINDOLE IS LESS PNEUMOTOXIC THAN 3-METHYLINDOLE.
DESMAL DEVELOPING MICE/SECOLONIC ACID D-INDUCED PALATAL CAMP AND cGMP CHANGES IN
DESMAL DEVELOPING OTOCYST IN CULTURE/EFFECT OF LEAD ON THE
DESMAL DEVELOPING RODENTS MORPHOLOGICAL AND B/1-TERT-BUTYL THEROXIDE (TBTO) TO THE
DESMAL DEVELOPING RAT CEREBRAL CORTEX/EFFECT OF CI02 ON THE MORPHOLOGY OF THE
DESMAL DEVELOPING RAT/ACUTE ETHANOL EXPOSURE AND BRAIN MALFORMATIONS IN THE
DESMAL DEVELOPMENT AND CHARACTERIZATION OF A MUSCULAR SCORING SYSTEM FOR ME
DESMAL DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF FISCHER 344 RATS FO/POSTNATAL
DESMAL DEVELOPMENT IN CF-1 MICE./EFFECTS OF INHALED ETHYL CHLORIDE ON FETAL
DESMAL DEVELOPMENT IN RATS./S OF CARBENDAZIM-INDUCED ALTERATIONS OF REPRODUCTIVE
DESMAL DEVELOPMENT OF LOCOMOTOR ACTIVITY IN YOUNG/ESSES T3 UPTAKE AND DELAYS THE
DESMAL DEVELOPMENT OF SEXUALLY DIMORPHIC HEPATIC ONE (DEX) TREATMENT ALTERS THE
DESMAL DEVELOPMENTAL MODEL OF ORGANOPHOSPHATE INDUCED PERSISTANT BRAIN ACETYLIC/A
DESMAL DEVELOPMENTAL TOXICITY EVALUATION OF INHALED N,N-DIMETHYLNAPHTALAMINE(DME
DESMAL DEVELOPMENTAL TOXICITY EVALUATION OF SOMAN IN CD RATS
DESMAL DEVELOPMENTAL TOXICITY OF 1,1,1-TRICHLOROETHANE (TEC) IN CD RATS
DESMAL DEVELOPMENTAL TOXICITY OF 4-NITRO-N-METHYL-PHTHALIMIDE IN RATS
DESMAL DEVELOPMENTAL TOXICITY OF DERMALLY APPLIED SLURRY /MATERNAL AND
DESMAL DEVELOPMENTAL TOXICITY OF INDUSTRIAL N-NITROSO COMPOUNDS IN VITRO
DESMAL DEVELOPMENTAL TOXICITY OF RHODAMINE 123 IN DROSOPHILA MELANOGASTER
DESMAL DEVELOPMENTAL TOXICITY STUDIES./ARGUS AND GALLIUM ARSENIDE (GA):
DESMAL DEVELOPMENTAL TOXICITY STUDY OF VIRGINAMICIN IN CR1:CD-1(ICR)BR MICE
DESMAL DEVELOPMENTAL TOXICITY/DERATION OF MATERNAL TOXICITY ASSESSMENT OF
DESMAL DEKAMETHASONE (DEX) TREATMENT ALTERS THE DEVELOPMENT OF SEXUALLY/NEONATAL
DESMAL DEKAMETHASONE(DEX) IN MICE./ALLOTHIONINE(MT)ISOFORMS BY ZINC, CADMIUM AND
DESMAL DEKAMETHASONE-SUPPRESSED RAT TESTICULAR HOLE, CYTOCHROME P-4/ PREVENTION OR
DESMAL DESAMINONDETHYL (CI-908), AN ANTICANCER AGENT, IN RA/TERATOGENIC POTENTIAL OF
DESMAL DFP AND SOMAN./OSPHATE COMPOUNDS IN RAT SKELETAL MUSCLE INTOXICATED WITH
DESMAL DFP ON THE ELECTROPHYSIOLOGICAL PROPE/ DIISOPROPYL PHOSPHOROFLUORIDATE (DIP
DESMAL DFP-INDUCED DELAYED NEUROPATHY IN THE CHICKEN/ATROPINE AND
DESMAL DI(2-ETHYHLXYL)PHTHALATE (DEHP) AND MONO(2-ETHYHLXYL)PHTHALATE/TRANSFER OF
DESMAL DI(2-ETHYHLXYL)PHTHALATE (DEHP) AND WY-14,643 (W/L PROLIFERATING AGENTS, 1029
DESMAL DI(2-ETHYHLXYL)PHTHALATE/ BY THE PEROXISOME PROLIFERATORS CLOBIFRATE AND
DESMAL DI-n-BUTYL-2,2-DICHLOROVINYL PHOSPHOSPHATE (BDCV) IN /IC DEFICITS INDUCED BY
DESMAL DIAGNOSTIC AID FOR NATURALLY-OCurrIN/CROM PROBE X-RAY ANALYSIS AS A
DESMAL DIAGNOSIS. SUPPRESSION OF LYMPHOCYTE PROLIFERATION BY HEXAMETHYLENE
DESMAL DIAMINODICHLORO-PLATINUMII (CIS-DFP)/DDUCT OF THE ANTITUMOR DRUG, CIS-
DESMAL DIAGNOSTIC SENSITIZATION DIAGNOSE TOXICITY OF POLYCHLORINATED
DESMAL DIAGNOSTIC SENSITIZATION IDENTIFICATION OF POLYCHLORINATED
DESMAL DIAPHRAGM MUSCLE OF SPRAGUE DAWLEY RATS./GY OF NEUROMUSCULAR JUNCTIONS IN
DESMAL DIBENZO-P-DIOXINS AND RELATED COMPOUNDS IN GU/TOXICITY OF POLYCHLORINATED
DESMAL DIBENZO-P-DIOXINS AND RELATED COMPOUNDS./ TOXIC EFFECTS OF POLYBROMINATED
DESMAL DIBENZOFURANS (PCDFS)/ADDITIONAL TERATOGENIC EFFECTS OF POLYCHLORINATED
DIBENZOFURANS IN THE JAPANESE MEDAKA EMBRYO/N THE TOXICITY OF DIOXINS AND
DIBROMOACETONITRILE (DBAN) WITH GLUTATHIONE (G) TOXICITY: INTERACTIONS OF
DIBROMOBENZENES METABOLISM AND COVALENT BIND/T(C) DUAL LABEL STUDY OF 1,2-
DICHLOOR-1,2,2,2-TETRACHLOROETHANE) A HERBICIDE TO XENOBIOIC PRODUCTS IN TIDATIVE ACTIVATION OF 3,3-
DICHLOORACETATE INCREASES CHLOROFROM TOXICITY IN FEMALE RATS
DICHLOORACETIC ACID/OTOXICITY IN RATS ASSOCIATED WITH CHRONIC EXPOSURE TO
DICHLOORANILINE IN RATS/A 2-WEEK SUBCHRONIC INHALATION STUDY ON 3,
DICHLOOROBENZENE./ION IN THE DIFFERENTIAL HEPATOTOXICITY OF THE ISOMERS OF
DICHLOOROBENZENES/TIDE MEDIA ON THE TOXICITY OF MONOCHLOROBENZENE, AND THE
DICHLOOROBENZIDINE PRETREATMENT IN THE RAT/TIC LIPID PEROXIDATION BY 3,3-
DICHLOOROETHANE (DCE)/THTHIONE (GSH) CONCENTRATION IN RATS EXPOSED TO 1,2-
DICHLOOROETHYLENE (DCE) TOXICITY AND A PUTATIVE TOXIC METABOLITE OF 1,1-
DICHLOOROMALATE (DCM) ON KIDNEY AND LIVER/EFFECTS OF
DICHLOOROPHENYL)-SUCINIMIDE-INDUCED NEPHROTOXICITY./NE EFFECTS ON N-3,5-
DICHLOOROPROPAINE (DCP)/ACUTE, SUBACUTE AND SUBCHRONIC PATHOLOGY OF 1,2-
DICHLOORPROPANONE AND CARBON TETRACHLORIDE./INTERACTION BETWEEN 1,3-
DICHLOORPROPENE/CID EXCRETION FOLLOWING ACUTE INHALATION EXPOSURE TO 1,3-
DICHLOORVINYL PHOSPHATE (DVCP) IN THE ADU/ITS INDUCED BY DI-N-BUTYL-2,2-
DIELDORIN./EASURE INHIBITED CELL-CELL COMUNICATION IN WB CELLS BY PB AND
DIELS EXHAUST, NITROGEN DIOXIDE AND OZ/S AFTER EXPOSURE TO CARBON BLACK,
DIET IN ESOPHAGEAL CARCINOGENESIS/TIME OF FEEDING ZINC DEFICIENT
DIET ON TRACE ELEMENT CONTENT OF RAT SERUM/THE EFFECTS OF LEAD AND
DIETARY ADMINISTRATION OF CAPTAN TO CD-1/PATHOLOGIC ALTERATIONS FOLLOWING
DIETARY ADMINISTRATION OF TWO SODIUM ALUMINA CONCENTRATION IN BONE FOLLOWING
DIETARY CHRONIC TOXICITY-Oncogenicity STUDY/6-CHLOROPOLINOLIC ACID: 2-YEAR
DIETARY CLOFIBRATE ON HYDROLYTIC ENZYMES/EFFECT OF
DIETARY COPPER AND SELENIUM ON ANTIOXID/1UM CARBOXTOXICITY: INFLUENCE OF 62-
DIETARY COPPER/N CEMENTO, COPPER, ZINC, CALCIUM AND/EFFECTS OF 66-
DIETARY COPPER/YMSE AND TRACE ELEMENTS IN RAT KIDNEY I. INFLUENCE OF HIGH
DIETARY EXPOSURE/ONE PROLIFERATION IN MALE B6C3F1 MICE FOLLOWING HIGH FAT
DIETARY FAT IN B6C3F1 MALE MICE./HEPATIC TUMOR PROMOTION BY
DIETARY FAT MODIFIES HEPATIC ACTIVATION OF COOKED-FOOD MUTAGENS IN THE RA
DIETARY FISH OILS./ICULAR FIBRILLATION IN THE RAT: INFLUENCE OF STRAIN OR
DIETARY FLUORIDE TO MINK./CHRONIC TOXICITY OF
DIETARY INCORPORATION/HLORO-1,3,3-BUTADIENE (HCBD) IN B6C3F1 MICE FOLLOWING
DIETARY INDIGOS: A NOVEL MECHANISM./IFICATION OF XENOBOTIC METABOLISM BY
DIETARY IRON, ATOM, AND TCH-DERIVED MUTAGENS IN HEPATIC LIPID PEROXIDATION
DIETARY PROTEIN LEVELS ON ESTERASE ACTIVITY IN RATS./INFUENCE OF
DIETARY QUERCETIN MODIFIES HEPATIC TRANSFORMATION OF COOKED-FOOD MUTAGENS
DIETARY RESTRICTION AND AGE ON LIVER ENZYME ACTIVITIES AND /INFUENCES OF
DIETARY RESTRICTION IN RATS/NEUROBEHAVIORAL EFFECTS OF
DIETARY SODIUM ON SELENIUM, CADMIUM, COPPER, ZINC, CALCIUM A/EFFECTS OF 62-
DIETARY SODIUM/S AND TRACE ELEMENTS IN RAT KIDNEY II. INFLUENCE OF HIGH
DIETARY STATUS: AN IMPORTANT FACTOR IN MODULATING THE HYPERLIPIDEMIC RESP
DIETARY TOXICITY OF 2 PYRIDYL/ETHERS IN RATS AND MICE./COMPARATIVE
DIETARY TOXICITY STUDY IN BEAGLE DOGS ADMINISTERED /RESULTS OF A ONE-YEAR
DIETARY TRIACINONOLONE ON DEVELOPMENT OF ORGANOPHOSPHORUS-INDUCE/EFFECT OF
DIETARY TRIIODOTHYRONINE (T3) AND SURGIC/GIC EVALUATION OF THE EFFECTS OF 48-
DIETARY TRIIODOTHYRONINE (T3) ON 2,3,7,8-TECHLOROBENZO/THE EFFECT OF
DIETARY TRIIODOTHYRONINE (T3) ON TCDD-INDUCED HEPATIC VITAMIN THE EFFECT OF
DIETARY VS. GAVAGE ADMINISTRATION OF FENOC/G COMPARATIVE PHARMACOKINETICS.
DIETYL maleate ON GLUTATHIONE METABOLISM IN RATS PRETREATED/INFUENCE OF
DIETYL-M-TOLUICAEID (DEET)/CARDIOVASCULAR EFFECTS OF N,
DIETHYLTHIOTHIOCARBAMIC ACID (DDC)/MPTP IN C57Bl MICE BY PRETREATMENT WITH
DIETHYLENE GLYCOL./E PERFORMANCE STUDY IN RATS WITH A TERATOLOGY PHASE ON
DIETHYLHEXYL PHTHALATE (DEHP)/HER 344 RATS FOLLOWING MATERNA EXPORUE TO
DIETHYLNITROSAMINE (DEN) INITIATION./RESISTANT HEPATOCYTES IN RESPONSE TO
DIETHYLNITROSAMINE (DEA) INITIATED B6C3F1 M1/HEPATIC TUMOR EXPRESSION IN
DIETHYLNITROSAMINE AND CONTINUOUS EXPOSURE TO/PRODUCED BY A SINGLE DOSE OF
DIETHYLSTILESTROL IN HAMSTER RENAL CORTEX./N THE IRREVERSIBLE BINDING OF
DIETHYLSTYRENE TO HUMAN METALLOTHIONEIN (MT) LIVE/EFFECT OF BULHYDRYL-DEFICIENT
DIETS/TCDD/INDUCED DIOXYGENATION (TCDD) IN COLD-ADAPTED RATS/PER DIFFERENT
DIFFERENTIATION ANGEL GLYCOSAMINOLGLYCAN SULF/ PROMYEOCYTIC LEUKEMIA CELL
DIFFERENTIATION OF HUMAN PROMYEOCYTIC LEUK/STONE PHOSPHORYLATION DURING
DIFFERENTIATION-INDUCING AGENTS PROTECT 3T3 CELLS FROM CADMIUM TOXICITY.
DIFFERENTIATION/AROMATIC HYDROCARBON ALTERATIONS IN B-CELL ACTIVATION AND
DIFFERENTIATION/TION IN THE RESPONSE TO THE REGULATOR SIGNALS CONTROLLING
DIGITONIN-PERFUSED RAT LIVER/NICATION BY DIFFERENCES IN ENZYME RELEASE IN
DINALOETHANES./OF PLASMA a1phal-PROTEINASE INHIBITOR BY EPOXIDES AND 1,2-
DINUTRITION NORMAL AND POSTSURGICAL PERITONEAL/EFFECTS OF TOLMETIN SODIUM
DIHYDRO-2,2,4-TRIMETHYL-QUINOLINE IN F344 RATS AND B6/AND POLYMER OF 1,2-
DIHYDRO-2,2,4,3-TRIMETHYLQUINOLINE IN FISCHER 344 RATS/ DISPOSITION OF 1,2-
DIHYDROBENZ(V)(A)PYRENE/ULFTE-DEPENDENT MUTAGENICITY OF 7,8-DIHYDRO-7,8-
DIHYDROXY-7,8-DIHYDROBENZO(A)PYRENE/ULFITE-DEPENDENT MUTAGENICITY OF 7,8-
DIHYDROXY-THIOPHENOL NEPHROTOXICITY: STRUCTURE ACTIVITY REQU/6-BROMO-2,5-
DIHYDROXY-THIOPHENOL/SYTHESIS OF 7,8-DIHYDROXY-THIOPHENOL NEPHROTOXICITY OF 7,8-
DIISOCYANATE (MDI). /GENS WHICH DETECT ANTIBODIES TO DIPHENYLHYDANTOIN 4,4'-
DIISOCYANATE (TDI) AND TRIMELLITIC ANHYDR/INHALATION EXPOSURE TO TOLUENE
DIISONYLON PHTHALATE (DINP) IN F-344 RATS/NIC AND CHRONIC ORAL STUDIES OF 405
DIISONYLON PHTHALATE (DINP) IN RATS. XICITY AND CARCINOGENIC POTENTIAL OF 378
DIISOPROPYL PHOSPHOROFLUORIDATE (DFP) ON THE ELECTROPHYSIOLOGIC/EFFECT OF 543
DIISOPROPYLAMINOETHYL) METHYLPHOSPHONITE (QL) NEUROTOXICITY/O-ETHYL-O'-
DIKETONES/PEROXIDATIVE BIOTRANSFORMATION OF CYCLIC 1,2-
DIMETHYLACETAMIDINE (DMA) AND REVERSAL OF 15175Y /EFFECTS OF FORMYCINS AND 943
DIMAC WITH SILVER SULFADIAZINE AS COMPARED TO SILVADE/ABSORPTION STUDY OF 584
DIMETHOXYBENZIDINE (DMOB) AND THEIR TERAOTGEN/DIMETHYLBENZIDINE (DMB) OR 38
DIMETHYL METHYLPHOSPHONATE (DMMP) NEUROTOXICITY IN HENS 765
DIMETHYL PHOSPHORODIOTHIOATE/HRONIC INHALATION TOXICITY STUDIES WITH 0,0'-
DIMETHYL PHOSPHORODIOTHIOATE/RTILITY AND REPRODUCTION STUDIES WITH 0,0'-
DIMETHYL-2,6-OCTADIENYL (CITRAL) IN RATS/STRIPIATION AND EXCRETION OF 3,7-
DIMETHYL-2,6-DIAMINO-1,4-THIAPENTHALONE (DTP) BY THE RABBIT 858
DIMETHYLPHOSPHONATE (DMO) OR DIMETHYLPHOSPHONATE/ERIVED FROM BENZIDINE (B), 691
DIMETHYLTHANOLAMINE(DEMA) IN FISCHER 344 RATS./VALUATION OF INHALED N,N-
DIMETHYLAMINO-N-FORMANYL-5-ROSYLAMINE FOLLOWING ETHANOL PRETREAT/IN VITRO MUTAGENICITY OF 38
DIMETHYLAMINO-M UTILIZATION OF IN VITRO CHANGES IN MACROPHAGE M6/MERGAMIC MECHANISMS FOR 37
DIMETHYLAMINOSUOMO - DISTRIBUTION STUDIES WITH 14C-L/IMMUNOTOXICITY BY 811
DIMETHYLAMINOSUOMO (DMSO) DOES NOT PROTECT AGAINST PULMONARY FIBROSIS 855
DIMORPHIC HEPATIC P-650'S IN THE R N/P ALTERS THE DEVELOPMENT OF SEXUALLY 560
DINITROBENZENE (DNB)/DOCIRME STATUS OF MALE RATS FOLLOWING EXPOSURE TO m-
DINITROBENZENE (DNB): EFFECTS OF REPEATED ORAL DOSING ON THE RAT TES/1,3-
DINITROBENZENE (DNB): EFFECTS ON FERTILITY IN THE RAT/1,3-
DINITROBENZENE (DNB): IN YOUNG ADULT AND A/REPRODUCTIVE TOXICITY OF 1,3-
DINITROBENZENE BY RAT TESTICULAR CELL CULTURES AND/NITROREDUCTION OF 1,3-
DINITROBENZENE(CDNB). / CYTOSKELETAL PERTURBATION INDUCED BY 1-CHLORO 2,4-
DINITROBENZENE BY MALE FISCHER-344 RATS./METABOLISM AND EXCRETION OF 457
DINITROBENZENE ISOMERIZATION AND C-ACETYLATION OF CYTOTOXICITY OF 923
DINITROBENZENE IN MICE/EVALUATION OF THE IMMUNOTOXIC POTENTIAL OF 935
DINOBEG, DETERMINED BY IN VIVO AND IN VITRO/PERCUTANEOUS PENETRATION OF 703
DIOCTYL SODIUM SULFOSUCCINATE (DSS) IN R/MERATION REPRODUCTION STUDY WITH 907
DIOCTYLTRIETHYLLAMINE (DOT) AT A CELLULAR LEVEL./EARLY THYMIC EFFECTS OF 850
DIONE (HD), AND 23187 PRODUCE DIFFEREN/YL-PHTHALATE (MDHP), 2,5-HEXANE-
DIOXYN (TCDD) ANTAGONIST IN RATS - AHH /S A 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD) AT DOSES ASSOCIATED WITH /M BY 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD) IN COLD-ADAPTED RATS PED / OF 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD) INDUCED LESIONS IN MALE S/ ON 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD) INDUCED LIPID PEROXIDATION/ ON 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD) ON PEER GROUP SOCIAL BEHAV/TO 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD) INDUCED TOXICITY IN RATS./ON 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD)-TREATED RATS./ IN TESTES FROM 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD) INDUCED CARCINOGENESIS IN 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD)/Y NOT BE A PRIMARY EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD)/R IN HAMSTERS ADMINISTERED 3H-2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD)/SSUE THERMOREGESIS INDUCED BY 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN (TCDD)/TION IN HAMSTERS TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN ANTAGONIST IN C57BL/6J AND DBA/2/S A 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN SUPPRESSION OF ESTROGEN REGULATED PLASMINOGEN ACTIVATOR ACTIVITY 502
DIOXYN TREATED RATS/TIONS IN THE TESTES FROM 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN, ANTAGONISM BY ARCOCLOR 1254./CITY 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN: ANTAGONISM BY ARCOCLOR 1254./MY 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXYN AND DIBENZOFURANS IN THE JAPANESE/IREV RUDIMENT IN THE TOXICITY OF 606
DIOXYN AND RELATED COMPOUNDS IN GUINEA /TY OF POLYCHLORINATED DIBENZO-P-
DIOXYN AND RELATED COMPOUNDS./TOXIC EFFECTS OF POLYBROMINATED DIBENZO-P-
DIOXYN AS LIGANDS FOR THE Ah RECEPTOR A/ITUTED-3,7,8-TRICHLORODIBENZO-P-
DIPHENYL ETHERS./EVIEWS: METHYL BROMIDE, CHLORINATED STYRENE, BROMINATED 731
DIPHENYLACETONE/1-ACETATE/CYSTEINS EXPOSED TO TAMOXIFEN, 4,4'-DIISOCYANATE (MDI)./IGENS WHICH DETECT ANTIBODIES TO 223
DIQUAT DISTRIBUTION OF IRON IN RAT LIVER./EFFECT OF 996
DISCRIMINATION TASK IN ADULT MONKEYS/LOW-LEVEL LEAD EXPOSURE ON A SPATIAL 446
DISODIUM CROMOGLYRATE (DSCG) INHIBITS THE FORMATION OF FREE RADICALS, POT 740
DISPOSAL OF MUNICIPAL SLUDGE/A RISK ASSESSMENT METHODOLOGY FOR OCEAN 117
DISPOSITION AND METABOLISM OF INHALED 1-CHLORO-2-PROANOL IN RATS. 431
DISPOSITION OF 1,2-DIHYDRO-2,2,4-TRIMETHYLMORPHOLENE/DERMAL ABSORPTION AND 638
DISPOSITION OF 2,3,4,7,8-PENTACHLORODIBENZOFLUORIDE (PCDF) IN T/TOXICITY AND 349
DISPOSITION OF 5-HYDROXYTRYPTAMINE IN THE PARA-METHYLBENZAMIDE ON THE
Disposition of a series of herbicides in the rat/comparative

Disposition of acetylaminophen (AA) in rats. Acid (UDP-GA) depletion on the

Disposition of n-butyl acrylate in male Fischer rats/metabolism and

Disposition of orally administered vinylcyclohexene (VCH) I/comparative

Disposition of reserpine, benzodiazepine, and scopolamine by sequestration and

Disposition of [14C]-ethyl-3-ethoxypropionate in the rat/the metabolism and

Disposition studies/acquisition and management of radiolabeled xenobiotic

Distribution after mercuric chloride exposure in C/time course of mercury

Distribution and elimination of 4-isopropylbiphenyl from the rat and mouse

Distribution and excretion of 3,7-dimethyl-2,6-octadienal (cital) in rat

Distribution and excretion of manganese AF/effect of complexing agents on

Distribution and excretion of 14C-Phenytoin/absorption and

Distribution and metabolism in the Fischer 344 rat/chlorpyrifos-

Distribution in rat dorsal root ganglion cells and in injured A/elemental

Distribution in rats/effects of ethanol pretreatment on morphine

Distribution of arsenic in the rabbit following subcutaneous admin/tissue

Distribution of cadmium/mercury and stress pretreatment on the subcellular

Distribution of Fe, Cu, Zn, and Hg in the mouse cerebellum

Distribution of iron in rat liver/effect of diquat on the

Distribution of iron, zinc and copper in r/on the hepatic and testicular

Distribution of lead/aging alters the tissue

Distribution of manganese in chronically exposed P344 rats and B6C3F1/tissue

Distribution of trans-4-hydroxy-2-hexenal and tandem mass spectrometric D

Distribution width in the rat/bocytopenia on platelet volume and relative

Dissolution studies with 14C-labeled DMN/city by dimethylnitrosamine -

Dithiane in the CD rat/subchronic toxicity study of 1,4-

Dithiocarbamate inhibition of cadmium-induced changes in spermatogenesis CHO

Dithiocarbamate/carcinogenicity of nickel

Dithiothreitol/protection of cadmium toxicity and enzyme inhibition by

DINSA or nocturnal ozone (03) exposure/in pulmonary response induced by

DMBA detected using flow cytometry/duction of IL-2 receptor expression by

DMEA in Fischer 344 rats/evaluation of inhaled N,N-dimethylethanolamine(

DMH and IPN upon microtubule cytoskeletal netwo/effects of ACR, 2,5-HD,

DMH exposed rats/plasmic transport in sciatic nerves of ACR, 2,5-HD and

DMH on neural and non-neural NA/DH-TR activity in/effects of ACR, HD, and

DMH upon the microtubule/mitosis and tub/toxic action of ACR, 2,5-HD and

DMP) neurotoxicity in Hens/dimethyl methylphosphonate (DMP)

DMN) induced changes in macrophage MNS/channels for dimethylnitrosamine -

DMN-induced immunosuppression/role of metabolism in

DMN/city by dimethylnitrosamine - distributional studies with 14C-labeled

DMPA/alpha decay products: polonium-210 decarboxylation by

DMSA chelation therapy/mobilization of lead over the course of

DMSO does not protect against pulmonary fibrosis/dimethylsulfoxide (DMF)

DNA adducts of the antitumor drug, cis-/ viral genome containing the major

DNA adducts after exposure to complex orga/an approach for characterizing

DNA binding of aflatoxin B1 in Fischer/GE on the metabolic activation and

DNA cross-linking by gravity-flow alkali elution/detection of

DNA damage and cell transformation/10 of magnesium against nickel-induced

DNA damage and lack of repair synthesis in rat hepatocytes following in V

DNA damage and repair in airway epithel/ and benzo(a)pyrene (BaP)-induced

DNA damage in hamster/oxidant and non-oxidant tumor cell lines

DNA damage is not positively correlated with glutathione (GSH) /hepatic

DNA double-strand damage and repair in isolated spermatogenic cells follo

DNA from mouse and L1210 tumor mitocho/cin-induced damage of mitochondria

DNA guanine/normal development of hydrazine-induced methylation of

DNA produced by trichloroacetic acid (TCA), chloroh (CH)/breaks in hepatic

DNA repair assay/mtugen and nonmutagenic in the rat spermatogenic cell

DNA repair system in the eukaryote, TE/OR evidence suggesting an inducible

DNA synthesis, ornithine decarboxylase/lymphoblast-13-acetyl (TFA)-induced

DNA synthesis (UDS) and s-phase synthesis induction of hepatic unscheduled

DNA synthesis (UDS) in xenografts containing hum/induction of unscheduled

DNA synthesis in primary cultures of h/MEHP inhibits the enhancement of

DNA synthesis in primary cultures of postnatal rat hepatocyte/protein and

DNA synthesis in rat hepatocytes by th/laminofluorine-induced unscheduled

DNA synthesis in vitro/ of me and NA on rat sertoli-germ cells co-culture

DNA synthesis, ornithine decarboxylase/lymphoblast-13-acetyl (TFA)-induced

DNA-binding in vivo of aflatoxin B1 in medaka (Oryzias lati/metabolism and

DNA-binding [3H] and [14C]formaldehyde (HCHO) in the nasal muc/analysis of

DNA-protein cross-linking in rat liver nuclei by halogenated acetaldehyde

DNA-protein interaction following chromat otre/analysis of alterations in

DNA/oxin B1, aflatoxins B1, aflatoxin M1, and aflatoxin M1 with purified

DNA/2-aminofluorene adducts/PCR analysis of 32P-postlabeled DNA

DNA/BINDING IN VIVO OF AFLATOXIN B1 IN MEDAKA (ORYZIAS LATI/METABOLISM AND

DNA-BOUND [3H]- AND [14C]FORMALDEHYDE (HCHO) IN THE NASAL MUC/MANAGEMENT OF

DNA-PROTEIN CROSS-LINKING IN RAT LIVER NUCLEI BY HALOGENATED ACETALDEHYDE

DNA-PROTEIN INTERACTION FOLLOWING CHROMATIDE RE/ANALYSIS OF ALTERATIONS IN

DNA/OXIN B1, AFLATOXIN B1, AFLATOXIN M1, AND AFLATOXIN M1 WITH PURIFIED

DNA/2-AMINOFLUORENE ADDUCTS/PCR ANALYSIS OF 32P-POSTLABLED DNA (DNB)

DNA/2-AMINOFLUORENE ADDUCTS/PCR ANALYSIS OF 32P-POSTLABLED DNA (DNB)

DNA/BINDING IN VIVO OF AFLATOXIN B1 IN MEDAKA (ORYZIAS LATI/METABOLISM AND

DNA-BOUND [3H]- AND [14C]FORMALDEHYDE (HCHO) IN THE NASAL MUC/MANAGEMENT OF

DNA-PROTEIN CROSS-LINKING IN RAT LIVER NUCLEI BY HALOGENATED ACETALDEHYDE

DNA-PROTEIN INTERACTION FOLLOWING CHROMATIDE RE/ANALYSIS OF ALTERATIONS IN

DNA/OXIN B1, AFLATOXIN B1, AFLATOXIN M1, AND AFLATOXIN M1 WITH PURIFIED

DNA/2-AMINOFLUORENE ADDUCTS/PCR ANALYSIS OF 32P-POSTLABLED DNA (DNB)

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DNA/BINDING IN VIVO OF AFLATOXIN B1 IN MEDAKA (ORYZIAS LATI/METABOLISM AND

DNA-BOUND [3H]- AND [14C]FORMALDEHYDE (HCHO) IN THE NASAL MUC/MANAGEMENT OF

DNA-PROTEIN CROSS-LINKING IN RAT LIVER NUCLEI BY HALOGENATED ACETALDEHYDE

DNA-PROTEIN INTERACTION FOLLOWING CHROMATIDE RE/ANALYSIS OF ALTERATIONS IN

DNA/OXIN B1, AFLATOXIN B1, AFLATOXIN M1, AND AFLATOXIN M1 WITH PURIFIED

DNA/2-AMINOFLUORENE ADDUCTS/PCR ANALYSIS OF 32P-POSTLABLED DNA (DNB)

DNA/2-AMINOFLUORENE ADDUCTS/PCR ANALYSIS OF 32P-POSTLABLED DNA (DNB)

DNA/BINDING IN VIVO OF AFLATOXIN B1 IN MEDAKA (ORYZIAS LATI/METABOLISM AND

DNA-BOUND [3H]- AND [14C]FORMALDEHYDE (HCHO) IN THE NASAL MUC/MANAGEMENT OF

DNA-PROTEIN CROSS-LINKING IN RAT LIVER NUCLEI BY HALOGENATED ACETALDEHYDE

DNA-PROTEIN INTERACTION FOLLOWING CHROMATIDE RE/ANALYSIS OF ALTERATIONS IN

DNA/OXIN B1, AFLATOXIN B1, AFLATOXIN M1, AND AFLATOXIN M1 WITH PURIFIED
ELIMINATION OF 4-ISOPROPYL-BIPHENYL FROM THE RAT AND MOUS/DISTRIBUTION AND METABOLISM
132 ELISA Method for the THIOCARBAMATE HERBICIDE MOL/REFINEMENT AND VALIDATION OF AN
196 ELISA TO DETECT HUMAN IgE ANTIBODY TO /ZYMIE LINKED IMMUNOSORBENT ASSAY (ELISA)
197 ELISA DETECTION OF DNA CROSS-CONNECTED BY GRAVITY-FLOW ALKALINE
198 ELUTION/RECOVERY OF PYRROLIZIDINE AND LARKSPUR ALKALOIDS DETECTED BY ALKALINE
202 EMBRYO (ORYZIAS LATIPES)/M IN THE TOXICITY OF CC24 ON THE JAPANESE MEDAKA
203 EMBRYO CELLS BY SODIUM ARSENITE./ TRANSFORMATION IN C3H1/10T1/2 CL 8 MOUSE
204 EMBRYO CULTURE SYSTEMS TO IDENTIFY FRENCH CATION OF POSTIMPLANTATION DENSITY
205 EMBRYO CULTURE/COMPARISON OF UMBILICAL PLIP V/C POSTIMPLANTATION DENSITY
207 EMBRYO DEVELOPMENT./RIZATION OF A MORPHOLOGICAL SCORING SYSTEM FOR MEDAKA
209 EMBRYO HEPATOCYTES TREATED WITH POLYH/CREASED/AL JAPANESE QUAIL AND CULTURED CHICK
212 EMBRYO LIVER SUPERNATANTS./EDIATED OXIDATION OF UROPHORPHIN OXID IN CHICK
213 EMBRYO RETINA CELL CULTURE AS AN IN VITRO TERATOGEN SCREEN./CHICK
214 EMBRYO./TOXICITY OF PARAOXAN IN THE CHICK
216 EMBRYO/S THE TOXICITY OF DIOXINS AND DIBENZOFURANS IN THE JAPANESE MEDAKA
217 EMBRYOS IN VITRO./I OF CHEMICALS TO ELICIT OPEN NEURAL TUBES (ONT) IN RAT
218 EMBRYOS EFFECTS OF APLAOKIN C1 AND G1 ON POSTIMPLANTATION DENSITY
220 EMBRYOTOXIC EFFECTS OF THE ANTIMETABOLITE ETHEL NOXY PHENOX ETHER ALKOIDS ARE AFFECTED
225 EMBRYOTOXICITY AND GLUTATHIONE (GSH) DEPLETION ELICITED BY INHIBITION OF
226 EMISSIONS AND COAL DUST, ALONE AND COMBINE/ADDRESSES EXPOSED TO DIESEL ENGINE
227 ENDOCRINE STATUS OF MALE RATS FOLLOWING EXPOSURE TO /DINITROBENZENE (DNB)
228 ENDOCRINE STATUS./ULATION OF ACETAMINOPHEN (AA) HEPATOTOXICITY IN RATS BY
229 ENDOPITHELIAL RETICULUM DURING MAMMARY CANCER/IONIC CHANGES IN HEPATIC
230 ENDOPITHELIAL RETICULUM./) ON INITIATION OF HEPATOCARCINOGENESIS AND ON THE
231 ENDOTHELIAL AND CANTHARIDIN ACID./HEPATIC BIOCHEMICAL RESPONSES OF MICE TO
233 ENDOTHELIAL CELL CULTURES/TOXIC EFFECTS OF MAMBA SNAKE VENOM IN PRIMARY
234 ENDOTHELIAL CELLS (BE). INHIBITION OF CHOLINERGIC AND INSULIN RESPONSES
235 ENDOTHELIN HYPERSENSITIVITY/USES OF THE MECHANISM OF 2,3,7,8-TCDD INDUCED
237 ENDOTOXIN IN GUINEA PIGS./ERMINA AND RESPIRATORY INHIBITION TO INHALATION OF
239 ENDOTOXIN AND PARAOXAN DURING PREGNANCY/TOXICITIES OF
241 ENERGETICS IN THE CATFISH DECREASE/IN M PENTACHLOROPHENATE ON FEEDING
242 ENERGY AND FAT METABOLISM AND HYPOTHALAMUS IN RA/INTERRELATIONSHIPS BETWEEN
245 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
248 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
252 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
255 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
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282 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
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288 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
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381 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
384 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN
387 ENERGY METABOLISM IN ISOLATED RAT RETINA/LEAD-INDUCED ALTERATIONS IN

FUNGICIDE./HYPERACTIVITY INDUCED BY TRIADIMEFON (TDF), A TRIAZOLE

G-6-Pase) Activity on Rat Liver/Hydrul Levels and Glucose-6-Phosphatase (G-6-Pase).

GALACTOSAMINE/ASSOCIATED WITH PROTECTION FROM HEPALECTOMY TOXICITY BY

GALUMINARSENIDE (GaAs)/RESISTANCE FOLLOWING INTRATRACHEAL INSTILLATION OF

GALUMINARSENIDE (GaAs)/ULTRASONIC PRODUCED BY INTRATRACHEAL INSTILLATION OF

GALUMINARSENIDE COMPARED TO SILICA AND/ISTELOGICAL PULMONARY EFFECTS OF

GAMMA IRRADIATION/GE AND REPAIR IN ISOLATED SPERMATOCYTIC CELLS FOLLOWING

GAMMA RADIATION EXPOSURE ON LUMOTOPE ACTIVITY IN SWISS/EFFECTS OF ACUTE

GAMMA-Glutamyltranspeptidase (GGTase) EXPRESSION IN PR/THROMBOMODULIN OF

GAMMACELLS AND IN INJURED AXONS/ERAL DISTRIBUTION IN RAT DORSAL ROOT

GAMMAHOSIDE EXPRESSION WITHIN NEONATAL MOUSE CEREBELLAR CELLS POL/ALTERED

GAP JUNCTION AND EARLY-LIVERS EXPRESSION QUANTIFICATION OF

GAS CHROMATOGRAPHIC (GC) ANALYSIS OF N/PECIFIC CLEAN-UP PROCEDURE FOR THE

GASES FOR VARIOUS EXPOSURE TIMES/LOGICAL EFFECTS OF PURE AND MIXED FIRE

GASES OR THERMAL DECOMPOSITION PRODUCTS/ICE FOLLOWING INHALATION OF THESE

GASES./A NOVEL INHALATION CHAMBER FOR EXPOSURE OF RATS TO OXIDANT

GASOLINE-INDUCED HYALIN DROPLETS AND alpha-2/RAPID POST-EXPOSURE DEATH OF

GASTRIC MUCOSA OF MINIATURE SWINE/RNITHINE DECARBOXYLASE ACTIVITY (ODC) IN

GASTRIC MUCOSAL DAMAGE BY ASPRIN IN DOGS. PROTEIN/ENDOSCOPY ASSESSMENT OF

GAVAGE ADMINISTRATION OF PENTOFLOXIN TO /IVE PHARMACOKINETICS: DIETARY VS.

GAVAGE STUDY OF 8X0XOCTYL ACETATE IN RATS/RESULTS OF A 90-DAY ORAL

GAVAGE TO MALE SPRAGUE-DAWLEY RATS./ENYL-3-PYRAZOLIDINONE ADMINISTERED BY

GAVAGE) ON RAT FORESTOMACH/THE EFFECTS OF ETHYL ACRYLATE (BY

GAVAGE/T OF VEHICLE ON THE RELATIVE UPTAKE OF HALOALKANES ADMINISTERED BY

GC/MS IN SPECIMENS FROM HORSES POISONED/ON OF CANTHARIDIN USING CAPILLARY

GMPB/OF PATHOGENICITY FOR GENETICALLY ENGINEERED MICROBIAL PESTICIDES (PG

GMPB EXPRESSION WITHIN SUBCELLULAR FRACTIONS/RAP-INDUCED ALTERATIONS OF RENAL

GMPB GENERATION REPRODUCTION STUDY IN CD RATS EXPOSED TO CYC/RESULTS OF A TWO-

GENERATOR FOR THE DELIVERY OF RESPIRABLE AEROSOLS./A LOW FLOW AEROSOL

GENETICALLY ENGINEERED MICROBIAL PESTICIDES /ABSENCE OF PATHOGENICITY FOR

GENETICALLY SENSITIVE MICE/ 2,3,7,8-TETRACHLORIN ENVIRONMENTAL SAMPLE USING

GENOTOXICITY OF 1,3-BUTADIENE (BD)./CARCINOGENICITY AND

GENOTOXICITY OF METICILIN-RESISTANT ESCHERICHIA COLI CE: EFFECTS ON TREATMENT OF

GENOTOXICITY OF PYRROLIZIDINE AND LARSGUR ALKALOIDS DETECTED BY ALKALINE

GENOTOXICITY OF SIX OXIME COMPOUNDS IN BACTERIA AND MAMMALIAN CELLS IN VITRO

GENOTOXICITY STUDIES ON 2-ETHYL-1,3-HEXANEDIOL./IN VITRO AND IN VIVO

GENOTOXICITY TESTING USING HUMAN TISSUES./SYSTEM FOR SHORT-TERM IN VITRO

GENOTOXINS IN VIVO AND IN VITRO./ON OF COOKED-FOOD MUTAGENS TO BACTERIAL

GENOTROPIC INDUCED INCREASES IN CYTOSOLIC CALCIUM [Ca2+] IN PIG KIDNEY

GENOTROPIC-INDUCED RENAL PHOSPHOLIPIDOSIS IN /ROLE OF PHOSPHOLIPASE C IN

GENOTROPIC VIOLET./THREE-GENERATION TOXICITY STUDY OF RATS INGESTING

GERM BRAIN./OXYGEN FREE RADICALS CAUSED BY IRREVERSIBLE TESTICULAR

GERM CELL MUTAGENS AND NONMUTAGENS IN THE RAT SPERMATOCYTIC C/ACTIVITY OF

GERM CELLS AS MALLS PROMOTIONAL AGENTS/EARLY CYCLASE (AC) IN ISOLATED MALE

GERMINAL EPITHELIAL DAMAGE./SERUM ANDROGEN BINDING PROTEIN (ABP) INDICATE

GEP BY ACYCHIN AND ANTI-GEP ANTIBODIES ON GAMMA-GlutAMYL TRANSPERASE (GGTase)

GEP EXPRESSION IN PRIMARY CULTURES O/F GAMMA-GlutAMYL TRANSPERASE (GGTase)

GI MICROFLORA/INVASIVE ASSAY TO DETERMINE ALTERED XENOBIOTIC METABOLISM BY

GINSENG ON ACUTE ALCOHOL INTOXICATION/EFFECTS OF P.

GLAND IN LACTATING RATS/EFFECTS OF DEHP ON MILK COMPOSITION AND MAMMARY

GLAND OF MICE./INFLUENCE OF PROLACTIN ON THE PORPHYRIN OF THE HARDERIAN

GLASS FIBERS IN A MURINE MODEL OF PNEUMONIC RESPONSE INDUCED BY EXPOSURE TO

GLOBULIN (alpha-2u) IN MALL FISCHER 344/LPENTANE (TMP) TO RENAL alpha-2u

GLOBULIN (alpha-2u)/BINDING OF 2,2,4-TRIMETHYLPENTANE (TMP) TO alpha-2u-

GLOBULIN (alpha-2u) ACCUMULATED IN THE MALL RENAL KID/ED HYALIN DROPLETS AND alpha-2u-

GLOMERULAR IMMUNE COMPLEXES (GIC) IN ASW/ANTI-CIRCULATING ANTIBODY (ACA) AND

GLUCOCORTICOID RECEPTOR BINDING IN MICE LI/XACACRIBIDINOBENZOIC ACID DECREASE

GLUCOSE METABOLISM/TRICHLOROACETATE EFFECTS ON LACTATE AND

GLUCOSE-6-PHOSPHATASE (G-6-Pase) ACTIVITY ON RA/ON SULFURHYDRYL LEVELS AND

GLUCOSE-6-PHOSPHATE DEHYDROGENASE./VANADIUM INHALATION OF YEAST

GLUCOSIDE/CIDE OF 2-METHOXYETHANOL(ME)-INDUCED DIGEST MALFORMATIONS BY D-

GLUCOSURIC ACID (UDP-6-GA) DEPLETION ON THE DISPOSABLE EFFECT OF HEPATIC UDP-

GLUCURONIC ACID (UDP-GA) DEVELOPMENT DURING PHOSPHOSULFATE (PAPS) AND UDP-

GLUCURONIC ACID REGULATION DURING CARBON/PHOSPHOSULFATE (PAPS) AND UDP-

GLUCURONIDATION AND SULFATION/L METABOLISM OF HARMOL (HA): DOSE-DEPENDENT

GLUCURONIDATION AND TOXICITY WITH AGE USING 4-4' THIODIS/ALTERATIONS IN

GLUCURONIDATION IN HAMSTERS TREATED WITH 2,3/YRIDONE METABOLISM AND THYROXINE
GLUCURONOSYL-TRANSFerase./RAT LIVER MICROSOMAL CYTOCHROME P-450 AND UDP-
GLUCURONYL TRANSFERASE (UDPGT) ACTIVITY AND ANTIESTROGENIC E/STEROID UDP-
GLUTAMATE (MSG) AS NEONATES EXHIBIT HYPERACTIVITY/RATS TREATED WITH MONOSODIUM
GLUTAMATE SEVERELY DISRUPTS FLASH EVOKED /AL ADMINISTRATION OF MONOSODIUM
GLUTAMATE TRANSFERASE (GOT) BY ACICIFICIN A/ELICITED BY INHIBITION OF GAMMA-
GLUTATHIONE (GSH) AND GLUTATHIONE-S-TRANSF/IBROMOACETONITRILE (DBAN) WITH
GLUTATHIONE (GSH) BY EXOGENOUSLY ADDED NAPHTH/DELETION OF HEPATOCELLULAR
GLUTATHIONE (GSH) CONCENTRATION IN RATS EX/NOT POSITIVELY CORRELATED WITH
GLUTATHIONE (GSH) DEPLETION CYTOSKELETAL PERTURBATION/ROLE OF
GLUTATHIONE (GSH) DEPLETION ELICITED BY INHIBITION OF /EMBRYOTOXICITY AND
GLUTATHIONE (GSH) DEPLETION ON ACTIVATION OF INORGANIC /EFFECT OF HEPATIC
GLUTATHIONE (GSH) IN OLTIPRAZ (OTP)-INDUCED PROTECTION IN ACETAM/ROLE OF
GLUTATHIONE (GSH) IN THE MOUSE/ IMMUNOMODULATOR ADMINISTRATION ON HEPATIC
GLUTATHIONE (GSH) STATUS AND THE ABILITY OF CHEMICALS TO EL/INTRACELLULAR
GLUTATHIONE AND MERCAPTURIC ACID EXCRETION FOLLOWING ACUTE INHALATION/TISSUE
GLUTATHIONE CONJUGATION PATHWAY/PERCHLOROETHYLENE METABOLISM BY THE
GLUTATHIONE CONTENT IN RATS/ALTERATIONS IN HEPATIC LIPOID PEROXIDATION AND
GLUTATHIONE DIMINISHED BY APOPTOSIS IN THE LIVER/CHARACTERIZATION OF
GLUTATHIONE DEPLETION IN THE DIFFERENTIAL /CYTOCHROME P-450 INDUCTION AND
GLUTATHIONE DISULFIDE (GSSG)/PATIC CANALICULAR MEMBRANE VESICLES (CMV) BY
GLUTATHIONE ENHANCE METHYLMERCURY UPTAKE BY THE BRAIN./CETYLENE AND
GLUTATHIONE LEVEL OF RAT LIVER/ITRILES ON THE MIXED FUNCTION OXIDASES AND
GLUTATHIONE METABOLISM IN RATS PRETREATED /INFLUENCE OF DIETHYL MALEATE ON
GLUTATHIONE ON MICROTUBULAR ARCHITECTURE/ICITY: THE PROTECTIVE EFFECTS OF
GLUTATHIONE ON MITOCHONDRIA IN M/R POST-EXPOSURE EFFECT OF LUNG
GLUTATHIONE ON THE IRREVERSIBLE BINDING OF DIETHYLTOLUIDINE I/EFFECT OF
GLUTATHIONE S-TRANSFERASES AND PHENOLIC ANTIOXIDANTS IN THE DETOX/ROLE OF
GLUTATHIONE S-TRANSFERASES?DEFLUORINATION BY HEPATIC
GLUTATHIONE TRANSFERASE ACTIVITY IN VIVO A/METHYLIN (TM) ON RAT HEPATIC
GLUTATHIONE, SULFITE, AND BENZO(a)PYRENE INTERACTIONS: A MATHEMATICAL MOD
GLUTATHIONE-S-TRANSFERENCE (GSHT) IX RATS/DBAN WITH GLUTATHIONE (GSH) AND
GLUTATHIONE-S-TRANSFERASE./OP BCNU ON ACETAMINOPHEN BIOTRANSFORMATION AND
GLYCEROLPHOSPHATE/IN VIVO AND IN VITRO INTERACTION OF METHYLCHLOROFORM WITH
GLYCEROL PHOSPHORYLASE IN VARIOUS PREPARATIONS/TOTAL AND ACTIVE FORMS OF
GLYCOL ETHER PHENOXY ETHANOL ARE AFFECTED/EMBRYOTOXIC EFFECTS OF THE ARYL
GLYCOL IN CD-1 RATS/SUBCHRONIC TOXICITY OF ETHYLENE
GLYCOL IN RATS/SUBCHRONIC INHALATION STUDY OF PROPYLENE
GLYCOL MONOBUTYL ETHER (2-BUTOXYETHANOL)/METABOLIC ACTIVATION OF ETHYLENE
GLYCOL MONOMETHYL ETHER (EGME) IN THE MALE /FERTILITY EFFECTS OF ETHYLENE
GLYCOL PHENYL ETHER (ESPE): TOXICOLOGICAL EFFECTS IN RABBITS AND/ETHYLENE
GLYCOL/E PERFORMANCE STUDY IN RATS WITH A THERATOLOGICAL PHASE ON DIETHYLENE
GLYCOL/OMEGY/IN VIVO AND IN VITRO INTERACTION OF METHYLCHLOROFORM WITH
GLYCOLOSATE IN THE MALE FISCHER RAT/ABSORPTION OF
GLYCOLOSATE./MUTAGENICITY STUDIES WITH THE HERBICIDE
GOATS. HASTENING WITHDRAWAL USING MINERAL OIL./PCBs IN
GONADOTROPIN (HCG)/450, 17-HYDROXYLASE AND 17,20-LYASE BY HUMAN CHORIONIC
GOSSYPOL IN MALE RATS/PHARMACOKINETICS AND BIOAVAILABILITY OF
GOSSYPOL ON SOLUBLE F1 ATPase FROM VERTEBRATE AND INHIBITORY ACTION OF
GRANULOCYTIC AND MONOCYTIC INDUCERS OF HUMAN PROMYELOCYTIC LEUKAEMIA CELL
GRAFTING./PATHOLOGICAL CHANGES IN RATS TO ACUTE INHALATION OF SYNTHETIC
GROWTH AND PROGRESSION OF NATURALLY OCC/PECT OF DEHP AND PHENOBARBITAL ON
GROWTH ARREST AND RECOVERY OF L5178Y CE/PECTS OF FORMYCNES AND DILAZEP ON
GROWTH FACTOR (EGF) BINDING./PSORALENS ARE POTENT INHIBITORS OF EPIDERMAL
GROWTH FACTOR (EGF)/HERSIS IN PRIMARY CULTURES OF HEPATOCYTES BY EPIDERMAL
GROWTH HORMONE IN Rhesus MONKEYS./IMMUNOREACTIVITY OF BIOSYNTHETIC HUMAN
GROWTH STUDIED BY FOURIER TRANSFORM INFRARED SPECTROSCOPY/CtEL
GSH DEPLETION ON THE UPTAKE OF ACRYLONITRILE (AN) VAPORS AND/INFLUENCE OF
GSH AND GLUTATHIONE-S-TRANSFERASE (GS/ONITRILE (DBAN) WITH GLUTATHIONE
GSH BY EXOGENOUSLY ADDED NAPHTHALENE/ON OF HEPATOCELLULAR GLUTATHIONE
GSH CONCENTRATION IN RATS EXPOSED TO /VEY CORRELATED WITH GLUTATHIONE
GSH CONTENT IN MODULATING CYTOSKELETAL PERTURBATION/ROLE OF GLUTATHIONE
GSH DEPLETION ELICITED BY INHIBITION OF/EMBRYOTOXICITY AND GLUTATHIONE
GSH DEPLETION ON ACTIVATION OF INORGANIC/EFFECT OF HEPATIC GLUTATHIONE
GSH IN OLTIPRAZ (OTP)-INDUCED PROTECTION IN ACETAM/ROLE OF GLUTATHIONE
GSH IN THE MOUSE/IMMUNOMODULATOR ADMINISTRATION ON HEPATIC GLUTATHIONE
GSH STATUS AND THE ABILITY OF CHEMICALS TO EL/INTRACELLULAR GLUTATHIONE
GSH-S-TRANSFERENCE (SGT) TOWARDS ATTOXIN B-9/ES TOWARDS ATTOXINS B-9 AND
GAAs)/LATION PRODUCED BY INTRANASAL INSTILLATION OF GALUJ URESE (GAAs)/SISTANCE PRODUCED BY INTRANASAL INSTILLATION OF GALUJ URESE
GTP-RESPONSIVE ADENYLATE CYCLASE (AC) IN ISOLATED MALE GERM/MODULATION OF
GUANINE/OF FORMALDEHYDE HYDRAZONE IN HYDRATOME-INDEPED MethylTHIONE OF DNA
GUAR GUM./STUDY OF THE TERATOGENIC POTENTIAL OF
GUINEA PIG (EGP) TEST IN THE ASSESSMENT OF PETROLEUM PRODUCT/THE EPILATED
GUINEA PIG ALVEOLAR MACROPHAGES (AM)/UPEROXIDE AMION (O2-)- PRODUCTION IN
GUINEA PIG AND HAMSTER IN UTOX AND T/ CYTOCHROME E-450. (O2-+450) IN THE
GUINEA PIG INTRANASAL TEST SYSTEM./RESPIRATORY ALLERGY: SUBCHRONIC
GUINEA PIG MODEL OF HALOTHANE HEPATITIS/HANE INDUCED IMMUNE RESPONSE IN A
GUINEA PIG MODEL OF HALOTHANE-ASSOCIATE/PHENOBARBITAL PRETREATMENT ON THE
GUINEA PIGS - STRUCTURE-ACTIVITY RELATI-/-DIOXINS AND RELATED COMPOUNDS IN
GUINEA PIGS AND HAMSTERS/IVY AND ANTIOXIDANT EFFECTS IN TCDD TREATED
GUINEA PIGS AND THREE STRAINS OF RATS/SPIRATORY SYSTEM UPTAKE OF OZONE IN
GUINEA PIGS BY INHALATION EXPOSURE TO TOLUENE DIISOC/ THE SENSITIZATION OF
GUINEA PIGS EXPOSED TO A COMBINATION OF ZNO-SO2/ THE PULMONARY RESPONSE OF
GUINEA PIGS EXPOSED TO TWO ISOTOPES/COMPAREN OF FLOW-VOLUME LOOPS IN
GUINEA PIGS IMMUNIZED BY INJECTING A Mixture OF HDI OR DES-N ACTIVATOR PU
GUINEA PIGS TO THE INHALATION OF PLATELET ACTIVATING FACTOR ((/RESPONSE OF
GUINEA PIGS UPON EXPOSURE TO SULFUR OXIDES ON THE SURFACE OF /RESPONSE OF
GUINEA PIGS WITH DNB AND BENZOCAIN/ NAL PERTINITION STUDY IN HAIRLESS
GUINEA PIGS./..ERMA AND RESPIRATORY RESPONSE TO INHALATION OF ENDOTOXIN IN
GUINEA PIGS./..IONIZING RADIATION INDUCES HYPOTERMIA IN
GUAM./STUDY OF THE TERATOGENIC POTENTIAL OF GUAR
GUTHION BY MOUSE LIVERS PERFUSED IN SITU/OLIC ACTIVATION OF THE PESTICIDE

H2O2 GENERATION BY FATTY ACIDS; EFFECT OF CHAIN LENGTH/STIMULATION OF
HAIRLESS GUINEA PIGS WITH DNCB AND BENZOCAIN/DERMAL SENSITIZATION STUDY IN
HALOCETONITRILES ACUTE TOXICITY: ESTABLISHES/STUDIES ON THE MECHANISM OF
HALOCETONITRILES ACUTE TOXICITY: INTERACTION/STUDIES ON THE MECHANISM OF
HALOALKANES ADMINISTERED BY GAVAGE/T OF VEHICLE ON THE RELATIVE UPTAKE OF
HALOSTYNYL-L-Cysteines/NU/IODIONS IN THE FORMATION AND REACTIONS OF S-(2-
HALOGENATED ACETALDEHYDES.-/A-PROTEIN CROSS-LINKING IN RAT LIVER NUCLE1 BY
HALOGENATED ACETONITRILES ON THE MIXED FUNCTION OXIDASES AN THE EFFECT OF
HALOTHANE EXPOSED RABBITS./...H OF HALOTHANE-INDUCED ANTIGEN EXPRESSION IN
HALOTHANE/CHARACTERIZATION OF ANTIBODIES PRODUCED IN HUMAN
HALOTHANE HEPATITIS./..CHARACTERIZATION OF ANTIBODIES PRODUCED IN HUMAN
HALOTHANE HEPATITIS/HANE INDUCED IMMUNE RESPONSE IN A GUINEA PIG MODEL OF
HALOTHANE-INDUCED IMMUNE RESPONSE IN A GUINEA PIG MODEL OF HALOTHANE HEPA
HALOTHANE-ASSOCIATED HEPATIC NECROSIS./EATMENT ON THE GUINEA PIG MODEL OF
HALOTHANE-INDUCED ANTIGEN EXPRESSION IN HALOTHANE EXPOSED R/CHROMATOLOGY OF
HAMSTER IN RESPONSE TO TCDD TREATMENT/-450 (cP-450) IN THE GUINEA PIG AND
HAMSTER CENTRAL CORTEX IRREVERSIBLE BINDING OF DIETHYLSIS-LBESTROL IN
HAMSTER./...ERENCES IN THE METABOLISM OF BENZO(a)PYRENE (BP) BY THE RAT AND
HAMSTER/PHEN (AAP) OR ALLYL ALCOHOL (AOH) HEPATOTOXICITY (HT) IN THE MALE
HAMSTERS ADMINISTERED 3H-2,3,7,8-TETRACHLOROMETHYL/.../CHROMOGRAM OF THE A ReCEPTOR IN
HAMSTERS TREATED WITH 2,3,7,8-TETRACHLOROETHANE AND THYROID GLUCORONIDATION IN
HAMSTERS/IVY AND ANTIOXIDANT EFFECTS IN TCDD TREATED GUINEA PIGS AND
HAMSTERS/N ACETAMINOPHEN- OR ALLYL ALCOHOL-INDUCED HEPATOTOXICITY IN MALE
HARDERIAN GLAND OF MOUSE./...USE OF PROLACTIN ON THE PORPHYRIN OF THE
HARMOL (HA): DOSE-DEPENDENT GLUCORONIDATION AND /INTESTINAL METABOLISM OF
Hazard RATINGS/ANOTHER APPROACH TO TOXIC
HCB) ALTERS THE DYNAMICS OF VITAMIN A / 3,4,5,3'-4'-5'-HEXACHLOROBIPHENYL
HC8) AND 2,3,7,8-TETRACHLOROBENZOP-4',4',5'-HEXACHLOROBIPHENYL
HC8) PORPHYRIA: STUDIES IN CONGENIC C5/RECEPTOR IN HEXACHLOROBENZENE
HC8) TREATED MICE./...PRESSOR CELLS IN 3,4,5,3',4',5'-HEXACHLOROBIPHENYL
HCBD) IN B6CF1 MICE FOLLOWING DIETARY /IES OF HEXACHLORO-1,3-BUTADIENE
HCG/50, 17-HYDROXYLASE AND 17,20-lysE BY HUMAN CHORIONIC GONADOTROPIN
HCND) IN THE NASAL MUCOSA OF F-344 RATS/UND [3H]- AND [14C]FORMALDEHYDE
HCND) AND BENZO(A)PYRENE (BAP)-INDUCED DNA DAMAGE AND REP/FORMALDEHYDE
HD, AND DMHD ON NEURAL AND NON-NEURAL NADH-TR ACTIVITY IN/EFFECTS OF ACR,
HD OR DES-N ACTIVATOR EXHIBIT PULMONA/Pigs IMMUNIZED BY INJECTION WITH
HEALTH EFFECTS ASSESSMENT METHODOLOGY FOR AIRBORNE CONTAMINANTS./CHEMICAL
HEALTH EFFECTS REVIEWS: MELON BROMIDE, CHLORINATED STYRENES, BROMINATED
HEALTH EVALUATION OF MERCURY CONTAMINATED SPORTFISH/HUMAN
HEALTH HAZARD ASSESSMENT BASED ON INTEST/FIED METHOD FOR PRELIMINARY HUMAN
HEART RATE IN RATS/SURE TO COMPLEX ORGANIC MIXTURES ON BLOOD PRESSURE AND
HEART UPON REOXYGENATION BY CHOLESTEROL/ELEASE FROM ISOLATED PERFUSED RAT
HEMATOLOGIC-BIOCHEMICAL EVALUATIONS IN A HETERO/G THE STATISTICAL POWER OF
HEMATOLOGIC AND HISTOPATHOLOGICAL EFFECTS/YL LEAD. PART 1. BIOCHEMICAL,
HEMATOPOTIC SYSTEM OF RATS AND MICE./..ARINE: ALTERATIONS IN THE
HEM OR LEAD NITRATE IN DIFFERENT RAT /CAL CHANGES IN RESPONSE TO COBALT-
HEMY, CYTOCHROME P-450, 17-HYDROXYLASE /THASONE-SUPPRESSED RAT TESTICULAR
HEMOLOGIC ANEMIA AND CYTOPENIAS ASSOCIATED WITH ETA/LACT/IMMUNE-MEDIATED
HEM/INDUCED BY DI-NBUTYL-2,2-DICHLOROVINYL PHOSPHATE (DCV) IN THE ADULT
HENS FOR ORGANOPHOSPHATE-INDUCED DELAY/3-WK EVALUATION IN LAYING CHICKEN
HENS/...H METHYL-PHOSPHONATE (MMP) NEUROTOXICITY IN
HENS/...LING AND DECREASED PHOSPHORYLATION OF NEUROfilaMENT PROTEINS IN
HENS/...METYL METHYLPHOSPHONATE (MMMP) NEUROTOXICITY IN
HENS/S/...ANAPHASE DELAYED NEUROPATHY (OPIDN) IN ADULT
HEPATECTOMIZED RATS INDUCED BY PHENOLIC ANT/MACH CARCINOMAS IN PARTIALLY-
HEPATECTOMIZED RATS/CHLORODECON INDUCED CC14 HEPATOTOXICITY IN PARTIALLY-

305
HEPATECTOMY/ION OF CHLORODEONE POTENTIATED CC14 HEPATOTOXICITY BY PARTIAL
HEPATECTOMY/OTECTION OF HEPATOTOXIC AND LETHAL EFFECTS OF CC14 BY PARTIAL
HEPATIC ACTIVATION OF COOKED-FOOD MUTAGENS IN THE RA/DIETARY FAT MODIFIES
HEPATIC AND EXTRAHEPATIC MICROSOMAL MONOOXYGENATION OF PHORATE.
HEPATIC AND CANCER METABOLISM OF MTH/DEFECTS OF GENETIC DAMAGE IN MICE
HEPATIC CAMP-DEPENDENT PROTEIN KINASE (CAMP-PK) ISOENZYME (MODULATION OF
HEPATIC CANALICULAR MEMBRANE VESELLES (C/AUROCHOLATE (TC) EFFLUX FROM RAT
HEPATIC CELLS/METABOLISM OF METHOTREXATE IN
HEPATIC CLEARANCE FOR THE FORMATION OF THE CH/A METHOD FOR ESTIMATING THE
HEPATIC CYTOCHROME P-450 ISOSYPE RESPONSIBLE/CHARACTERIZATION OF THE DOG
HEPATIC CYTOCHROME P-450 ISOSYPE-P-450, CHLOROPHORPHOLINE ACTIVITY.
HEPATIC DNA DAMAGE IS NOT POSITIVELY CORRELATED WITH GLUTATHIONE (GSH) CO
HEPATIC DNA PRODUCED BY TRICHLOROACETIC ACID (TCA), CHLORAL (CH/Breaks IN
HEPATIC DRUG-METABOLIZING ENZYMES/FECT ON Picrotoxin-INDUCED SEIZURES AND
HEPATIC ENZYMATIC ACTIVITY OF C/N AMMAY/ MAMMARY TUMOR/HETEROLOGOUS TUMORS IN
HEPATIC ENZYMES TO MUTAGENIC PRODUCTS IN/O/N OF BENZENE CONSUMERS BY RAT
HEPATIC ESTROGEN RECEPTOR LEVELS IN RATs/E DOWN REGULATION OF UTERINE AND
HEPATIC FIBROSIS IN THE RATS/ULATION DURING CARBON TETRACHLORIDE-INDUCED
HEPATIC FIBROSIS IN THE RATS/ULATION DURING CARBON TETRACHLORIDE-INDUCED
HEPATIC FIBROSIS IN THE RATS/ULATION DURING CARBON TETRACHLORIDE-INDUCED
HEPATIC GLUTATHIONE s-TRANSFERASES/DEFLUORINATION BY
HEPATIC GLUTATHIONE s-TRANSFERASE ACTIVITY/ECT OF TRIMETHYLNIT (TMN) ON RAT
HEPATIC INDUCERS ON TESTICULAR XENOBIOTIC METABOLISM IN RODENT/EFFECTS OF
HEPATIC ISO-METALLOTHIONEINS (MTs) IN IMMATURE /ONTOGENY AND INDUCTION OF
HEPATIC LIPID PEROXIDATION AND GLUTATHIO/ AND TCDD-INDUCED ALTERATIONS IN
HEPATIC LIPID PEROXIDATION AND GLUTATHIO/ AND TCDD-INDUCED ALTERATIONS IN
HEPATIC METALLOTHIONEINS (MTs) LEVELS s/OF SULPHUR-DEFICIENT DIETS ON
HEPATIC METALLOTHIONEIN-I (MT-1) AND METALLOTHIONEIN-II (MT/REGULATION OF
HEPATIC MICROXOMA ALDEHYDE DEHYDROGENASE/ATION PRODUCTS BY PURIFIED RAT
HEPATIC MICROXOMA ALDEHYDE DEHYDROGENASE/ATION PRODUCTS BY PURIFIED RAT
HEPATIC MICROXOMA MIXED FUNCTION OXIDAS/NALDEHYDE (2-BUTENAL) OF THE RAT
HEPATIC MICROXOMA PROTEINS OF C57BL/6 N/DIOXYGENPHENYL (MDP) COMPOUNDS ON
HEPATIC MICROXOMA FROM PHENOBARBITAL TR/METABOLITES WITH PHOSPHOLIPID OF
HEPATIC MITOCHONDRIAL RESPIRATION/TAMINOPHEN (APAP)-INDUCED INHIBITION OF
HEPATIC MITOCHONDRIA ACTIVITY OF ACUTE ABSURDITY ALTERNATIVE OR
HEPATIC MIXED FUNCTION OXIDASE ACTIVITI/OF IMMUNE REACTION IN DEPRESSING
HEPATIC MONOXOGENASE INDUCERS ON LEVELS OF mRNA IN/THAT EFFECT OF VARIOUS
HEPATIC MONOXOGENASE SYSTEM BY 2,3,7,8-TCDD/2,3,7,8-TCDD/2,3,7,8-TCDD/2,3,7,8-TCDD
HEPATIC NACROSIS./EATMENT ON THE GUINEA PIG MODEL OF HEPATOTOXICITY ESPECIALLY
HEPATIC NUCLEOSIDE/ATION IN NORMAL LIVERS AND CHEMICALLY-INDUCED
HEPATIC OXGEN UPTAKE IN THE ISOLATED PB/E AND IRREVERSIBLE INHIBITION OF
HEPATIC OXGEN UPTAKE IN THE ISOLATED PB/E AND IRREVERSIBLE INHIBITION OF
HEPATIC PALMITATE OXIDATION IN VITRO IN / ACID (PFPD)-INDUCED INCREASE IN
HEPATIC PEROXISOME INDUCTION/L PHTHALATE (DNP) IN F-344 RATS: EFFECTS ON
HEPATIC PEROXISOME PROLIFERATION IN MALE B6C3F1 MICE FOLLOW-INDUCTION OF
HEPATIC PLASMA MEMBRANES/NDING PROPERTIES OF SINUSOIDAL AND CANCILLARIAL
HEPATIC TRANSFORMATION OF COOKED-FOOD MUTAGENS/DIETARY QUERCETIN MODIFIES
HEPATIC TUMOR EXPRESSION IN DIETHYLMITRO/BARBITAL (PB) ADMINISTRATION ON
HEPATIC TUMOR PROMOTION BY DIETARY FAT IN B6C3F1 MALE MICE.
HEPATIC UDP/GLUCURONIC ACID (UDP-GA) DEPLETION ON THE DISPOSITI/EFFECT-INDUCED
HEPATIC UNSCHEDULED DNA SYNTHESIS (UDS) A/EXAMINATION OF THE INDUCTION OF
HEPATIC VITAMIN A DEPLETION./ETYARY TRIODOOTHRONEIN (T3) ON TCDD-INDUCED
HEPATICITIS/.CHARACTERIZATION OF ANTIBODIES PRODUCED IN HUMAN HEPATITIS
HEPATICITIS/HANE INDUCED IMMUNE RESPONSE IN A GUINEA PIG MODEL OF HEPATITIS
HEPATOBLINARY FUNCTION IN RATS FOLLOWING SUB-CHRONIC EXPOSURE TO AMIODARONE
HEPATOCARCINOGENESIS AND ON THE EPIDEMIC CLINICAL RE/S (CBOA) ON INITIATION OF
HEPATOCARCINOGENESIS./D 3H-ETHYNYLSTRADEOL IN THE RAT TWO-STAGE MODEL OF
HEPATOCELLULAR CALCIUM HOMEOSTASIS IN FEMALE/ONINE AND CCL4 INTERACTION ON
HEPATOCELLULAR GLUTATHIONE (GSH) BY EXOGENOUSLY ADDED NAPHTH/DEPLETION OF
HEPATOCELLULAR NECROSIS AND BIOCHEMICAL CHANGES IN RESPONSE/COMPARISION OF
HEPATOCELLULAR TOXICITY BY GALACTOSAMINE/ ASSOCIATED WITH PROTECTION FROM
HEPATOCTYE ASSAYS./IVATIVES IN THE SALMONELLA TYPHIMURIUM, CHO/HPRT, AND
HEPATOCTYE CULTURE/YLHYDROXYMIC ACIDS OF 4-NITROSOBIPHENYL IN PRIMARY RAT
HEPATOCTYE CULTURE/YLHYDROXYMIC ACIDS OF 4-NITROSOBIPHENYL IN PRIMARY RAT
HEPATOCTYE FUNCTION IN VIVO AND IN VITRO/E OF CHLORGONATED HYDROCARBONS ON
HEPATOCTYE INTERCELLULAR COMMUNICATION BY/PREVENT THE INHIBITION OF MOUSE
HEPATOCTYE MODEL FOR THE INVESTIGATION OF INTRACELL/DEVELOPMENT OF A FISH
HEPATOCTYE PHOSPHOLIPID IN VIVO AND IN/SFORMATION IN THE ACTIVATION OF
HEPATOCTYE PRIMARY CULTURE (HPC)/DNA REPAIR TEST./VALIDATION OF THE HUMAN
HEPATOCTYES (IRH)/17G) AND 3H-TAUCOLATE (TC) INTO ISOLATED MALE RAT
HEPATOCTYES (R)/XYACIL COA DEHYDROGENASE (EC) mRNA BY MT-1,4,643 IN RAT
HEPATOCTYES AFTER INCUBATION WITH PEROXISOMAL PEROXIDATION (LP) IN PRIMARY
HEPATOCEYES AND A COMPARISON OF METABOLISM/NE METABOLISM IN HUMAN AND RAT
HEPATOCEYES AND S-9/FRICHLOREHYLENE METABOLISM IN HUMAN AND RAT
HEPATOCEYES BY A TETRAZOLE-SUBSTITUENT ALK/ACID OXIDATION IN ISOLATED RAT
HEPATOCEYES BY A PEPTIDAL GROWTH FACTOR (EGF/THESIS IN PRIMARY CULTURES OF
HEPATOCEYES BY NONCOETOXIC ENVIRONMENTAL /SIS IN PRIMARY CULTURES OF RAT
HEPATOCEYES BY PHTHALATE MONOESTERS/EXPRESSION IN PRIMARY CULTURES OF RAT
HEPATOCEYES BY THE PEROXISOME PROLIFERATOR/SCHEDULED DNA SYNTHESIS IN RAT
HEPATOCEYES EXPOSED TO INDOMETHACIN./MORPHOMETRIC ANALYSIS OF CULTURED
HEPATOCEYES EXPOSED TO TAMOXIFEN, DIPHENYLPHOSPHATASE (AP) FROM CULTURED
HEPATOCEYES FOLLOWING IN VIVO EXPOSURE TO /ACK OF REPAIR SYNTHESIS IN RAT
HEPATOCEYES FOLLOWING CHEMICAL FCNATE SUCINATE TREATMENTS
HEPATOCEYES FROM RAT, CYMOLUS MONKEY AND /OR ENZYMES IN PRIMARY CULTURES
HEPATOCEYES IN RESPONSE TO DIETHYLTHIOTRISAM/LY IDENTIFICATION OF RESISTANT
HEPATOCEYES ISOLATED FROM MOUSE RATS/HANISMS OF TOXICITY OF COCAINE IN
HEPATOCEYES MAINTAINS CYTOCHROME P-450 LEVELS IN PRIMARY CULTURES OF RAT
HEPATOCEYES OBTAINED FROM CHLORACIDE(CD)/C14 HEPATOXICITY IN ISOLATED
HEPATOCEYES TREATED WITH POLYHALOGENATED A/UL AND CULTURED CHICK EMBRYOS
HEPATOCEYES USING THE NEUTRAL RED DYE ASSA/CYTOTOXICITY TEST FOR CULTURED
HEPATOCEYES./CITY OF CDC12 AND CD-METALLOTHIONEIN (CD-MT) IN ISOLATED RAT
HEPATOCEYES./MECHANISM OF SKF 101772-INDUCED CYTOTOXICITY TO ISOLATED RAT
HEPATOCEYES./IDE DEPLETION AND PHOSPHORYLASE A-ACTIVATION IN ISOLATED RAT
HEPATOCEYES./OTIEN AND DNA SYNTHESIS IN PRIMARY CULTURES OF POSTNATAL RAT
HEPATOCEYES/ BY CCL4 EXPOSURE IN RAT LIVER AND IN PRIMARY CULTURES OF RAT
HEPATOCEYES/ ON THE INDUCTION OF P450 BY 3-METHYLCHOLANTHRENE IN CULTURED
HEPATOCEYES/CRYOPRESERVATION OF RODENT AND PRIMATE
HEPATOCEYES/ND OXYGEN CONCENTRATION ON MERCANE TOXICITY IN ISOLATED RAT
HEPATOCEYES/ A SYSTEM FOR SHOWING THE EFFECT OF ACETYLAMINOLEURNE BY INTACT
HEPATOCEYES: ROLE OF CYTOCHROME P-450 IN D/TY OF 4- AZASTEROIDS TOWARD RAT
HEPATOCEANS OF TWO MID-ATLANTIC BIVALVE /ND ANTIOXIDANT ENZYMES IN THE
HEPATOPOCARCINOGENESIS IN RATS BY SIMULTANEOUS ADMINISTRATOR/ENHANCEMENT OF
HEPATORENAL TOXICITY IN HYPERTENSIVE RATS./DIFFERENCES IN STYRENE-INDUCED
HEPATOGENE TOXICITY OF STYRENE OXIDE DUE TO REPEATED EXPOSURE/STUDIES ON
HEPATOGENE TOXICITY OF STYRENE OXIDE IN R/ OF DOSE ON THE METABOLISM AND
HEPATOGENE AND LETHAL EFFECTS OF CC14 BY PARTIAL HEPATECTO/PROTECTION OF
HEPATOGENE EFFECTS OF ACROLEIN IN MALE SPRAUGE-DAWLEY RATS
HEPATOGENE RESPONSE TO ACUTE COCAINE ADMINISTRATION IN MALE AND FEMALE M
HEPATOTOXICITY (HT) IN THE MALE HAMSTER/PHEN (AAP) OR ALLYL ALCOHOL (AOH)
HEPATOGENE AND METABOLISM IN RATS./CARBAMAZEPINE AND STYRENE ON THEIR
HEPATOGENE BY BUTYLATED HYDOXYTOLEUENE /ATION OF RETINYL ACETATE (RA)
HEPATOGENE BY CYANAMIDE/POTENTIATION OF ALLYL-ALCOHOL-INDUCED
HEPATOGENE BY KETONES/ OF THE CC14 DOSEAGE ON THE POTENTIATION OF CC14
HEPATOGENE BY PARTIAL HEPATECTOMY/ION OF CHLORACIDE POTENTIATED CC14
HEPATOGENE EFFECTS OF ALLOXAN IN ISOLATED HEPATOCEYES OBTAINED FROM CHLORACIDE(CD)/CC14
HEPATOGENE IN ISOLATED HEPATOCEYES OBTAINED FROM CHLORACIDE(CD)/CC14
HEPATOGENE IN MALE HAMSTERS/ACETAMINOPHEN- OR ALLYL-ALCOHOL-INDUCED
HEPATOGENE IN MICE: CORRELATIONS WITH RAPID EFFECTS ON CALCIUM U/CC14
HEPATOGENE IN PARLIALY HEPATECTOMIZED RATS/CHLOROACIDE INDUCED CC14
HEPATOGENE IN RATS BY ENDOCRINE STATUS./ULATION OF ACETAMINOPHEN (AA)
HEPATOGENE IN RATS./XGINOSUCINATE LYASE): A SENSITIVE INDICATOR OF
HEPATOGENE OF A NOVEL ANINEOPLASTIC AGENT, SKP IN VIVO AND IN VITRO
HEPATOGENE /ACTIVITY OF ACETAMINOPHEN (ACETAMINOPHEN) IN THE BROWN BULLHEADED CATFISH (ACETAMINOPHEN)
HEPATOGENE OF APLATOXIN B1 (APB1) IN TH/OLITIPRAZ PROTECTS AGAINST THE
HEPATOGENE OF CARBON TETRACHLORIDE (CC13/URAL DOING VEHICLES ON ACUTE
HEPATOGENE OF CC14 AND TRICHLOROETHYLENE: ANY ROLE FOR EN/INTERACTIVE
HEPATOGENE OF COMPLEX WASTE MIXTURES./LETHALITY AND
HEPATOGENE OF PYRAZOLE IN MICE./SEX AND STRAIN SPECIFICITY IN
HEPATOGENE OF THE ISOMERS OF DICHLORO/METABOLISM IN THE DIFFERENTIAL
HEPATOGENE OFFERED BY AN E1 AND AN E2 P/OTECTION AGAINST ANIT INDUCED
HEPATOGENE - SENSITIVE INDEX OF THE REVERSAL OF ACETAMINOPHEN-INDUCED
HEPATOGENE/ST TO OTHER INDICES OF CARBON TETRACHLORIDE (CC14)-INDUCED
HEPATOGENE/ETRICHLOROETHYLENE METABOLISM AND
HEPATOGENE/TINED ELECTROPHILE PRODUCTION DURING ACETAMINOPHEN (APAP)
HEPATOGENE PRODUCED BY BLUE-GREEN ALGA/AMINO ACID FROM A CYCLIC PEPTIDE
HEPATOGENE./VITAMIN A POTENTIATION OF CARBON TETRACHLORIDE
HEPATOGENE/ACETOL CONTAMINATED LIVESTOCK/DECONTAMINATION STRATEGIES FOR
HERBICIDE GHOSPALATE./MUTAGENICITY STUDIES WITH THE
HERBICIDE HANCE/S /MENT AND VALIDATION OF AN ELISA FOR THE THIOCARBATE
HERBICIDE PEROXISOME PROLIFERATING PROTEIN REGULATORY PROTEIN (PPR), (2,2,2-TRICHLORO-ETHYL)OXIRANE), A
HERBICIDE SC-1084 IN THE CHICKEN./METABOLISM OF THE PYRIDE/PHENYL ETHER
HERBICIDE, IN RATS AND RABBITS/ALUATIONS OF AMETRYN TECHNICAL, A TRIAZINE
HERBICIDE./ IN RHESUS MONKEYS FOLLOWING TOPICAL ADMINISTRATION OF MACHETE
HERBICIDE/CIES DIFFERENCES IN MALE REPRODUCTIVE TOXICITY OF A PYRROLIDINE
HERBICIDES IN THE RAT/COMPARATIVE DISPOSITION OF A SERIES OF
HERBICIDES./CES IN STIMULATION OF MICROSMAL NADPH OXIDATION BY BIPYRIDYL
HERBICIDES SIMILARITIES EXIST BETWEEN FACTOR-9 OF THE BLADDER CARCINOGENIC RESPONSE TO
HETEROCYCULAR ANALOGS OF PHENCYCLIDINE (PCP/HIBITION OF CYTOCHROME P450 BY
HETEROPNEUSTES FOSSILIS (BLOCH)./ATE ON FEEDING ENERGETICS IN THE CATFISH
HEXABROMOBIPHENYL IN WB CELLS./CELLULAR COMMUNICATION BY 2,2',4,4',5,5'-  
HEXABROMOBIPHENYL/WB-P344) CELLS BY HEPATIC TUMOR PROMOTER 2,2',4,4',5,5'-  
HEXACHLORO-1,3-BUTADIENE (HCBD) IN B6C3F1 MICE P/IC TOXICOLOGY STUDIES OF  
HEXACHLOROBIPHENYL (HCB) POISONING OF LAB RATS IN/RODENT CONSUMPTION IN  
HEXACHLOROBENZENE ON CALCIUM HOMEOSTASIS AND BONE IN FISHER 3/F EFFECTS OF  
HEXACHLOROBIPHENYL (6-CB) FROM LACTATING MICE/MINATON OF 2,4,5,2',4',5'-  
HEXACHLOROBIPHENYL (HCB) ALTERS THE DYNAMICS /C EXPOSURE TO 3,4,5,3',4'5'-  
HEXACHLOROBIPHENYL (HCB) AND 2,3,7,8-TETRACHL/STRATION OF 2,4,5,2',4',5'-  
HEXACHLOROBIPHENYL (HCB) TREATED MICE./UPPRESSOR CELLS IN 3,4,5,3',4'5'-  
HEXACHLOROBIPHENYL IN YOUNG AND ADULT RATS./OKINETICS OF 2,4,5,2',4',5'-  
HEXACHLOROBIPHENYL./YME RESPONSIBLE FOR THE METABOLISM OF 2,4,5,2',4',5'-  
HEXACHLORODIBENZO-P-DIOXIN (2,3,7,8-TCDD) IN PERSIST IN RABBIT CORNEAL SLEEVES./ICICITY OF CYP2A5 IN THE TREATMENT OF 2,3,7,8-TCDD AND  
HEXACHLOROCYCLOHEXANE (beta-HCH) IN THE B6C3F1 /OLOGICAL EFFECTS OF BETA-  
HEXACHLORODIBENZOFURAN DECREASE GLUCOCORTICOID /NIFURAN AND 1,2,3,4,7,8-  
HEXACHLOROBIPHENYL ETHYL IN THE RAT/SUBACUTE TOXICITY OF 2,2',3,4,4',6-  
HEXAMETHYlene DIAMINE./SUPPRESSION OF LYMPHOCYTE PROLIFERATION BY  
HExANE AND METHYL ISO-BUTYKETONE (MIBK) PRO/SIMULTANEOUS EXPOSURE TO N-  
HExANE-DIONE (HD), AND A23187 PRODUCE DPP/LHEXYL)-PHTHALATE (MEHP), 2,5-  
HExANEDIOL./IN VITRO AND IN VIVO GENOTOXICITY STUDIES OF 2-ETHYL-1,1-  
HExAxENIDEON (2,5-HD) EXPOSED RATS/TIONS IN OPTIC TRACT (OT) AXONS IN 2,5-  
HExAXENIDONE AND PERDEUTERIO-2,5-HEXANEDION ROLE-FORMING POTENTIAL OF 2,5-  
HExAXENIDONE CAUSES IRREVERSIBLE TESTICULAR GERM CELL LOSS AT NEUROTO/2,5-  
HExAXENIDONE IN THE RAT/ POTENTIAL OF 2,5-HEXANEDION AND PERDEUTERIO-2,5-  
HExAXENIDONE ON MITONCHONDRIAL RESPIRATION/EFFECOtS OF ACRYLAMIDE AND 2,5-  
HEXENAL AND TELANDE MHis SPECTROMETRIC DE/STRIUTION OF TRANS-4-HYDROXY-2-  
HEXOBARBITAL IN PRIMARY CULTURES OF RAT HEPATOCYTES MAINTAINS/PRESENCE OF  
HG IN MEHGE- EXPOSED MOUSE KIDNEY./C DISTRIBUTION OF ORGANIC VS INORGANIC 30  
HG IN THE HOMO DISTRIBUTION OF FE, CU, DISTRICT IN A CULTURED RAT  
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)/ASAL MUCOSA OF F-344 RATS BY  
HIPPURIC ACID/L MALEATE ON GLUTATHIONE METABOLISM IN RATS PRETREATED WITH  
HISTOCHEMICAL LOCALIZATION OF THALLIUM INDUCED LIPID PEROXIDATION IN THE  
HISTOCHEMICAL EVALUATION OF THE EFFECTS OF DIETARY TRIDIOOTHYRONINE (T3) AND  
HISTOLOGICAL PULMONARY EFFECTS OF GALLIUM ARSENIDE COMPAR/BIOCHEMICAL AND  
HISTONE PHOSPHORYLATION DURING DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEU  
HISTOPATHOLOGICAL ALTERATIONS FOLLOWING DENTAL ADMINISTRATION OF CAPTAN TO  
HISTOPATHOLOGICAL CHANGES IN RATS EXPOSED TO CARBON MONOXIDE  
HISTOPATHOLOGICAL EFFECTS/YL LEAD. PART 1. BIOCHEMICAL, HEMATOLOGICAL AND  
HISTOPATHOLOGICAL EVALUATION OF THE REPRODUCTIVE STATUS OF SELENIUM-EXPO  
HISTOPATHOLOGY AND LAVAGE CYTOLOGY/BIOCHEM/IONS IN RAT RESPIRATORY TRACT  
HISTOPATHOLOGY/EFFECTS OF INHALED NOSEGONE ON PULMONARY BIOCHEMISTRY AND  
HLC-60 CELLS INDUCED BY A PHORBAL DIESTE/N OF HUMAN PROMYELOCYTIC LEUKEMIA  
HOC1 OR CI02 WITH PATTY ACIDS UNDER AQUEOUS CONDITION/REACTIONS INVOLVING  
HOMAPUS AMERICANUS/SULFADIMETHOXINE DISPOSITION IN THE LOBSTER.  
HOMOSTASIS AND BONE IN FISHER 344 RATS/OF HEXACHLOROBENZENE ON CALCIUM  
HOMESTASIS IN FEMALE RATS./ND CCL4 INTERACTION ON HEPATOCELLULAR CALCIUM  
HORMONAL RESPONSIVENESS OF RAT SERTOLI C/HYHELIX)-PHTHALATE (MEHP) ON THE  
HORMONAL STATUS IN 2,3,7,8-TETRACHLOROBENZOP-DIOXIN (TCDD)-TREATED RAT  
HORMONE IN RHEUS MONKEYS./IMMUNOGENICITY OF BIOSYNTHETIC HUMAN GROWTH  
HORMONE SECRETION./DIRECT EFFECT OF HEAVY METAL IONS ON PITUITARY  
HORMONES AND THYROXINE GLUCRONIDATION IN HAMSTERS TREATED WITH 2/THYROID  
HORSE'S POISONED BY INGESTION OF BILSTER/CAPILLARY GC/MS IN SPECIMENS FROM  
HOST RESISTANCE AND IMMUNITY IN MICE EXPOSED TO ALDICARB./EVALUATION OF  
HOST RESISTANCE/POSTEXPOSURAL INSTILLATION OF GALACTOSURUBIAE OF  
HOST RESISTANCE TO PLASMODIUM YOELII AND P. /EFFECTS OF BENZO(A)PYRENE ON  
HOST RESISTANCE/COMBINED INJURY INDUCED PERTURBATIONS IN NON-SPECIFIC  
HOSUE FLY (MUSCA DOMESTICA)/F-450 FROM AN INSECTICIDE RESISTANT STRAIN OF  
HPLC ANALYSIS OF 32P-POSTLABELED DNA-2-AMINOPHENOLINE ADDUCTS.  
HPLC ASSAY FOR FOUR HYDROXYLATION ENZYMES TOWARDS APLAT/DEVELOPMENT OF AN  
HPLC)/L MUCOSA OF F-344 RATS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (  
HRS) BY DELTA-9-TETRAHYDRO-CANNABINOL/RESPONSE TO HERPES SIMPLEX VIRUS 2 (  
HRS INDUCED CYTOPATHOLOGY (ARI) AN/ECT OF CYCLOSPORIN A (Csa) ON  
HUMAN AND RAT ALVEOLAR EPITHELIAL CELLS./NICKEL TOXICITY IN  
HUMAN AND RAT HEPATOCYTES AND A COMPARISON OF BENZO(A)PYRENE METABOLISM IN  
HUMAN AND RAT HEPATOCYTES AND S-9/TRICHLOROETHYLENE METABOLISM IN  
HUMAN AND RAT LAVAGE FLUID BIOCHEMISTRY/T OF ACUTE OZONE (03) EXPOSURE ON  
HUMAN AND RAT LIVER/ACETYLATION/DEACETYLATION (AC/DC) OF ARYL AMINES IN  
HUMAN AND RAT METABOLISM AND MUTAGENESIS OF 2-ACETYLAMINOFUORENE BY INTA  
HUMAN AND RODENT AN RECEPTOR./PHYSICOCHEMICAL PROPERTIES OF THE  
HUMAN ABDOMINAL EPITHELIAL CELLS/PHENOTYPIC CHANGES (UDS) IN-VENTROGERMINAL  
HUMAN BY HYPOLIPIDEMIC DRUGS/ HEPATOCYTES FROM RAT, CYMOMOLUS MONKEY AND  
HUMAN CHORIONIC GONADOTROPIN (HCG)/450, 17-HYDROXYLASE AND 17,20-LYASE BY  
HUMAN CUTANEOUS EPIDERMIS, IN VITRO/GENERATION OF A  
HUMAN ERYTHROCYTES (RBG) IN VITRO/UPTAKE OF INORGANIC MERCURY (HG) BY  
HUMAN EXPOSURE ASSESSMENTS FOR LAUNOY PRODUCTS  
HUMAN GM-CSF IN RHEUS MONKEYS/IMMUNOGENICITY OF CHO CELL-DERIVED I DNA  
HUMAN GROWTH HORMONE IN RHEUS MONKEYS./IMMUNOGENICITY OF BIOSYNTHETIC
IN VITRO STUDIES OF HYDROCARBON-INDUCED MALE RAT NEPHROPATHY
IN VITRO STUDIES WITH MOUSE SKIN IN O/CUTANEOUS FATE OF TOPICAL STEROIDS:
IN VITRO STUDY ON CARBON TETRACHLORIDE AND METALLOTHIONEIN INTERACTION
IN VITRO SYSTEM FOR PROLONGED INCUBATION OF IS/DEVELOPMENT OF AN IMPROVED
IN VITRO TETRAGON SCREEN/CHICK EMBRYO RETINA CELL CULTURE AS AN
IN VITRO TEST BATTERY/ OF 14F REFERENCE CIGARETTE SMOKE CONDENSATE IN AN
IN VITRO TO IN VITRO/LAL CELL CAMID TOXICITY DATA FOR EXTRAPOLATION FROM
IN VITRO TOXICITY OF A BONE REPLACEMENT MA/THE EFFECTS OF SOLVENTS ON THE
IN VITRO TOXICITY OF CEPHALORIDINE AND HEXACHLOROBUTADIENE IN RABBIT CORT
IN VITRO.ION OF COOKED-FOOD MUTAGENS TO BACTERIAL GENES/COLONIC VIVO AND
IN VITRO../OVALENT BINDING OF 14C-BROMOBENZENEO METABOLITES TO LIVER SLICES
IN VITRO./PER OXIDES TO RAT, MOUSE AND DOG PULMONARY ALVEOLAR MACROPHAGES
IN VITRO./ROXIDE (O2) RELEASE FROM STIMULATED, RAT PERITONEAL NEUTROPHILS
IN VITRO./THE EFFECT OF PARAOXON ON MURINE T AND B CELL PROLIFERATION
IN VITRO./THE MURINE ANTIBODY RESPONSE IN VIVO AND MITOGEN RESPONSIVENESS
IN VITRO./TO INHIBIT CELL-CELL COMMUNICATION: MECHANISM OF IMMUNOTOXICITY
IN VITRO/HELICOTHERMIA OF CHEMICALS TO ELICIT OPEN NEURAL TUBES (ONT) IN RAT EMBRYOS
IN VITRO/CORTICAL SLICE SYSTEM FOR ASSESSMENT OF CHEMICAL-INDUCED INJURY
IN VITRO/ OF ME AND MA ON RAT SERTOLI-GERM CELLS CO-CULTURE DNA SYNTHESIS
IN VITRO/ALATE (MEHP) ON THE HORMONAL RESPONSIVENESS OF RAT SERTOLI CELLS
IN VITRO/DEVELOPMENTAL TOXICITY OF INDUSTRIAL N-NITROSO COMPOUNDS
IN VITRO/E OF CHLORINATED HYDROCARBONS ON HEPATOCYTE FUNCTION IN VIVO AND
IN VITRO/EFFECTS OF METHYLEMERCURY ON NEURONAL CYTOSKELETON
IN VITRO/GENERATION OF A HUMAN CUTANEOUS EPIDERMIS,
IN VITRO/TOXICITY OF SIX OXIME COMPOUNDS IN BACTERIA AND MAMMALIAN CELLS
IN VITRO/RESISTANCE OF THE MURINE 12, 14 RB HEPATIC LIVERCELLS TO CEPHALORIDINE TOXICITY
IN VITRO/ION OF THE ACTIVATION OF HEPATOCYTE PHOSPHOLIPASE C (PLC) IN RAT LIVER AND
IN VITO/TMT ON RAT HEPATIC GLUTATHIONE TRANSFERASE ACTIVITY IN VIVO AND
IN VITRO/UPAKTUE OF INORGANIC MERCURY (HG) BY HUMAN ERYTHROCYTES (HBC)
IN VIVO AND MITOGEN RESPONSIVENESS IN/XIN ON THE MURINE ANTIBODY RESPONSE
IN VIVO FOR THE INDUCTION OF CARCINOIDS AND SMALL CELL CANCER/A NOVEL
IN VITRO NO2 EXPOSURE/G CELLS FROM NITROGEN DIOXIDE (NO2)-EXPOSED RATS TO
IN VITRO SYSTEM TO STUDY THE EFFECTS OF LOW LEVELS OF LEAD ON T/USE OF AN
IN VITRO TEST FOR ASSESSING THE SKIN COR/NTRA-LABORATORY VALIDATION OF AN
IN ACTIVATION OF CYTOCHROME P-450 ISOGENES BY ANALOGS OF C/METABOLISM-BASED
IN ACTIVATION OF FOUR RAT LIVER MICROSOMAL ANDROSTENEDIONOGENE HYDRO/SELECTIVE
INACTIVATION MEDIUM ON THE CRYPTOFESSION/AGING RATES, CRYPTOAUTOTRANT AND
INDOLE: A NOVEL MECHANISM./IFICATION OF XENOBIOTIC METABOLISM BY DIETARY
INDOMETHACIN.-MORPHOMETRIC ANALYSIS OF CULTURED HEPATOCYTES EXPOSED TO
INDUCE PHENOBARBITAL-INDUCIBLE CYTOCHROMES AZ ASSOCIATED WITH ITS ABILITY TO
INDUCERS AND INHIBITORS OF P-450-DEPENDENT/BOW TROUGHT, SALMO GARDNERI, WITH
INCREASED INHIBITORS (4-ACETAMIDINOPHEN (AA): EFFECT OF CYTOCHROME P-450
INDUCERS OF CYTOCHROME P450A./INHIBITION OF 2-AMINOFUROANON MUTAGENESIS BY
INDUCERS ON LEVELS OF mRNA IN RAINBOW TR/R OF VARIOUS HEPATIC MONO-OXGENASE
INDUCERS ON TESTICULAR XENOBIOTIC METABOLISM IN RODENT/EFFECTS OF HEPATIC
INDUCTION AND GLUTATHIONE DEPLETION IN TH/TO THE ROLE OF CYTOCHROME P-450
INDUCTION AND THYMUS ATROPHY/C57BL/6J AND INDB2 J3 MICE - EFFECTS ON ENZYME
INDUCTION IN HEPATOCYTES FROM RAT, CYNO/OLOGY STUDIES OF PEROXISOMAL ENZYME
INDUCTION OF CARCINOIDS AND SMALL CELL CANCER/A NOVEL IN VIVO MODEL FOR THE
INDUCTION OF CYTOCHROMES P-450 BY XENOBIOTICS/MECHANISM OF
INDUCTION OF DRUG METABOLIZING ENZYMES BY CHLORTIMAZOL/DOSE DIFFERENTIATED
INDUCTION OF HEPATIC PEROXISOME PROLIFERATION IN MALE BEAGLE MICE/VARIOUS
INDUCTION OF HEPATIC UNSCHEDULED DNA SYNTHESIS (UDS) A/EXAMINATION OF THE
INDUCTION OF IL-2 RECEPTOR EXPRESSION BY DMBA DETECTED USIN/INHIBITION OF
INDUCTION OF MICROSONAL CHOLESTEROL EPIDOXONE HYDROLASE BY CHLORDFRATE
INDUCTION OF OXYGEN TOLERANCE IN MICE
INDUCTION OF P450 BY 3-METHYLCORONANTHRENE/7-ALPHA-EHYXYLESTRADIOL ON THE
INDUCTION OF SUPPRESSOR CELLS IN 3, 5, 5' / TOTOCIC T CELL ACTIVITY WITHOUT
INDUCTION OF THE HEPATIC MONOXGENASE SYSTEM BY 2, 3, 3', 8-TETRACLOROBENZI
INDUCTION./,8-TETRACLOROBENZENO-90-DIOXIN (TCDD) ANTAGONIST IN RATS - AMH
INDUCTION/L PHTHALATE (DINP) IN F-344 RATS: EFFECTS ON HEPATIC PEROXISOME
INDUSTRIAL CHEMICALS/SHORT-TERM TOXICITY OF 9
INDUSTRIAL COMPOUNDS IN THE MOUSE AND GUI/E SENSITISING POTENTIAL OF SOME
INDUSTRIAL N-NITROSO COMPOUNDS IN VITRO/DEVELOPMENTAL TOXICITY OF SOME
INERT PESTICIDE INGREDIENTS/PERSPECTIVES ON TOXICITY OF
INFLUENTE WILLAM MONKEYS./EFFECT OF VOMITOXIN (DEOXYVINALENOL) ON
INHIBITION OF DIOXIN (TCDD) ON BEHAVIORAL PERCEPTUAL BEHAVIOR OF RHESUS MONKEY
INFECTIONS ON THE DEVELOPMENT OR SURVIVAL OF B6C3F1 M/INFLUENCE OF VITAL
INFLAMMATION MEASURED BY BRONCHOALVEOLAR L/O CADMIUM IN RATS AND MICE CAUSED
INFLAMMATORY AND FIBROTIC POTENTIAL OF SYNTH/A MURINE MODEL TO ASSESS THE
INFLAMMATORY POTENTIAL OF SURGICAL IMPLANTS/A NEW MODEL TO STUDY THE
INFLAMMATORY RESPONSE INDUCED BY EXPOSURE TO GL/CHRONOGRAPHY OF A PULMONARY
INFLAMMATORY RESPONSES TO INHALED CARBONYL/F BALO ALVEOLAR CLEARANCE AND
INFLUENZA IN MICE/EFFECT OF O3 EXPOSURE ON SUSCEPTIBILITY TO

311
INHALED PHOSGENE ON PULMONARY BIOCHEMISTRY AND HISTOPATHOLOGY/EFFECTS OF 48
INHALED POLYVINYLCHLORIDE (PVC) SMOKE IN NON/ACUTE RESPIRATORY EFFECTS OF 114
INHALED SUBMICRON CARBON BLACK PARTICLES/RETENTION AND CLEARANCE OF 39
INHIBIT MYELOPOIESIS IN FIBROBLASTOID ST/AL CELLS EXPOSED TO HYDROQUINONE 210
INHIBIT THE NEURON PERIKARYAL RESPONSE TO ANATOMY./VINCA ALKALOIDS 193
INHIBITION AND REACTIVATION OF MAMMALIAN BRAIN ACETYLCHOLINES/KINETICS OF 265
INHIBITION BY BROMOTHOROL/PROTECTION OF CADMIUM TOXICITY AND ENZYME 191
INHIBITION BY THREE OP‘S/PERIPHERAL AND CENTRAL ENZYME 191
INHIBITION IN BLOOD AND BRAIN REGIONS./ TOXICITY AND CHOLINESTERASE (CHE) 129
INHIBITION OF 2-AMINOFUORENE MUTAGENESIS BY INDUCERS OF CYTOCHROME P450a 861
INHIBITION OF AMINOPYRINE METABOLISM IN THE ISOLA/CARBON MONOXIDE-INDUCED 431
INHIBITION OF BENZO(A)PYRENE SKIN TUMOR INITIATING ACTIVITY BY COMPLEX OR 251
INHIBITION OF CADMIUM-INDUCED CHANGES IN SPERMATOZOA AL CO/DITHIOCARBAMATE 571
INHIBITION OF CARNITINE PALMITOYL TRANSFERASE I IN RAT LIVER MITOCHONDRIA 251
INHIBITION OF CYTOCHROME P450 BY HETEROCYCLIC ANALOGS OF /MECHANISM-BASED 657
INHIBITION OF CYTOXIC CELL ACTIVITY WITHOUT INDUCTION OF CYTOKINES BY MULTIPLE-AMINOACID/METALLOTHIONEIN (MT) INFLUENCES 271
INHIBITION OF ELECTRIC EEL ACETYLCHOLINES/D COMPETITIVE INHIBITORS ON THE 1104
INHIBITION OF ENZYME RELEASE FROM ISOLATED PERFUSED RAT HEART UPON ROXOGY 372
INHIBITION OF FATTY ACID OXIDATION IN ISOLATED RAT HEPATOCYTES BY A TETRA 252
INHIBITION OF FC-MEDIATED PHAGOCYTOSIS IN PULMONARY ALVEOLAR MACROPHAGES 52
INHIBITION OF GAMMA-Glutamyl TRANSFERASE /ONE (GSH) DEPLETION ELICITED BY 724
INHIBITION OF HEPATIC AND ENDOHYDROXYNE DEIODINASE BY IODINATED ISOMERS 951
INHIBITION OF HEPATIC MITOCHONDRIAL RESPIR/ON ACETAMINOPHEN (APAP)-INDUCED 458
INHIBITION OF HEPATIC OXIDATION OF THE IS/REVERSIBLE INHIBITION AND IMMUNOLOG. 458
INHIBITION OF INDUCTION OF IL-2 RECEPTOR EXPRESSION BY DMBA DETECTED USIN 103
INHIBITION OF INTERCELLULAR COMMUNICATION OF THE 'FRAP' ASSAY TO MEASURE 653
INHIBITION OF LIPOAMIDO DEHYDROGENASE (LpDH) AND CYTOCHOR/NEURON-SPECIFIC 527
INHIBITION OF METABOLIC COOPERATION IN RAT EPITHELIAL (WB/-DOSE-DEPENDENT 305
INHIBITION OF MG2+-ATP-DEPENDENT CALCIUM TRANSPORT IN ISOLATED RAT L/LEAD 536
INHIBITION OF MOUSE HEPATOCYTE INTERCELLULAR/ CYCLIC AMP AGONISTS PREVENT THE 276
INHIBITION OF NEUROTOXIC ESTERASE BY TFP AN/CORRELATION BETWEEN PROLONGED 276
INHIBITION OF PROSTAGLANDIN H SYNTHASE-MEDIATED COXIDATION /MECHANISM OF 1104
INHIBITION OF RAT LIVER MICROSONAL CYTOCHROME P450c IN VIVO 145
INHIBITION OF SICEROXIDE ANION RADICAL PRODUCTION IN STIMULATED PHAGOCYTI 592
INHIBITION OF THE BLASTOGENIC RESPONSE TO HERPES SIMPLEX VIRUS 2(HSV2) BY 99
INHIBITION OF YEAST GLUCOSE-6-PHOSPHATE DEHYDROGENASE./VANADIUM 330
INHIBITION/HOSPHATE INDUCED PERSISTANT BRAIN ACETYLCHOLINES/ERASE (ACHE) 681
INHIBITOR BY EXPOXIDE AND 1,2-DIHALOTHAN/ION OF PLASMA alphal-PROTEINASE 681
INHIBITORS OF EPIDERMAL GROWTH FACTOR (EG/OACTIVATED PSORALENS ARE POTENT 276
INHIBITORS OF NERVE TERMINAL Ca2+ REGULATION WITH METHYM/INTERACTIONS OF 676
INHIBITORS OF P-450-DEPENDENT MONOXIGE/MO GAIRDNERI, WITH INDUCERS AND 1104
INHIBITION ON THE INHIBITION OF ELECTRIC /S OF SUBSTRATES AND COMPETITIVE 463
INHIBITORS./ACETAMINOPHEN (AA): EFFECT OF CYTOCHROME P-450 INDUCERS AND 1104
INHIBITORY ACTION OF GOSSYPOL ON SOLUBLE F1 ATPase FROM VERTEBRATE AND IN 99
INITIATING ACTIVITY BY COMPLEX ORGANIC MI/ON OF BENZ0(A)PYRENE SKIN TUMOR 431
INITIATION/OXTERD CARCINOGENESIS AND ON/CHLORINATED BIPHENYLS (PCBs) ON 39
INITIATION/RESISTANT HEPATOCYTES IN RESPONSE TO DIETHYLNITROSAMINE (DEN) 954
INJECTIONS OF A NOVEL ENKEPHALINE ANALOG./ IN THE RABBIT FOLLOWING SINGLE 15
INJURIES TO ORGANIC Hg IN HZO/- EXPOSURE TO HEPATOCYTE, DAP, EPIDERMAL 893
INSECTICIDE ACTIVATION BY RAT BLOOD MONOXYGENS./PHOSPHOROTHIONATE 61
INSECTICIDE RESISTANT STRAIN OF HOUSE FLY /MS OF CYTOCHROME P-450 FROM AN 1104
INSECTICIDE TOXICITY./NEUROPSYCHOLOGICAL EVALUATION OF CYCLODINE 99
INSECTICIDES IN Xenopus laevis/TERATOGENIC EFFECTS OF ORGANOPHOSPHORUS 699
INSTILLATION OF GALLIUM ARSENIDE (GaAs)/RESISTANCE FOLLOWING INTRATRACHEAL 898
INSTRUCTION FOR NON-TOXICOLOGISTS./DING CHEMICAL EXPOSURE: COMPUTER-BASED 165
INSULIN RECEPTOR BINDING TO ENDOTHELIAL /ES SURFACE MEMBRANE FLUIDITY AND 15
INTERACTION OF BENZENE METABOLITES REPRODUCES THE MYELOTOXICITY OBSERV/AN 90
INTERACTION OF METHACRYLONITRILE WITH GLUTATHIONE./IN VIVO AN IN-VITRO 193
INTERACTIONS IN EPIDERMIS EFFECTS AND NATUR/NICKEL- MAGNesium 160
INTERACTIONS INVOLVING MULTIPLE TARGET ORGANS/EVALUATION OF TOXIC 1049
INTERACTIONS OF INHIBITORS OF NERVE TERMINAL Ca2+ REGULATION WITH METHYM 376
INTERACTIONS OF MONENSIN AND OUJABAIN ON AN ISOLATED SENSORY NEURON/THE 165
INTERCELLULAR COMMUNICATION BY 2,2',4,4',5,5'/SSAY TO MEASURE INHIBITION OF 653
INTERFERON GAMMA IN THE RHEUSUS MONKEY AFTER I/INETICS OF RECOMBINANT HUMAN 937
INTERLEUKIN-2 IN RODENTS./SERVATIONS ON THE TOXICITY OF RECOMBINANT HUMAN 100
INTERMEDIATE DURING THE REDUCTION OF 5-RIT/ON OF A REACTIVE HYDROXYLAMINE 431
INTERMITTENT USE OF TOXICOLOGICAL METHODS IN THE USE OF BACKGROUND DATA 143
INTERSTITIAL CELLS TO CADMIUM/RENTAL RESPONSE OF PRIMARY RAT SERTOLI AND 6
INTESTINAL METABOLISMS OF HARMOL (HA): DOSE-DEPENDENT GLUCURONIDATION AND 474
INTESTINAL MICROFLORA./ABOLISHM OF 1,-3- AND 6-NITROBENZO(A)-PYRENE BY RAT 280
INTESTINES./TRANSPORT ACROSS BRUSH BORDER MEMBRANE VESICLES FROM RAT SMALL 563
INTRACELLULAR GLUTATHIONE (GSH) STATUS AND THE ABILITY OF CHEMICALS TO EL 304
INTRACELLULAR LEAD-CALCIUM RELATIONSHIPS 600
INTRACELLULAR METAL REGULATION/ HEPATOCYTE MODEL FOR THE INVESTIGATION OF 937
INTRAMUSCULAR AND SUBCUTANEOUS ADMINISTRATION/SUS MONKEY AFTER INTRAVENOUS, 313
KERATIN EXPRESSION ARE INDUCED BY POTENT/INSTINCTIVE PATTERNS OF EPIDERMAL
KERATIN MONOCLONAL ANTIBODY USED TO PROBE FOR PERTURBATION IN THE D/ANTI-
KERATINOCYTES AS A MODEL SYSTEM FOR THE STUDY OF IRRIT/CULTURED MAMMALIAN
KERATINOCYTES TO BIS-(B-CHLOROSTYLL) SULFID/SS INDUCED BY EXPOSURE OF RAT
KETAMINE PROFUNDELY ALTERS RAT FLASH EVOKED POTENTIALS.
KETONE (MIBK) PRODUCED CROSSLINKING AND TO N-HEXANE AND METHYL ISO-2UTYL
987 KETONE IN SPRAGUE DAWLEY RATS/SUBCUTANEOUS TOXICITY OF METHYL ISOBUTYR
980 KETONES/ OF THE CC14 DOSAGE ON THE POTENTIATION OF CC14 HEPATOTOXICITY BY
981 KEVLAR ARAMID FIBERS IN RATS/DEPOSITION & CLEARANCE OF INHALED
983 KIDNEY AND LIVER CELLS TO CEPHALORIDINE TOX/COMPARATIVE SUSCEPTIBILITY OF
984 KIDNEY AND LIVER/EFFECTS OF CHLOROMETHATE (DCM) ON
985 KIDNEY CELLS (LLC-PK1) INDUCED INCREASES IN CYTOSOLIC CALCIUM [Ca2+]i IN PIG
986 KIDNEY INFLUENCE OF INFLUENCE OF HIGH DIOXANE CONCENTRATIONS AND TRACE ELEMENTS IN RAT
987 KIDNEY II. INFLUENCE OF HIGH DIOXANE CONCENTRATIONS AND TRACE ELEMENTS IN RAT
988 KIDNEY IN MALE P344 RATS INITIATED BY N/AL (Na-Ba) ON URINARY BLADDER AND
989 KIDNEY SYNTHESIZES LESS METALLOTHIONEIN (MT) THAN LIVER IN RESPONSE TO CCl4
990 KIDNEY TISSUE FOLLOWING ADMINISTRATION /LOCALIZATION OF RADIOIODINE IN RABBIT
991 KIDNEY/ C DISTRIBUTION OF ORGANIC VS INORGANIC HG IN MEHCG - EXPOSED MOUSE
992 KIDNEY/ YES OF METAL ACTION ON PROXIMAL AMINO ACID REABSORPTION BY RABBIT
993 KIDNEY/ HYALIN DROPLETS AND ALPHA-2U-GLOBULIN ACCUMULATED IN THE MALE RAT
994 KIDNEY/ACUTE TOXIC EFFECTS OF PARAQUAT ON THE RABBIT
995 KIDNEY/AR WEIGHT NON-METALLOTHIONEIN LEAD-BINDING PROTEIN (PbBP) FROM RAT
996 KIDNEY/FECTS THE BASAL LAMINA AND EXTRACELLULAR MATRIX OF THE FETAL MOUSE
997 KILLER CELL ACTIVITY BY OCTROXIN(A/ DEPRESSION OF NATURAL
998 KILLER CELL ACTIVITY/EFFECT OF OZONE ON PULMONARY NATURAL
999 KILLER CELL ACTIVITY/EFFECT OF PHOSGENE ON LUNG NATURAL

100 KILLER CELLS INVOLVEMENT/IONS IN CARCINOGENESIS: DOSE EFFECTS AND NATURAL
101 KINASE (CAMP-PK) ISOENZYME (12Z) ACTIV/OF HEPATIC CAMP-DEPENDENT PROTEIN
102 KINASE ACTIVITY. A NEW METHOD FOR THE MEASUREMENT OF HEPATIC APs
103 KINASE ACTIVITY II ACTIVITY IN BRAIN CYTOSOL OF CHICKENS TREATED/ALTERED CALMODULIN
104 KINDLING FOLLOWING TREATMENT WITH FORMAMID/MED SUSCEPTIBILITY TO AMYGDALOID
105 KINETIC AND MECHANISMS OF ISOYCYANIDE BINDING TO PURIFIED RAT LIVER C/TH
106 KINETIC BUT SLIGHTLY ALTERED CYTOCHROME/YL HYDROCARBON HYDROXYLASE (ASH)
107 KINETIC IN RATS EXPOSED TO SUB-LETHAL CONCENTRATION/ELIMINATION AND ELIMINATION
108 KINETIC IN VIVO/ F HYPEROXIA ON TUMOR CELL ANTIOXIDANT DEFENSES AND CELL
109 KINETIC OF ELEMENTAL MERCURY OXIDATION BY A SUSPENSION OF WASHED ERTHRO
110 KINETICS IN INHALED MC12 AEROSOLS: INFLUENCE OF CONCENTRATION
111 KINETICS OF INHIBITION AND REACTIVATION OF MAMMALIAN BRAIN ACETYLCHOLINES
112 KINETICS OF ORGANOPHOSPHATES, STEROIDS, CAFFEINE/PERCUTANEOUS ABSORPTION
113 KINETICS OF THE A2 RECEPTOR IN HAMSTERS ADMINISTRED 3H-2,3,7,8/LONG-TERM
114 KINETICS OF THE PULMONARY CLEARANCE OF COBALT IN RATS
115 KIRSTEN-RAS (KI-RA) ONCogene IN THE LIVER OF THE MALE B6C3/STATUS OF THE
116
117
118 LI210 TUMOR MITOCHONDRIA/INDEED DAMAGE OF MITOCHONDRIA DNA FROM MOUSE AND
119 L5178Y CELLS,. S OF FORMYCYN AND PILAZEP ON GROWTH ARREST AND RECOVERY OF
120 LABELED DNA/ITY BY DIMETHYLAMINOPHENOL - DISTRIBUTIONAL STUDIES WITH 14C-
121 LAC-ANTIBODY ADMINISTRATION/MIA AND CYTOPENIAS ASSOCIATED WITH beta-
122 LACTATE AND GLUCOSE METABOLISM/THIRLACETATE EFFECTS ON
123 LACTATE AND PYRUVATE PRODUCTION AS SPECIFIC INDICES OF ALTERED SERTOLI CE
124 LACTATING MICE/IMMINATION OF 2,4,5,2',4',5'-HEXACHLOROBIPHENYL (6-CB) FROM
125 LACTATING RATS/D EFFECTS OF DEHP ON MILK COMPOSITION AND MAMMARY GLAND IN
126 LACTATIONAL TRANSFER OF TRICHLOROETHYLENE (TCE) AND ITS METABOLIT/MODELING
127 LARKSPUR ALKALOIDS DETECTED BY ALKALINE /EMOTOXICITY OF PYRROLIZIDINE AND
128 LAUNDRY PRODUCTS/HUMAN EXPOSURE ASSESSMENTS FOR
129 LAVAGE CYTOLOGY/BIOCHEMISTRY FOLLOWING/PRIARY TRACT HISTOPATHOLOGY AND
130 LAVAGE FLUID BIOCHEMISTRY/T OF ACUTE OZONE (O3) EXPOSURE ON HUMAN AND RAT
131 LAVAGE FLUIDS FOLLOWING THORACIC X-IRRA/R AND BIOCHEMICAL CHANGES IN LUNG
132 LAVAGE/MIUM STIMULATES PULMONARY INFLAMMATION MEASURED BY BRONCHOALVEOLAR
133 LC50S/D OF SUDDEN INHALATION EXPOSURE BY EXTRAPOLATION OF TLV'S AND ACUTE
134 LEACHATE IMMUNOSUPPRESSION/LOVE CANAL
135 LEAD (Pb)/S INHIBITION OF DELTA-AMINOLEVULINIC ACID DEHYDRATASE (ALAD) BY
136 LEAD (TMBP) IN THE RAT/NEUROPATHOLOGY THYMETHY
137 LEAD ACETATE/TOXICITY OF MIXTURES OF CC14, MONOCHLOROBENZENE, AND
138 LEAD AND DIET ON TRACE ELEMENT CONTENT OF RAT SERUM/THE EFFECTS OF
139 LEAD AND ZINC CONCENTRATIONS IN HUMANS: COMP/EFFECTS OF EDTA CHELATION ON
139 LEAD BEHAVIORAL TOXICITY IN THE MONKEY/SENSITIVE PERIODS FOR
140 LEAD CONTAMINATED SOIL/RISK ASSESSMENT FOR
141 LEAD EXPOSURE ON A SPATIAL DISCRIMINATION TASK IN AD/EFFECTS OF LOW-LEVEL
142 LEAD FROM PRETREATED RAT DAMS./IN UTERO MOBILIZATION OF
143

315
LEAD IN INTACT LEGS BY L X-RAY FLUORES-/INVASIVE DETECTION OF Tibial BONE
LEAD IN THE PLASMA OF HIGH RISK POPULA/MACKEKNIC PREDICTIONS OF UNSOUND
LEAD INCLUSION DEVELOPMENT IN MOUSE Ti/INTRAVENOUS ADMINISTRATION MODE FOR
LEAD INHIBITION OF MG2+/ATP-DEPENDENT CALCIUM TRANSPORT IN ISOLATED RAT L
LEAD LEVEL IN PLUMBERS USING PLASMA ATOMIC/DIRECT DETERMINATION OF BLOOD
LEAD NITRATE IN DIFFERENT RAT STRAINS/NGES IN RESPONSE TO CORALIS-HEME OR
LEAD NITRATE TREATMENT IN MOUSE LIVER INDUCE BIOCHEMICAL PATTERNS SI/DOES
LEAD ON SEIZURE SPREAD AND MORTALITY/EFFECT OF
LEAD ON THE BLOOD-BRAIN BARRIER/TEM TO STUDY THE EFFECTS OF LOW LEVELS OF
LEAD ON THE DEVELOPING OTOCYST IN CULTURE./EFFECT OF
LEAD OVER THE COURSE OF DMSA CHELATION THERAPY./MOBILIZATION OF
LEAD-BINDING PROTEIN (PbBP) FROM RAT KIDNE/LAR WTNESS NON-METALLOTHIOINEIN
LEAD-CALCIUM RELATIONSHIPS/INTRACELLULAR
LEAD-INDUCED ALTERATIONS IN ENZYME ACTIVITY OF ISOLATED RAT RETINA
LEAD-INDUCED ALTERATIONS OF RENAL GENE EXPRESSION WITHIN SUBCELLULAR COMP
LEAD. PART 1. BIOCHEMICAL, HEMATOLOGICAL AND/SUBCHRONIC TOXICITY OF ALKYL
LEAD. PART 2. MICROSCOPIC AND ULTRASTRUCTURAL/SUBCHRONIC TOXICITY OF ALKYL
LEAD. AGING ALTERS THE TISSUE DISTRIBUTION OF
LEAD/ DOPAMINERGIC PRESYNAPTIC RECEPTOR FUNCTION IN RAT CNS AFTER CHRONIC
LEAD/ERYTHROCYTE MEMBRANE FLUIDITY AS RELATED TO BLOOD
LEARNING BEHAVIOR IN WATER-FILLED MULTIPLE/NEW STATISTICAL MODELING FOR
LEUKEMIA CELL DIFFERENTIATION ALTER GLYC/ INDUCERS OF HUMAN PROMYELOCYTIC
LEUKEMIA HL-60 CELLS INDUCED BY A PHORBO/ENTiation OF HUMAN PROMYELOCYTIC
LEUKOCYTE-MEDIATED LIPID PEROXIDATION AND LUNG INJURY./POLYMORPHONUCLEAR
LEUKOTRIENES IN PULMONARY OXYGEN TOXICITY.
LEWISlie WITH OR WITHOUT BRITISH ANTI-LE/G SUBCUTANEOUS ADMINISTRATION OF
LH AND TESTOSTERONE/UCED BY SAH 59-801 IN THE DOG: DECREASE IN SERUM FSH,
LIMONEN AND DECALONON ON MALE FERTILITY/EFFECTS OF d-
LIMONEN IN MALE FISCHER-344 RATS./THRESHOLD FOR THE NEPHROTOXICITY OF D-
LINDANE EFFECT ON Picrotoxin-INDUCED SEIZURES/TEMPORAL RELATIONSHIP BETWEEN
LINDANE IN THE RAT./THE EFFECT OF DOSE AND AGE ON THE METABOLISM OF
LINKING BY GRAVITY-PLOW ALKALINE ELUTION./DETECTION OF DNA CROSS-
LIPID FLUIDITY AND PHAGOCYTOSIS OF RAT/UNSATURATED ALDEHYDES ON MEMBRANE
LIPID FLUIDITY, AND MEMBRANE SULPHHYDRYL PRODUCTION, PHAGOCYTOSIS MEMBRANE
LIPID METABOLISM IN THE RAT/PEROXECANCIC ACID (PPOA) AND
LIPID PEROXIDATION (LP) IN PRIMARY HEPATOCYTES AFTER INCUBATION WITH PERO
LIPID PEROXIDATION AND CELLULAR DAMAGE CAUSED BY THE PYRROLIZIDINE ALKALO
LIPID PEROXIDATION AND GLUTATHIONE CONT/INDUCED ALTERATIONS IN HEPATIC
LIPID PEROXIDATION AND LUNG INJURY./POLYMORPHONUCLEAR LEUKOCYTE-MEDIATED
LIPID PEROXIDATION BY 3,3'-DICHLOROBENZENIDE PRET/ENHANCEMENT OF HEPATIC
LIPID PEROXIDATION IN MICE/ATION AND AGE ON LIVER ENZYME ACTIVITIES AND
LIPID PEROXIDATION IN MTPP-INDUCED CYTOSOME ROLES FOR OXIDATIVE STRESS AND
LIPID PEROXIDATION IN THE BRAIN AND COR/ LOCALIZATION OF THALLIUM INDUCED
LIPID PEROXIDATION IN TRICHLORETHYLENE-INDUCED NEPHROTOXICITY/ROLE OF
LIPID PEROXIDATION PRODUCTS BY PURIFIED RAT HEPATIC/OXIDATION OF ALDEHYDE
LIPID PEROXIDATION/ ON 2,3,7,8-TETRAChLOROdIBENZO-P-DIOXIN (TCDD) INDUCED
LIPID PEROXIDATION/Y OF CC14 AND TRICHLORETHYLENE: ANY ROLE FOR ENHANCED
LIPIDS./CYANIDE AND PEROXIDATION OF BRAIN
LIPOAMIDE DEHYDROGENASE (LpDH) AND CYTCHRON/NEURON-SPECIFIC INHIBITION OF
LIPOIC ACID (LA) ON BILARY EXCRETION OF METALS./PARADOXICAL EFFECT OF
LIPOSOMAL MEMBRANE/HEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION OF
LIPOSOMES. /E OF NO2 PEROXIDATION OF POLYSATURATED FATTY ACIDS (PUFA) IN
LIQUID CHROMATOGRAPHY (HPLC)/AL MUCOSA OF P-344 RATS BY HIGH-PERFORMANCE
LIVER AND BRAIN PIG MICROSOHS/A COMPARISON BETWEEN
LIVER AND IN PRIMARY CULTURES OF RAT HE/ PRODUCED BY CCL4 EXPOSURE IN RAT
LIVER AND PLACENTAL CYTOSOL./ASE GLUCOCORTICOID RECEPTOR BINDING IN MOUSE
LIVER BY ACETYLMONOP/EPATIC OXYGEN UPTAKE IN THE ISOLATED PERFUSED RAT
LIVER CELL CULTURES FROM DRUG-INDUCED INJURY BY FLAVON/PROTECTION OF RAT
LIVER CELLS TO CEPHALORidine TOXICITY I/TIVE SUSCEPTIBILITY OF KIDNEY AND
LIVER CYTOCHROME P-450./ THE MAJOR PHENOBARBITAL-INDUCIBLE ISOMYZE OF DOG
LIVER CYTOCHROME P-450/D MECHANISMS OF ISOCYANIDE BINDING TO PURIFIED RAT
LIVER ENZYME ACTIVITIES AND LIPID PEROX/DIETARY RESTRICTION AND AGE ON
LIVER HYPERTROPHY./50 (P-450P-B), AMINOPYRINE N-DEMETHYLASE ACTIVITY AND
LIVER IN MEDIATING THE ACUTE TOXICITY OF METHYL PARATHION/THE ROLE OF THE
LIVER IN RESPONSES TO cdc1 AND C3-METAL/SES LESS METAL TOXICITY INDUCED INHIBITION OF
LIVER INDUCE BIOMARKER PATTERNS SIMI/S LEAD NITRATE TREATMENT IN MOUSE
LIVER MEMBRANES./ OF MG2+/ATP-DEPENDENT CALCIUM TRANSPORT IN ISOLATED RAT
LIVER MICROSOmal AND MITOCHONDRIAL ALDE/2-NONENAL (T-4HN) BY PURIFIED RAT
LIVER MICROSOmal AND ANDROSTENEDIONE HYDROX/ELECTIVE INACTIVATION OF FOUR RAT
LIVER MICROSOmal CYTOCHROME P-450/LATION BY THE PCN-INDUCIBLE FORM OF RAB
LIVER MICROSOmal CYTOCHROME P-450b WITHOUT THE USE OF/PURIFICATION OF RAB
LIVER MICROSOmal CYTOCHROME P-450d AND UDP-GlCURONOSY/REGULATION OF RAB
LIVER MICROSOmal CYTOCHROME P-450a IN VIVO/INHIBITION OF RAB
LIVER MICROSOmes USING TANDEM MASS SPEC/E ALKALOID METABOLITES FROM MOUSE
LIVER MICROSOmes/LATION OF N-NITROGuDIMETHYLAMINE (NDMA) CATALYZED BY PIG

316
LUNGS/E MECHANISM OF THIOL UTILIZATION BY SMOKE CHALLENGED RAT AND RABBIT
LUNGS/OMBOXANE IN PHORBOL MYRISTATE ACETATE-INDUCED EDEMA IN ISOLATED RAT
LYASE BY HUMAN CHORIONIC GONADOTROPIN (/P-450, 17-HYDROXYLASE AND 17,20-
LYASE TO OTHER INDICES OF CARBON TETRAC/MPARISON OF ARGININOSUCINIC ACID
LYASE: A SENSITIVE INDICATOR OF HEPATOTOX/SEUM ASAL (ARGININOSUCINATE
LYMPHOCYTE INFIltrATION OF MOUSE LUNG FOLLOWING EXPOSURE TO OZONE/T-
LYMPHOCYTE NUMBERS AND SUBPOPULATIONS FOLLOW/I ALTERNATIONS IN MEDIA STAIN
LYMPHOCYTE PROLIFERATION BY HEXAMETHYLENE DIAMINE/ SUPPRESSION OF
LYMPHOCYTE RESPONSE/SEGNE EXPOSURE ON THE INFLUENZA PULMONARY CYTOTOXIC T
LYMPHOCYTES AS A MODEL FOR SULFUR MUSTARD TOXICITY./HUMAN

LYMPHOCYTES FROM MONKEYS EXPOSED TO DIESEL/ AND CHROMOSOME ABERRATIONS IN
LYMPHOCYTES.//TOGEN RESPONSIVENESS AND ANTIBODY PRODUCING ABILITY OF HUMAN
LYMPHOCYTE POPULATIONS FOLLOWING ACUTE ADM/ ANALYSIS OF THYMIC AND SPLENIC
LYMPHOCYTE POPULATIONS/DROUGHT-INDUCED ALTERATION OF STROMAL CELL DEPENDENT B-
LYSINE PRODUCTION BY PERITONEAL CELLS: RATION OF DISEASE INFLUENCE BETWEEN DNA AND
LYSOPHOSPHATIDYLCHOLINE IN TYPE II CELLS AND MAC/C FATE OF 1-PALMITOL-
LYSOSOMAL FUNCTION/EFFECT OF TRICYANOHEXANE ON RENAL

MACHETE HERBICIDE./ IN RUSHE MONKEYS FOLLOWING TOPICAL ADMINISTRATION OF
MACROCYCLIC TRICHOTHECENES./TOXICOLOGICAL STUDIES OF A NEW CLASS OF
MACRODANTIN(R) IN MICE/ONCOCENICITY STUDY OF
MACRODANTIN(R) IN RATS/TWO YEAR ONCOCENICITY STUDY OF
MACROPHAGE MELOPOIESIS: ALTERATION IN TH/SAMINE (DN) INDUCED CHANGES IN
MACROPHAGE SUPEROXIDE ANION RADICAL PRODUCTION INHABITS RABBIT ALVEOLAR
MACROPHAGE VIABILITY AND FUNCTION/T OF ARAMID AND METAPHOSPHATE FIBERS ON
MACROPHAGE-LIKE STROMAL CELLS EXPOSED TO HYDROQUINONE INHIBIT MELOPOIESI
MACROPHAGES (AM)/SUPEROXIDE ANION (O2- ) PRODUCTION IN GUINEA PIG ALVEOLAR
MACROPHAGES PROLIFERATION AND NUCLEAR CHANGES IN MACROPHAGES OF PULMONARY ALVEOLAR
MACROPHAGES AFTER EXPOSURE TO CARBON BLACK/CHLOROGEN IN TYPE II CELLS AND
MACROPHAGES IN VITRO./PER OXIDES TO RAT, MOUSE AND DOG PULMONARY ALVEOLAR
MACROPHAGES STIMULATED BY TUMOR PROMOTERS/ID RELEASE BY MURINE PERITONEAL
MACROPHAGES TO RELEASE NEUTROPHIL AND MONO/ONE STIMULATES RABBIT ALVEOLAR
MACROPHAGES/OZONE INHALATION DECREASES CYTOCHROME b245 IN RABBIT ALVEOLAR
MACROPHAGES/S ON MEMBRANE LIPID FLUIDITY AND PHAGOCYTOSIS OF RABBIT ALVEOLAR
MAGNESIUM AGAINST NICKEL-INDUCED DNA DAMAGE AND CELL/PROTECTIVE EFFECT OF
MAGNESIUM INTERACTIONS IN CANCERGENESIS: DOSE EFFECTS AND NATURE/NICKEL -
MAGNETIC RESONANCE./IMAGING MANGANESE IN THE PRIMATE BRAIN WITH
MALE RAT KIDNEY/ HYALIN DROPLETS AND alpha-2u-GLOBULIN ACCUMULATED IN THE
MALE REPRODUCTIVE TOXICITY OF 2-METHOXYETHANOL APPLIED DERMALLY TO OCCLUD
MALE REPRODUCTIVE TOXICITY OF A PYRROLIDINE HERBIC/IPARTS DIFFERENCES IN
MALE WASTE WORKERS/REPRODUCTIVE ASSESSMENT OF
MALE BATS ON GLUTATHIONE METABOLISM IN RATS PRETREATED/INFLUENCE OF DIETHYL
MALEIC ACIDHYDE, METHYL METHACRYLATE, /AS ASSESSMENT OF MONOCHOROETHANE,
MACROPHAGE MELOPOIESIS/DEMONSTRATIONS OF 2-METHOXYETHANOL/ME-INDUCED DIGIT
MALFORMATIONS IN THE DEVELOPING RAB/ACUTE ETHANOL EXPOSURE AND BRAIN
MALFORMATIONS OF RODENT- RABBIT SPECIES./MATERIAL TOXICITY AND FETAL
MALONDIALDEHYDE (MDA) FORMATION INDUCED BY IRON-BLEOMYCIN C/ASSESSMENT OF
MAMMALIAN BRAIN ACETYLCHOLINESTERASE (ACHE)/ INHIBITION AND REACTIVATION OF
MAMMARY CANCER IN THE RAT/CHANGES IN HEPATIC ENDOPLASMIC RETICULUM DURING
MAMMARY GLAND IN LACTATING RATS/EFFECTS OF DEHP ON MILK COMPOSITION AND
MAX./ASSESSING THE PREDICTIVE VALUE OF ANIMAL TOXICITY TESTS FOR
MANAGEMENT/RISK COMPARISON AND RISK
MANCOZEB IN RATS/TWO-WEEK WHOLE BODY AND NOSE-ONLY INHALATION TOXICITY OF
MANGANESE AFTER INHALATION TO 54McCl2./S ON DISTRIBUTION AND EXCRETION OF
MANGANESE IN CHRONICALLY EXPOSED F344 RATS AND B6C/TISSUE DISTRIBUTION OF
MANGANESE IN THE PRIMATE BRAIN WITH MAGNETIC RESONANCE./IMAGING
MARKER OF LUNG RESPONSE TO O3 AND NO2/DECARBOXYLASE ACTIVITY: A SENSITIVE
MARROW NEUTROPHILS/ON OF INCREASED OXIDANT GENERATION BY DBA/2 MOUSE BONE
MASS SPECTROMETRIC DETECTION OF ITS UR/ANS-4-HYDROXY-2-HEXENAL AND TANDEM
MAS SPECTROMETRY/ID METABOLITES FROM MOUSE LIVER MICROSONES USING TANDEM
MATERNAL AND DEVELOPMENTAL TOXICITYOF DERMALLY APPLIED CHLORIPED SLUBBY
MATERNAL EXPOSURE TO 2,3,7,8-TETRACHLORIDIBENZOP-DIO/EFFECTS OF PREVIOUS
MATERNAL EXPOSURE TO DIETHYLBENZYL PHTHALATE/CE OF FISCHER 344 RATS FOLLOWING
MATERNAL IRON RESTRICTION ON NITRITE INDUCED NEONATAL ANEMIA/EFFECTS OF
MATERNAL TOXICITY AND FETAL MALFORMATIONS OF RODENT- RABBIT SPECIES.
MATERNAL TOXICITY IN THE ASSESSMENT OF DEVELOPMENTAL TOX/CONSIDERATION OF
MATING TRIALS IN MALE F-344 RATS/OF BUTYL BENZYL PHTHALATE AND MODIFIED
MATURE RAB BIT BRAIN AND SUB/EXPRESSION OF CHLORIDEION OF CHLORIDE OF
MCDF AS A 2,3,7,8-TETRACHLORIDIBENZO-P/HY/1,3,8-TRICHLORIDIBENZOFURAN (MCF-7 BREAST CANCER CELLS/GEN REGULATED PLASMINOGEN ACTIVATOR ACTIVITY OF
MCMT) AND RELATED IMMUNE FUNCTION TESTS/ILIITY TO MURINE CYTOMEGALOVIRUS (MD)
GENS WHICH DETECT ANTIBODIES TO DIPHENYLTHANE 4,4'-DIISOCYANATE (MDP) COMPOUNDS ON HEPATIC MICROSOMAL P/ EFFECTS OF METHYLDIETHOXYPHENYL (MDEP)
MEAT AFTER ORAL ADMINISTRATION TO CHIC/E MYCOTOXIN CYCLOPIAZONIC ACID, IN MECHANISM OF 2,3,7,8-TCDD INDUCED ENDOTOXIN HYPERSENSITIVITY/STUDIES OF THE MECHANISM OF CYANIDE-INDUCED CYTOTOXICITY

MECHANISM OF HALOCETONITRILES ACUTE TOXICITY: ESTABLISHMENTS/STUDIES ON THE MECHANISM OF HALOCETONITRILES ACUTE TOXICITY: INTERACTION/STUDIES ON THE MECHANISM OF CYANIDE DOPAMINEMETITIC IN VITRO TO INHIBIT MITOCHONDRIAL COMMUNICATION.

MECHANISM OF INDUCTION OF CYTOCHROMES P-450 BY XENOBOTOS.

MECHANISM OF INHIBITION OF PROSTAGLANDIN H SYNTHASE-MEDIATED COOXYDATION

MECHANISM OF INTERACTION OF CADMIUM AND METHYL MERCURY WITH OUABAIN-RECEPTORS.

MECHANISM OF SKF 101772-INDUCED CYTOTOXICITY TO ISOLATED RAT HEPATOCYTE.

MECHANISM OF THIOL UTILIZATION BY SMOKE CHALLENGED RAT AND RABBIT LUN/RESP.

MECHANISM-BASED INACTIVATION OF CYTOCHROME P-450 ISOZYMES BY ANALOGS OF C

MECHANISM-BASED INHIBITION OF CYTOCHROME P-450 BY HETEROCYCLIC ANALOGS OF C

MECHANISM OF 4-HYDROXYMICANEGISTIC TOXICITY BY DICTAMNUS.

MECHANISMS FOR DIMETHYLHYDRAZINE (DMN) INDUCED CHANGES IN MACrophage MY

MECHANISMS FOR PROCAZINE-INDUCED SPERMATOTOXICITY AND CHEMOTHERAPEUTIC.

MECHANISMS FOR THE RELEASE OF ALKALINE PHOSPHATASE (AP) FROM CULTURED HEP.

MECHANISMS OF CA2+-DEPENDENT CELL INJURY./DIFFERENTIAL

MECHANISMS OF FORMATION OF POTENT CARCINOGENS DUR/NUTRITIONAL TOXICOLOGY:

MECHANISMS OF ISOCYANIDE BINDING TO PURIFIED RAT LIVER C/ THE KINETICS AND

MECHANISMS OF ORGANOMETAL TOXICITY/AMINATION OF THE POTENTIAL BIOCHEMICAL

MECHANISMS OF TOXICITY OF COCAINE IN HEPATOCYTES ISOLATED FROM MOUSE AND

MEDAKA EMBRYO (ORYZIAS LATIPES)/LIM AND DNA-BINDING IN VIVO OF APLATOXIN B1.

MEDAKA EMBRYO (ORYZIAS LATIPES)/M IN THE TOXICITY OF CC14 IN THE JAPANESE

MEDAKA EMBRYO DEVELOPMENT./RIZATION OF A MORPHOLOGICAL SCORING SYSTEM FOR

MEDAKA EMBRYO/N THE TOXICITY OF DIOXINS AND DIBENZOFURANS IN THE JAPANESE

MEDIA ON THE TOXICITY OF MONOCHLOROBENZENE/ THE EFFECT OF DIFFERENT CULTURE

MEH - EXPOSED MOUSE KIDNEY./C DISTRIBUTION OF ORGANIC VS INORGANIC HG IN

MEHP INHIBITS THE ENHANCEMENT OF DNA SYNTHESIS/2-ETHYLMETHYLPHTHALATE (MEHP) THROUGH RABBIT MILK AND EFFECTS OF D/N MONO-(2-ETHYLMETHYL) PHTHALATE (MEHP), 2,5-HEXANE-DIONE (HD), AND A23187/MONO-(2-ETHYLMETHYL)-PHTHALATE (MEHP), 2,5-HEXANE-DIONE (HD), AND A23187/MONO-(2-ETHYLMETHYL)-PHTHALATE (MEHP).

MELATONIN/CHRONOPHARMACOLOGY OF PHENYLUNITAZONE: INFLUENCE OF

MEMBRANE AND ATROPIONE/ICITY OF ORGANOPHOSPHATE (OP) COMPOUNDS IN RAT BY

MEMBRANE BILAYERS/CHOLESTEROL AND PHOSPHOLIPID PEROXIDATION IN

MEMBRANE FLUIDITY AND INSULIN RECEPTOR B/ DIOXIDE (NO2) DECREASES SURFACE

MEMBRANE FLUIDITY AS RELATED TO BLOOD LEAD/ERYTHROCYTE

MEMBRANE FLUIDITY EFFECTS/RECONVOLVANT AND ANTICONVOLVANT

MEMBRANE LIPID FLUIDITY AND BETA-UNSATURATED ALDEHYDES ON

MEMBRANE LIPID FLUIDITY, AND MEMBRANE SU/RADICAL PRODUCTION, PHAGOCYTOSIS

MEMBRANE SULFHYDROL GROUP STATUS/HAGOCYTOSIS MEMBRANE LIPID FLUIDITY, AND

MEMBRANE TRANSPORT/MEHRIC AND CHROMATE ION EFFECTS ON RENAL VESICLE

MEMBRANE VESICLES (CMV) BY GLUTATHIONE D/LUX FROM RAT HEPATIC CANALICULAR

MEMBRANE VESICLES FROM RAT SMALL INTESTI/UM TRANSPORT ACROSS BRUSH BORDER

MEMBRANE, p-XYLENE'S ALTERATION OF THE RAT LUNG MICROSMAL

MEMBRANES./OF MG2+-ATP-DEPENDENT CALCIUM TRANSPORT IN ISOLATED RAT LIVER

MEMBRANES/HEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION OF LIPOSOMAL

MEMBRANES/INDICATIONS FOR THE FORMATION OF CLONAL AND CANALICULAR HEPATIC PLASMA

MEMBRANES/EFFECTS OF p-XYLENE ON CEREBRAL

MENADIONE TOXICITY IN ISOLATED RAT HEPATO/ENT AND OXYGEN CONCENTRATION ON

MERCAPTAN/FECTS IN RATS OF REPEATED INHALATION EXPOSURE TO PERCHLOROMETHYL

MERCAPTOPURINE (6MP) AND 6-THIOGUANINE (6TG) COM/PROTECTIVE EFFECTS OF 6-

MERCAPTURIC ACID EXCRETION FOLLOWING ACUTE INHALATION/TISSUE GLUTATHIONE AND

MERCAPTURIC ACID IN THE RAT./ MASS SPECTROMETRIC DETECTION OF ITS URINARY

MERCAPTURIC ACIDS IN RATS EXPOSED TO ACYL-1/A ON THE URINARY EXCRETION OF

MERCAPTURIC AND CHROMATE ION EFFECTS ON RENAL VESICLE MEMBRANE TRANSPORT

MERCAPTURIC ACID/EXPOSURE TO CULTURED R/SOE OF MERCURY DISTRIBUTION AFTER

MERCURIC CHLORIDE INDUCED CYTOTOXICITY I/NZYMSE LEAKAGE AS AN INDICATOR OF

MERCURY (HG) BY HUMAN ERYTHROCYTES (RBC) IN VITRO/UPTAKE OF INORGANIC

MERCURY CONTAMINATED SPORTFISH/HUMAN HEALTH EVALUATION OF

MERCURY CYTOTOXICITY: THE PROTECTIVE EFFECTS OF GLUTATHIONE ON MIC/METHYL

MERCURY DISTRIBUTION AFTER MERCURIC CHLORIDE EXPOSURE IN C/TIME COURSE OF

MERCURY ON BRAIN ATPases OF FISH AND RAT/NATIVE STUDIES ON THE EFFECTS OF

MERCURY OXIDATION BY A SUSPENSION OF WASHED ERYTHRO/KINETICS OF ELEMENTAL

MERCURY OXIDATION OF INTERACTION OF MC2+/CHROMATE AND METHYL

METABOLIC ACTIVATING SYSTEM/RO NEUTRAL RED CYTOTOXICITY ASSAY WITH AN S-9

METABOLIC ACTIVATION AND DNA BINDING OF APLATOXIN B1/EFFECT OF AGE ON THE

METABOLIC ACTIVATION OF 14C-BENZO(a)PYRENE ADSORBED ON/ LUNG RETENTION AND

METABOLIC ACTIVATION OF ETHYLENE GLYCOL MONOBUTYL ETHER (2-BUTOXYETHANOL)

METABOLIC ACTIVATION OF THE PESTICIDE GUTHION BY MOUSE LIVERS PERFUSED IN

METABOLIC ASSAY/ATNE AND NONVOLATILE CARCINOGENS ON THE V79

METABOLIC COOPERATION IN RAT EPITHELIAL (WB/-DOSE-DEPENDENT INHIBITION OF

METABOLIC FATE OF 1-PALMITOYL-L-LYSOPHOSPHATIDYLCHOLINE IN TYPE II CELLS

METABOLIC FATE OF [U-14C-PHENYL]-1-PHENYL-3-PYRAZOLIDINONE ADMINISTERED BY
METHYLINDOLE IS LESS PNEUMOTOXIC THAN 3-METHYLINDOLE./DEUTERATED 3-
METHYLINDOLE IS LESS PNEUMOTOXIC THAN 3-
METHYLINDOLE (MeHg) INDUCED DOPAMINE RELEASE FROM SUPERFUSED RAT STRIATA
METHYLINDOLE (MeHg) ON SPONTANEOUS RELEASE/TERMINAL Ca2+ REGULATION WITH
METHYLINDOLE IN MAMMALIAN CELLS: PNEUMOTOXICITY OF
METHYLINDOLE IN MICE./B AND TESTOSTERONE ON THE RENAL CARCINOGENICITY OF
METHYLINDOLE IN RATS./E DEPENDENT DIFFERENCE IN CHEMICAL FORM OF BILIRARY
METHYLINDOLE UPTAKE BY THE BRAIN./CYSTEINE AND GLUTATHIONE ENHANCE
METHYLINDOLE./NATAL MOUSE CEREBELLAR CELLS FOLLOWING IN VIVO EXPOSURE TO
METHYLPHOSPHINATE (YL) NEUROTOXICITY IN HENS./ETHYL
METHYLPHOSPHONATE (DMMP) NEUROTOXICITY IN HENS/DIMETHYL
METHYLPHOSPHONATE (QL) NEUROTOXICITY IN PIG/E=(-2-DIISOPROPYLAMINOETHYL)
METHYL/THIOBENZAMIDE ON THE DISPOSITION OF 5-HYDROXYTRYPTAM/ EFFECT OF PARA-
METALSULFURYL METHYL IN THE RABBIT/SUBCONSCIOUS DERMAL TOXICITY OF
MFO ACTIVITY AND AGE ON ACETAMINOPHEN/EFFECT OF MIXED FUNCTION OXIDASE /
MG2+-ATP-DEPENDENT CALCIUM TRANSPORT IN ISOLATED RAT L/LEAD INHIBITION OF
MICROBIAL PESTICIDES (GEMPS)/OF PATHOGENICITY FOR GENETICALLY ENGINEERED
MICROCYSTIN FROM MICROCYSTIS AERUGINOSA IN RATS: MORPHOLOGIC /TOXICITY OF
MICROCYSTIS AERUGINOSA IN RATS: MORPHOLOGIC /TOXICITY OF MICROCYSTIN FROM
MICROFLORA./ABOLITION OF 1-,3- AND 6-NITROBENZO(A)-PYRENE BY RAT INTESTINAL
MICROFLORA/ACTIVE ASSAY TO DETECT THE ALTERED XENOBIONT METABOLISM BY GI
MICROSCOPES FOR POLARIZED LIGHT MICROSCOPY/EROSION OF UPRIGHT AND INVERTED
MICROSCOPIC AND ULTRASTRUCTURAL NEUROPATHO/OXICITY OF ALKYL LEAD. PART 2.
MICROSCOPY./RISION OF UPRIGHT AND INVERTED MICROSCOPES FOR POLARIZED LIGHT
MISCELLANEOUS ACETANILIDNE HYDROXYLASE ACTIVI/AND SENSITIVE METHOD TO MEASURE
MISCELLANEOUS ALDEHYDE DEHYDROGENASE/DATION PRODUCTS BY PURIFIED RAT HEPATIC
MISCELLANEOUS AND MITOCHONDRIAL ALDEHYDE DEH/L (T=4HN) BY PURIFIED RAT LIVER
MISCELLANEOUS ANDROSTENEDIONE HYDROXYLASES/B/ INACTIVATION OF FOUR RIVER
MISCELLANEOUS CHOLINE RENAME/RE-NOSIDE BY CLOFIBRATE/INDUCTION
MISCELLANEOUS CYTOCHROME P-450/LATION BY THE PCN-INDUCIBLE FORM OF RIVER
MISCELLANEOUS CYTOCHROME P-450 WITHOUT THE USE OF/PURIFICATION OF RIVER
MISCELLANEOUS CYTOCHROME P450 AND UDP-GLUCUROSONYL/REGULATION OF RIVER
MISCELLANEOUS CYTOCHROME P450 IN VIVO/INHIBITION OF RIVER
MISCELLANEOUS DECHLORINATION OF CHLOROPROPAINES.
MISCELLANEOUS MEMBRANE./P-XYLENE'S ALTERATION OF THE LUNG
MISCELLANEOUS MIXED FUNCTION OXIDASE SYSTEM/E (2-BUTENAL) OF THE RAP HEPATIC
MISCELLANEOUS MONOOXYGENATION OF PHENATE./HEPATIC AND EXTRAHEPATIC
MISCELLANEOUS NADH/DEHYDROGENASES IN SPIKES IN STIMULATION OF
MISCELLANEOUS OXIDATION OF NIFEDIPINE/EVIDENCE FOR REGIOSELECTIVITY IN THE
MISCELLANEOUS OXIDATION AND ANTIOXIDANT ENZ/EFFECT OF PARACETAMOL ON
MISCELLANEOUS PROTEINS OF C57BL/6 AND D2/ENYL (MDP) COMPOUNDS ON HEPATIC
MISCELLANEOUS PROTEINS./CEPHALOSPORIN NERPHOTOXICITY: EFFECTS ON RENAL
MISCELLANEOUS FROM PHENOBARBITAL TREATED RAT/ES WITH PHOSPHOLIPID OF HEPATIC
MISCELLANEOUS USING TANDEM MASS SPECTROMETRY/ID METABOLITES FROM MOUSE LIVER
MISCELLANEOUS/A COMPARISON BETWEEN LIVER AND BRAIN PIG
MISCELLANEOUS/A COMPARISON BETWEEN LIVER AND BRAIN PIG LIVER
MICROTUBULAR ARCHITECTURE/ICTY: THE PROTECTIVE EFFECTS OF GLUTATHIONE ON
MICROTUBELE CYTOSKELETAL NETWORKS./CTS OF ACR, 2,5-HD, DMHD AND IDP UPON
MICROTUBULE REASSEMBLY BY CADMIUM, ARSENITE, AND METHYLMERC/INHIBITION OF
MICROTUBULE: MITOSIS AND TUBULIN CROSSLINK/P ACR, 2,5HD AND DMHD UPON THE
MILK AND EFFECTS OF DEHP ON MILK COMPO/EXPLOY PHTHALATE (MEHP) THROUGH RAT
MILK COMPOSITION AND MAMMARY GLAND IN GR RAT MILK AND EFFECTS OF DEHP ON
MILK SECRETION RATES: A COMPARISON OF EXCRETION OF 1,2-NITRILE INTO
MILK SAMPLING/INTO MILK OF LACTATING RATS: A COMPARISON OF TWO METHODS OF
MINER THER FERRED INDUCED RTh PEURAL TUMORS/AL AND CYTOGENETIC EVALUATION OF
MINERAL OIL./PCB'S IN GOATS. HASTENING WITHDRAWAL USING
MINERAL/CA/DIUM AND OVARIETOMY-INDUCED LOSS OF BONE
MINK./CHRONIC TOXICITY OF DIETARY FLUORIDE TO
MIREX (MX/K) ISOENZYME (IEZ) ACTIVITY AND [3H]- CMP BINDING IN VITRO BY
MIREX DECREASES THE UPTAKE OF 3H-ESTRADIOL-17beta-D-GLUCURONIDE (E217G) A
MIREX ON HYPOTHALAMUS, PANCREAS, ADRENALS, ADIPOSE AND LIVER/EFFECTS OF
MISOMIDASOLE-INDUCED DNA DAMAGE IN HYPOXIC AND NORMALLY AERATED TUMOR CEL
MITOCHONDRIA BY A TETRAZOLE-SUBSTITUTED AL/OYL TRANSFERASE I IN RAT LIVER
MITOCHONDRIA DNA FROM MOUSE AND LL210 TUMOR M/BLEOMYCIN-INDUCED DAMAGE OF
MITOCHONDRIA EXPOSED TO NEUROFILAMENTOUS AXONOPAT/ATP PRODUCTION BY BRAIN
MITOCHONDRIA/DNOCAD DAMAGE OF MITOCHONDRIA DNA FROM MOUSE AND LL210 TUMOR
MITOCHONDRIAL ALDEHYDE DEHYDROGENASES (ALDH)/FIED RAT LIVER MICROSONAL AND
MITOCHONDRIAL RESPIRATION/EFFECTS OF ACRYLAMIDE AND 2,5-HEXANEDIONE ON
MITOCHONDRIAL RESPIRATION/TAMINOPHEN (APAP)-INDUCED INHIBITION OF HEPATIC
MITOCHONDRIAL STRUCTURE/FUNCTION./ACUTE ARSENITE /ALTERATION OF HEPATIC-
MITOGEN RESPONSIVENESS AND ANTIBODY PROD/IN VITRO EFFECTS OF T-2 TOXIN ON
MITOGEN RESPONSIVENESS IN VITRO./THE MURINE ANTIBODY RESPONSE IN VIVO AND
MITOPLASTS/PARTIAL PURIFICATION OF A BENZENE HYDROXYLASE FROM RAT LIVER
MITOSIS AND TUBULIN CROSSLINKING./R, 2,5HD AND DMHD UPON THE MICROTUBULE:
MIXED FUNCTION OXIDASE (MFO) ACTIVITY AND AGE ON ACETAMINOPHEN /EFFECT OF

322
MIXED FUNCTION OXIDASE ACTIVITIES AFTER NE REACTION IN DEPRESSING HEPATIC 596 MIXED FUNCTION OXIDASE ACTIVITY IN BROWN BULLHEAD FROM A CONTAMINATED NEU 597 MIXED FUNCTION OXIDASE ACTIVITY AND REACTION IN HEPATIC MICROSOMAL 598 MIXTURES ON BLOOD PRESSURE AND HEART RATE/RML EXPOSURE TO COMPLEX 599 ORGANIC 600 MOBILIZATION OF LEAD FROM PRETREATED RAT DAMS./IN UTERO 601 MOBILIZATION OF LEAD OVER THE COURSE OF CMSA CHOLESTEROL THERAPY. 602 MODEL COMPOUND. S.J./GE USING 4-4'-THIOBIS(6-t-BUTYL-m-CRESOL) (TBBC) AS A 603 MODEL FOR ASSESSING MUSCLE IRRITATION DUE TO PARENTERAL ANTIB. IN VITRO 604 MODEL FOR PHYSIOLOGIC AND BEHAVIORAL EVALUATION OF ANIMAL PERFORMANCE D/A 605 MODEL FOR SULFUR MUSTARD TOXICITY./HUMAN LIMPHOCYTES AS A 606 MODEL FOR TEST OF TOXICITY IN RATS/CITRAL: 607 MODEL FOR THE INDUCTION OF CARCINOIDS AND SMALL CELL CANCRA/V NOVEL IN VIVO 608 MODEL FOR THE INVESTIGATION OF INTRACELL/DEVELOPMENT OF A FISH HEPATOCYTE 609 MODEL FOR THE STUDY OF METHANOL VISUAL NEUROTOXICITY/A RAT 610 MODEL FOR VINYLIDINE CHLORIDE./PHYSIOLOGICALLY-BASED PHARMACOKINETIC 611 MODEL IN RATS/SOME CHARACTERISTICS OF AN OZONE (O3) EXPOSURE-EFFECT 612 MODEL IN THE RABBIT AND OvariECTOMIZED /INAL IRRITATION/SUBACUTE TOXICITY 613 MODEL OF HALOTHANE HEPATITIS/HOLD INDUCED IMMUNE RESISTANCE IN A GUINEA PIG 614 MODEL OF HEPATOCARCINOMA. D 3H-ETHYNYLESTRADIOL IN THE RAT TWO-STAGE 615 MODEL OF OPIN./ AND SOMATOSENSORY EVOKED POTENTIALS (SSEP) IN THE RODENT 616 MODEL OF ORGANOPHOSPHATE INDUCED PERSISTANT BRAIN ACETYLIC/A DEVELOPMENTAL 617 MODEL OF PNEUMOCONIOSIS./INDUCED BY EXPOSURE TO GLASS FIBERS IN A MURINE 618 MODEL SYSTEM FOR THE STUDY OF IRRITANTS/URED MAMMALIAN KERATOCYTES AS A 619 MODEL TO ASSESS THE INFLAMMATORY AND FIBROGENIC POTENTIAL OF SINTH/A MURINE 620 MODEL TO STUDY THE INFLAMMATORY POTENTIAL OF SURGICAL IMPLANTS./A NEW 621 MODEL./OF VINYLIDENE FLUORIDE (VDF) IN RATS SIMULATED BY A PHYSIOLOGICAL 622 MODEL./TATTOO/ANO (A)PYRENE INTERACTIONS ARE MATHEMATICAL 623 MODELING FOR LEARNING BEHAVIOR IN WATER-FILLED MULTIPLE/A NEW STATISTICAL 624 MODELING LACTATIONAL TRANSFER OF TRICHLOROETHYLENE (TCE) AND ITS METABOLI 625 MODELS FOR ASSESSING CARDIOVASCULAR TOXICITY/IN VIVO AND IN VITRO 626 MODELS FOR INDIRECT CARCINOGENS./PHARMACOKINETIC 627 MODULATION OF GTP-RESPONSIVE ADENYLA CYCLASE (AC) IN ISOLATED MALE GERM 628 MOLINATE/EMENT AND VALIDATION OF AN ELISA FOR THE THIOCARBAMATE HERBICIDE 629 MOLLUSCS./IDENT ENZYMES IN THE HEPATOPANCREAS OF TWO MID-ATLANTIC BIVALVE 630 MONKEYS AND OUBAIN ON AN EXCLUSIVE SENSORY NEURON/INTERACTIONS OF 631 MONKEYS AND HUMAN BY HYPOLIPIDEMIC DRUGS/ HEPATOCYTES FROM RAT, CYNOLOGY 632 MONKEY INFANTS./-P-DIOXIN (TCDD) ON PEER GROUP SOCIAL BEHAVIOR OF RHEUS 633 MONKEY TESTES./ACARACTERIZATION OF A NOVEL METAL-BINDING PROTEIN FROM PATA 634 MONKEY/POSITION OF 2,3,4,7,8-PENTACHLORODIBENZOFURAN (PCDF) IN THE RHEUS 635 MONKEY/SENSITIVE PERIODS FOR LEAD BEHAVIORAL TOXICITY IN THE 636 MONKEYS EXPOSED TO DIESEL ENGINE EMISSION/ ABERRATIONS IN LIMPHOCYTES FROM 637 MONKEYS FOLLOWING CHROMIC EXPOSURE./DIOXIN (TCDD) FROM BODY FAT OF RHEUS 638 MONKEYS FOLLOWING TOPOICAL ADMINISTRATION/ABSORPTION OF BUTACHLOR IN RHEUS 639 MONKEYS/ OF INHALED METHANOL: A COMPARISON BETWEEN F-344 RATS AND RHEUS 640 MONKEYS./EFFECT OF VOMITOXIN (DEOXYVINALENOL) ON INFANT CYNOLOGY 641 MONKEYS./IGHT-WEEK INHALATION TOXICITY STUDY OF SEVOFLURANE IN CYNOLOGY 642 MONKEYS./IMMUNOCOMPETENCY OF CHO CELLS-DErIVED IDNA HUMAN GM-CSF IN RHEUS 643 MONKEYS/LOW-LEVEL OF LEAD EXPOSURE ON A SPATIAL DISCRIMINATION TASK IN ADULT 644 MONO-/2-ETHYLHEXYL) PHthalate (MHP) THROUGH R/HEXYL PHthalate (DEHP) AND 645 MONO-(2-ETHYLHEXYL)PHthalate (MHP) ON THE HORMONAL RESPONSIVE/EFFECT OF 646 MONOBUTYL ETHER (2-BUTOXYETHANOL BE) VIA/C ACTIVATION OF ETHYLGLYCOL 647 MONOCHLOROBENZENE, AND LEAD ACETATE./TOXICITY OF MIXTURES OF CCl4, 648 MONOCHLOROBENZENE, AND THE DICHLOROBENZENES/TUBE MEDIA ON THE TOXICITY OF 649 MONOCHLORoETHANE, MALEIC ANHYDRIDE, METHYL ME/EALTH EFFECTS ASSESSMENT OF 650 MONOCALON ANTIBODY USED TO PROBE FOR PERTURBATION IN THE DI/ANTI-KERATIN 651 MONOCORTALINE PYRROLE-TREATED RATS./ COPPER AND PULMONARY HYPERTENSION IN 652 MULTIPLE CHLORATE ACTIVITY./OLAR MACROPHAGES TO RELEASE NEUTROPHIL AND 653 MONOCYTIC INDUCERS OF HUMAN PROMYELOCYTIC LEUKEMIA CELL./GRANULOCYTIC AND 654 MONOMETHYL TATTOO/EFFECTS OF 1,2-DICHLOROBENZENE ON THE MOUSE 2,4/ RATIVE TOXICOLOGY STUDIES OF THE 655 MONONUCLEAR CELL LEUKEMIA AND PHECROMOCYTOMA IN/DECREASED INCIDENCES OF 656 MONOXOGENASE INDUCERS ON LEVELS OF mRNA IN/THE EFFECT OF VARIOUS HEPATIC 657 MONOXOGENASE SYSTEM BY 2,3,7,8-TETRACHLORODIBENZ/INDUCTION OF THE HEPATIC 658 MONOXOGENASES/ON THE IN VITRO BIOTRANSFORM/INHIBITORS OF P-450-DEPENDENT 659 MONOXOGENASES/PHOSPHORYLATION INSECTS BEHAVIOR BY RAPID BRAIN 660 MONOXOGENASES/PHOSPHORATE/HEPATIC AND EXTRAPLETHAL MICROSOMAL 661 MONOSODIUM GLUTAMATE (MSG) AS NEONATES EXHIBIT HYPERACT/RATS TREATED WITH 662 MONOSODIUM GLUTAMATE SEVERELY DISRUPTS FLASH E/NEONATAL ADMINISTRATION OF 663 MORPHINE ADMINISTRATION ON RAP/PHARMACOLOGICAL EFFECTS OF REPEATED FENTANYL OR 664 MORPHINE DISTRIBUTION IN RATS/EFFECTS OF ETHANOL PRETREATMENT ON 665 MORPHOFUNCTIONAL CHANGES IN HEPATIC ENDOPLASMIC RETICULUM DURING MAMMARY 666 MORPHOLOGIC AND SERUM CHEMISTRY ALTERATION/ICRYSTIS AERUGINOSA IN RATS; 667 MORPHOLOGICAL AND BIOCHEMICAL INDICATORS OF/ TO THE DEVELOPING RAT ALTERS 668 MORPHOLOGICAL AND CYTOGENETIC EVALUATION OF MINERAL FIBER INDUCED RAT PLE
NEUROPATHY (OPIDN) IN ADULT HENS/S DURING ORGANOPHOSPHATE-INDUCED DELAYED
NEUROPATHY (OPIDN) IN CHICKENS./PMENT OF ORGANOPHOSPHORUS-INDUCED DELAYED
377 NEUROPATHY AND SOMATOSENSORY EVOKE POTENTIAL/A CORRELATION OF SPINAL CORD
392 NEUROPATHY IN HUMANS./RATION SENSE AS A MEANS OF TESTING FOR PERIPHERAL
393 NEUROPATHY IN THE CHICKEN/ATROPIONE AND DFP-INDUCED DELAYED
407 NEUROPATHY/TARGET ENZYME/ SOLUBILIZATION AND CHROMATOGRAPHY OF
421 NEUROPATHY/INAL DEFICIT EARLY IN beta,beta'-IMIDOPROVINITRILE (IDPN)
436 NEUROPHYSIOLOGICAL RESPONSE OF RHESUS MACAQUES TO ATROPINE AND PYRIDOSTIGM
451 NEUROPATHOLOGICAL EVALUATION OF CYCLODIENE INSECTICIDE TOXICITY.
465 NEUROTOXIC BICYCLOPHOSPHATE ESTERS FOLLOWING THERMAL DECOMP/PRODUCTION OF
480 NEUROTOXIC DOES./EDONE CAUSES IRREVERSIBLE TESTICULAR GERM CELL LOSS AT
495 NEUROTOXIC EFFECTS./A DATABASE FOR COMPUTER ANALYSIS OF REPORTED
510 NEUROTOXIC ESTERASE BY TTP AND TOCP AND T/BETWEEN PROLONGED INHIBITION OF
525 NEUROTOXIC POTENTIAL OF ORTHO-,META-, AND PARA-CRESOLS
540 NEUROTOXICITY [MON/OXYGEN FOR ORGANOPHOSPHATE-INDUCED DELAYED-
555 NEUROTOXICITY AND FYROLE- FORMING POTENTIAL OF 2,5-HEXADION/COMPARATIVE
570 NEUROTOXICITY IN HENS./ETHYL METHYLPHOSPHATE (YL)
585 NEUROTOXICITY IN HENS/DIMETHYL METHYLPHOSPHONATE (DMMP)
600 NEUROTOXICITY IN PIGEONS./2-DISOPROPLAMINOETHYL METHYLPHOSPHONITE (GL)
615 NEUROTOXINS IN RATS ASSOCIATED WITH CHRONIC EXPOSURE T/REPRODUCTIVE AND
630 NEUROTOXICITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (M/ENHANCED
645 NEUROTOXICITY OF PYRIDOETH/BIOLOGICAL LETHALITY VERSUS THE AU/AGE-DEPENDENT
660 NEUROTOXICITY./ING RAT ALTERS MORPHOLOGICAL AND BIOCHEMICAL INDICATORS OF
675 NEUROTOXICITY/A RAT MODEL FOR THE STUDY OF METHANOL VISUAL
690 NEUROTOXINS IN YOUNG CHICKENS/F ADMINISTRATION OF ORGANOPHOSPHATE DELAYED
705 NEUROTOXINS/MITOCHONDRIA EXPOSED TO NEUROFILAMENTOUS AXONOPATHY PRODUCING
720 NEURAL RED CYTOXICITY ASSAY WITH AN S-9 METABOLIC ACTIVATING /IN VITRO
735 NEUTROPHIL AND MONOCYTOCHEMOTACTIC ACTIV/ALVEOLAR MACROPHAGES TO RELEASE
750 NEUTROPHIL- DERIVED OXIDANTS/ISM OF BENZG(A)PYRENE-7,8-DIHYDRODIOL BY LUNG
765 NEUTROPHILS AS IMMUNOTOXICOLOGICAL TARGETS/MURINE POLYMORPHONUCLEAR
780 NEUTROPHILS IN MURINE POLYMORPHONUCLEAR LEUKOCYTES FROM TUMOR/PHENOLIC T-PECTONEAL
795 NEUTROPHILS/ON OF INCREASED OXIDANT GENERATION BY DBA/2 MOUSE BONE MARROW
810 NICKEL - MAGNESIUM INTERACTIONS IN CARCINOGENESIS: DOSE EFFECTS AND NATUR
825 NICKEL DITHIOCARBAMATE-/CARCINOGENICITY OF
840 NICKEL IN RATS DURING CONTINUOUS INTRAVENOUS INFUSION OF/TISSUE UPTAKE OF
855 NICKEL ON SPECIFIC PROTEIN BINDING TO MOUSE SATELLITE DRA./THE EFFECT OF
870 NICKEL OXIDE, NICKEL SULFATE, AND NICKEL SULPHATE/COMPARATIVE TOXICITY OF
885 NICKEL OXIDES AND NICKEL-COMPETITIVE CYTOTOXICITY OF
900 NICKEL REDUCE CADMIUM-INDUCED MORPHOLOGICAL CHANGES IN 3T3 FIBRO/ Zinc AND
915 NICKEL SUBSULFIDE IN THE F344/N RAT AND NICKEL OXIDE, NICKEL SULFATE, AND
930 NICKEL SUBSULFIDE TO F344/N RATS AND B6C3F1 MICE E/INHALATION TOXICITY OF
945 NICKEL SULFATE, AND NICKEL SULFIDE 1/AARATIVE TOXICITY OF NICKEL OXIDE,
960 NICKEL SULFIDE (NS) AND METHYLCODRANTHR/ ASSOCIATED WITH TUMORGENESIS BY
975 NICKEL TOXICITY IN HUMAN AND RAT ALVEOLAR EPITHELIAL CELLS.
990 NICKEL COPPER OXIDES TO RAT, MOUSE AND DOG /TOXICITY OF NICKEL OXIDES AND
324 NICKEL-INDUCED DNA DAMAGE AND CELL TRANSFER/E EFFECT OF MAGNESIUM AGAINST
1009 NICKEL UPTAKE OF NICKEL IN RATS DURING CONTINUOUS INTRAVENOUS INFUSION OF
1024 NICOTINE ADMINISTRATION AND WITHDRAWAL: EFFECTS ON RADIAL ARM MAZ/CHRONIC
1039 NICOTINE AND COTININE IN SMALL VOLUMES O/CHROMATOGRAPHIC (GC) ANALYSIS OF
1054 NIFEDIPINE/EVIDENCE FOR REGIOSPECIFICITY IN THE MICROSONAL OXIDATION OF
1079 NIFLURIDE INTO MILK OF LACTATING RATS: A COMPARISON O/EXCRETION OF 14C-
1094 NITRITE INDUCED NEONATAL ANEMIA/EFFECTS OF MATERNAL IRON RESTRICTION ON
1109 NITRITE OR HYDROXYLAMINE/IN VIVO OXIDATION OF CARDIAC HRGLOBIN BY SODIUM
1124 NITRO-3-METHYL-4-PHENYL-1-HYDROXYPYRIDINE IN RATS/DEVELOPMENTAL TOXICITY IN 4-
1139 NITRO-N-METHYLPHENYLHYDRAZINE [CARBONYL-14C] (4-NPI) / PHARMACOKINETICS OF 4-
1154 NITROTEANOL (PNA) IN THE RAT./Y AND POTENTIAL REPRODUCTIVE EFFECTS OF P-
1169 NITROAROMATIC CATALYSIS OF SUPERKIDNE GENERATION IN THREE SPECIES OF FRE
1184 NITROBENZO(A)PYRENE BY RAT INTESTINAL MICROPL/METABOLISM OF 1-,3- AND 6-
1199 NITROFURAZONE (NF) IN CD-1 MICE./TERATOLOGIC EVALUATION OF
1214 NITROGEN DIOXIDE (NO2) DECREASES SURFACE MEMBRANE FLUIDITY AND INSULIN RE
1229 NITROGEN DIOXIDE (NO2) TOXICITY./EXERCISE POTENTIATES
1244 NITROGEN DIOXIDE (NO2)-EXPOSED ISOLATED LUNG CELLS FROM
1259 NITROGEN DIOXIDE AND OZONE/TER UPTAKE TO CARBON BLACK, DIESEL EXHAUST,
1274 NITROGEN DIOXIDE AND RESPIRABLE AEROSOLS./SYNERGISTIC INTERACTION BETWEEN
1289 NITROGEN DIOXIDE-/NG ACUTE EXPOSURES TO RELATIVELY HIGH CONCENTRATIONS OF
1304 NITROGUANIDINE (NG) AND NITROSOGUANIDINE (NSG)/MUTAGENICITY STUDIES OF
1319 NITROIMIDAZOLES/IVE HYDROXYLAMINE INTERMEDIATE DURING THE REDUCTION OF 5-
1334 NITROREDUCTION OF 1,3-DINITROBENZENE BY RAT TESTICULAR CELL CULTURES AND
1349 NITROSLOODS IN VITRO/DEVELOPMENTAL TOXICITY OF INDUSTRIAL N-
1364 NITROSO COMPOUNDS/STRUCTURE-ACTIVITY RELA/HUMORAL IMMUNOSUPPRESSION BY N-
1379 NITROPHENYL IN EFFICACY RATE HEPATOCELLAR PCY/OCYLOWHYDROAMIC ACIDS OF 4-
1394 NITROSODIMETHYLAMINE (NDMA) CATALYZED BY PIG LIVER /N-DEMETHYLATION OF N-
1409 NITROSOGUANIDINE (NSG)/MUTAGENICITY STUDIES OF NITROGUANIDINE (NG) AND
1424 NMR STUDIES/TION BY ACUTE ARSENITE EXPOSURE: ULTRA-STRUCTURAL AND IN VIVO
1439 NMR)/RATE OF ABSORPTION AS MONITORED BY 31P-NUCLEAR MAGNETIC RESONANCE (N
1454 NO2 EXPOSURE ON THE UPTAKE OF VITAMIN E BY LUNG./EFFECT OF
Oxidant generation by DBA/2 mouse bone marrow /demonstration of increased oxidative activities after treatment with /expressing hepatic mixed function /oxidation by a suspension of washed erythrokinetic lymphocytes /stimulation of microsomal NADPH /oxidation in isolated rat hepatocytes by a tetra/inhibition of fatty acid /oxidation in vitro in male F344 rats/induced increase in hepatic palmitate /oxidation of nifedipine/evidence for regiospecificity in the microsomal /oxidation of unoprophyrinogen by chick EM/4'-tetrachlorobiphenyl-mediating /oxidation to formaldehyde in the eye and /in vitro evaluation of methanol /oxidative desulfuration and dearylation of peniitrophen by /comparison of carboxylic acids of microsomal NADPH /oxidative stress and lipid peroxidation in mptp-indu/abense of roles for /oxides and nickel-copper oxides to rat, comparative cytotoxicity of nickel /oxides to rat, mouse and dog pulmonary /P nickel oxides and nickel-copper /oxide inm bacteria and mammalian cells in VI/genotoxicity of nickel /oxidase peroxidisome proliferator/hloro)-2(2,2,2-trichloro-ethyl) /oxo-1-imidazolidinyl)ethyl]-N-phenylurea/Ted phagocytic cells by N-[2-(2- /oxoacetyl acetate in rats/results of a 90-day oral gavage study of /oxothiolins-4-carboxylic acid (Otca) on the urinary excretion of /oxacyclic, arylic, and phenylcyclopropanes /acetylation in acute and chronic /acetylation of it in the eye and /in vitro evaluation of methanol /activity of microsomal NADPH /activity with simultaneous stimulation of microsomal NADPH /activity of the two reactions /activity of the two reactions /activity of the two reactions /activity of the two reactions

Oxidative/concentration on menadione toxic of phenobarbital pre-treatment and /oxygen free radical mediated ischemia-reperfusion damage to gerbil brain /oxygen metabolism and thromboxane in phorbol myris/involvement of active /oxygen species inducing mutations in mammalian cell/evidence for reactive /oxygen tolerance in mice/induction of /oxygen toxicity/leukotrienes in pulmonary
OXYGEN UPTAKE IN THE ISOLATED PERFUSED /REVERSIBLE INHIBITION OF HEPATIC
OZONE (O3) EXPOSURE OF RATS/NARY RESPONSE INDUCED BY DIURNAL OR NOCTURNAL
OZONE EXPOSURE/HUMAN AND RAT LAVAGE FLUID BIOCHEM/EFFECT OF ACUTE
OZONE (O3) EXPOSURE-EFFECT MODEL IN RATS/SOME CHARACTERISTICS OF AN
OZONE EXPOSURE/EPIDEMIOLOGY Lymphocyte NUMBERS AND SUBPOPULATIONS FOLLOWING
OZON IN GUINEA PIGS AND THREE STRAINS OF RA/RESPIRATORY SYSTEM UPTAKE OF
OZONE INHIBITION AFFECTS RAPIDLY ALVEOLAR MACROPHAGE SUPEROXIDE ANION RADICAL
OZONE INHIBITION DECREASES MACROPHAGE b245 IN RAT ALVEOLAR MACROPHAGES
OZONE ON COLLAGEN METABOLISM IN RAT LUNG/EFFECTS OF CHRONIC EXPOSURE TO
OZONE ON PULMONARY NATURAL KILLER CELL ACTIVITY/EFFECT OF
OZONE STIMULATES RABBIT ALVEOLAR MACROPHAGES TO RELEASE NEUTROPHIL AND MO
OZONE-INDUCED FIBROSIS IN MICE./ST-EXPOSURE EFFECT OF LUNG GLUTATHIONE ON
OZONE./METABOLISM AND PROTEINOLYSIS AFTER REPEATED INHALATION EXPOSURE TO
OZONE/EXPOSURE TO CARBON BLACK, DIESEL EXHAUST, NITROGEN DIOXIDE AND
OZONE/T-LYMPHOCYTE INFILTRATION OF MOUSE LUNG FOLLOWING EXPOSURE TO

-P-

643 P-450 (CP-450) IN THE GUINEA PIG AND HA/DEETHYLASE (EROD), AND CYTOCHROME
141 P-450 (P-450) CATALYZED METABOLISM OF 2,2,2-TRIFLUOROETHANOL A/CYTOCHROME
425 P-450 (P-450PB-B), AMINOPYRINE N-DEMETH/OBARBITAL-INDUCIBLE CYTOCHROME(S)
1058 P-450 ENHANCES THE EFFECT OF CARBON TETRA/P-XYLENE INDUCES CYTOCHROME
737 BY XENOBIOTICS/Mechanism of induction of CYTOCHROME
61 INSECTICIDE RESISTANT L/MULTIPLE FORMS OF CYTOCHROME
858 A/STEROIDS TOWARD RAT HEPATOCYTES: ROLE OF CYTOCHROME
463 INDUCERS AND INHIBITORS./P ACETAMINOPHEN (AA): EFFECT OF CYTOCHROME
87 INDUCTION AND GLUTATHIONE DEPLETION/IONS INTO THE ROLE OF CYTOCHROME
84 ISOZYME RESPONSIBLE FOR THE METABOLISM OF THE DOG HEPATIC CYTOCHROME
85 ISOZYMES BY ANALOGS OF CHLORAMPHE/BLACK INACTIVATION OF CYTOCHROME
608 ISOZYMES FROM UNTREATED RAINBOW T/IZATION OF TWO HEPATIC CYTOCHROME
858 ISOZYMES./SULFURATION AND DEARYLATION OF PENTOTHAL BY CYTOCHROME
858 LEVELS AND DRUG METABOLIZING ENZYME T/ Hepatocytes Maintains CYTOCHROME
546 P-450, 17-HYDROXYLASE AND 17,20-LYASE B/D RAT TESTICULAR HEMO, CYTOCHROME
601 P-450-DEPENDENT W/oXGENASES ON THE IN VITRO INDUCERS AND INHIBITORS OF
81 P-450./ THE MAJOR PHENOBARBITAL-INDUCIBLE ISOZYME OF DOG LIVER CYTOCHROME
89 P-450/D MECHANISMS OF ISOCYANIDE BINDING TO PURIFIED RAT LIVER CYTOCHROME
875 P-450/LATION BY THE PCN-INDUCIBLE FORM OF RAT LIVER MICROSONAL CYTOCHROME
875 P-450/OCARBON HYDROXYLASE (AHH) KINETICS BUT SLIGHTLY ALTERED CYTOCHROMES
83 P-450b WITHOUT THE USE OF NON-IONIC DET/P RAT LIVER MICROSONAL CYTOCHROME
82 P-450p AND UDP-GlUCURONOSYL-TRANSFERASE/P RAT LIVER MICROSONAL CYTOCHROME
144 P450 BY 3-METHYLCARBAZOLE IN CULTURE/HYDROXYSTEROIDOL ON THE INDUCTION OF
857 P450 BY HETEROCYCLIC ANALOGS OF PHENCY/ISM-BASED INHIBITION OF CYTOCHROME
129 P450d./INHIBITION OF 2-AMINOFUORENE MUTAGENESIS BY INDUCERS OF CYTOCHROME
145 P450p IN VIVO/INHIBITION OF RAT LIVER MICROSONAL CYTOCHROME
779 PAF/NSE OF GUINEA PIGS TO THE INHALATION OF PLATELET ACTIVATING FACTOR ()
8 Palatal CAMP AND CAMP CHANGES IN DEVELOPING MICE/SECALONIC ACID D-INDUCED
1080 PALMITATE OXIDATION IN VITRO IN MALE F344/DA)-INDUCED INCREASE IN HEPATIC
820 PALMITOYL-L-lysophosphatidylcholine IN TYPE II CELLS A/TABOLIC PATE/1-
52 PAM FROM MICE EXPOSED TO MAINSTREAM C/N PULMONARY ADRENA sympathetic MACROPHAGES (
184 PANCREAS, ADRENAL, ADIPOSE AND LIVER/EFFECTS OF MIREX ON HYPOTHALAMUS,
1065 PAPS) AND UDP-GlUCURONIC ACID (UDPGA) S/ 3'-PHOSPHATE 5'-PHOSPHOSULFATE (,
1064 UDP-GlUCURONIC ACID REGULATION/3'-PHOSPHATE 5'-PHOSPHOSULFATE (,
349 PARA-METHYLPALOMAZIDE ON THE DISPOSITION OF 5-HYDROXYTRYPTAM/EFFECT OF
920 PARAOXON ON MURINE T AND B CELL PROLIFERATION IN VITRO/THE EFFECT OF
783 PARAQUAT AEROSOLS/RESPIRATORY EFFECTS OF REPEATED EXPOSURE TO
182 PARAQUAT DURING PREGNANCY/TOXICITIES OF ENDRIN AND
1 PARAQUAT IN THE CHICKEN EMBRYO/TOXICITY OF
595 PARAQUAT ON MICROSONAL OXYGEN REDUCTION AND ANTIOXIDANT ENZ/THE EFFECT OF
361 PARAQUAT ON THE RAT KIDNEY/ACUTE TOXIC EFFECTS OF
663 PARAQUAT-INDUCED CYTOSKELETAL INJURY IN CULTURED LUNG EPITHELIAL CELLS
180 PARATHION./HE ROLE OF THE LIVER IN MEDIATING THE ACUTE TOXICITY OF METHYL
562 PARENTERAL ANTIMICROB./ITRO MODEL FOR ASSESSING MUSCLE IRRITATION DUE TO
116 PARTICLES CLEARING FROM THE PLEURAL SPACE/SUE AS A TRANSLOCATION SITE FOR
53 PARTICLES./CLEARANCE AND INFLAMMATORY RESPONSES TO INHALED CARBONYL IRON
795 PARTICLES/INDUCTION OF INHALED 14C-BENZ0(A)-PYRENE ADSORBED ON CARBON BLACK
794 PARTICLES/BOLIC ACTIVATION OF 14C-BENZ0(A)-PYRENE ADSORBED ON CARBON BLACK
114 PARTICLES/RETENTION AND CLEARANCE OF INHALED SUBMICRON CARBON BLACK
915 PARTICULATE ABSORBED APLATOXIN B1 IN THE R/INHIBITED MICROCRYSTALLINE AND
593 PATHOGENICITY FOR GENETICALLY ENGINEERED MICROBIAL PESTICIDES/ABSENCE OF
796 PATHOLOGICAL RESPONSES OF RATS TO ACUTE INHALATION OF SYNTH/PULMONARY AND
548 PATHOLOGY OF THE REPEATED DOSE TOXICITY OF SALICYLALDOSULFAPYRIDINE IN PIS
825 PBA AND DEIDRIN./EASURE INHIBITED CELL-CELL COMMUNICATION IN WB CELLS BY
831 PBNA IN F344/N RATS AND B6C3F1 MICE G1/CTS OF N-PHENYL-2-NAPHTHIOLAMINE (,
1086 PCBs IN GOATS. HASTENING WITHDRAWAL USING MINERAL OIL.)
PCBx) ON INITIATION OF HEPATOCARCINOGEN/ PURE POLYCHLORINATED BIPHENYLS (368) PCDF) IN THE RHEUS MONKEY/OSSITION OF 2,3,4,7,8-PENTACHLOROBENZOFURAN (367) PCDF): TOXICOKINETICS AND METABOLISM IN/3,4,7,8-PENTACHLOROBENZOFURAN (366) PCDFs)/ADITIVE TERATOGENIC EFFECTS OF POLYCHLORINATED DIBENZOFURANS (367) PCNF-INDUCIBLE FORM OF RAT LIVER MICROSONAL /TOXIN BI HYDROXYLATION BY THE (365) PCB)/RATION OF CYTOCHROME P450 BY HETEROCHROMIC ANALOGS OF PHENYLCLIDINE (392) PENETRATION AND PHARMACOKINETICS OF 2,4,5,7'-IN VIVO AND IN VITRO DERMAL (392) PENETRATION OF DINOSEB, DETERMINED BY IN VIVO AN/AGE RELATED PERCUTANEOUS (397) PENETRATION POTENTIAL OF 14C-POLYMERS JR400 AND LR400 FOLLOWING A SI/SKIN (397) PENTACHLOROBENZOFURAN (PCDF) IN THE RHEUS MON/ISPOSITION OF 2,3,4,7,8- (367) PENTACHLOROBENZOFURAN (PCDF): TOXICOKINETICS AND METABOLISM /3,4,7,8- (397) PENTACHLOROBENZOFURAN AND 1,2,3,4,7,8-HEXACHLOROBENZOFURAN/2,3,4,7,8- (397) PENTACHLOROPHENATE ON FEEDING ENERGETICS IN THE CATFISH/ EFFECT OF SODIUM (358) PERIFERICAL REACT TO 3,4,7,8-TRIMETHYLPTA Ex/IN VITRO TRANSPORT OF (358) PENTAVALENT TO TRIVALENT ARSENIC AS DETRIM/AGE AND METABOLIC REDUCTION OF (359) PEPTIDE HEPATOTOXIN PRODUCED BY BLUE-GREY/ DEHYDROAMINO ACID FROM A CYCLIC (374) PERACETIC ACID IN RATS/ A 2-WEEK SUBCHRONIC INHALATION STUDY ON (1077) PERCHLOROETHYLCYCLIC METABOLISM BY THE GLUTATHIONE CONJUGATION PATHWAY (761) PERCHLOROETHYLETHYLEFERENCE AND METABOLISM AFTER INHALATIONAL EXPOSURE TO (762) PERCHLOROMETHANOL/FLASHS IN RATS OF REPEATED INHALATION EXPOSURE TO (756) PERCUTANEOUS ABSORPTION KINETICS OF ORGANOPHOSPHATES, STEROIDS, CAFFEINE, (975) PERCUTANEOUS EXPOSURE TO 2,4-D/PROPYLENE (RAP) FROM IN VITRO MICRODIALYSIS APP (1094) PERCUTANEOUS ABSORPTION OF CAPTAPOL/DY ON THE EFFECT OF ANIMAL AGE ON THE (397) PERCUTANEOUS ABSORPTION STUDIES/USE OF EXCISED HUMAN SKIN FOR IN VITRO (976) PERCUTANEOUS FATE OF TOPICAL STEROIDS: IN VITRO STUDIES WITH MOUSE SKIN I (976) PERCUTANEOUS PENUMINATION OF DINOSEB, DETERMINED BY IN VIVO AN/AGE RELATED (976) PERFLUORO-N-DECANOIC ACID (PFDA)-INDUCED INCREASE IN HEPATIC PALMITATE (OX (1080) PERFLUOROCHEMICAL ACIDITY/TOXICITY OF (1081) PERFLUOROCHEMICAL ACIDITY/TOXICITY OF (1081) PERFLUOROCHEMICAL ACIDITY/TOXICITY OF (1081) PERFLUOROALKYL ETHYL METHACRYLATE/TOXICITY OF (1081) PERFLUOROALDEHYDE IN VIVO AND IN VITRO METABOLISM OF THE (823) PERFLUORODECANAL/FIGNATOR AND HYPOTHALAMUS IN RATS TREATED WITH (823) PERFORMANCE DEGRADATION FOLLOWING THE INHA/STRUMENTED RATS TO ASSESS WORK (123) PERFORMANCE DURING TOXIC INHALATION EXPOSURE/BEHAVIORAL EVALUATION OF ANIMAL (1005) PERFORMANCE IN RATS./ISTRATION AND WITHDRAWAL: EFFECTS ON RADIAL ARM MAZE (1000) PERFORMANCE IN RATS./L DOSES OF IONIZING RADIATION ON SCHEDULE-CONTROLLED (999) PERFORMANCE IN THE MOUSE./ INTRAPERITONEAL CARBON MONOXIDE ON FIXED-RATIO PERFORMANCE./AN INDEX OF EPILEPSY-INDUCED CONVULSIONS AND CHANGES IN OPERANT (121) PERFUSED NASAL CAVITY/THE ISOLATED LUNWAY/ (179) PERFUSED IN SITU./LIC ACTIVATION OF THE PESTICIDE GUTHION BY MOUSE LIVERS (442) PERFUSED MOUSE LUNG/ DAMAGE TO CLARA CELLS BY NAPHTHALENE IN THE ISOLATED (975) PERFUSED PORCINE SKIN PLAP (TPSIP)/./IN, AND BENZOIC ACID IN THE ISOLATED (861) PERFUSED RABBIT LUNG./INHIBITION OF AMINOPYRINE METABOLISM IN THE ISOLATED (327) PERFUSED RABBIT HEART UPON RESUSCITATION OF THE DAJAL ACID RELEASE FROM ISOLATED (349) PERFUSED RABBIT LUNG./AMIDE ON THE DISPOSITION OF 5-HYDROXYTRYPTAMINE IN THE (327) PERKINS NEUTRALS TO 3,4,7,8-TRIMETHYLPHTHALIC ACID INHIBIT PLATELET-MEDIATED APPL (694) PERINATALLY TO CHLORINE DIOXIDE./CTIVE EFFECTS IN LONG-EVANS RATS EXPOSED (992) PERIPHERAL NEUROPATHY IN HUMANS./IBRATION SENSE AS A MEANS OF TESTING FOR (972) PERITONEAL CELL FUNCTION./IN SODIUM DIHYDRATE ON NORMAL AND POSTSURGICAL (511) PERITONEAL MACROPHAGES STIMULATED BY TUMO/CHIDONIC ACID RELEASE BY MURINE (506) PERITONEAL NEUTROPHILS IN VITRO./ROXIDE (02) RELEASE FROM STIMULATED, RAT (434) PEROXIDASE CATALYZED OXIDATION OF CATECHOL/ELPEROXIDASE AND HORSEPERIDASE- (434) PEROXIDASE CATALYZED OXIDATION OF CATECHOL/ELPEROXIDASE AND HORSEPERIDASE- (434) PEROXIDATION IS AN IMPORTANT PROCESSES AFTER INJURY OR PERTURBATION OF A (616) PEROXIDATION AND CELLULAR DAMAGE CAUSED BY THE PYRROLIZIDINE ALKALOID/LIPID (495) PEROXIDATION AND GLUTATHIONE CONTENT IN RA/D ALTERATIONS IN HEPATIC LIPID (97) PEROXIDATION AND LUNG INJURY./POLYMORPHONUCLEAR LEUKOCYTE-MEDIATED LIPID (494) PEROXIDATION BY 3,3'-DICHLOROBENZENE PRETR/ENHANCEMENT OF HEPATIC LIPID (494) PEROXIDATION IN MEMBRANE BILAYERS/CHOLESTEROL AND PHOSPHOLIPID (688) PEROXIDATION IN MICE/RCTION AND AGE ON LIVER ENZYME ACTIVITIES AND LIPID (656) PEROXIDATION IN MPP-INDUCED CITOTOXICITY/ FOR OXIDATIVE STRESS AND LIPID (656) PEROXIDATION IN THE BRAIN AND CORRELATION/ATION OF THERMAL INDUCED LIPID (1076) PEROXIDATION IN TRICHLOROETHYLENEM-MEDIANE TOXICITY/ROLE OF LIPID (665) PEROXIDATION OF BRAIN LIPIDS./CYANIDE AND (258) PEROXIDATION OF ISOLATED RAT LIVER NUCLEI./NT INHIBITION OF NADPH INDUCED (749) PEROXIDATION OF LIPOSOMAL MEMBRANES./HEROL AND ASCORBATE ON NO2-INITIATED (748) PEROXIDATION OF POLYUNSATURATED FATTY ACIDS (PUFA) 1/REACTION RATE OF NO2- (889) PEROXIDATION PRODUCTS BY INDUCED MPT-INDUCED CITOTOXICITY/OF ALDHYDE LIPID (435) PEROXIDATION/ OXIDATION OF BENZO-P-DIOXINS TO ALDHYDE LIPID (1057) PEROXIDATION/Y OF CCL4 AND TRICHLOROETHYLENE: ANY ROLE FOR ENHANCED LIPID (1057) PEROXIDATIVE ACTIVATION OF 3,3'-DICHLORO-BENZENEDINE TO MUTAGENIC PRODUCTS (437) PEROXIDATIVE BIORTRANFORMATION OF CYCLIC 1,3-DIKETONES (437) PEROXIDE, A NON-PHORBOL TUMOR PROMOTER, FAILS TO INHIBIT CELL-CELL/BENZOYL (134) PEROXIDE-MEDIATED CHROMOSOME DAMAGE IN VITRO BY PHE/INDUCTION OF HYDROGEN (255) PEROXISOMAL ENOYL-CoA HYDRATASE : 3-HYDROX/TRANSCRIPTIONAL STIMULATION OF (253) PEROXISOMAL ENZYME INDUCTION IN HEPATIC ENZYMES FROM A/COMPARATIVE STUDIES OF (658) PEROXISOMAL PROLIFERATIVNS AGENTS, DI-(2-ETHYLB/COMPARATIVE EFFECTS OF THE (225) PEROXSOME INDUCTION/L PHTHALATE (DIP) IN P-444 RATS: EFFECTS ON HEPATIC (761) PEROXISOME PROLIFERATION AND METABOLISM AFTER INH/SPECIES DIFFERENCES IN
POOPLATIONS WITH TIME-DEPENDENT EXPOSURE P/EAD IN THE PLASMA OF HIGH RISK
242 PORCINE LIVER SLICE./-MT AND INCUBATION MEDIUM ON THE CRYOPRESERVATION OF
975 PORCINE SKIN FLAP (IPPSF)./INE, AND BENZOIC ACID IN THE ISOLATED PERFUSED
640 PORPHYRIA STUDIES IN CROCODILE (P. MILI/PORT) IN HEPHIDOBENZENE (HCB)
556 PORPHYRIN OF THE HARDIER GLAND OF MICE./INFLUENCE OF PROLACTIN ON THE
580 POSTNATAL DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF FISCHER 344 RATS PO
243 POSTNATAL RAT HEPATOCYTES./OTEIN AND DNA SYNTHESIS IN PRIMARY CULTURES OF
390 POTENTIAL ONTOGENY./P MONOSOMAL GLUTAMATE SEVERELY DISRUPTS FLASH EVOKED
381 POTENTIALS (SEE) IN THE RODENT MODEL OF /OPATHY AND SOMATOSENSORY EVOKED
391 POTENTIALS./ SUSTAINED AND TRANSIENT COMPONENTS OF RAT PATTERN EVOKED
389 POTENTIALS./ KETAMINE PROPIONDLY ALTERS RAT FLASH EVOKED
46 POTENTIATES NITROGEN DIOXIDE (NO2) TOXICITY./EXERCISE
1050 POTENTIATION OF 2-ACETYLAMINOPHEN-INDANCED UNRAPPED DNA SYNTHESIS I
222 POTENTIATION OF ALLYL-ALCOHOL-INDUCED HEPATOTOXICITY BY CYANAMIDE
520 POTENTIATION OF CHARON TETRACHLORIDE HEPATOTOXICITY./VITAMIN A
1050 POTENTIATION OF CC14 HEPATOTOXICITY BY KET/ENCE OF THE CC14 DOSAGE ON THE
264 POTENTIATION OF RETINYL ACETATE (RA) HEPATOTOXICITY BY BUTYLATED HYDROXYT
231 POTENTIATION OF TAURULITHIC ACID-INDUCED/IN MELAHE ISOBUTYL KETONE
812 PPO DUST IN RATS./inhilation TOXICITY STUDIES WITH POLYPHENYLENE OXIDE (I
588 PRE-IMPLANTATION LOSS IN F-344 RATS BY METHY/TOXICITY IN THE INDUCTION OF
846 PRECLINICAL TOXICITY/DRUG METABOLISM DAT/A NOVEL SCENARIO FOR OBTAINING
432 PRECOCENCE II./TOXICOLOGIC SIGNIFICANCE OF THE BENZYLIC DOUBLE BOND OF
620 PRECONVULSANT AND ANTICONVULSANT MEMBRANE FLUIDITY EFFECTS.
733 PREDICTIVE VALUE OF ANIMAL TOXICITY TESTS FOR MAN./ASSESSING THE
72 PRENISOLONE 21-ACETATE/CYTES EXPOSED TO TAMEXIFEN, DIPHENYLHYDANTOIN, OR
722 PREGNATAL CLASS DRUGS./SAFE FACTORS APPLIED TO VARIOUS FDA
182 PREGNANCY/TOXICITIES OF ENDURIN AND PARAQUAT DURING
701 PREGNANT RATS/THE EFFECT OF PHENYLETHYL ALCOHOL APPLIED DERMALLY TO
713 PREIMPLANTATION LOSS IN RATS/ACRYLAMIDE (ACR) INDUCED
1013 PRENATAL KETAMINE./NEUROBEHAVIORAL DEVELOPMENT IN RATS AFTER
563 PRETREATMENT OF HUNOUS/HAZARDOUS W/SCXO CULTURE SYSTEMS TO IDENTIFY
17 PRESS INFORM THE PUBLIC: RISK REPORTING FOR JOURNALISTS/HELPING THE
680 PRETREATED WITH HIPPURIC ACID/L MALEATE ON GLUTATHIONE METABOLISM IN RATS
467 PRETREATED WITH STYRENE (S)./ ADMINISTRATION OF ACETAMINOPHEN (A) IN RATS
660 PRETREATMENT AND OXYGEN CONCENTRATION ON MENA/THE EFFECT OF PHENOBARBITAL
131 PRETREATMENT IN RATS./AGENICITY OF DIMETHYLTRIO-SAMINE FOLLOWING ETHANOL
601 PRETREATMENT OF RAINBOW TROUT, SALMO GARIOREI, WITH INDUCERS A/EFFECT OF
942 PRETREATMENT ON MORPHINE DISTRIBUTION IN RATS/EFFECTS OF ETHANOL/INDUCED
222 PRETREATMENT ON THE METABOLISM OF HALOTHANE/EFFECTS OF PHENOBARBITAL
478 PRETREATMENT ON THE METABOLISM OF BIS(2-ME/L OR BIS(2-METHOXYETHYL) ETHE
281 PRETREATMENT ON THE SUBCELLULAR DISTRIBUTION/INFLUENCE OF METAL AND STRESS
618 PRETREATMENT WITH DIELTHLIDITHIOCARBAMIC AC/RIDINE (MPTP) IN C57Bl MICE BY
855 PRETREATMENT ON THE METABOLISM OF ACRYLONITRILE (ACN) /EFFECT OF VARIOUS
829 PRIMATE BRAIN WITH MAGNETIC RESONANCE./IMAGING MANGANESE IN THE
687 PRIMATE HEPATOCYTES/CYRORESVOLUTION OF RODENT AND
770 PRIMATE NASAL CAVITY./MORPHOMETRIC ANALYSIS OF THE
808 PRIMATES./INJECTION OF POLYVINYLCHLORIDE (PVC) SMOKE IN NONHUMAN
767 PRIMATES/DROGEN CHLORIDE ON PULMONARY FUNCTION AND MORPHOLOGY IN NONHUMAN
451 PROCARBAZINE-INDUCED SPERMATOXICITY AND CHEMOTH/SEPARATE MECHANISMS FOR
164 PROGRAMMING TO COMMUNICATE TOXICITY TO NO/MOTION PICTURE AND SLIDE-TAPE
556 PROLACTIN ON THE PORPHYRIN OF THE HARDIER GLAND OF MICE./INFLUENCE OF
654 PROMOTER 2,2',4,4',5,5'-HEXABROMOBIPHENYL/(WB-F344) CELLS BY HEPATIC TUMOR
420 PROMOTER, FAILS TO INHIBIT CELL-CELL COMM/L PEROXIDE, A NON-PHOBOL TUMOR
336 PROMOTORS./ODEL SYSTEM FOR THE STUDY OF IRRITANTS, CARCINOGENS AND TUMOR
519 PROMOTORS./OF MOUSE HEPATOCYTE INTERCELLULAR COMMUNICATION BY LIVER TUMOR
511 PROMOTORS./ID RELEASE BY MURINE PERITONEAL MACROPHAGES STIMULATED BY TUMOR
425 PROMOTING ACTIVITY OF PHENOBARBITAL IS ASSOCIATED WITH ITS AB/LIVER TUMOR
411 PROMOTING EFFECTS OF SODIUM SALTS OF PHENOBARBITAL (Na, Pb/TOXIC AND TUMOR
144 PROMOTION BY DIETARY FAT IN B6C3F1 MALE MICE./HEPATIC TUMOR
395 PROMOTION OF TRANSFORMATION IN C3H/101/F2/ORPHOLOGICAL TRANSFORMATION AND
410 PROMOTION ON MOUSE SKIN./RHININE DECARBOXYLASE (ODC) INDUCTION AND TUMOR
674 PROLAMINagic/AGENTS/LATE CYCLASE (AC) IN ISOLATED MALE GERM CELLS (MGC) BY
1096 PROMOBLASTIC LEUKEMIA CELL DIFFERENTIATION/D MONOCYTIC INDUCERS OF HUMAN
674 PROMOBLASTIC LEUKEMIA HL-60 CELLS INDUCED /RING DIFFERENTIATION OF HUMAN
1097 PROTOPLANT TOXICITY OF SALMELLA TYPHIMURIUM, CHO/E TOXICITY OF
120 PROPAHOL AND ACETONE VAPORS IN THE NASAL CAV/DEPOSITION AND METABOLISM OF
117 PROPAHOL IN RATS./DISPOSITION AND METABOLISM OF INHALED 1-CHLORO-2-
936 PROPEPTAMPHS IN RATS./PHARMACOKINETICS OF
758 PROPERYLINE GLYCOC IN RATS/SUBCHRONIC INHALATION STUDY OF
662 PROSTAGLANDIN FORMATION BY THE DIRECT ADDITION OF DOXORUBIC/ALTERNATION OF
18 PROSTAGLANDIN H SYNTHASE-MEDIATED COXIDATION /MECHANISM OF INHIBITION OF
227 PROSTAGLANDIN/INSTANT ANTI INHIBITED HEPATOTOXICITY OFFERED BY AN E1 AND AN E2
811 PROTECT AGAINST PULMONARY INJURY/DIMETHYLSULFOXIDE (DMSO) DOES NOT
227 PROTECTION AGAINST ANTI INHIBITED HEPATOTOXICITY OFFERED BY A COMPARISON OF
250 PROTECTION FROM HEPATOCYTOLOGICAL TOXICITY &/ETASE ACTIVITY ASSOCIATED WITH
289 PROTECTION OF CADMIUM TOXICITY AND ENZYME INHIBITION BY DITHIOREIOTOL.
PROTECTION OF CHLOROENCEA POTENTIATED CC14 HEPATOTOXICITY BY PARTIAL HEPA

PROTECTION OF HEPATOTOXIC AND LETHAL EFFECTS OF CC14 BY PARTIAL HEPATECTO

PROTECTIVE EFFECT OF MAGNESIUM AGAINST NICKEL-INDUCED DNA DAMAGE AND CELL 25000-PROTECTIVE EFFECTS OF GLUTATHIONE ON NICKEL/EthyL MERCURY CYTOTOXICITY; THE PROTEIN (ABP) INDICATE GERMINAL EPITHEL/HANGES IN SERUM ANDRONE FI BRING

PROTEIN (PDBP) FROM RAT KIDNEY/AR WEIGHT NON-METALLOTHIONEIN LEAD-BINDING

PROTEIN ACCUMULATION AS THE INITIAL EVENT IN TRICYANOHYDRO INDUCED NEPHR

PROTEIN AND DNA SYNTHESIS IN PRIMARY CULTURES OF POSTNATAL RAT HEPATOCYTE

PROTEIN AND TOXICITY TO RABBIT RENAL PRO/DRYQUINONE (BHQ)-EQUIVALENTS TO

PROTEIN BINDING TO MOUSE SATELLITE DNA./THE EFFECT OF NICKEL ON SPECIFIC

PROTEIN CROSS-LINKING IN RAT LIVER NUCLEI BY HALOGENATED ACETALDEHYD/DNA-

PROTEIN FROM PATAS MONKEY TESTES AND POSITIONING OF A DNA-BINDING PROTEIN INTERACTION FOLLOWING CHROMATE TR/ANALYSIS OF ALTERATIONS IN DNA-

PROTEIN INTERACTIONS: STRUCTURE ACTIVITY-ROMATIC HYDROCARBON - 45 BINDING

PROTEIN KINASE ([AMP]-PK) ISOENZYME (II2)/LATION OF HEPATIC [AMP]-DEPENDENT

PROTEIN LEVELS ON ESTERASE ACTIVITY IN RATS./INFLUENCE OF DIETARY

PROTEIN METABOLISM PROVIDES A SENSITIVE INDEX OF THE REVERSAL OF ACETAMIN

PROTEIN(S) FROM RAT LIVER./SFFINITY PURIFICATION OF CLOFIBRIC ACID BINDING

PROTEIN INHIBITOR BY /POUTHE AND 1,2-/ INK ACTIVATION OF PLASMA alpha-R

PROTEINOLYSIS AFTER REPEATED INHALATION EXP/ES IN COLONIC STI MULATING

PROTEINS IN GH3 PITUITARY CELLS/ORYLATION OF DISTINCT RIBOSOME-ASSOCIATED

PROTEINS IN HENS./SLINKING AND DECREASED PHOSPHORYLATION OF NEUROFILAMENT

PROTEINS OF C57BL/6 AND DBA/2 MICE./(MDP) COMPOUNDS ON HEPATIC MICROSMAL

PROTEINS./CEPHALOSPORIN NEPHTOTOXICITY: EFFECTS ON RENAL MICROSMAL

PROTEINS/IMMUNOCHEMICAL DETECTION OF ACETAMINOPHEN-BOUND LIVER

PROTEINS ARE POTENT INHIBITORS OF EPIDERMAL GROWTH FACTOR/PHOTOACTIVATED

PUBLIC: RISK REPORTING FOR THE PUBLIC/HHELPING THE PATIENT

PUBLIC: THE ENVIRONMENTAL AND OCCUPATIONAL-BASED APPROACH TO INFORMING THE

PULMONARY ALVEOLAR MACROPHAGES (PAM) FROM/ OF FC-MEDIATED PHAGOCYTOSIS IN

PULMONARY ALVEOLAR MACROPHAGES IN VITRO./PER OXIDES TO RAT, MOUSE AND DOG

PULMONARY AND PATHOLOGICAL RESPONSES OF RATS TO ACUTE INHALATION OF SYNTH

PULMONARY BIOCHEMISTRY AND HISTOPATHOLOGY/EFFECTS OF INHALED PHOSGENE ON

PULMONARY CLEANSING OF COBALT IN RATS/KINETICS OF THE

PULMONARY LUNG/THYMIC RESPONSE/GENE EXPOSURE ON THE INFLUENZA

PULMONARY EFFECTS OF GALIUM ARISPER RAS/PHARMACOLOGICAL AND HISTOLOGICAL

PULMONARY ENZYMES AND METABOLISM IN RATS/INHALATION STUDY TO O3 AND NO2 ON

PULMONARY EXPOSURE TO BENZO(A)PYRENE ON IGA IMM/S EFFECTS OF SUBCHROMIC

PULMONARY FIBROSIS/DIMETHYLSULFOXIDE (DMSO) DOES NOT PROTECT AGAINST

PULMONARY FUNCTION AND MORPHOLOGY IN NONH/EFFECTS OF HYDROGEN CHLORIDE ON

PULMONARY EFFECTS ON RATS EXPOSED TO SUB-LETHAL CONCENTRATIONS OF

PULMONARY RESPONSE IN THE OUTBREAK OF A LITTLE WHITE MOUSE AFTER 6 WEEKS OF 5.0-

PULMONARY HYPERTENSION IN RABBIT/ROLE OF HEPARIN/REPLACEMENT OXIDATION

PULMONARY INFLAMMATION MEASURED BY BRONCH/OF HUMANS TO CADMIUM STIMULATES

PULMONARY INFLAMMATORY RESPONSE INDUCED BY EXPOSURE TO GL/CHRONOLOGY OF A

PULMONARY NATURAL KILLER CELL ACTIVITY/EFFECT OF OZONE ON

PULMONARY OXYGEN TOXICITY./LEUKOTRIENES IN

PULMONARY RESPONSE INDUCED BY DIURNAL OR NOCTURNAL OZONE /DIFFERENCES IN

PULMONARY FUNCTION OF GUINEA PIGS EXPOSED TO A COMBINATION OF ZNO-502/ THE PULMONARY SENSITIVITY RESPONSES/CATION WITH HDI OR DES-N, N-DIACETYL

PULMONARY ULTRASTRUCTURE/HALATION AND INTRAVASCULAR ROUTES OF EXPOSURE ON

PURIFICATION AND CHARACTERIZATION OF CYTOSOLIC EPOXIDE HYDROLYSE FROM HUMA

PURIFICATION OF CLOFIBRIC ACID BINDING PROTEIN(S) FROM RAT LIVER/AFFINITY

PURIFICATION OF RAT LIVER MICROSOMAL CYTOCHROME P-450 WITHOUT THE USE OF

PURIFICATION OF THE LOW MOLECULAR WEIGHT NON-METALLOTHIONEIN LEAD-BINDING

PURINES, SPERINE, SPERINE AND SPERINE BY RAT/QUESTRATION AND DISPOSITION OF

PVF SMOKE IN NONHUMAN PRIMATES./Y EFFECTS OF INHALED POLYVINYLCHLORIDE ( PV

PYRINE AND AMBUSH IN MALE MICE./BEHAVIORAL EFFECTS OF

PYRAZOLE IN MICE./SEX AND STRAIN SPECIFICITY IN HEPATOTOXICITY OF

PYRAZOLIDINONE ADMINISTERED BY GAVAGE TO MA./ [U-14C-PHENYL]-1-PHENYL-3-

PYRETHROID (BIFENTHRIN) ON THE SCIATIC NERVE O/THE EFFECTS OF A SYNTHETIC

PYRETHROIDS AND THE STRIATAL DOPAMINERGIC SYSTEM IN VIVO.

PYRETHROIDS: ACUTE LETHALITY VERSUS THE AU/AGE-DEPENDENT NEUROTOXICITY OF

PYRROLIDINES/PHENYL ETHER HERBICIDE SC-1084 IN THE CHICKEN./METABOLISM OF THE

PYRRODITICINE BROMIDE IN RAT LIVER CYTOCHROME P-450/EFFECT OF

PYRRODITICINE BRONZE ON THE MORPHOLOGY OF NEUROMUSCULAR JUNC/EFFECT OF

PYRRODITICINE ON TETANUS CONTRACTURE IN INNERVATED VS. DENERVA/EFFECT OF

PYRRODITICINE USING A VAGAL TONE MONITOR/HSUS MACAQUES TO ATROPINE AND

PYRIDOFENYL-ETHERS IN RATS AND MICE./COMPARATIVE DIETARY TOXICITY OF 2

PYRROLE-FORMING POTENTIAL OF 2,5-HEXANEDIONE/OMPARATIVE NEUROTOXICITY AND

PYRROLIDINE HERBICIDE/CISS DIFFERENCES IN MALE REPRODUCTIVE TOXICITY OF

PYRROLIZIDINE ALKALOID METABOLITES FROM MOUSE LIVER MICR/DETERMINATION OF

PYRROLIZIDINE ALKALOID SENECHONINE, THE ALK/CELLULAR DAMAGE CAUSED BY THE

PYRROLIZIDINE AND LARKSPUR ALKALOIDS DETECTED BY ALKALINE/GENOTOXICITY OF

PYRUVATE PRODUCTION AS SPECIFIC INDICES OF ALTERED SERTOLI CK/LACTATE AND

334
QUAIL AND CULTURED CHICK EMBRYO HEPATOC/IN ACCUMULATION IN MICE, JAPANESE
QUALITY OF FERRITS SELECTED FOR BIOLOGIC/ING PROCEDURES FOR IMPROVING THE
QUERCETIN MODIFIES HEPATIC TRANSFORMATION OF COOKED-FOOD MUTAGENS/DIETARY
QUINOLINE IN F344 RATS AND B6C3F1 MICE (/OF 1,2-DIHYDRO-2,2,4-TRIMETHYL-
QUINOME DETOXIFICATION./DT-DIAPHRAGASE AND
QUINONE REDUCTASE (QR) IN IN VIVO ETHANOL (EtOH) METABOLISM./ROLE OF
RABBIT AIRWAYS AFTER REPEATED EXPOSURES TO SOL/A MORMETRIC ANALYSIS OF
RABBIT ALVEOLAR MACROPHAGES TO RELEASE NEUTROPHIL AND MC/OZNE STIMULATES
RABBIT AND OVARIETOMIZED RAT./IRRITATION/SUBACUTE TOXICITY MODEL IN THE
RABBIT AND RAT SKIN FOR ASSESSING SKIN IRRITATION/A COMPARISON OF EX-VIVO
RABBIT CORTICAL SLICES./CITY OF CEPHALORSIDINE AND HEXACHLOROBUTADIENE IN
RABBIT FOLLOWING SINGLE INJECTIONS OF A NOVEL ENKPH/MUSCLE DAMAGE IN THE
RABBIT FOLLOWING SUBCUTANEOUS ADMIN/UE DISTRIBUTION OF ARSENIC IN THE
RABBIT KIDNEY./TES OF METAL ACTION ON PROXIMAL AMINO ACID REABSORPTION BY
RABBIT LUNG./INHIBITION OF AMINOPYRINE METABOLISM IN THE ISOLATED PERFUSED
RABBIT LUNGS/E MECHANISM OF THIOL UTILIZATION BY SMOKE CHALLENGED RAT AND
RABBIT RENAL PROXIMAL TUBULES (RPT)/QUIVALENTS TO PROTEIN AND TOXICITY TO
RABBIT SPECIES./MATERNAL TOXICITY AND FETAL MALFORMATIONS OF RODENT-
RABBIT/AC WITH SILVER/ICIDENT TO SILVADENE CREAM AS COMPARED TO SILVADENE CREAM IN THE
RABBIT/CURE ORAL TOXICITY OF CHEMICALS IN THE RAT AND THE
RABBIT/SUBCHRONIC DERMAL TOXICITY OF METSULFURON METHYL IN THE
RABBITS AND RATS-1/E GLYCOL PHENYL ETHER (EGPE): TOXICOLOGICAL EFFECTS IN
RABBITS GIVEN A DETERGENT BUILDER./CE OF TERATOGENIC RESPONSE IN RATS AND
RABBITS WITH CARBOFURAN TECHNICAL./REPEATED DOSE DERMAL TOXICITY STUDY IN
RABBITS./ES OF VANCOMYCIN (V) ADMINISTERED INTRAVENOUSLY (IV) TO RATS AND
RABBITS./OGY OF HALOTHANE-INDUCED ANTIGEN EXPRESSION IN HALOTHANE EXPOSED
RABBITS./SUBCHRONIC DERMAL TOXICITY STUDIES OF ETTHON TECHNICAL IN
RABBITS./ALIQUANTS OF AMETRYN TECHNICAL, A TRIAZINE HERBICIDE, IN RATS AND
RABBITS/CHARACTERISTICS OF LATE BRONCHIAL AND EARLY ALVEOLAR CLEARANCE IN
RABBITS/ENT EXPOSURE TO SULFURIC ACID AEROSOL ON MUCOCILIARY CLEARANCE IN
RABBITS./OF 4-NITRO-3-N-METHYLPHENALIMIDE [CARBONYL-14C] (4-NP) IN RATS AND
RABBITS/TENTAL OF DEZAGUANINE (CI-908), AN ANTICANCER AGENT, IN RATS AND
RADIATION EXPOSURE ON LOCOMOTOR ACTIVITY IN SWISS-EEFFECTS OF ACUTE GAMMA
RADIATION ENHANCES HYPOTHERMIA IN GUINEA PIGS./IONIZING
RADIATION ON SCHEDULE-CONTROLLED PERFORMA/OF SUBLETHAL DOSES OF IONIZING
RADIANT MEDIATED ISCHEMIA-REPERFUSION DAMAGE TO GERSIL BLOOD./OXYGEN FREE
RADIATION PRODUCTION AND ARACHIDONIC ACID RELEASE BY MURINE PERITONEAL /OXY
RADIATION IN STIMULATED PHAGOCYT/INHIBITION OF SUPEROXIDE ANION
RADIATION PRODUCTION, PHAGOCYTOSIS MEMBRAN/OLAR MACROPHAGE SUPEROXIDE ANION
RADIATION, POTENT REACTIVE INTERMEDIATES./INHIBITS THE FORMATION OF FREE
RADIOALLERGOSORBENT TEST (RAST) AND SKIN TEST /IN A; CORRELATION WITH THE
RADIOISOTOPIC LABEL IN RABT KIDNEY TISSUE FOLLOWING ADM/SCUBULAR LOCALIZATION OF
RADIOISOTOPE CAST AND METABOLIC STUDIES./ACQUISITION AND MANAGEMENT OF
RADON DECAY PRODUCTS: POLONIUM-210 DECORPORATION BY DMA.
RAST) AND SKIN TEST IN OCCUPATIONALLY E/TH THE RADIOALLERGOSORBENT TEST (}
RATINGS/ANOTHER APPROACH TO TOXIC HAZARD
rDNA HUMAN GM-CSF IN RHESUS MONKEYS/IMMUNOGENICITY OF CHO CELL-DERIVED
REABSORPTION BY RABBIT KIDNEY./TES OF METAL ACTION ON PROXIMAL AMINO ACID
REACTIVATION OF MAMMALIAN BRAIN ACETYLCHOLINES/KINETICS OF INHIBITION AND
RECEPTOR AND IN VIVO EFFECTS./LORDIBENZO-P-DIOXINS AS LIGANDS FOR THE Ah
RECEPTOR BINDING IN MOUSE LIVER/FLAC/CEPT}-${OUIRED OF GLUCOCORTICOID
RECEPTOR BINDING OF ENDOTHelial CELLS (E/CE MEMBRANE FLUIDITY AND INSULIN
RECEPTOR EXPRESSION BY DMA DETECTED USIN/INHIBITION OF INDUCTION OF IL-2
RECEPTOR FUNCTION IN RAT CNS AFTER CHRON/INISHED DOPAMINERGIC PRESYNAPTIC
RECEPTOR IN HAMSTERS ADMINISTERED 3H-2,3,7,8/LONG-TERM KINETICS OF THE Ah
RECEPTOR IN HEXACHLOROBENZENE (HCB) PORPHYRIA: STUDIES/ THE ROLE OF THE Ah
RECEPTOR IN THE DOWN REGULATION OF UTERINE AND HEPATIC EST/ROLE OF THE Ah
RECEPTOR LEVELS IN RATS./ DOWN REGULATION OF UTERINE AND HEPATIC ESTROGEN
RECEPTOR SITE./OF INTERACTION OF CADMIUM AND METHYL MERCURY WITH OVARAIN-
RECEPTOR./F) - SYNTHESIS AND CHARACTERIZATION AS A RADIOLIGAND FOR THE Ah
RECEPTOR./PHYSICOCHEMICAL PROPERTIES OF THE HUMAN AND RODENT Ah
RECOMBINANT HUMAN INTERFERON-ALPHA IN THE RHESUS MONKEY/PHARMACOKINETICS OF
RECOMBINANT HUMAN interleukin-2 in RODENTS/BSERVATIONS ON THE TOXICITY OF
RECOVERY OF LS171Y CELLS./S OF FORMYCINS AND DILAEZEP ON GROWTH ARREST AND
REDUCTASE (CCR) BY ACRYLAMIDE/AMIDE DEHYDROGENASE (LpDH) AND CYTOCHROME c
REDUCTASE (QR) IN IN VIVO ETHANOL (EtOH) METABOLISM./ROLE OF QUINONE
REDUCTION OF 5-NITROIMIDAZOLES/TIVE HYDROXYLAMINE INTERMEDIATE DURING THE
REDUCTION OF PENTVALENT TO TRIVALENT ARSEN/L CELLULAR UPTAKE AND METABOLIC
REFLEXES PRODUCED BY SOMAN/EPINES ANTAGONIZE THE FACILITATION OF THE SPINAL
REGENERATION./GLUCURONIC ACID (UDPGA) SYNTHESIS DURING LIVER NECROSIS AND
RNF-
S-9 METABOLIC ACTIVATING SYSTEM/NO NEUTRAL RED CYTOTOXICITY ASSAY WITH
S-9/PHENOLISOLATED METABOLISM IN HUMAN AND RAT HEPATOCYTES AND
SAFETY FACTORS APPLIED TO VARIOUS FDA PREGNANCY CLASS DRUGS.
SALICYLACETOSULFAPYRIDINE IN FISCHER-344 RATS/NO REPEATED DOSE TOXICITY OF
SALICYLACETOSULFAPYRIDINE TO RATS AND MICE/REPEATED DOSE TOXICITY OF
SALICYLACETOSULFAPYRIDINE IN RATS/NO LEVELS OF RNA IN RAINBOW TROUT
SALMO GAIFENI, WITH INDUCERS AND INHIBITION OF PRETREATMENT OF RAINBOW TROUT,
SALMONELLA TEST/BENEFICIALS BY RAT HEPATIC ENZYMES TO MUTAGENIC PRODUCTS IN THE
SALMONELLA TYPHIMURIUM TEST/CHLORO-BENZIDINE TO MUTAGENIC PRODUCTS IN THE
SAMPLE SIZES OF INCREASING THE STATISTICAL POWER OF THE EPIDEMIOLOGIC/REQURED
SCREEN CHICK EMBRYO RETINA CELL CULTURE AS AN IN VITRO TERATOGEN
SCREENING DATA IN TOXICOLOGY./AN APPROACH FOR THE STATISTICAL ANALYSIS OF
SCREEN AND FISCHER-344 (F344) RATS/LEIN: COMPARISON BETWEEN SPRAGUE DAWLEY
SECRATAL ACID D-INDUCED PULPAL CAM AND CFM CHANGES IN DEVELOPING MICE
SECRETION/DIRECT EFFECT OF HEAVY METAL IONS ON PITUITARY HORMONE
SEIZURES AND HEPATIC DRUG-METABOLIZING E/N EFFECT ON PICTOTOXIN-INDUCED
SELENIUM ACCUMULATION AND MORPHOLOGICAL CHANGES IN LIVERS OF ENVIRONMENTAL
SELENIUM ON ANTI-OXIDANT ENZYMES AND TRAC/ INFLUENCE OF DIETARY COPPER AND
SELENIUM ON SODIUM, CADMIUM, COPPER, ZINC, CALCIUM A/EFFECTS OF DIETARY
SELENIUM, CADMIUM, COPPER, ZINC, CALCIUM A/EFFECTS OF DIETARY SELENIUM ON
SELENIUM, COPPER, ZINC, CALCIUM AND IRON /S OF DIETARY COPPER ON CADMIUM,
SELENIUM-EXPOSED FRESHWATER FISH./VALUATION OF THE REPRODUCTIVE STATUS OF
SELENIUM/USING THE MUTATIONS OF CIGARETTE SMOKE CONSTITUTE TO THE
SENSITIVITY POTENTIAL OF SOME INDUSTRIAL COMPOUNDS IN T/COMPARISON OF THE
SENSITIVITY RESPONSES/TATION WITH HDL OR DES-N-ACETIVATOR EXHIBIT PULMONARY
SENSITIZATION STUDY IN HAIRLESS GUINEA PIGS WITH DCNB AND BENZOCAL/DERMAL
SENSITIZATION TO SUBLITISIN THROUGH INHALAT/SPONSOR FOLLOWING EXPERIMENTAL
SENSITIZERS BASED ON DATA FROM SEVERAL TES/F RELATIVE POTENCIES OF DERMAL
SENSORY NEURON/THE INTERACTIONS OF MONESSIN AND OUABAIN ON AN ISOLATED
SEIRA/DECREASED SUPEROXYDE ELABORATION IN THE ABSENCE OF NORMAL
SIKTO/INTERSTITIAL CELLS TO CADMIUM/EVENTUAL RESPONSE OF PRIMARY RAT
SIKTO CELL FUNCTION IN VITRO AFTER THE/N AS SPECIFIC INDICES OF ALTERED
SIKTO CELLS IN PRIMARY CULTURE./PRODUCE DIFFERENT SPECTRA OF EFFECTS ON
SIKTO CELLS IN VITRO/ALATE (MEHP) ON THE HORMONAL RESPONSIVENESS OF RAT
SIKTO-GERM CELLS CO-CULTURE DNA SYNTHEIS IN/EFFCRETE OF ME AND MA ON RAT
SERUM ANDROGEN BINDING PROTEIN (ABP) INDICATE GERMINAL EPITHEL/CHANGES IN
SERUM ASAL (ARGININOSUCINATE LYASE): A SENSITIVE INDICATOR OF HEPATOTOXIN
SERUM CHEMISTRY ALTERATIONS/CROCVYTSIS AERUGINOSA IN RATS: MORPHOLOGIC AND
SERUM COPPER AND PULMONARY HYPERTENSION IN MONOCOTILALINE PYRROLE-TREATED
SUPEROXIDE GENERATION IN THE PRESENCE OF NORMAL SERA./DECREASED
SUPEROXIDE GENERATION IN THREE SPECIES OF FREES/NITROAROMATIC CATALYSIS OF
SUPPORTER CELLS IN 3,4,5,3',4',5'-HEXACHLORODIBENZ
SURLAFTANTS./ACTE FOR EVALUATING THE SKIN IRRITATION POTENTIAL OF
SURGICAL IMPLANTS./A NEW MODEL TO STUDY THE INFLAMMATORY POTENTIAL OF
SURGICAL THYROIDECTOMY IN 2,3,7,8-TETRA/CATARY TRIPODOTHIONINE (T3) AND
SURVIVAL OF 16632 MICE/LUENCE OF VIRAL INFECTIONS ON TUMOR INCIDENCE AND
SUSCEPTIBILITY TO INFILTRATION IN MICE/EFFECT OF 03 EXPOSURE ON
SWINHE/RHINITE DECARBOXYLASE ACTIVITY (ODC) IN GASTRIC MUCOSA OF MINATURE
SWINE: COMPARISON OF INHALATION AND INTRAVASCULAR ACUTE T-2 MYCOTOXICOSIS IN
SWISS-WEBSTER MICE/CUTE GAMMA RADIATION EXPOSURE ON LOCOMOTOR ACTIVITY IN
SYNAPTOSOMES/(mMg) INDUCED Dopamine release FROM SUPERFUSED RAT STRIATAL
SYNERGISTIC INTERACTION BETWEEN NITROGEN DIOXIDE AND RESPIRABLE AEROSOLS.
SYNTHASE-MEDIATED COOCCIDATION OF XENOBIOTICS /HIBITION OF PROSTAGLANDIN H
SYNTHESIS AND HEPATOCYTIC HEMIOPHLOGENIC AND 6-BROMO-2,5-DIHYDROXO-THYIOPHEN
SYNTHESIS FOLLOWING AKOTOME./DECREASED AS NOVO ACHE
SYNTHESIS IN PRIMARY CULTURES OF HEPATOCY/INHIBITS THE ENHANCEMENT OF DNA
SYNTHESIS IN PRIMARY CULTURES OF POSTNATAL RAT HEPATOCYTE/PROTEIN AND DNA
SYNTHESIS IN RAT HEPATOCYES BY THE PEROX/LUORINE-INDUCED UNSCHEDULED DNA
SYNTHESIS IN RAT HEPATOCYES FOLLOWING IN V/DNA DAMAGE AND LACK OF REPAIR
SYNTHESIS IN VITRO/ OF ME AND MA ON RAT SERTOLI-GERM CELLS CO-CULTURE DNA
SYNTHESIS, ORNITHINE DECARBOXYLASE (ODC) /SIG-13-ACETATE (TPA)-INDUCED DNA

T AND B CELL PROLIFERATION IN VITRO./THE EFFECT OF PARAOXON ON MURINE
T CELL ACTIVITY WITHOUT INDUCTION OF SUPPRESSOR C/INHIBITION OF CYTOTOXIC
T LYMPOCYTE RESPONSE/SIGNE EXPOSURE ON THE INFLUENZA PULMONARY CYTOTOXIC
T-2 MYCOTOXICOSIS IN SWINE: COMPARISON OF INHALATION AND INTRAVASCULAR ACUTE
T-2 TOXIN IMMUNOMODULATION IS MEDIATED BY A NEUT-ILIKE RESPONSE.
T-2 TOXIN ON MITOTEN RESPONSIVENESS AND ANTIBODY PROD/IN VITRO EFFECTS OF
T-2 TOXIN ON THE MURINE ANTIBODY RESPONSE IN VIVO AND MITOTEN /EFFECTS OF
T-LYMOPHOCYTE INFILTRATION OF MOUSE LUNG FOLLOWING EXPOSURE TO OZONE
T3 UPTAKE AND DELAYS THE DEVELOPMENT OF LOCOMOTOR/CHLORINE DIOXIDE DEPRESSES
TAMOXYFIN, DIPHENYLHYDANTOIN, OR PREDNISOL/CULTURED HEPATOCYES EXPOSED TO
TAPE PROGRAMMING TO COMMUNICATE TOXICOLOGY TO N/MOTION PICTURE AND SLIDE-
TARGET SPECIFIC REAGENIC REAGENIC ACTIVITY: A DOSE RESPONSE STUDY.
TAUROCHOLATE (TC) EFFLUX FROM RAT HEPTIC CANALICULAR PUMP.
TAUROCHOLATE (TC) INTO ISOLATED FEMALE RAT-/-GLUCURONIDE (E217G) AND 3H-
TAUROLITHIOLIC ACID-INDUCED CHOLESTASIS/ISOBUTYL KETONE POTENTIATION OF
TBBC) AS A MODEL COMPOUND. S.J/4 USING 4'-THIOBIOS (4-t-BUTYL-m-CRESOL) AND
TBBC/AL EVALUATION OF THE ANTIOXIDANT 4,4'-THIOBIOS (4-t-BUTYL-m-CRESOL)
TBTO) TO THE DEVELOPING RAT ALTERS MORPH/NISTRATION OF TRIBUTYL TIN OXIDE
TC) IN THE RAT/ETHYLENE (TCE) AND ITS METABOLITE, TRICHLOROACETIC ACID
TC), CHLORAL (CH) AND TRICHLOROETHYLEN/REDUCED BY TRICHLOROACETIC ACID
TCDD EFFECTS THE BASAL LAMINA AND EXTRACELLULAR MATRIX OF THE FETAL MOUSE
TCDD IN AN ENVIRONMENTAL SAMPLE USING GENETICALLY /RESOLUTION OF 2,3,7,8-
TCDD IN THREE STRAINS OF MICE/INTERACTION OF ESTRADIOL AND
TCDD INDUCED ENDOOTOXIN HYPERSENSITIVITY/UDIES OF THE MECHANISM OF 2,3,7,8-
TCDD ON THE HEPATIC AND TESTICULAR DISTRIBUTION OF IRON, ZINC A/EFFECT OF
TCDD PRODUCES HYDRONEPHROSIS IN FETAL MICE BY INDUCING HYPERPLASIA OF THE
TCDD TREATED GUINEA PIGS AND HAMSTERS/IVIVITY AND ANTIESTROGENIC EFFECTS IN
TCDD TREATMENT/450 (CP-450) IN THE GUINEA PIG AND HAMSTER IN RESPONSE TO
TCDD ANTAGONIST IN RATS - AH INDUC/7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD AT DOSES ASSOCIATED WITH ACUTE TO/7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD FROM BODY FAT OF RHESUS MONKEYS P/7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD IN COLD-ADAPTED RATS FED DIFFER/7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD INDUCED LESIONS IN MALE SPRAGUE-D/7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD INDUCED LIPID PEROXIDATION/ON 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD ON PEER GROUP SOCIAL BEHAVIOR OF /7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD-INDUCED TOXICITY IN RATS/-/2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD-TREATED RATS/-/IN TESTES FROM 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD-TREATED RATS/ONAL STATUS IN 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD/HEXACHLOROBIPHENYL (HCB) AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD/Y NOT BE A PRIMARY EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD/R IN HAMSTERS ADMINISTERED 3H/2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD/SUITE THERMOCINESS INDUCED BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCDD/ION IN HAMSTERS TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
TCCH AND ALTERNATION IN HEPATIC LIPID PEROXIDATION IN HEPATIC LIPID PEROXIDATION IN
TCDD-INDUCED HEPATIC VITAMIN A DEPLETION./IETARY TRIODOTHYRONINE (T3) ON
TCDF - SYNTHESIS AND CHARACTERIZATION/2,3,7,8-TETRACHLORODIBENZOFURAN
TCE AND ITS METABOLITE, TRICHLOROACETAL TRANSFER OF TRICHLOROETHYLENELYTL
TCE IN RATS/ACID KINETICS OF INHALED AND INGESTED TRICHLOROETHYLENE
TCCE/BY TRICHLOROACETIC ACID (TCA), CHLORAL (CH) AND TRICHLOROETHYLENE
TETRACHLOROETHYLENE METABOLISM AND HEPATOTOXICITY

TETRACARBONYLPHOSPHORUS-13-ACETATE (TCA) - INDUCED DNA SY/MITATE (AP) ON 12-0-
75 TETRAFLOUROVOLENE IN RATS AND MICE/Ts OF 13-WEEK INHALATION EXPOSURE TO
TETRABORHYDO-FUROVOLENE/RESERVE SIMPLEX VIRUS 2 (HSV2) BY DELTA-9-
29 TETRABORHYDO-PHOSPHINOL ON RAT NEUROECTODERMAL TUMORS/EFFECT OF DELTA-9-
618 TETRAHYDROPYPYRIDINE (MTPP) IN C57BL MICE BY PR/ 1-METHYL-4-PHENYL-1,2,3,6-
1038 TETRAHEMEN PYRIFORMIS/G AN INDUCIBLE DNA REPAIR SYSTEM IN THE EUKARYOTE,
3 TETRALIN) RANOGICIS OF RETINOIC ACID./ SIDE-CHAIN MODIFIED (TETRAMETHYLATED
25 TETRABIS/SUBSTITUTED ALKYOXYACETOPHENONE/I IN RAT LIVER MITOCHONDRIA BY A
25 TETRABIS/SUBSTITUTED ALKYOXYACETOPHENONE/IN ISOLATED RAT HEPATOCYTES BY A
327 THALLIUM INDUCED LIPID PEROXIDATION IN THE /HISTOCHEMICAL LOCALIZATION OF
242 THAWING RATES, CRYOPROTECTANT AND INCUBATION MEDI/EFFECTS OF FREEZING AND
777 THEORETICAL POLARIZATIONS OF TOXIC INFLAMMATION (T)
300 THERAPY./ADMINISTRATION OF LEWISITE WITH OR WITHOUT BRITISH ANTI-LEWISITE
312 THERAPY./MOBILIZATION OF LEAD OVER THE COURSE OF DMA SCHALETION
805 THERMAL DECOMPOSITION OF SYNTHETIC LUBRI/ICYCLOPHOSPHATE ESTERS FOLLOWING
47 THERMAL DECOMPOSITION PRODUCTS OF POLYME/INGHALATION OF THESE GASES OR
494 THERMOGENESIS INDUCED BY 2,3,7,8-TETRACHLOR/ANGES OF BROWN ADIPOSE TISSUE
192 THERMOREGULATORY PROCESSES BY CHLORDEcone IN/EMENT IN THE MODIFICATION OF
17 THIRIUM IONS IN THE FORMATION AND REACTIONS OF S-(2-HALOETHYL)-L-CYSTE
750 THIOIS (6-T-BUTYL-6-CRESOL) (TBBC) AS A MODEL COMP/ITY WITH AGE USING 4-4'
899 THIOIS (6-T-BUTYL-6-CRESOL) (TBBC)/AL EVALUATION OF THE ANTIOXIDANT 4,4'-
199 THIOCARBAMATE HERBICIDE MOLINATE/EMENT AND VALIDATION OF AN ELISA FOR THE
879 THIOCYANATE (SCN) URINARY EXCRETION./YLONITRILE (AN) VAPORS AND RESULTING
734 THIOGUANINE (6T) COMBINED OR OF IRRADIATION/ 6-MERCAPTOPURINE (6MP) AND 6-
808 THIOI UTILIZATION BY SMOKE CHALLENGED RAT AND RABBIT LUN/THE MECHANISM OF
22 THIOPHENOL NEPHROTOXICITY: STRUCTURE ACTIVITY REQU/6-BROMO-2,5-DIHYDROX-
23 THIOPHENOL/SYNTHEIS AND NEPHROTOXICITY OF 6-BROMO-2,5-DIHYDROX-
101 TIOGLUTATHIONE IN RATS/AUSTRO TOXICITY OF 2-
836 TIOGLUTATHIONE OF BUTYRALDEHYDE (BA) IN F344 RAT
935 THIRTEEN WEEK SUBCHRONIC TOXICITY STUDY OF CROTONALDEHYDE (CA) IN F344 RA
51 THORACIC X-IRRADIATION./OCHEMICAL CHANGES IN LUNG LAVAGE FLUIDS FOLLOWING
703 THREE-GENERATION REPRODUCTION STUDY WITH DICOTYL SODIUM SULFOSUCCINATE (D
960 THREE-GENERATION TOXICITY STUDY OF RATS INJECTING GENTIAN VIOLET.
953 THRESHOLD FOR THE NEPHROTOXICITY OF D-LIMONENE IN MALE /DOSE AND TEMPORAL
1022 THRESHOLD OF CNS STIMULANTS IN RATS./T OF PERTUSSIS VACCINE ON CONVULSIVE
956 THROMBOCYTOPENIA ON PLATELET VOLUME AND RELATIVE D/EFFECT OF DRUG-INDUCED
505 THROMBOXANE IN PHORBOL MYRISTATE ACETATE-1/ ACTIVE OXYGEN METABOLITES AND
295 THYMIC AND SPLENIC POPULATION LISTS/CYTOME/ETRIC ANALYSIS OF
486 THYMIC ATROPHY/5786/6J AND DBA/2J MICE - EFFECTS ON ENZYME INDUCTION AND
905 THYMIC EFFECTS OF DIETHYL GLUCYLTHIONE (DOCT) AT A CELLULAR LEVEL./EARLY
551 THYROID CELLS IN CULTURE./ DIOXIDE ON IODINE UPTAKE AND ORGANIZATION BY
491 THYROID HORMONES AND THYRONINE GLUCRONIDATION IN HAMSTERS TREATED WITH 2-
488 THYROIDECTOMY ON 2,3,7,8-TETRACHLOROBENZO-P-DIOXIN (TCDD) IND/EFFECT OF
490 THYROIDECTOMY ON 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN/THYRONINE (T3) AND SURGICAL
486 THYMIC DEATH MECHANISMS/DIOXIN/ENHANCEMENT OF HEPATIC AND RENAL
489 THYMIC GLUCRONIDATION IN HAMSTERS TREATED WITH 2/THYROID HORMONES AND
1015 TIME DEPENDENT EFFECTS IN THE RAT./ ACOUSTIC STARTLE RESPONSE: DOSAGE AND
742 TIME FOR POST-EXPOSURE EFFECT OF LUNG GLUTATHIONE ON OZONE-INDUC/CRITICAL
108 TIME-DEPENDENT DEFICITS IN DELAY CONDITIONING PRODUCED BY TRIMETHYLIN.
309 TIME-DEPENDENT EXPOSURE PATTERNS/THE PLASMA OF HIGH RISK POPULATIONS WITH
803 TIMES./LOGICAL EFFECTS OF PURE AND MIXED FIRE GASES FOR VARIOUS EXPOSURE
116 TISSUE AS A TRANSFER SITE FOR PARTI/RETROSTERNAL, CAUDAL MEDIASTINAL
534 TISSUE CARBOXYLASE (ca2) AND CHOLI/2-ZIDOXOAPHTHSORNIC OXIDE (CBP) ON
300 TISSUE DISTRIBUTION OF ARSENIC IN THE RABBIT FOLLOWING SUBCUTANEOUS ADMIN
307 TISSUE DISTRIBUTION OF LEAD./AGING ALTERS THE
682 TISSUE FOLLOWING ADMINISTRATION OF COMP/ATION OF RADIOLABEL IN RAT KIDNEY
60 TISSUE GLUTATHIONE AND MERCAPTURIC ACID EXCRETION FOLLOWING ACUTE INHALAT
235 TISSUE SLICES FOR SPECIES COMPARISONS./ FOR OBTAINING METABOLICALLY ACTIVE
415 TISSUE SPECIFIC ONCOGENE ACTIVATION./CARCINOGEN AND
162 TISSUE UPTAKE OF NICKEL IN RATS DURING CONTINUOUS INTRAVENTRINAL INFUSION OF
630 TISSUE/BISMUTH ACUMULATION IN MAMMAL BRAIN
10 TISSUES./SYSTEM FOR SHORT-TERM IN VITRO GENOTOXICITY TESTING USING HUMAN
58 TISSUES./EFFECT OF CYCLODIENE COMPOUNDS ON CALMODULIN IN RAT
310 TISSUES./NOUS ADMINISTRATION MODE FOR LEAD INCLUSION DEVELOPMENT IN MOUSE
260 TLV'S AND ACUTE LC50S./D OF SUDDEN INHALATION EXPOSURE BY EXTRAPOLATION OF
303 TOXICITY OF TOLUENE DIISOCYANATE (TDI) AND TRIMELLITIC ANHYDRIDE (7
749 TOCOPHEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION O/EFFECT OF alpha-
536 TOCOPHEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION O/EFFECT OF alpha-
31 TOCOPHEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION O/EFFECT OF alpha-
215 TOCOPHEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION O/EFFECT OF alpha-
31 TOCOPHEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION O/EFFECT OF alpha-
31 TOCOPHEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION O/EFFECT OF alpha-
2 TOCOPHEROL AND ASCORBATE ON NO2-INITIATED PEROXIDATION O/EFFECT OF alpha-
109 TOLERANCE IN MICE/INDUCTION OF OXYGEN
95 TOLERANCE TO TRIMELLITIC ANHYDRIDE DURING A 13-WEEK /ACQUIRED IMMUNOLOGIC
TOLMETIN SODIUM DIHYDRATE ON NORMAL AND POSTSURGICAL PERITONEAL/EFFECTS OF
TOLUENE DIISOCYANATE (TDI) AND TRIMELLIT/A PIGS BY INHALATION EXPOSURE TO
TOLUENE IN ORALLY EXPOSED MALE RATS./ADSORPTION ALTERS BIOAVAILABILITY OF
TOLUENE ON MOTOR ACTIVITY IN RATS: DEPENDENCE OF /EFFECTS OF p-XYLENE AND
TOXIC EFFECTS OF CD-1 MOUSE AND NEUROCHEMICAL EXAMINATIONS OF
TOPOCAL ADMINISTRATION OF MACHELE HERBIC/HOR IN BUSSER MONKEYS FOLLOWING
TOPOCAL STEROIDS: IN VITRO STUDIES WITH MOUSE SKIN I/PERCUTANEOUS PATE OF
TOXAPHENE: A HIGH PERFORMANCE LIQUID CHRO/OF RAT BRAIN BIOPHISIC AMINES BY
TOXIKINETIC STUDIES OF BEAGLE DOGS EXPOSED BY INHALATION TO ERYLLIUM O
TOXIKINETICS AND METABOLISM IN THE RAT/-PENTACHLORODIBENZOFURAN (PDCF):
TOXIN IMMUNOMODULATION IS MEDIATE BY A STRESS-LIKE RESPONSE./ T-2
TOXIN ON MICE TUMOR RESPONSIVE/IN VITRO EFFECTS OF T-2
TOXIN ON THE MURINE ANTIBODY RESPONSE IN VIVO AND MITOGEN /EFFECTS OF T-2
TPA/-INDUCED DNA SYNTHESIS, ORNITHINE DECA/RADECANYLPHORBOL-13-ACETATE (T
TPP AND TCP AND THE RATE OF ABSORPTION/HIBITION OF NEUROTOXIC ESTERASE BY
TPP AND TRI-ORHO-CRESYL PHOSPHATE (T/COMPARISON OF TRIPHENYL PHOSPHITE (T
TRACE ELEMENT CONTENT OF RAST SERUM/THE EFFECTS OF LEAD AND DIET ON
TRACT (OT) AXONS IN 2,5-HEXANEDIONE (2,-TY-DEPENDENT ALTERATIONS IN OPTIC
TRAINING PROGRAM IN TOXICOLOGY./AUDIOVISUAL
TRANS-4-OM-2-HEXENAL, AND RELATED ALKENALS./LID SENECTION, THE ALKENAL
TRANS CISIONAL STIMULATION OF PEROXISOMAL ENOYL COA HYDRATASE : 3-HYDROX
TRANSFER ASSAY TO MEASURE INHIBITED CELL/TATION OF THE SCRAPE-LOADING/DYE
TRANSFERASE (GGT) BY ACVICININ AND ANTI-GGT/Y INHIBITION OF GAMMA-GUMTAMYL
TRANSFERASE (GST) TOWARDS AFLICTOXIN B-8,-/OWARDS AFLICTOXIN B1 AND GSH-S-
TRANSFERASE ACTIVITY IN VIVO AND IN VITRO/TMT ON RAT HEPATIC GLUTATHIONE
TRANSFERASE I IN RAT LIVER MITOCHONDRIA BY/IBITION OF CARNITINE PALMITOYL
TRANSFERASE./P SEQUENCE ON ACETAMINOPHEN AND BIOTRANSFORMATION AND GLUTATHIONE
TRANSFERASE./RAT LIVER MICROSOMAL CYTOCHROME P-450 AND UDP-GLUCURONOSYL-
TRANSFERASES AND PHENOLIC ANTI-OXIDANTS IN THE DETO/ROLE OF GLUTATHIONE S-
TRANSFERASES//DEFLOURINATION BY HEPATIC GLUTATHIONE S-
TRANSFORMATION AND PROGRESSION OF TRANSFORMATION IN C3H/10T1//MORPHOLOGICAL
TRANSFORMATION IN C3H/10T1/2 CL 8 MOUSE EMB/ANFORMATION AND PROMOTION OF
TRANSFORMATION/T TO TRIVALENT ARSENIC AS DETERMINANTS OF CYTOXICITY AND
TRANSFORMING ONCOGENES IN RAT TUMORS PRODU/ENCE OF DELECTABLE ACTIVATED
TRANSCRIPTION FACTOR FOR PARTICUL MEDISTINAL TISSUE AS A
TRANSPLANTED TUMORS./TION OF MINERAL FIBER INDUCED RAT PLEURAL TUMORS
TRANSPORT ACROSS BRUSH BORDER MEMBRANE VESICLES FROM RAT SMALL IN/CADMIUM
TRANSPORT IN ISOLATED RAT LIVER MEMBRANES/H OF MG2+-ATP-DEPENDENT CALCIUM
TRANSPORT IN SCIATIC NERVES OF ACR, 2,5,-HD AND D/ATP, CF, AND AXOPLASMIC
TRANSPORT OF DOXORUBICIN (Dox)/AXONAL DEGENERATION FOLLOWING RETROGRADE
TRANSFUSIONS OF PENTANOCIC ACIDS DERIVED FROM 2,2,4-TRIMETHYLPENTAN/THE RENAL
TRANSPORT/MERCU AND CHROMATE ION EFFECTS ON RENAL VESICLE MEMBRANE
TRENOR IN INTRATECHALLY PLANTED RATS/NARY STUDIES OF MOTOR ACTIVITY AND
TRENOR IN INTRASENTALLY PLANTED RATS/NARY STUDIES OF MOTOR ACTIVITY AND
TRIUMPHPHORETHANE (TECM) IN CD RATS/DEVELOPMENTAL TOXICITY OF 1,1,1-
TRICHLORETHANE EFFECTS ON LACTATE AND GLUCOSE METABOLISM
TRICHLOROACETIC ACID (TCA) IN THE RAT/EETHYLENE (TCE) AND ITS METABOLITE,
TRICHLOROACETIC ACID (TCA), CHLORAL (CH) AND/S IN HEPATIC DNA PRODUCED BY
TRICHLOROBENZO-P-DIOXINS AS LIGANDS FOR THE Ah REC/2-SUBSTITUTED-3,7,8-
TRICHLOROBENZOFURAN (MCDF) AS A 2,3,7,8-TETRACHLORODIBENZP-METHYL-1,3,8-
TRICHLORETHANE (TRI) IN INHALATION AND ORAL/KE AND ELIMINATION OF 1,1,1-
TRICHLORETHYLENE (TCE) IN RATS./PHARMACOKINETICS OF INHALED AND INGESTED
TRICHLORETHYLENE (TCE).//BY TRICHLOROACETIC ACID (TCA), CHLORAL (CH) AND
TRICHLORETHYLENE METABOLISM IN HUMAN AND RAT HEPATOCYTES AND S-9
TRICHLORETHYLENE-INDUCED NEPHROTOXICITY/ROLE OF LIPID PEROXIDATION IN
TRICHLORETHYLENE: ANY ROLE FOR ENHANCED LIP/HETAPOTOXICITY OF CC14 AND
TRICHLORETHYLENE-10-DAY TOXICITY STUDY FOR L.2-3-
TRICHOTOCESCES ON THE IMMUNE SYSTEM./THE IN VITRO EFFECTS OF
TRICHOTOCESCES./TOXICOLOGICAL STUDIES OF A NEW CLASS OF MACROCYCLIC
TRICYANOHANEXE INDUCED NEPHROPATHY IN MALE /ATION AS THE INITIAL EVENT IN
TRICYANOHANEXE ON RENAL LYSOSOMAL FUNCTION./EFFECT OF
TRICYCLIC ALCOHOL ETHOXYSATE IN RATS./SUBCHRONIC ORAL TOXICITY STUDY OF
TRIDIPHANE (2,-5-DICHLORO)-2(2,2,2-TRICHLORO-ETHYL)OXIRANE), A HERBIC DE
TRIDIPHANE (2,-5-DICHLORO)-2(2,2,2-TRICHLORO-ETHYL)OXIRANE), A HERBIC DE
TRIPLOMETANOL AND ITS RELATIONSHIP TO TOX/TLYZED METABOLISM OF 2,2,2-
TRIIODOTHYRONINE (T3) AND SURGICAL THYROIDC/ON OF THE EFFECTS OF DIETARY
TRIIODOTHYRONINE (T3) ON 2,3,7,8-TETRACHLORODIBENZO/EFFECT OF DIETARY
TRIIODOTHYRONINE (T3) ON TCDD-INDUCED HEPATIC VITAM/THE EFFECT OF DIETARY
TRILOSTANE/ACTH-INDUCED ADRENAL ADENOMAS IN RATS TREATED WITH

344
TRIMELLITIC ANHYDRIDE (TMA). /N EXPOSURE TO TOLUENE DISOCYANATE (TDI) AND
TRIMELLITIC ANHYDRIDE DURING A 13-WEEK INH/JURED IMMUNOLOGIC TOLERANCE TO
TRIMETHYL LEAD (TMB) IN THE RAT/NEUROPATHOLOGY OF
TRIMETHYL PHOSPHOROTHIOATE/TIONS FOLLOWING ACUTE ADMINISTRATION OF 0.05-
TRIMETHYLMAGNATE (TMB) METABOLITE BOUND TO M/IDENTIFICATION OF THE 2,2,4-
TRIMETHYLTRANSE (TMB) METABOLITES IN MALE FISH/NEPHROTOXICITY OF 2,2,4-
TRIMETHYLTRANSE (TMB) TO alpha-2U-GLOBULIN /F COVALENT BINDING OF 2,2,4-
TRIMETHYLTRANSE (TMB) TO RENAL alpha-2U GLO/REVERSIBLE BINDING OF 2,2,4-
TRIMETHYLTRANSE./ RENAL TRANSPORT OF PENTANOIC ACIDS DERIVED FROM 2,2,4-
TRIMETHYLMAGNILEIN IN FISHER 344 RATS./DISPOSITION OF 1,2-DIHYDRO-2,2,4-
TRIMETHYLMAGNILEIN (TMB) ON RAT HEPATIC GLUTATHIONE TRANSFERASE ACTIV/EFFECT OF
TRIMETHYLMAGNILEIN/RATS: EFFECTS OF DELAY, INTERMITTENT INTERVAL REVERSAL, AND
TRIMETHYLMAGNILEIN./TIME-DEPENDENT DEPILIS OF INTERVAL CONDITIONING PRODUCED BY
TRIPHENYL PHOSPHITE (TPP) AND TRI-ORTHO-CRESYL PHOSPHATE /A COMPARISON OF
TRITIUM/CARBON-14 (T/C) DUAL LABEL STUDY OF 1,2-DIBROMOBENZENE METABOLISM
TRIVALENT ARSENIC AS DETERMINANTS OF CYTO/LIC REDUCTION OF PENTAVALENT TO
TROUT (SALMO GAIREDNERI)/NOOXGENASE INDUCERS ON LEVELS OF RNA IN RAINBOW
TROUT MARKEDLY ALTERED ARYL HYDROCARBON/ATTACHMENT (TA) OF RAINBOW
TROUT, SALMO GAIREDNERI, WITH INDUCERS A/EFFECT OF PRETREATMENT OF RAINBOW
TROUT/ION OF TWO HEPATIC CYTCHROME P-450 ISOZYMES FROM TREATED RAINBOW
TROUT/PHARMACOKINETICS OF SULFAPRIMETHOXINE IN FREE SWIMMING RAINBOW
TRYPAN BLUE EXCLUSION: A POOR INDICATOR OF CELLULAR INJURY
TUBULAR SEGMENTS IN RENAL CORITICAL SLICE/VE IN VITRO NECROSIS OF PROXIMAL
TUBULE/ CELLS/ AFTER MERCURIC CHLORIDE EXPOSURE IN CULTURED RENAL PROXIMAL
TUBULE/ CELLS/ MERCURIC CHLORIDE INDUCED CYTOTOXICITY IN CULTURED PROXIMAL
TUBULE SEGMENTS./HYDROPEROXIDE-INDUCED TOXICITY IN RENAL PROXIMAL
TUBULES (RPT)/QUIVANTS TO PROTEIN AND TOXICITY TO RABBIT RENAL PROXIMAL
TUBULES/ CYCLOMETHYLMAGNILEINE ON ORGANIC ION TRANSPORT BY RAT RENAL
TUBULES/ PROVED IN VITRO SYSTEM FOR PROLONGED INCUBATION OF ISOLATED RENAL
TUBULIN CROSSTALKING/R./, 2,5HD AND DMED UNDER THE MICROSCOPIC MITOSIS AND
TUMOR CELL ANTIOXIDANT DEFENSES AND CELL KINETICS/EFFECTS OF HYPOXIA ON
TUMOR CELLS./ONITIZOLE-INDUCED DNA DAMAGE IN HYPOXIC AND NORMALLY AERATED
TUMOR EXPRESSION IN DIETHYLNLITROSAMINE /AL (PB) ADMINISTRATION ON HEPATIC
TUMOR INCIDENCE AND SURVIVAL OF B6C3F1 M/INFLUENCE OF VIRAL INFECTIONS ON
TUMOR INITIATING ACTIVITY BY COMPLEX OR/INHIBITION OF BENZO[ALPYRENE SKIN
TUMOR MICRONODIA/INDUCED DAMAGE OF MITOCHONDRIA DNA FROM MOUSE AND L1210
TUMOR PROMOTER 2,2',4,4',5,5'S-HEXABROMO/HYTAL (WB-F344) CELLS BY HEPATIC
TUMOR PROMOTER, FAILS TO INHIBIT CELL-CEL/BENZYL PERACETATE, A NON-PHORBOL
TUMOR PROMOTERS/ OF MOUSE HEPATOCYTE INTERCELLULAR COMMUNICATION BY LIVER
TUMOR PROMOTORS/ID RELEASE BY MURINE PERITONEAL MACROPHAGES STIMULATED BY
TUMOR PROMOTING ACTIVITY OF PHENOBARBITAL IS ASSOCIATED WITH ITS AB/LIVER
TUMOR PROMOTING EFFECTS OF SODIUM SALTS OF PHENOBARBITAL (Na.PB/TOKIC AND
TUMOR PROMOTION BY DIETARY FAT IN B6C3F1 MALE MICE./HEPATIC
TUMOR PROMOTION ON MOUSE SKIN./RNIQUENE DECARBOXYLASE (ODC) INDUCTION AND
TUMORIGENESIS BY NICKEL SULFIDE (NS) AND ME/SOMAL CHANGES ASSOCIATED WITH
TUMORS AND TRANSPLANTED TUMORS./TION OF MINERAL FIBER INDUCED RAT PLEURAL
TUMORS PRODUCED BY A SINGLE DOSE OF DIE/TED TRANSFORMING ONCOGENES IN RAT
TUMORS./TAL ON GROWTH AND PROGRESSION OF NATURALLY OCCURRING RODENT LIVER
TUMORS./TION OF MINERAL FIBER INDUCED RAT PLEURAL TUMORS AND TRANSPLANTED
TUMOR PROMOTORS./ODEL SYSTEM FOR THE STUDY OF IRITANTS, CARCINOGENS AND
TWO YEAR FEEDING STUDY-A COMPARISON OF/ ON THE SCIAITIC NERVE OF RATS IN A
TWO YEAR ONCOGENICITY STUDY OF DRODPANT(R) IN RATS
TWO YEARS WITH PHENYLEPHRINE HCL./OMYCOTMA IN MALE F344/R RATS DOSED FOR
TWO-GENERATION REPRODUCATION STUDY IN CD RATS EXPOSED TO CYCL/RESULTS OF A
TWO-GENERATION REPRODUCTION STUDY IN RATS WITH VIRGINIAMYCIN./A
TWO-WEEK GAVAGE ADMINISTRATION OF ETHYL ACYLATE IN CD RATS: EFFECTS OF THE FORESTOMA
TWO-WEEK WHOLE BODY AND NOSE-ONLY INHALATION TOXICITY O/COMPARISON OF THE

U-40461: ROLE OF EXTRA-CELLULAR CALCIUM/ THE FROG SLOW SKELETAL MUSCLE TO
UDP-GA ULTRAGENIC ACID (UDG) DEFLECTION ON THE DISPOSITION OF /OF HEPATIC UDP-GLUCRONIC ACID (UDG)
UDP-GLUCRONIC ACID (UDPGA) SYNTHESIS DURING 5'-PHOSPHOSULFATE (PAPS) AND
UDP-GLUCRONIC ACID REGULATION IN PAPS/5'-PHOSPHOSULFATE (PAPS) AND
UDP-GLUCURONOSYL TRANSFERASE./ RAT LIVER MICROSOMAL CYTCHROME P-450p AND
UDP-GLUCURONYL TRANSFERASE (UDPGT) ACTIVITY AND ANTIESTROGENIC EP/STEROID
UDPGA SYNTHESIS DURING LIVER NECROSIS / (PAPS) AND UDP-GLUCURONIC ACID (UDPGT)
ACTIVITY AND ANTIESTROGENIC EP/ROID UDP-GLUCURONYL TRANSFERASE (UDPGT) AND s-PHASE SYNTHESIS (SPS)
POLLO/EPATIC UNSCHEDULED DNA SYNTHESIS (UDS) IN XENOGRAFTS CONTAINING HUMAN BR/ON UNSCHEDULED DNA SYNTHESIS (UDS)
ULTRA-STRUCTURAL AND IN VIVO NMR STUDIES/TION BY ACUTE ARSENITE EXPOSURE:
ULTRASTRUCTURAL NEUROPATHOLOGICAL EFFECTS/L LEAD. PART 2: MICROSCOPIC AND
ULTRASTRUCTURE/HALATION AND INTRAVASCULAR ROUTES OF EXPOSURE ON PULMONARY

345
UNSATURATED ALDEHYDES ON MEMBRANE LIPID FLUIDITY A/EFFECT OF ALPHA, BETA-
UNSCHEDULED DNA SYNTHESIS (UDS) AND S-PHAS/ON OF THE INDUCTION OF HEPATIC
UNSCHEDULED DNA SYNTHESIS (UDS) IN XENOGRAFTS CONTAINING HUM/INDUCTION OF
UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYT/ 2-ACETYLAMINOFLUORENE-INDUCED
Aktivit/iten in liver subcellular /ons with rat hepatic effects on calcium
UPTAKE AND ELIMINATION OF 1,1,1-TRICHLOROETHANE (TCE) IN INHALE/RELATIVE
UPTAKE AND METABOLIC REDUCTION OF PENTAVALENT TO TRIVALENT ARSENIC/CELLULAR
UPTAKE AND ORGANIZATION BY THYROID CY/ECEPT OF CHLORINE DIOXIDE ON IODINE
UPTAKE BY RAT RENAL TUBULES./CRYLONITRILE AND BROMOBENZENE ON ORGANIC ION
UPTAKE BY THE BRAIN./CYSTEINE AND GLUTATHIONE ENHANCE METHYL/ALKY
UPTAKE OF 3H-ESTRADIOL-17beta-D-GLUCURONIDE (E217g) A/MIREX DECREASES THE
UPTAKE OF ACRYLONITRILE (AN) VAPORS AND INFLUENCE OF OSH DEPLETION ON THE
UPTAKE OF HALOALKANES ADMINISTERED BY G/EFFECT OF VEHICLE ON THE RELATIVE
UPTAKE OF NICKEL IN RATS DURING CONTINUOUS INTRAVENOUS INFUSION OF/TISSUE
UPTAKE OF VITAMIN E BY LUNG./EFFECT OF NO2 EXPOSURE ON THE
URETERIC EPITHELIUM/EPIPHYSIS IN FETAL MICE BY INDUCING HYPERPLASIA OF THE
URICOSURIC DRUG BENZBROMARONE./PEROXISOME PROLIFERATION INDUCED BY THE
URINARY BLADDER AND KIDNEY IN MALE F344 / (NA.PB) AND BARBITAL (NA.BB) ON
URINARY EXCRETION OF ACETAMINOPHEN (AA) /SPECIES VARIATION IN BILARY AND
URINARY EXCRETION OF MERCAPTURIC ACIDS 1-/4-CARBOXYLIC ACID (OTCA) ON THE
URINARY EXCRETION /YLNITRILE (AN) VAPORS AND RESULTING THIOCYANATE (SCN)
URINARY MERCAPTURIC ACID IN THE RAT./ MASS SPECTROMETRIC DETECTION OF ITS
URINARY METABOLITES OF ETHOXYQUIN./OVINE
URINARY METABOLITES OF ISOPROPYLCYCLOHEXANE IN MALE F-344 RATS WITH HYDRO
URINARY METABOLITES OF N-NITROSOTHIAZOLIDINE (N-NT)
UROPORPHYRIN ACCUMULATION IN MICE, JAPANES/N DECARBOXYLASE ACTIVITIES AND
UROPORPHYRINogen BY CHICK EMBRYO LIVER SUPER-PHENYL-MEDIATED OXIDATION OF
UROPORPHYRINogen DECARBOXYLASE ACTIVITIES AND UROPORPHYRIN /COMPARISON OF
UTERINE AND HEPATIC ESTROGEN RECEPTOR LE/CEPTOR IN THE DOWN REGULATION OF
UV-LIGHT/IZATION OF BACILLUS THURINGIENSIS STRAIN HD1-1 AND HD2-1 AGAINST

V79 METABOLIC COOPERATION ASSAY./ATILE AND NONVOLATILE CARCINOGENS ON THE
VACCINE ON CONVULSIVE THRESHOLD OF CNS STIMULANTS IN /EFFECT OF PERTUSSIS
VAGAL TONE MONITOR/REHESUS MACAQUES TO ATROPINE AND PYRIDOSTIGMINE USING A
VAGOTONIC INITIATING /TRIGGER MODEL IN THE RABBIT AND G/A RELEVANT
VAGINALLY ADMINISTERED (14) LABELED NONOX/E DISPOSITION AND METABOLISM OF
VALIDATION OF AN ALTERNATIVE TEST SYSTEM: THE MOU/DESIGN, DEVELOPMENT AND
VALIDATION OF AN ELISA FOR THE THIOCARBAMATE HERBICIDE MOL/REPELS AND
VALIDATION OF AN IN-VITRO TEST FOR ASSESSING THE SKIN CO/INTRA-LABORATORY
VALPROIC ACID TOXICITY IN PRECISION-CUT LIVER SLICES.
VANADIUM INHIBITION OF YEAST GLUCOSE-6-PHOSPHATE DEHYDROGENASE.
VANCYCLINE (v) ADMINISTERED INTRAVENOUSLY (IV) TO R/TERATOLOGY STUDIES OF
VAPORS AND RESULTING THIOCYANATE (SCN) / THE UPTAKE OF ACRYLONITRILE (AN)
VAPORS IN THE NASAL CAVITY./SITION AND METABOLISM OF PROPANOL AND ACETONE
VEHICLE ON THE RELATIVE UPTAKE OF HALOALKANES ADMINISTERED BY G/EFFECT OF
VEHICLES ON ACUTE HEPATOTOXICITY OF CARBON TETRACHLORIDE/EFFECT OF ORAL DOSING
VEHICLES ON THE PHARMACOKINETICS OF ORALLY ADMINISTERED /EFFECT OF DOSING
VENOMS IN PRIMARY ENDOTHELIAL CELL CULTU/ THE TOXIC EFFECTS OF MAMBA SNAKE
VENOMS IN PRIMARY MYOCARDIAL CELL CULTUR/ THE TOXIC EFFECTS OF MAMBA SNAKE
VENTRICULAR FIBRILLATION IN THE RABBIT: INFLUENCE OF S/ISOPROTERENOL-INDUCED
VESICLE MEMBRANE TRANSPORT/MERCURY AND CHROMATE ION EFFECTS ON RENAL
VESICLES (CMV) BY GLUTATHIONE DISULFIDE /RAT HEPATIC CANALICULAR MEMBRANE
VESICLES FROM RAT SMALL INTESTINE./TRANSPORT ACROSS BRUSH BORDER MEMBRANE
VIBRATION SENSE AS A MEANS OF TESTING FOR /RELIABILITY AND SENSITIVITY OF
VINCA ALKALOIDS INHIBIT THE NEURON PERIKARYAL RESPONSE TO AXOTOMY.
VINYL CYCLOHEXENE (VCH) IN RATS AND MICE/OPTION OF ORALLY ADMINISTERED 4-
VINYLIDENE FLUORIDE (VDF) IN RATS SIMULATED BY A PHYSIOLOGICAL/UPAKE OF
VITAMIN A DEPLETION I/ETARY TRIOIODOTHYRONINE (T3) ON TCDD-INDUCED HEPATIC
VITAMIN A METABOLISM/4'-5'-HEXACHLOROBIPHENYL (HCB) ALTERS THE DYNAMICS OF
VITAMIN A POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY.
VITAMIN E BY LUNG./EFFECT OF NO2 EXPOSURE ON THE UPTAKE OF
VITAMIN E SUCCINATE PROTECTS HEPATOCYTES FROM CHEMICAL TOXICITY.
VITAMIN K IN DOGS/PHARMACOKINETICS AND BIOAVAILABILITY OF

346
VOLATILE ANESTHETICS ON THE BILIARY CLEARANCE OF CHOLEPHILIC CH/EFFECT OF
VOMITOXIN (DEOXYNIVALENOL) ON INFANT CYTOMOLGUS MONKEYS./EFFECT OF

WASTE DISCHARGE ON AN AQUATIC ECOSYSTEM./IMPACT OF A PAPER MILL
WASTE MIXTURES./LETHALITY AND HEPATOTOXICITY OP COMPLEX
WASTE SITES/RE SYSTEMS TO IDENTIFY PRENATAL TOXIC COMPONENTS OF HAZARDOUS
WASTE WATER WORKERS/REPRODUCTIVE ASSESSMENT OF MALE
WATER QUALITY CRITERIA FOR MUNITION COMPOUNDS.
WATER THROUGH SHOWERING, BATHING OR USE/BESTOS FROM CONTAMINATED DRINKING
WATER WORKERS/REPRODUCTIVE ASSESSMENT OF MALE WASTE
WATER (ON RAT FORESTOMACH/THE EFFECTS OF ETHYL ACRYLATE (IN DRINKING
WATER./HEALTH EFFECTS OF CORROSION PRODUCTS IN DRINKING
WB CELLS BY PEB AND DIELDRIN./EASURE INHIBITED CELL-CELL COMMUNICATION IN
WB-P344) CELLS BY HEPATIC TUMOR PROMOTER/COOPERATION IN RAT EPITHELIAL
WEEK NOSE-ONLY INHALATION STUDY IN FISCHER 344 RATS./CHLOREPYRIFOS: 13-
WISTAR RAT BY ZINC TREATMENT: DOSE AND / OF CADMIUM CARCINOGENESIS IN THE
WORK PERFORMANCE DEGRADATION FOLLOWING/OVING INSTRUMENTED RATS TO ASSESS
WORKERS EXPOSED TO OXADIAZON./ASSESSMENT OF CANCER RISK IN
WORKERS FOR ORGANO-PHOSPHORUS INDUCED DELAYED NE/SCREENING PESTICIDE PLANT
WORKERS/REPRODUCTIVE ASSESSMENT OF MALE WASTE WATER

X-IRRADIATION./OCHEMICAL CHANGES IN LUNG LAVAGE FLUIDS FOLLOWING THORACIC
X-RAY ANALYSIS AS A DIAGNOSTIC ADJUNCT FOR /APPLICATION OF ELECTRON PROBE
X-RAY FLUORESCENCE./IVE DETECTION OF TIBIAL BONE LEAD IN INTACT LEGS BY L
XENOBIOLOGICS BY MITHIMAGOLE/OSTAGLANDIN H SYNTHASE-MEDIATED COOXIDATION OF
XENOGRAFTS CONTAINING HUMAN BRONCHIAL EPI/CHELED DNA SYNTHESIS (UDS) IN
Xenopus laevis/TERATOGENIC EFFECTS OF ORGANOPHOSPHORUS INSECTICIDES IN
XYLENE AND TOLUENE IN MOTOR ACTIVITY IN RATS: DEPENDENCE OF/EFFECTS OF P-
XYLENE INDUCES CYTOCHROME P-450 AND ENHANCES THE EFFECT OF CARBON TETR/P-
XYLENE ON CEREBRAL MEMBRANES/EFFECTS OF P-
XYLENE'S ALTERATION OF THE RAT LUNG MICROSOMAL MEMBRANE./P-
XYLENE/ATION-DEPENDENT CONDITIONED FLAVOR AVersions INDUCED BY INHALED p-

YEAR DIETARY TOXICITY STUDY IN BEAGLE DOGS ADMINISTERED/RESULTS OF A ONE-
YEAST GLUCOSE-6-PHOSPHATE DEHYDROGENASE./VANADIUM INHIBITION OF
YOELII AND P. BERGHEI IN B6C3F1 MICE/ENE ON HOST RESISTANCE TO PLASMODIUM

ZINC AND COPPER IN RATS/ THE HEPATIC AND TESTICULAR DISTRIBUTION OF IRON,
ZINC AND NICKEL REDUCE CADMIUM-INDUCED MORPHOLOGICAL CHANGES IN 3T3 FIBRO
ZINC CONCENTRATIONS IN HUMANS: COMPART/ECTS OF EDTA CHELATION ON LEAD AND
ZINC DEFICIENT DIET IN ESOPHAGEAL CARCINOGENESIS/TIME OF FEEDING
ZINC TREATMENT: DOSE AND ROUTE DEPENDENCE/ARCINOCINEIISIS IN THE WISTAR RAT BY
ZINC, CADMIUM AND DEXAMETHASONE (DEX) IN/OF METALLOTHIONEIN (MT) ISOFORMS BY
ZINC, CALCIUM AND IRON IN THE EYE OF CA/UM ON SELENIUM, CADMIUM, COPPER,
ZINC, CALCIUM AND IRON IN THE EYE OF CA/PER ON CADMIUM, SELENIUM, COPPER,
ZINC./) AND METALLOTHIONEIN-II (MT-II) LEVELS FOLLOWING ADMINISTRATION OF
ZN, AND HG IN THE MOUSE CEREBELUM/DISTRIBUTION OF FE, CU,
ZNOSO2 AEROSOLS/ SULFUR OXIDES ON THE SURFACE OF FRESHLY GENERATED ULTRAFINE
ZNOSO2 AEROSOLS FOLLOWING THE 'WORKEWEEK/PIGS EXPOSED TO A COMBINATION OF
ZONISAMIDE IN BEAGLE DOGS/CHRONIC TOXICITY OF THE ANTICONVULSANT
<table>
<thead>
<tr>
<th>Name</th>
<th>Team</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butler, L.E.</td>
<td>Butternut, B.</td>
<td>194</td>
</tr>
<tr>
<td>Bowman, A.J.</td>
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</tr>
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<td>39</td>
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</table>
Harbison, R.D. 1088
Hardisty, J.E. 297
Hardy, C. 794
Harkema, J.R. 742
Harmsen, A.G. 318
Harrington, R.M. 551
Harrington, W.W. 781
Harris, C. 563,724
Harris, J.E. 209
Harris, M. 4,71
Harris, M.W. 298,636
Harrison, J.R. 1000
Hartman, H. 545
Hartsoy, M.A. 53
Harvis, C.A. 612
Haschek, W.M. 228,811
Haschek-Hock, W. 787
Hasegawa, N. 1026
Haseman, J.K. 122
Hasewaga, L. 853
Haskins, E. 298
Hastings, C.E. 817
Hathe, S.S. 40
Hatherill, J.R. 97
Hatt, N.S. 95,338
Hawkins, D.R. 949
Hawkins, N.J. 949
Hayes, H.T. 902
Hayes, J.R. 1048
Hayes, T.J. 100
Hazelette, J.R. 260,261,262
Hazeltine, G.A. 817
Heath, J.R. 685,1083
Heck, H.D.A. 187
Heffelf, R.H. 698
Heidelberg, N. 891
Heidkand, R.E. 558,559,560
Hein, J.F. 448
Heinl, S.W. 41
Heisterkamp, S. 630
Heitmeyer, S.A. 321,987,1012
Heitmannik, M.R. 723,983
Hendel, T.J. 1093
Henderek, B.M. 743
Henderson, E.E. 539
Henderson, J.D. 608
Henderson, M.C. 429
Henderson, R. 117,119,125,161,742
Hendricks, J. 605
Henningsen, G. 453,454
Henry, E.C. 491
Henwood, S. 703
Hermansky, S.J. 488
Heitz, D.W. 1004
Hertzberg, V. 721
Hertzberg, U. 609
Hesse, E.J. 502,633,643
Heisterberg, T.W. 1033
Hewett, A.A. 506
Hewitt, W.R. 351
Hickem, P.M. 30,761
Heydens, W.F. 692,765,786
Hicks, J. 329
Higgins, C.V. 72
Higgins, R.J. 539
Hight, R.J. 23
Higet, S. 965
Higuchi, K.M. 932
Hilaski, R. 796
Hill, D.M. 880
Hillebrand, D.A. 32
Himel, C.M. 202
Hincks, J.R. 66,132
Hinders, R.K. 767,807
Hinson, J.A. 16,187
Hirano, M. 402
Hjelle, J.J. 518,860,1064,1065
Hjelle, J.T. 518
Hofb, C.H. 799,790
Hofb, E.J. 768,827
Hobberman, A.M. 707
Hobson, D.W. 805
Hochstein, J.R. 1101
Hochstein, P. 63
Holcolm, M. 642
Holloom, I.L.M. 915
Holten, A. 666
Holley, D.C. 555
Hollinger, J.O. 817
Hollingsworth, R. 865
Holohan, P.D. 359
Holsapple, M.P. 37,37,899,909,910,916,917
Holscher, M.A. 325
Holt, R. 803,804
Homan, R.E. 691
Hood, R.D. 561
Hosmer, H. 797
Hook, J.B. 110,350
Hooser, S.B. 228
Hoover, M.D. 118
Horada, R.A. 743
Horim, M.G. 451
Horton, V.L. 932
Howard, L.C. 934
Howard, W.B. 9
Howell, S.R. 272,466,878
Hsta, M.T. 432,1029
Hsieh, G.C. 990
Hsieh, A.W. 510
Hsieh, D.P. 133
Hsieh, L.S. 133
Huang, M.T. 410
Huang, X.S. 585
Hubbard, A.K. 798,799,924,925,926
Hubner, S.H. 844
Hudnall, H.K. 389
Hudnett, L.H. 718
Hughes, E.W. 718
Hui, J.M. 951
Huijer, J.C. 26
Hulin, T.A. 264
Hampehryes, J.E. 777
Hunt, W.A. 1024
Hussein, G.I. 586,588,778
Huston, T.B. 452,594
Hutchinson, A.P. 851
Hwang, K.K. 904
Hyde, E.G. 943
Hysmith, R.M. 1104
Iba, M.M. 371
Ichida, T. 136,449,1040
Iglehart, J.D. 26
Illegwuxwenu, F.I. 138
Illing, J.W. 98
Imamichi, T. 1026
Imamura, T. 853
Imura, N. 323
Inamura, T. 767,807
Inoan, Y.M. 974
Iorio, K.R. 998
Irvin, R. 562,565
Irvin, R. 1012
Ison, G.E. 665
Istok, A. 885
Itu, N. 419
Iverson, F. 826
Jackowski, S.J. 522
Jacobs, A.J. 150
Jamil, A. 204,285,286,287,288,627,628
James, M.D. 610
James, R.C. 1088
Jameson, C.W. 902
Jane, J.D. 623
Jarvis, B.B. 830
Jaskol, R.H. 48,745
Jayskakara, S. 34,626
Jeffery, E.H. 894
Jenkins, W.L. 484,576
Jensen, R.K. 413,1089
Jersey, R. 1018
Ji, S. 455,549,736,737
Jianxing, F. 349
Jimerson, V.R. 530,534
Jin, R. 29
Jinna, R.R. 289,604,622
Johannsen, F.R. 107,108,499,499
Johnson, M.B. 367
Johnson, G.L. 704,705,760
Johnson, J.B. 702,719
Johnson, K.A. 107,108,587,935
Johnson, K.W. 861
Johnson, D.E. 39
Johnson, D.E. 948
Joiner, R.L. 300
Jones, A.D. 482,947
Jones, K.W. 302,303
Jones, L.S. 378
Jones, M.M. 325,571
Jones, R.C. 1037
Jones, S.G. 571
Jones, W.G. 814
Jortner, B.S. 910,917
Joseph, X. 367,374
Joshi, U.M. 808,1051,1056
Jovanovich, L. 184
Juchau, M.R. 563,724
Junghans, T. 164
Jurek, M.A. 490,492
Kacew, S. 113
Kado, Y.N. 133
Kajimura, T. 556
Kafel-Erraz, I.A. 308
Kalf, G.P. 870
Kallman, M.J. 1007
Kaminsky, M. 1026
Kaminsky, N.E. 910
Kaminsky, L.S. 91,141,635
Kamps, C. 71
Kamras, A.A. 169
Kandasamy, S.B. 1024
Kanekal, S. 442
Kao, J. 976
Kapezhic, I.C. 824
Kaphalia, B.S. 1068
Kapp, H.L. 767,807
Kapp, M.C. 410
Kaplowitz, N. 256
Kapp, R.W. 825
Karaszewicz, G. 870
Kari, F.W. 547,548