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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 25th Anniversary Meeting of the Society of Toxicology, held at the Hyatt Regency Hotel, New Orleans, LA, March 3–7, 1986.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 319. 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), appears on pages 409–421.

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INCREASED PROTEIN COVALENT BINDING OF BENZO(A)-PYRENE (BaP) IN BUTHIONINE SULFOXIMINE (BSO) TREATED HUMAN T47D CELLS. D.A. Merrick and J.K. Selkirk, Biology Division, Oak Ridge Natl. Lab., P.O. Box Y, Oak Ridge, TN 37831. Spons: R.G. Schnell

Metabolism of BaP in cell culture results in covalent protein adduct formation in cells and with extracellular (EC) protein in culture medium. This study examined the effect of BSO and BaP (cold) treatments upon (1) cellular glutathione (GSH) levels and (2) intracellular protein covalent binding (PCB) after 3-48 hr exposure to 3H-BaP in human mammary epithelial tumor cultures. BSO (0.5 mM) depleted cellular GSH levels to 52% after 6 hr and to <10% after 48 to 72 hr. BaP (4μM), however, increased GSH levels which remained elevated >50% of control at 24-72 hr. Next, GSH levels were depleted or elevated with BSO or BaP vs control for 48 hr prior to receiving 3H-BaP (6 Ci/mole, 4μM) with PCB measured from 3-48 hr afterward. Peak specific 3H-BaP PCB occurred after 18 hr of incubation according to the order: BSO (100%) > BaP (100%) > control (50%). EC-PCB continued to increase up to 48 hr according to the order: BaP (35%) > control (100%) > BSO (93%). 3H-BaP PCB took place in control and BaP treated cells despite adequate levels of GSH. These studies suggest that not only are GSH depleted cells at risk to BaP electrophiles but also GSH adequate cells are susceptible to BaP adduct formation as well (Research sponsored by U.S. DOE, No. DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.)

4-BROMOCATECHOL (CAT) FORMATION FROM BROMOBENZINE IN RATS IN VIVO, IN PERFUSED LIVER, AND IN ISOLATED HEPATOCYTES. D.A. Dankovic and R.E. Billings, Univ. of Texas Med. School, Pharmacology Dept., P.O. Box 20708, Houston TX 77225.

Catechols are potentially reactive metabolites of aromatic compounds, and they may be formed by either hydroxylation of phenolic metabolite or dehydrogenation of a dihydrodiol (DHD). We have previously shown that CAT is formed from bromobenzene (BB) primarily via DHD, in isolated rat hepatocytes. By examining the retention of deuterium in metabolites of 4-deuterio-BB, we have now determined the route of CAT formation in various experimental models. Formation of CAT via DHD is expected to remove the deuterium completely, while CAT formed by two successive hydroxylations should retain 50% of the label found in 4-bromophenol (4-OH). GCMS determination of the deuterium content of 4-OH and CAT formed from 4-deuterio-BB indicates that the percent of CAT formed by two successive hydroxylations is < 5% in isolated hepatocytes, < 6% in isolated perfused liver (either single-pass or recirculating perfusion), and 34% in vivo. These results indicate that CAT is formed in the liver primarily via the DHD. The discrepancy between the results in vivo and those obtained with isolated hepatocytes or isolated perfused liver suggests that extrahepatic tissues may be involved in BB metabolism in vivo. Supported by NIH grants ES06286 and HD0646.

METABOLIC ACTIVATION OF PHENOL BY HUMAN MYELOPEROXIDASE. D.A. Eastmond, D. Ross, L.O. Buro, and M.T. Smith, School of Public Health and Department of Entomology, University of California, Berkeley, CA

The myeloperoxidase-mediated conversion of benzene metabolites to more reactive products within the bone marrow may be involved in the hematopoietic toxicity observed in occupational exposure to benzene. A series of experiments were conducted to determine the metabolism and protein-binding of phenol during peroxidase-dependent metabolism. Incubations containing 14C-phenol and peroxidase (human myeloperoxidase or horseradish peroxidase) showed considerable H2O2, dependent binding of phenol to boiled rat liver protein. The time course for the observed protein-binding paralleled the removal of phenol from the incubations. This covalent binding was 80-100% inhibited by the addition of glutathione or ascorbic acid. These results indicate the involvement of free radicals in the formation of the reactive metabolites and that the binding metabolites are early reaction products. Further studies showed diphenoxquinone and 4,4'-biphenol to be the principal identifiable metabolites with very small amounts of 2,2'-biphenol also being observed. The peroxidase-mediated formation of reactive metabolites from phenol and possibly other benzene metabolites in the bone marrow could therefore be involved in benzene's hematopoietic toxicity.

HEME-DERIVED PROTEIN ADDUCTS AS A MECHANISM OF CARBON TETRACHLORIDE AND 2-ISOPROPYL-4-PENTENAMIDE-INDUCED INACTIVATION OF CYTOCHROME P-450. H.W. Davies, S.G. Briti and L.P. Pohl, Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, Bethesda, MD 20892

Known mechanism for the irreversible inactivation of cytochrome P-450 by xenobiotic compounds include the initial conversion of the compound by cytochrome P-450 to reactive metabolites that covalently bind to either the protein or the prosthetic heme of the enzyme. The present study has uncovered another mechanism for the irreversible destruction of cytochrome P-450. When CCl4 was incubated with liver microsomes from phenobarbital treated rats in air or anaerobically, over 69% of the heme moiety of cytochromes P-450 was destroyed. At least 45% of the degraded heme under both reaction conditions was accounted for as heme-derived products irreversibly bound to microsomal proteins. Furthermore, 33% of the irreversibly bound products were bound specifically to a 54 kilodalton form of cytochrome P-450. A structurally different compound, 2-isopropyl-4-pentenamide, also destroyed the heme moiety of cytochromes P-450 and produced heme-derived adducts to microsomal proteins. These results show that reactive metabolites of xenobiotics can inactivate cytochromes P-450 by causing irreversible binding of heme-derived products to cytochrome P-450 protein. If this binding is at the active site, the addition of heme would likely not reanimate the cytochrome P-450.
MECHANISM OF ETHANOL PROTECTION AGAINST ACETAMINO- 
PHEN HEPATOTOXICITY. K.E. Thumel, J.T. 
Sletteny and S.D. Nelson, Depts. of Pharmaceutics and 
Medicinal Chemistry, U. of Washington, 
Seattle, WA.

Ethanol (E), administered to rats and mice (1-3g/kg) protects against acetaminophen (A) 
hepatotoxicity. E inhibits binding of A to 
rat liver microsomes (Ki=50M) implying that the 
protective effect is due to an inhibition of A 
oxidation. We have evaluated a second mechanism. 
E metabolism causes elevations in the 
hepatocellular NADH:NAD ratio. In vitro studies 
have shown NADH to be an effective reductant of the 
putative reactive A intermediate, N-acetyl p-benzoquinoneimine. In rate, 2g/kg E was found to 
increase the NADH:NAD ratio 3-fold until E 
concentrations fell below 2-3 mM. In a second 
study, E was infused into 3MC induced rats to a 
steady state conc. of 4-5 mM. E infusions, 
maintained for the period of A elimination, 
decreased 24 h plasma ALT by 68% compared to 
saline infusion. In a parallel infusion study, 
hepatic GSH and NADH:NAD were 45% and 73% greater in the +E+ group than the -E- group 3 h after A 
administration. Both A groups showed marked GSH 
depletion. +E- animals showed no GSH depletion and a 
3.8-fold increase in NADH:NAD in contrast to 
a 1.8-fold increase for the -E- group (both 
compared to -E-). The protective effect of E 
could be due to the shift in NADH:NAD, or to 
inhibition of A oxidation at very low levels of E 
relative to the Ki.

PROTECTION OF ACETAMINOPHEN 
INDUCED HEPATOTOXICITY IN MICE BY ASCORBIC ACID 
ESTERS. A. Mitra, S. L. Morris and D.R. 
Bourguier, Division of Pharmacology and 
Toxicology, School of Pharmacy, Northeast 
Louisiana University, Monroe, LA. Sponsor: 
J.P. Natchman

Ascorbic acid is an active reducing agent 
which exhibits a protective action on the 
hepatotoxicity of the reactive metabolite 
of acetaminophen (APAP). In the present 
study, the protective effects of palmitate 
(ASC-P) and stearate (ASC-S) esters of 
ascorbic acid were studied. LD-50 and liver 
function tests were performed on mice 
administered APAP (200-600 mg/kg p.o.) alone 
or in combination with ASC-P or ASC-S (600 
and 900 mg/kg, p.o.). It was observed that 
the LD-50 curve was shifted to the right 
upon addition of ASC-P to the APAP dose 
(APAP alone=400 mg/kg; APAP + ASC-P= 640 
mg/kg), however, the ASC-P protection was 
not as great as that produced by a 600 
mg/kg i.p. dose of N-acetyl cysteine (LD-50 
= 1500 mg/kg ). Addition of ASC-B (400 
mg/kg) to APAP (600 mg/kg) resulted in a 
reduction in mean SGDT levels by more than 
50% (APAP=207 units; APAP + ASC-P = 81 
units). Change from the aqueous vehicle to 
propylene glycol appeared to increase the 
protective effects of both ASC-P and ASC-S, 
the mechanism for this is currently being 
investigated. Ascorbic acid esters produce 
a dose related protective effect against the 
hepatotoxicity of APAP.

STUDIES ON THE MECHANISM OF NITROSOUREA-INDUCED 
HEPATOTOXICITY VII: EFFECT OF PHYTOHORMONE 
ON CONNU 1-[2-(chloroethyl)-3-cyclohexyl-1-
nitrosourea] BILARY EXCRETION; H. Farrish, 
& A.E. Ahmed. The University of Texas Medical 
Branch, Galveston, TX 77550

Experiments were undertaken to investigate the 
mechanism of phytohormone (Pb) protection 
against CONU-induced bile duct injury in rats. 
Our objective was to determine if the protective 
effect was due to 1) a washout of CONU 
and/or its toxic metabolites via increased 
excretion in the bile, or 2) a change in the 
metabolic profile of CONU via the induction 
of hepatic enzymes. At 0, 24, 48, and 120 
hours after CONU (50 mg/kg, p.o.) administration, 
the rat bile duct was cannulated, and 
bile flow was monitored for 6 hours. Pb 
increased bile flow. At 48 hours, the CONU 
only treated animals displayed a 50% reduction 
in bile flow, whereas those treated with Pb and 
CONU had bile flow rates similar to control. 
To examine changes in CONU biliary excretion 
rate and/or metabolic profile, [3-CONU 1-c 
chloroethyl] was administered and bile 
was collected every 15 minutes for 6 hours. 
The amount of the dose excreted in the bile at 3 °C 
during this period was significantly decreased 
by Pb pretreatment. This study suggest that 
Pb pretreatment alters CONU metabolism and divert 
toxic metabolite excretion away from the bile.

STUDIES ON THE MECHANISM OF NITROSOUREA-INDUCED 
HEPATOTOXICITY VIII: EFFECT OF GLUTATHIONE 
TURNOVER ON CONU-INDUCED ALTERATION OF LIVER 
FUNCTION IN RATS. Ahmed, A.E., Hagie, S., 
and Farrish, H. The University of Texas Medical 
Branch, Galveston, Texas 77550

CONU 1-[2-(chloroethyl)-3-cyclohexyl-1-nitro-
sourea] is a hepatotoxic antineoplastic nitro-
sourea derivative. Previous studies in our 
laboratory have shown that CONU-induced 
localized severe bile duct damage and this effect 
was altered by pretreatments which modulate 
objectives are to study the role of glutathione 
turnover in CONU-induced hepatic injury in 
rats. GSH levels were measured in rats treated 
by i.p. administration of the GSH precursor, 
a) 1-2-oxothiazolidine-4-carboxylate (OTZ), 
(2mM/kg) or b) N-acetylcysteine (250 mg/kg) or 
the GSH deploter diethylmaleate (0.6 ml/kg), 1 
hour prior to CONU. The animals were sacrifici 
ced 12 hours following CONU administration (50 
mg/kg, p.o.). A time course study was also 
conducted to determine GSH levels following 
each treatment. Our studies indicate that both 
induction and depletion of GSH levels have 
suppressed CONU-induced hepatotoxicity. A 
dearth of GSH prevents a toxic intermediate 
from injuring the liver, as does an increase. A 
critical glutathione concentration, however, 
may allow the toxic intermediate to exist and 
consequently induce liver damage.

A di-substituted glutathione (GSH) conjugate of 2-bromohydroquinone (2-BHQ) is a more potent nephrotoxin than either of three mono-substituted isomers. The reason for this differential toxicity is unknown. We now report that 2-BHQ-GSH conjugates inhibit both organic anion (p-aminophenol) and organic cation (tetraethylammonium) accumulation by freshly isolated kidney slices in both a time and concentration dependent manner. Moreover, the rate of uptake of 2-Br-3,5- or 6-(diGSy1)HQ, 2-Br-3-(GSy1)HQ, 2-Br-5-(GSy1)HQ and 2-Br-6-(GSy1)HQ by kidney slices is 11.9, 4.8, 4.0 and 1.6 mmoles/mg protein/60 min, respectively. γ-Glutamyl transpeptidase (GGT) activity in intact kidney slices accounted for 5-8% of that detected in subsequently homogenized kidney slices. AT-125 (0.5mM), an inhibitor of GGT, inhibited GGT in intact and homogenized kidney slices by 50% and 92%, respectively. Moreover, AT-125 decreased the accumulation of the isomeric [25S]-conjugates by 49%, 25a, 25b and 29%, respectively. The data suggest that the transport of 2-BHQ-GSH conjugates into isolated kidney slices may be mediated by GGT within the basolateral membrane and that the more extensive renal uptake of the di-substituted conjugate may be partially responsible for its enhanced nephrotoxicity.


Lung cytotoxic chromes P-450 bioactivation of 3-methylindole, 3-methylfurane, 4-pomeanol, or perilla ketone (PK, (3-furyl) isoamyl ketone) leads to a reactive metabolite responsible for pulmonary edema and rapid mortality in susceptible species. PK is the most potent edemagenic agent in mice, having a 48 h LD50 of 5 mg/kg (30 ± 1.8 pg/mg/kg) after ip injection. In contrast, the 2,5-dimethyl-3-furyl derivative g was far less toxic to mice (48 h LD50 of 2,238 ± 1,424 pg/mg/kg) suggesting that substrate bioactivation occurs at either the #2 or #5 furan ring carbon. To determine the position of bioactivation, the 2-methyl-3-furyl- and 3-methyl-3-furyl- conjugates were synthesized. Although the 2-methyl-3-furyl derivative (3) has a 48 h mouse LD50 (190 + 19 pg/mg/kg) near that predicted, the 3-methyl-3-furyl derivative (4) was comparatively non-toxic (8,807 ± 3,710 mg/mg/kg). Thus, bioactivation likely occurs at the #3 carbon position and confirms previous work. In addition, it was discovered that a single sublethal ip administration (ip) of 4 substantially reduced the LD50 of 1 administered (ip) 1 hr after pretreatment. This protection persisted up to 2 days after pretreatment. No significant protection occurred after pretreatment with 3; a slight effect with 2 seemed dose-dependent. Additive toxicity was observed with pretreatment by 1 or 3, followed by challenge with 1.


Administration of mono- and di-substituted glutathione (GSH) conjugates of 2-bromohydroquinone (2-BHQ) to rats causes elevations in blood urea nitrogen (BUN) and extensive renal necrosis. However, the mechanism(s) by which these conjugates elicit toxicity is unclear. We now report that the isomeric [25S]-conjugates covalently bind to rat kidney 10000xg homogenates in the order 2-Br-6-GSY1HQ > 2-Br-5-GSY1HQ > 2-Br-3-GSY1HQ > 2-Br-3,5- or 6-(diGSy1)HQ. In contrast the di-GSH adduct is the most potent nephrotoxin. AT-125 (0.4mM) an inhibitor of γ-glutamyl transpeptidase (GGT) decreased covalent binding by 25%, 17%, 33% and 28% respectively. Aminooxyacetic acid (AOA; 0.1mM) an inhibitor of cysteine conjugate α-lyase, inhibited covalent binding by 28%, 10%, 17% and 17% respectively. Ascorbic acid (10mM) inhibited covalent binding by 63%, 87%, 92% and 28% respectively. AT-125 inhibited 2-BHQ-GSH mediated elevations in BUN by 80% but not that of 2-BHQ cysteine(CYS). In contrast, AOA did not inhibit either 2-BHQ-GSH or 2-BHQ-CYS mediated elevations in BUN. The data suggest that the covalent binding is mediated preferentially by redox cycling of the quinone moiety although the formation of reactive thiol cannot be excluded. The kidney specific uptake of 2-BHQ-GSH conjugates by renal GGT followed by the redox cycling of the quinone moiety may be the cause of kidney toxicity.

SPECIES AND ORGAN SELECTIVITY OF 3-METHYLINDOLE BIOACTIVATION. G.S. Yost, M.R. Nocerini, and J.R. Carlson. College of Pharmacy and Department of Animal Sciences, Washington State University, Pullman, WA.

3-Methylindole (3MI) is selectively pneumotoxic to ruminants, horses, and mice but is not toxic to rats. The glutathione (GSH) status in the lungs of susceptible species can modulate toxicity and an adduct of GSH and oxidized 3MI is produced from goat lung microsomal incubations. The rates of production of the GSH-3MI adduct were measured by HPLC analyses of the supernatants of microsomal incubations which contained 3MI, a NADPH-generating system, and GSH. Lung and liver microsomes from goats, horses, monkeys, mice, and rats were evaluated for the ability to form the GSH-3MI adduct. Pulmonary microsomes produced the adduct in decreasing order of species susceptibility, i.e., the goat was highest (1.3 pmol/min/mg) and the rat the lowest (0.04 pmol/min/mg). In contrast, hepatic microsomal production of the GSH-3MI adduct was highest in the rat (0.24 pmol/min/mg) and lowest in the horse (0.05 pmol/min/mg). Addition of cytosolic glutathione S-transferases increased goat pulmonary adduct formation by only 30%. Thus, the major reason for organ- and species-selectivities of 3MI toxicity appears to be a proficient bioactivation of 3MI to the toxic intermediate in the lungs of susceptible species.
13 EFFECT OF OZONE INHALATION ON SUPEROXIDE ANION RADICAL PRODUCTION BY MOUSE ALVEOLAR MACROPHAGES J. Ryer, G. Neitz, B.D. Goldstein and M. Amoroso UNDNI-Rutgers Medical School/Rutgers University, JGPI, Piscataway, NJ 08854

The ability of ozone (O₃) to potentiate pulmonary infections in laboratory animals has been well documented. Previous work from our laboratory has suggested that this potentiation might be due in part to an interference in the ability of alveolar macrophages (AM) from O₃-exposed animals to produce active bacterial species such as superoxide anion radical (O₂⁻). Studies in our laboratory have shown that following exposure to O₃, stimulated rat AM produce less O₂⁻ than controls. In the classic infectivity model however, the mouse rather than the rat was the most susceptible to potentiation of bacterial infection by O₃. When AM from O₃-exposed mice were stimulated with phorbol myristate acetate, there was a dose-dependent decrease in O₂⁻ production (as measured by NBT reduction) which ranged from 87% of control at 0.35 ppm O₃ to 11% of control at 1.56 ppm O₃. The viability of the AM as determined by trypan blue dye was greater than 90% for both the O₃-exposed and control mice. Therefore, the decreased ability of the AM to produce O₂⁻ cannot be attributed to a pollutant effect on cell viability. These results demonstrate that mouse AM are at least 3 times more sensitive to the inhibitory effects of O₃ with respect to O₂⁻ production than rat AM.


1-Nitropyrene (1-NP) was activated to mutagenic products in the nitroreductase deficient S. typhimurium TA98 Salmonella typhimurium lung microsomes from rabbit, rat, and hamster in the presence of NADPH. H-1-NP was metabolized to covalently protein bound products at a rate of 82 and 10 pmol/mg protein in rabbit and hamster control lung microsomes, respectively, whereas no binding was detected in rat lung microsomes. This correlated well with the formation of hydroxylated 1-NP products in lung microsomes measured by HPLC. Covalent binding in lung microsomes was increased by PCB-pre-treatment in hamster and rat, but decreased in rabbit. 1-NP was activated to covalently protein bound products in isolated rabbit lung cells, with rates in Clara cells > cell digest > type II cells. In contrast, covalent binding in cells isolated from rat lung was very low. 1-NP was not activated to mutagenic products in cells isolated either from rabbit or rat lung.


A 2 year rat carcinogenicity study was carried out on low activity histamine H₂-receptor antagonist SRF 93479. A histological examination of stomachs from rats surviving 22 months or longer was performed. Five groups of animals were examined: placebo dose controls, SRF 93479 1000mg/kg/day in 0.9% SRF 93479 200mg/kg/day, SRF 93479 400mg/kg/day and undosed controls.

Oxyntic mucosa from rats in the high dose group showed focal and areas of hyperplasia of pale-staining neuroendocrine cells in 62% of high dose males and 42% of high dose females. In nearly half of these cases there was progression to infiltration through the muscularis mucosae of foci of eosinophilic granular chief cells and fourteen discrete carcinoid tumours were also observed in the same high dose group. No lesions of these types were seen in mid- or low dose or control animals.

An increased neuroendocrine cell component of the oxyntic mucosa was demonstrated after 35 days treatment at both 1000mg/kg/day and 200mg/kg/day p.o. A dose-related diffuse progressive increase in the oxyntic neuroendocrine cell component of the mucosa was evident with increasing duration of treatment. These changes were considered to result from prolonged stimulation of more tumours were found in the rat producing hypergastrinaemia with consequent tropic effects on the oxyntic mucosa and neuroendocrine ECL cells in particular.

16 MODIFICATION OF GASTROINTESTINAL TUMOR DEVELOPMENT BY DIETARY BUTYLATED HYDROXYTOLUENE.

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The influence of butylated hydroxytoluene (BHT) on development of gastrointestinal tumors was studied. Balb/c mice were treated with six sc injections of 1,2-dimethylhydrazine (DMH), 20 mg/kg, and then fed a diet containing 0.5% or 0.05% BHT. Ten months later, colon tumor incidence in control animals was 10%. In the animals given 0.5% BHT it was 32% (p < 0.05). In mice fed 0.05% BHT, colon tumor incidence was 0%. BHT did not influence GI tract tumor incidence in mice treated with three intrarectal instillations (4.5 mg total) of N-nitro-n-methylene. 334 rats were given 2 sc injections of DMH, 40 mg/kg. They were fed diets containing 0.5% BHT, 0.1% BHT, or 0.5% butyalted hydroxyanisole (BHA). Seven months later it was found that 0.1% BHT had significantly increased the incidence of GI tumors at all sites (incidence controls: 50%; BHT 0.5% 66%; BHT 0.1% 78%; BRA 70%). BHT did not influence the development of colon tumors, but in animals fed 0.5% or 0.1% BHT, significantly more tumors were found in the small intestine than in animals fed the control diet or 0.5% BRA. BHT may enhance GI-tract tumor development, provided exposure to BHT begins after exposure to the carcinogen. (Operated by Martin Marietta Energy Systems with the U.S. Dept. of Energy. RCL supported by the postdoctoral program of ORAU).

Sponsor: P. A. de la Iglesia

The transplantable R3230AC mammary adenocarcinoma model and the DMBA-induced mammary tumor model were used to investigate the effects of extrahepatic neoplasia on liver endoplasmic reticulum (ER) function in rats. The presence of mammary cancer decreased Phase I components of the hepatic ER drug metabolism system and increased Phase II components. Progesterone content and specific progesterone binding were also increased in hepatic microsomes. A mammary tumor burden of at least 2% was required for the onset of hepatic function changes. Furthermore, the changes could be induced earlier by increasing the rate at which the critical tumor burden is reached or reversed by resecting of the mammary tumor. The changes in hepatic microsomal protein composition during the presence of mammary neoplasia have been characterized by SDS-polyacrylamide gel electrophoresis. These studies suggest that liver drug metabolizing ability may be impaired in patients with nonhepatic neoplasia, such as mammary cancer, and that the design of successful chemotherapeutic regimens should take into consideration the altered metabolism.


Changes in hepatic biochemistry and ER binding during EE2-mediated promotion of diethylnitrosamine (DEN) induced liver tumors is being investigated in whole liver and isolated cells. Adult ovariectomized rats were administered a single dose of DEN (200 mg/kg, i.p.) or saline (S). After one week, silastic capsules containing EE2 (90 μg EE2/kg/day) or cholesterol (C) were implanted s.c. and replaced every 28 days. A progression of liver lesions (nodules, foci, tumors) was observed in DEN-EE2 animals over 40 weeks. The area of GST(6-glutamyl)transpeptidase-positive foci was greater in DEN-EE2 animals than DEN-C, and few foci were present in the S-EE2 group. Parenchymal cells isolated from the DEN-EE2 animals displayed the greatest increase in GST. Nuclear ER occupancy increased from 5% in SC to 29% in DEN-C, and 40% for S-EE2 and DEN-EE2 in whole liver, and to 33%, 39% and 30% respectively in parenchymal cells. Therefore, a single dose of DEN may produce a change in the capacity of the nucleus to retain estrogens. Enriched liver cell subpopulations at various stages of promotion will be evaluated for changes in ER, growth factor receptors and oncogene expression. (ES05268-03)

DEVELOPMENT OF AFLATOXIN B1 (AFB1) INDUCED HEPATIC GAMMA-GLUTAMYL TRANSPEPTIDASE POSITIVE (GGT+) FOCI IN RATS OVER TIME. G.E. Dunaf and T.C. Campbell, Field of Environmental Toxicology, Cornell University, Ithaca, NY.

The identification and quantitation of enzyme-altered foci, including GGT+ foci, as indicators of early neoplasia, has been gaining increasing acceptance as a methodology for the study of chemical carcinogenesis. In this present study, we have characterized and quantitated the development of GGT+ foci in livers of Fischer 344 rats at several time points for 21 weeks following the p.o. administration of 250 μg AFB1/kg/day (10 doses over 12 days). Emergence of GGT+ foci, as measured by number and percent of liver volume occupied, increased in a curvilinear trend from Weeks 0 to 13, and then, decreased to Week 21 post dosing. The percentage of GGT+ foci undergoing differentiation to an adult liver phenotype (i.e. remodeling) increased throughout the entire study period, explaining the trend towards a decreasing number and percent of GGT+ foci after Week 13. In our laboratory we have used this in vivo model to study the role of dietary protein level in the development of preneoplastic liver lesions. The results of this study indicate that the 13-week postdosing period presently employed in our model can be shortened to approximately 10 weeks without significantly compromising the sensitivity of this bioassay. (Supported by NIH grants CA 34205/ES07052)


Phenobarbital has been shown to promote thyroid follicular tumors initiated by dihydroxyprogesterone-5-nitrosamine (DHPN) in the rat (Hisa et al. Carcinogenesis, 3, 1187, 1982). An experiment was performed to determine the mode of action of phenobarbital in this model by determining the effect of L-thyroxine feedback on phenobarbital thyroid tumor promotion.

Rats (20/sex/group) were injected SQ with saline (control) or 800 mg/kg of DHPN once a week for 5 weeks. DHPN treated rats were maintained for 15 weeks on a control diet, a diet containing 500 PPM of phenobarbital or a diet containing 500 PPM of phenobarbital plus L-thyroxine (50 μg/kg/day). Phenobarbital markedly increased the incidence of follicular tumors in DHPN treated rats [35% (15/45)] as compared to the group treated with DHPN alone [33% (6/18)]. Treatment of rats with L-thyroxine blocked the promoting action of phenobarbital since the thyroid tumor incidence was similar to that noted with DHPN alone [26% (5/18)]. In female rats no thyroid tumors were observed with DHPN and no promoting action of phenobarbital was observed. The effect of thyroxine feedback suggests that the promoting action of phenobarbital is pituitary mediated as a result of an effect of phenobarbital on the hepatic clearance of thyroxine.
PHENOTYPIC CHARACTERIZATION OF 1,3-BUTADIENE (BD)-INDUCED THYMIC LYMPHOMA IN MALE B6C3F1 MICE. R.D. Irons, W.S. Stillman, R.S. Shah, M.S. Morris and M. Higuchi. Chemical Industry Institute of Toxicology, R. T. P., NC 27709.

BD is a colorless gas used as a copolymer in the manufacture of synthetic rubber. Lymphomas have been recently reported to be the major cause of death in B6C3F1 mice chronically exposed to BD (Huff et al., 1985). The present study was undertaken to characterize the mechanisms of BD toxicity and was not originally designed to quantify tumor incidence. However, preliminary results confirmed lymphocytic leukemia to be the major cause of death in male B6C3F1 mice following chronic inhalation of BD. Presently, 11 thymic lymphomas have been identified in 144 mice exposed to 1250 ppm BD for 28-45 weeks. This represents the minimum incidence of leukemia resulting from these exposure conditions. No similar lesions have been observed in control animals. All lymphomas originated as mediastinal masses with varying degrees of systemic involvement and were characterized by the uniform proliferation of poorly differentiated lymphoblastic cells. Flow cytometric analysis revealed these cells to possess surface markers indicative of early T lymphocytes and to demonstrate variable but elevated amounts of murine leukemia virus (MuLV) as determined by measurement of surface expression of MuLV gp71 env protein.

SIGNIFICANCE OF MINERAL FIBERS IN MESOTHELIOMA INDUCTION AND V79 CYTOTOXICITY L.D. Palekar* and D.L. Coffin**, Northrop Services, Inc. **U.S. Environmental Protection Agency, RTP, NC

It has been generally accepted that long thin fibers play an important role in tumorigenesis by fiber mineral fibers. The results from our studies indicate that erionite, a non-asbestos alluminosilicate is an exception to this general belief. A fiber analysis of erionite, UICC chrysotile and UICC crocidolite was made for the categories of aspect ratio > 3, (NOSH Standard, L≥8um, W≥25um (Stanton Criterion) and L≥5um, W≤0.1um (superfine particles). The number of fibers in each category were in increasing order for erionite, crocidolite and chrysotile. When these samples were injected in F344 rats by intraperitoneal injections, a significantly higher incidence of mesotheliomas was observed in the erionite treated animals than those treated with chrysotile or crocidolite, thus showing that fewer fibers of erionite produced a higher incidence of mesotheliomas. The reactivity of the three minerals was also investigated in the V79 cytotoxicity assay, which showed similar results. From these studies, it appears that consideration of dimension and number of fibers are not enough to determine the potential tumorigenicity or cytotoxicity of fibrous minerals and the high reactivity of erionite is due to factor(s) other than the fiber dimensions.


The purpose of the study was to determine the chronic toxicity & carcinogenic potential of celiprolol in rats. Groups of 50 rats/sex/dosage level were fed a celiprolol & diet mixture for 2 years. The dosage levels were 100, 300 & 900 mg/kg/day for the first 45 weeks, & thereafter, were lowered to 30, 100 & 300 mg/kg/day to obtain appropriate body weight ranges. Body weight, food consumption, clinical signs, palpable masses & mortality were recorded at appropriate intervals. Histomorphologic evaluations were conducted on rats at the end of the two year study. Dose-related decreases in body weight gain & food consumption were observed. Neither mortality nor incidence rates for palpable masses were decreased in the treated rats as compared with the control rats. An increased incidence of focal atrophy of the lungs was observed in the treated rats. Other treatment-related findings were: 1) decreased incidence of chronic nephritis; 2) decreased incidence of myositis & arthritis. Exposure to celiprolol did result in treatment-related increases in any type of neoplasms. It was concluded that celiprolol was not carcinogenic for rats in this study.

EMBRYOTOXIC AND POTENTIAL TUMORIGENIC EFFECTS IN JAPANESE QUAIL INCUBATED IN OVO BY CHEMICAL CARCINOGENS AND NONCARCINOGENS. G. Hatch1, E. Edens2, L. Rogers1, B. Most1, and E. Berman2, Environmental Sciences Toxicology Div., Northrop Services1, Experimental Biology Div., U.S. EPA2, Research Triangle Park, NC and Poultry Sciences Dept., North Carolina State University1, Raleigh, NC. Sponsor D.E. Gardner.

Avian in ovo systems provide an experimental model to determine toxic health effects of hazardous chemicals in sensitive fetal tissues. The carcinogens benzo(a)pyrene (BP) and 2-Acetylaminofluorene (2AAF) and their putative non-carcinogenic structural analogs pyrene (PY) and 4-Acetylaminofluorene (4AAF), were injected into the yolk sac of 72 hr-incubated Japanese quail embryos and assayed for embryo survival and post hatch production of tumors. 2AAF was slightly more toxic than 4AAF producing significant embryo toxicity at 0.3 vs 1.2 moles/kg egg wc, respectively. BP was approximately 100 fold more toxic than PY producing embryotoxicity at 3 vs 500u moles/kg. BP, PY, and 4AAF significantly reduced body weight persisting through at least day 29 post hatch. Microscopic examination of liver and lung tissues of 3-month adult quail surviving in ovo inoculations of severe to moderate embryotoxic doses of BP, PY, and 4AAF revealed no tumor pathology, while 2AAF produced hyperplastic nodule(s) in liver. Tissue evaluations of liver, spleen, and lung from quail surviving in ovo chemical inoculations 12 months hatch are in test.
A series of long-term inhalation studies with emissions of incomplete combustion was carried out. Some results are reported: The exposure to a mixture of the effluent of pyrolysed pitch and coal furnace exhaust containing high concentrations of PAH (polycyclic aromatic hydrocarbons)*, 90 µg BaP (benzo(a)pyrene)/m³, 6 µg particles/m³, 80 h/week induced squamous cell lung carcinomas in 21 out of 116 female Wistar rats; no lung tumors were found in the control group. Three groups of female NMR mice were exposed to the same mixture of emissions for different periods of time (50-90 µg BaP/m³). The incidence of lung adenomas and the number of adenomas per lung were significantly higher than in the controls. An additive tumorigenic effect in the lung was found after inhalation of the emissions and the additional treatment with well-known carcinogens. Rats, mice, and hamsters were exposed to diluted diesel engine exhaust (*40 ng BaP/m³, 4 ng particles/m³, 90 h/week). Lung tumors were detected in 13 out of 95 exposed rats, but in none of 95 controls. Also the lung carcinoma incidence in mice increased significantly. No lung tumors were found in the Diesel-exposed hamsters.

Inhaled MEC caused dose-related increases in lung and liver tumors in B6C3F1 mice (NTP, 1986). MEC is metabolized by two pathways: one dependent upon mixed Function Oxidases (MFO) and the other upon glutathione (GSH) conjugation. In vivo kinetic constants for each pathway were estimated in mouse, rat, hamster, and human lung and liver by direct experiments or from the literature. A physiologically-based pharmacokinetic model (PB-PK) was developed to estimate target tissue doses of MEC and metabolites. Tumor incidence correlated with the amount of MEC metabolized by the GSH pathway but not with metabolism by MFO. The MFO pathway was saturable, but the GSH pathway was not (up to 10,000 ppm). Consequently, the levels of GSH metabolites increase disproportionately at high concentrations of MEC. Human target tissue doses were calculated by the PB-PK. Internal doses in humans were lower than expected from linear extrapolation of high external doses in mice. PB-PK can strengthen the scientific basis of risk assessment, improve experimental design, and structure selection on quantitative metabolic requirements required for risk assessment, irrespective of the mechanism of action.

Obese yellow A/J mice have generally been found to be more susceptible to formation of strain-specific spontaneous neoplasms than their non-yellow siblings. Perezino et al. (J. Natl. Cancer Inst. 51: 1349, 1973) reported increased prevalence of hepatocellular neoplasms in mice of the susceptible C3H strain after administration of phenobarbital (Pb). We compared the susceptibility to spontaneous liver neoplasms of yellow A/J and agouti A/a (C3H x VIY) F-1 hybrid males and their respective responses to promotion with Pb (0.05% mixed in Purina 5010M diet). There were no differences in the prevalence of spontaneous hepatocellular adenomas (A/J: 12%, A/a:16%) and carcinomas (A/J: 14%, A/a: 15%). However, there was a marked differential response of the two genotypes to Pb treatment, with an increased prevalence of animals with adenomas (A/J: 65%, A/a: 28%) and of animals with multiple adenomas (A/J: 43%, A/a: 6%). The potential usefulness of yellow mice for identification of promoting characteristics of environmental agents is indicated by the Pb-induced prevalence of multiple liver tumors (Pb: 43%, cont: 12%).

Eighty-one carcinogenicity studies carried out by the National Toxicology Program were evaluated to determine how the utilization of statistical analyses based on the proportions of tumor-bearing animals or animals with malignant neoplasms would have affected the interpretation of the data. Fewer carcinogenic effects were detected by this approach: approximately half of the carcinogens detected by site-specific analyses would not be judged carcinogenic based on total tumor or malignant tumor analyses. Three problems are associated with the latter approach: (1) it is difficult to detect an increase over the high background rates of total or malignant tumors, (2) most carcinogenic responses are site-specific, and the pooling of various tumor types reduces study sensitivity for detecting these effects, and (3) the biological relevance of combining the incidences of tumors of varying morphologies and topographies is questionable. Thus, despite the potential advantages of this approach (e.g., simplicity; reducing concerns regarding false positives) primary emphasis should continue to be on site-specific carcinogenicity.
ABSENCE OF CHANGE IN SENSITIVITY TO INHALED METHACHOLINE IN ASTHMATICS EXPOSED TO NO₂

Several reports have indicated that asthmatics show increased sensitivity to inhaled bronchoconstrictors (carbachol, cold air) after exposure to nitrogen dioxide (NO₂). We exposed 12 mild asthmatics (male, 18-36 yr, non-smoking) to NO₂ in a chamber (natural breathing, 20°C, 40% RH) for 75 min with three 10-min periods of moderate exercise (V̇E=42 liters/min). Each subject was exposed to 0.0, 0.15, 0.30 and 0.60 ppm NO₂ (random, double-blind) with exposures separated by a week. Two hr after each exposure, bronchial challenge to inhaled methacholine aerosol was performed. Specific airway resistance (sRAW) was measured and the dose (in cumulative inhalation units, CIU) needed to double the baseline sRAW (the doubling dose) was determined. One CIU corresponded to about 0.04 mg methacholine inhaled. The doubling doses (means±SEM) were 3.9±1.0, 3.5±0.9, 3.7±1.0, and 3.2±0.9 CIU after the 0.0, 0.15, 0.30 and 0.60 ppm NO₂ exposures respectively. These were not different by signed-rank tests and paired t-tests. We conclude that exposure to NO₂ during moderate exercise either does not cause increased airway sensitivity to methacholine, or if it does, sensitivity returns to baseline by two hr after exposure.


Quantitative extrapolation of animal toxicity data to humans requires an understanding of the differences in both tissue dose and tissue sensitivity between species. Previous studies suggest that tissue levels of glutathione (GSH), ascorbic acid (AA) and α-tocopherol (AT) are important in determining sensitivity of lung tissues to inhaled toxicants. The present study employs electrochemical detection of HPLC fractions of lavage fluid (LF) from healthy Sprague-Dawley rats and normal human volunteers. LF is separated centrifugally into cells, high speed pellet, and supernatant. Each fraction is further treated with an ethanol-heptane-surfactant mixture which allows determination of all of the above antioxidant substances in material from approximately 10 ml of LF. Large amounts of AA and some GSH are detectable in the LF supernatant, and the high speed pellet also contains these substances. AT is easily detectable in the LF cells and high speed pellets. Several unknown peaks are also observed in all fractions. The effects of O₃, NO₂, and COCl₂ inhalation on all detectable peaks are being determined. The human and rat LF antioxidant profiles appear similar. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
ALAN E. SULLIVAN, D.E. 

Altered mucosal permeability, histopathology & scanning electron microscopy (SEM) were applied to identify nasal epithelial injuries following exposure of resting or exercising SD rats to pollutant gases. For permeability measurements rats were tracheotomized under anesthesia & nasal cavities were isolated by biopsy oropharynx with dental impression cream. 99mTc-DTPA (492 d) & 125I-BSA (99,000 d) were instilled into the nose & % of tracers appearing in blood were measured at 1, 24 & 48 hrs after gas exposures. In air exposed controls, 0.13 to 0.31% of DTPA & 0.15 to 0.41% of BSA transferred from nose to blood. A 4 hr exposure of resting rats to 10 ppm HCHO increased DTPA in blood to 0.83% (p<0.05) & BSA to 0.53% (p<0.05). Percent transfer of 48 hrs after HCHO exposure were similar to controls. Histologically, necrotic cells, cell sloughing & increased 3H-第 labeling of nasal cells resulted after above exposure. A 3 day x 4 hr exposure to HCHO produced greater injury & hyperplasia in naso- & maxillo-orbitol turbinates. By SEM, single HCHO exposure randomly altered cell morphology & cilia. Injury was more severe after 3 d x 4 hr exposure to HCHO. O₃ (0.0 ppm), NO₂ (12 ppm) or their combination did not change nasal permeability in rats exposed at rest or during exercise. Supported by NIH#5-21-2, NIES#1018803521-01 & EPA#RF1956-1.


ADAPTATION TO OZONE IN F-344 RATS. J. G. W. E. R. E. R. D. T. K. M. R. C. R. O. O. M. "The adaptation of ozone in humans after the first or second day of exposure has been termed "adaptation." A rat analogue of ozone adaptation has been developed by exposing rats to ozone on five consecutive days. Each day between 15 min clean air pre- and postexposure periods, rats were exposed to clean air, 0.5 or 1 ppm ozone for 2.25 hr. Simultaneous with exposure, 8% CO₂ was added to the test atmosphere during alternate 15 min periods to stimulate ventilation. Results indicate that the prior exposure did not affect any variable throughout the daily preexposure period. However, during exposure, a concentration related response was observed for tidal volume, frequency of breathing, and inspiratory and expiratory times. Adaptation occurred in these measurements at 0.5 ppm on the third day of exposure, and some attenuation with exposure to 1 ppm ozone was observed on day 4, but not on day 5. When CO₂ was concurrent with exposure, adaptation was seen with all variables at 0.5 ppm and similar trends were observed at 1.0 ppm. Although morphological changes were observed at 0.5 ppm this attenuation did not accelerate recovery from ozone. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
The effect of hyperoxia on lung tumor development was examined in mice and rats. Male strain A/J mice received 1000 mg/kg of uracil and were kept for 4 months in 70% O_2. When the animals were removed from the O_2, they had an average of 5 ± 1 tumors per lung whereas the animals kept in air had 20 ± 1 tumors per lung. The difference persisted up to one year after urethan. As long as the animals were exposed to O_2, pulmonary levels of ornithine decarboxylase were significantly higher than in controls. No changes were found in pulmonary glucose-6-phosphate dehydrogenase or superoxide dismutase. Continuous labelling of pulmonary cells with tritiated thymidine during O_2 exposure showed an initial burst of cell proliferation followed by a maintained doubling of the alveolar labelling index. No parenchymal lesions were visible under the light microscope; the only change found was hyperplasia of the bronchiolar epithelium. Male F344 rats received a single intracheal instillation of 5 mg of 3-methylcholanthrene and one week later were placed into 40% or 70% O_2. Seven weeks later the O_2-exposed animals had significantly fewer tumors per lung. It is concluded that continuous hyperoxia is tumoricidal. (Oper. by the Martin Marietta Energy System with USDoe. RCL supported by the post-doc. program of ORAU.)

Pulmonary injury has been reported in man and in experimental animals after breathing O_2 at concentrations commonly found in urban areas. For critical assessment of injury, and to help extrapolate animal data to man, quantitative knowledge of O_3 dose is needed. We have measured O_3 dose for 30 adult rats in head out pressure plethysmographs. Rats were exposed nose only for 1 hr to 0.3, 0.6 or 1.0 ppm O_3. Resting breathing measurements, ozone, oxygen and carbon dioxide levels in the airstream were recorded and O_3 dose computed once/min. To verify that respiration was the only contributing factor in changing gas concentrations, rats were killed "in situ" with pentobarbital. Gas levels promptly returned to baseline. To determine the pattern of the dose over time (expressed as percent O_3 uptake and as O_3/mg retained min), linear regressions were fit to data from individual animals. The estimated parameters were then tested for O_3 concentration related differences in a multivariate ANOVA. Preliminary results indicate that Sprague Dawley rats retain 40±11 percent of inhaled O_3. The percent O_3 uptake does not vary over time nor is it affected by O_3 concentration up to 1 ppm. The actual dose of O_3 (μg/min) increases proportionally with the concentration. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

The acute inhalation toxicity of combustion products of wood (Douglas Fir) and some filling materials (cotton, polyurethane, neoprene foams) used in upholstered furnitures was compared using the NBS test methods. Rats were exposed head only for 30 min to flaming or nonflaming combustion products for varying concentrations of test materials. The exposure chamber CO, CO_2 and O_2 concentrations and the blood carboxyhemoglobin levels were measured during the exposure. The surviving animals were kept for 14 days for post-exposure observation. The LC50 values were calculated from the mortality data during the exposure and the post-exposure period. The results indicate that the degree of toxicity of combustion products generated from the above test materials is similar to Douglas Fir in either mode of combustion. The CO concentrations and the blood carboxyhemoglobin levels indicate that in most cases CO alone cannot be the sole cause of mortality. (Opinions expressed here are those of the authors and they do not necessarily represent the official views of the U.S. Consumer Product Safety Commission.)
Subchronic inhalation studies were conducted with 2,4-PD to assess the potential hazards following repeated exposure. Mean vapor concentrations in the 9-day study (6 hr/day, 5 days/wk for 9 exposures) were 805, 418, 197, and 0 (control) ppm. No deaths occurred, and the only clinical signs were of sensory irritation at 805 ppm. Also decreased body weight, decreased organ weight, increased white blood cell count, and inflammatory lesions in the nasal mucosa were observed at 805 ppm. The incidence and severity of these findings progressively decreased in the 418 and 197 ppm groups. A 14-week study (6 hr/day, 5 days/wk) was conducted at mean concentrations of 650, 307, 101, and 0 (control) ppm. All females and 10/30 males of the 650 ppm group died during the first 6 weeks of exposure. Noteworthy tissue lesions in rats that died were acute degeneration in the brain and acute lymphoid degeneration in the thymus. Survivors of the 650 ppm group had brain malacia and mild squamous metaplasia in the nasal mucosa. There were no deaths, abnormal clinical signs, or histologic lesions at 307, 101, or 0 ppm. Minor alterations in body weight gain and clinical pathology parameters occurred at 307 ppm; however, these alterations were not present following a 4-week recovery. The no observable effect level was 100 ppm.


Four groups of male and female Sprague-Dawley rats, male Hartley guinea pigs and male Syrian Golden hamsters were each exposed to benzyl chloride (BzC1) vapor six hours each weekday for five weeks (24 exposures) at analytical exposure levels of 0, 60, 180, or 530 mg/m³. Signs of toxicity were observed in all species at 530 mg/m³, and included decreased body weight, respiratory difficulties and eye, nasal and dermal irritation. Mild pulmonary alveolar edema and more marked alveolar distension were seen in mid and high exposure guinea pigs. These results indicate “no-effect” levels of 180 mg/m³ for rats and hamsters and 60 mg/m³ for guinea pigs. A six-month inhalation study (129 exposures) was then conducted in male and female rats and male guinea pigs at analytical levels of 0, 5, 62 or 148 mg/m³ of BzC1 in air. Signs of toxicity included a decreased mean corporeal volume and increased renal weights in upper exposure group guinea pigs and increased splenic weights in high level female rats. No tissue lesions were observed. The highest “no-effect” levels for rats and guinea pigs exposed to BzC1 for 6 months were 62 and 5 mg/m³, respectively. It was evident in both studies that the male guinea pig was the species most sensitive to BzC1.


The conjugation of xenobiotics in vitro has been shown to be energy dependent. This may be due to the requirement of energy for the synthesis of co-substrates used in conjugation. This hypothesis was examined in vivo. Pharmacokinetics of acetaminophen (AA), a drug which is conjugated with gluconic acid, sulfate and GSH, were analyzed in rats when the hepatic energy state (ADP/ATP and phosphorylation potential) had been decreased with ethanol (ET) or fructose (FR). ET reduced ATP/ADP 30-65%, whereas FR caused a 56-70% reduction. Phosphorylation potential was reduced 50-60% and 65% by ET and FR, respectively. Hepatic co-substrate levels (GSH, UDP-gluconic acid and PAPS) were decreased by 10-50%. During the period of decreased energy state, biliary and urinary excretion of AA (300 mg/kg, iv) and its conjugates were reduced; total excretion was decreased 57% by ET and 65% by FR. The decreased excretion of AA-conjugates probably was largely due to their reduced synthesis owing to decreased levels of co-substrate levels. These results suggest that hepatic conjugation reactions (and drug disposition) may be impaired when energy production is severely depressed. (Supported by USPHS grants ES-03192, ES-07079 and a Stueffer Fellowship).

EFFECTS OF ENZYME INDUCERS ON THE BILIARY EXCRETION OF ACETAMINOPHEN AND VALPROIC ACID. C.-P. Siegers, K. Haase, R. Pentsz, and M. Younes. Institute of Toxicology, Medical University of Lübeck, FRG

Male rats anesthetized with urethane (1.2 g/kg ip) excreted 26.5% of an iv dose of acetaminophen (AA, 100 mg/kg) into bile within 5 hrs. Phenobarbital (PB) induction reduced total AA to 14.5%, mainly due to a decrease in the sulfate (2.3% vs. 5.4% in controls) and the glucuronide fraction (4.4% vs. 16.5%), whereas AA-GSH was increased (7% vs. 3.7%). Methylcholanthrene (MC)- induced rats showed an increased biliary elimination of AA-GSH (11%), which compensated for decreases in the sulfate (3.4%) and glucuronide (10.4%) fractions. Pretreatment with trans-stilbene oxide (T5O) markedly reduced the total amount of AA in bile to 9.8%, due to a decrease in the sulfate (1.4%), and the glucuronide (3.4%). Biliary excretion of valproic acid (VPA, 50 mg/kg iv) amounted to 43.6% of the dose within 5 hrs, 40.8% as the glucuronide and 2.8% as unconjugated drug. Both PB- and T5O-pretreatment markedly reduced the amount of VPA-glucuronide in bile, whereas MC did not. It is concluded that microsomal enzyme induction by PB or T5O seems to favour the sinusoidal export of conjugates, mainly the glucuronides of AA and VPA, whereas MC exclusively stimulates the formation of canalicular export of GSH-conjugates of AA.
SUICIDE INACTIVATION OF MICROSONAL OXIDATION BY CIS- AND TRANS-DICHLOROETHYLENE (C-DCE AND T-DCE) IN MALE FISCHER RATS IN VIVO. M.E. Andersen, M.L. Gargas, and H.J. Clewell III, AAMRL/THB, Wright-Patterson APF, OH.

Families of gas uptake curves for C-DCE and T-DCE could not be described by a simple physiologically-based pharmacokinetic (PB-PK) model containing a single saturable pathway for oxidative metabolism. Uptake rates decreased with exposure time indicative of loss of metabolizing capacity. Enzyme inactivation was experimentally demonstrated by the inhibition of trichloroethylene metabolism after pre-exposure to 10-20 ppm T-DCE. Uptake was successfully described by a PB-PK model in which the rate of enzyme inactivation was proportional to a second order rate constant (k_d) times the square of the instantaneous rate of metabolism. A term also was included for enzyme resynthesis during exposure. Resynthesis was dependent on the instantaneous extent of inhibition from basal levels and independent of substrate with a maximum value of 5% \( V_{\text{max}}/\text{hr} \). With C-DCE, \( V_{\text{max}} = 1.06 \text{ mg/hr} (250 \text{ g rats}) \); \( K_m = 0.3 \text{ mg/L} \); and \( k_d = 1.25 \). With T-DCE, \( V_{\text{max}} = 1.06 \text{ mg/hr} \); \( K_m = 0.1 \text{ mg/L} \); and \( k_d = 400 \). Obviously, T-DCE is a much better suicide inhibitor than C-DCE (400 vs 1.5). Mechanistically, the square dependence on instantaneous rate suggests an interaction between a metabolite and the enzyme-substrate complex in the rate-limiting step for enzyme inactivation.

THE INHIBITION OF MICROSONAL CYTOCHROME C REDUCTACE ACTIVITY BY A SERIES OF \( \alpha,\beta \)-UNSATURATED ALDEHYDES. K.O. Cooper, C.W. Hines, and W.W. Yost. Pharmacology/Toxicology Program, Washington State University, Pullman, WA.

We have previously shown that chronic ethanol pretreatment of rabbits results in a substrate-selective induction of the microsomal UDP-glcuroynyltransferase (UDP-GT) activities: 1-naphtol- and oxazepam-GT, but not steroid- or bilirubin-GT activities. Ethanol induction also causes a shift in the stereoselective glucuronidation of (-) oxazepam. In this study, UDP-GT forms with differing substrate specificities (estrogen-GT vs. 1-napthol-GT) and enantiomeric preference of oxazepam glucuronidation were isolated with the use of mon-exchange chromatography from untreated and ethanol pre-treated rabbits. The estrogen-GT fraction from untreated and ethanol-treated rabbits appeared identical in substrate selectivity and SDS-gel electrophoresis, the 1-napthol-GT fraction from ethanol-treated rabbits provided a much greater yield of 1-napthol activity than that from the untreated microsomes. The electrophoretic patterns of this fraction revealed a new protein in the ethanol-treated fraction. Thus, ethanol pretreatment selectively enhanced the level of UDP-GT protein in the 1-napthol-GT fraction, accounting for the substrate-selective induction of 1-napthol-GT and the shift in enantiomeric preference of oxazepam glucuronidation that was observed in microsomes.

45 METABOLISM IN VITRO OF 2,6 DICHLORO-4-NITROANILINE (DCNA) IN MICROSOMES FROM FEMALE RATS. L.A. Basting, C.M. Witmer and M.A. Gello Joint Program of Toxicology, UMDNJ-Rutgers Medical Sch./Rutgers Univ. Piscataway, N.J.

The fungicide/herbicide DCNA was given to female Sprague-Dawley rats per os at 1000 mg/kg for 5 days. Cytochrome P450 was significantly increased from 0.83 to 1.09 moles/mg hepatic microsomal protein. SDS gel electrophoresis of these proteins showed an increase in bands that co-migrated with cytochrome P450c (MW 60,000); P450b or d (MW 52,000) and epoxide hydrolase. Ouchterlony immunodiffusion analysis showed an increase in cytochromes P450b, P450c and P450d and epoxide hydrolase. Aerobic metabolism of DCNA produces 3,5 dichloro-p-aminophenol which is increased six fold in hepatic microsomes from treated rats as compared to microsomes from control rats. This metabolite is not detected if oxygen or NADPH are deleted from the in vitro assay and is inhibited by 60% in the presence of monospecific antibody to cytochrome P450d. These results confirm that DCNA induces its own metabolism and that the metabolism is due, in part, to cytochrome P450d.
Homologous, steroid-inducible forms of cytochrome P-450 which may play a critical role in protection from toxic compounds have been identified in the rat (P-450sp), rabbit (LM3c), and human (HLp). We obtained a specific monoclonal antibody (Mab 1G8) that reacts with purified P-450sp but not HLp. On immunoblots Mab 1G8 reacted with 2 proteins in liver microsomes isolated from untreated male rats. One co-migrated with P-450sp; the other (P-450p4), had a lower apparent molecular weight. No 1G8-reactive proteins were detected in female microsomes. A second Mab, 13-1-13 that recognized HLp but not P-450sp, reacted with single proteins of different mobility in male (P-450p3) and female (P-450p4) microsomes. Quantitative immunoblots of microsomes isolated from female rats demonstrated that triacetyloleandomycin (TAO) was the most efficacious inducer of 1G8-reactive protein (p3p4), followed by chlorobane (CD), dexamethasone (DEX), and cyanopregnenolone (PCN). In contrast, 13-1-13-reactive proteins (p3p4) were induced to the greatest extent by DEX, followed by PCN and TAO, but not all by CD. We conclude that the rat liver steroid-inducible family of cytochromes P-450 is composed of at least four members that are under quantitatively and qualitatively distinct regulatory control. Supported by NIH grant AM 18976.

**Influence of human lipoproteins on the disposition of 2,2',4,4',5,5'-hexabromobiphenyl in 3T3L1 adipocytes and pseudoblood.** A. L. Kraus and I. A. Bernstein, Toxicology Program. The University of Michigan, Ann Arbor, Michigan 48109-2029.

This study concerned the elucidation of mechanisms by which hexabromobiphenyl (HBB) was removed from adipocytes, an important first step in ultimate removal of this lipophilic xenobiotic from the body. Adipocytes derived from the 3T3L1 cell line of mouse fibroblasts were used to conduct studies in vitro. Addition of human lipoprotein to extracellular medium both increased removal of HBB from precooled adipocytes (18 to 80 times greater than other blood proteins) and decreased the deposition of HBB in adipocytes (effect was LDL > HDL > VLDL). These and other results support the contention that human lipoproteins act as a depot by binding HBB in the culture medium. Rate of removal of HBB was correlated with concentrations of lipoprotein cholesterol, cholesterol ester, and phospholipid in the pseudoblood (r = 0.95). Total lipoprotein fractions from individuals with high levels of serum cholesterol increased removal of HBB from precooled adipocytes when compared with fractions from normal human serum. These results are consistent with the hypothesis that cholesterol, and/or cholesterol esters in the blood play an important role in removal of HBB from the adipose tissue.

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**TERP-BUTYL HYDROPEROXIDE METABOLISM AND STIMULATION OF THE PENTOSE PHOSPHATE PATHWAY IN ISOLATED RAT HEPATOCYTES.** G. P. Rush and D. Alberts, Department of Investigative Toxicology, Smith Kline and French Laboratories, Philadelphia, PA.

THF (0.25 mM) metabolism by the glutathione peroxidase/reductase enzyme system in isolated rat hepatocytes resulted in a rapid oxidation of hepatocyte NADPH from 2.85±0.32 to 0.55±0.24 mmol/10^6 cells which rapidly returned to 3.58±0.27 mmol NADPH/10^6 cells following complete THF metabolism. Isolated rat hepatocytes exposed to THF (0.5 mM) for 30 min, metabolised more [1-14C]glucose to 14CO_2 than control (63.2±96.2 vs. 306.9±69.5 dpm/10^6 cells) whereas 14CO_2 evolution from [6-14C]glucose was unchanged indicating that THF increases the activity of the pentose phosphate pathway and not glycolysis. Inhibition of the pentose phosphate pathway with 6-aminoaminonitrosamide (70 mg/kg; 5 hrs prior to hepatocyte isolation) inhibited THF-stimulated 14CO_2 evolution from [1-14C]glucose and decreased the rate of NADPH reduction.

Hepatocytes isolated from 6-aminoaminonitrosamide-treated animals were more susceptible to THF-induced cell injury than were control hepatocytes. These results suggest that the regeneration of NADPH by the pentose phosphate pathway may play a significant role in protecting hepatocytes from THF-induced damage.

**Role of flavin monooxygenase in the metabolism of pyrrolizidine alkaloids to their N-oxides.** D. R. Bhuler, B. Kedzierski, B. S. S. Masters, and D. E. Witters*, Oregon State University, Corvallis, OR and Medical College of Wisconsin, Milwaukee, WI.

Pyrrolizidine alkaloids (PAs) are metabolized in vitro to form highly reactive pyrrolic metabolites and PA N-oxides. The relative importance of the cytochromes P-450 and flavin monooxygenase (FMO) pathways in the in vitro metabolism of the PAs lasiocarpine (Lc) and senecionine (Sn) has been examined in rat liver microsomes by a new HPLC method (Anal. Biochem., in press). Metabolism of PAs to the pyrrole, 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolozone (DHP), and PA N-oxides was significantly inhibited by SKF-525A, metyrapone, or rabbit antibodies (Ab) against NADPH-cytochrome P-450 reductase, suggesting that P-450s may be involved in both oxidations. While DHP formation was completely inhibited at high Ab concentrations, N-oxidation leveled off at 20% of the control activity. When oxidations were performed in a reconstituted system with purified pig liver FMO (kindly provided by Dr. D. M. Ziegler), there was a quantitative conversion of Lc and Sn to their corresponding N-oxides. The Kms and Vmax for pig liver FMO with Sn was 6.6±3×10^4 moles/liter/min, respectively. Studies to delineate the relative contributions of the rat liver FMO and P-450 systems to microsomal PA metabolism are ongoing. (Supported by NIH grants CA-22524, ES00040 and GM-31296.)
Metabolism of R-(+)-nicotine in guinea pigs affords a minor polar metabolite of unknown structure, which can also be detected as a major biotransformation product of N-methylnicotinum ion (NNM), indicating that its formation probably results from subsequent biotransformation of the primary nicotine metabolite NNM. The new metabolite was isolated from the urine of guinea pigs treated with R-(+)-NNM. Male Hartley guinea pigs (552 ± 10 g) were injected i.p. with R-(+)-NNM (60 mg in divided doses) over a 10 h period and urine collected over 48h. Total 48h urine was desalted by cation-exchange chromatography and the NNM metabolites were isolated by preparative HPLC on a magnum 9 Partisil 10 SCX column (50x0.9 cm) using a NaOAc-MeOH-triethylamine (TEA) buffer. A band corresponding to the new metabolite was collected and rechromatographed using a MeOH:EtO:TEA mobile phase. Isolation of the pure metabolite and removal of solvent afforded approximately 0.5 mg of product. Structural analysis of the new metabolite by UV spectroscopy, and NMR and mass spectrometry indicated it to be a mixture of the cis 1S, 2R- and trans 1R, 2R-isomer of N-methyl-N'-oxonicotinum ion (NNNO) formed in the ratio 1:1.6. [Aided by a grant from the Tobacco and Health Research Institute, Lexington, KY, 40536-0053.]

**Effect of streptozotocin (STZ) on the biliary excretion of cholephilic anions. J.B. Watkins and H. Noda, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN.**

The effect of insulin deficiency on the plasma clearance and biliary excretion of xenobiotics was examined in male Sprague-Dawley rats pretreated with 45 mg STZ/kg iv. Four to five weeks later, diabetic rats exhibited elevations in serum and biliary gluconate, phenol, 3,6-dibromophenylamine, disulfiram and sulfobromophthalein. The biliary excretion of amaranth was decreased 30% in diabetic rats. There were no differences in bile flow rate in control or diabetic rats after administration of these four anions. In contrast, bromcresol green, indocyanine green and rose Bengal did not depress bile flow in diabetic rats as was observed in control rats, and their rate of maximal biliary excretion was decreased by 151, 240, 320, and 390%, respectively. In bile acid-depleted diabetic rats, rose Bengal was cholestatic and no increase in maximal biliary excretion was observed. These data indicate that diabetes-induced alterations in bile acid excretion influence the biliary elimination of cholephilic anions. (Supported by Indiana Affiliate of American Diabetes Association.)

**Tissue distribution of 14C-2, 4, 5, 5', 6', 6'-hexa-chlorobiphenyl (6CB) or 14C-Kepone in rats following 4-aminopyrazolo[4, 3-d]pyrimidine (4APP) treatment. L.A. Bau, J.J. Ring, M.J. Vodicnik. Medical College of Wisconsin, Milwaukee, WI.**

Female S/D rats (180-220 g) received saline or 10 mg/kg 4APP ip daily for 4 days. 4APP treatment caused a dramatic decrease in plasma triacylglycerol (TG) and cholesterol (C). The tissue and plasma lipoprotein distribution of iv 6CB or Kepone at 30 min post-injection were determined. Following 4APP treatment, the distribution of 6CB among lipoproteins changed from 37% in very low density lipoproteins (VLDL), 44% in low density lipoproteins (LDL) and 19% in high density lipoproteins (HDL) to 3% in VLDL, 85% in LDL and 12% in HDL. Increases (p<0.01) in 6CB tissue content (ng/g) following treatment occurred in liver (2517±179 vs 4430±271) and adipose tissue (254±15 vs 476±43). Decreases (p<0.01) occurred in skin (170±12 vs 102±12) and adrenal (426±274 vs 295±232). The distribution of Kepone changed from 56% in LDL and 44% in HDL to 90% in LDL and 10% in HDL following 4APP treatment. Kepone content (ug/g) increased (p<0.01) in adipose tissue (286±50 vs 62±105), adrenal (104±149 vs 203±146) and ovary (107±104 vs 139±149) following 4APP treatment. The dramatic decrease in TG and C with 4APP treatment changed the distribution of 6CB and Kepone among the lipoprotein classes and consequently altered the initial tissue distribution of these chemicals. (ES083493, MO015-9.)

**Effect of CCl4 on progesterone binding and cytochrome P-450 in rat liver microsomes. M.W. Rouni, R.C. Cameron, and C. Feuer. Depts. of Pathology and Clinical Biochemistry. University of Toronto, Toronto, Ontario.**

Recent studies showed a significant correlation between specific microsomal progesterone binding and cytochrome P-450 content suggesting a close association between these two parameters. To test this hypothesis, male Fischer rats were dosed with CCl4 (2 ml/kg) and killed at 1, 2, 3, 7 and 14 days. Necrosis of the liver was clearly evident at 24 hr and serum aspartate aminotransferase (AST) activities were markedly elevated at this time. AST levels returned to normal by one week and paralleled the histologic evidence of necrosis. CCl4 caused a rapid loss of cytochrome P-450 from 24 hr until the end of 7 days. The recovery of cytochrome P-450 was gradual and restored to normal levels completely by two weeks. Aminopyrine N-demethylase showed similar changes. Specific progesterone binding was decreased at the end of 24 hr, remained low until 7 days and returned to normal levels by 2 weeks. Specific progesterone binding and cytochrome P-450 showed a positive correlation. These results suggest that progesterone may have some modulatory role in drug metabolizing enzyme function.
IMMUNOLOGIC DEFINITION OF TRIMELLITIC ANHYDRIDE-INDUCED LUNG INJURY. C.L. Leach, N.S. Hatoum, H.V. Ratizczak, R.L. Sherwood, I.C. Roger, and P.J. Garvin. IIT Research Institute and Amoco Corporation, Chicago, IL.

Trimellitic anhydride (TMA) causes immunologically-mediated respiratory syndromes in humans. Previous TMA inhalation studies using a rat model showed similarities between rat and human lung lesions with high titers of TMA-specific serum antibody. Studies were undertaken to define the mechanisms involved in TMA lung injury. (I) Rats were exposed to 95 ug/m³ of TMA for 2 weeks and received daily injections of the immunosuppressant cyclophosphamide (Cy) or saline. The TMA-exposed/saline rats exhibited the usual TMA-induced lung lesions, whereas the TMA-exposed/Cy rats showed no lesions. Lack of spleen cell response in miogens confirmed that Cy eliminated T- and B-cell function. (II) Two of six naive rats which received serum from TMA-sensitized rats exhibited lung lesions following a 6-hour TMA inhalation challenge. Recipients of lymphatic cells, cells plus serum, or liver cells did not develop lung lesions. (III) Rats were exposed to 10, 20, 50, and 500 ug/m³ of TMA for 2 weeks and the lung lavage fluid was analyzed. There were no effects in the 20 ug/m³ group, but a dose-related increase in total cells and macrophages was observed in the 50 and 500 ug/m³ groups. Differential cell counts showed an increase in neutrophils and lymphocytes. Alveolar macrophage phagocytosis of 51Cr-RBC was unchanged.


Inhalation of toluene diisocyanate (TDI) has been associated with respiratory sensitivity. Using 14C-TDI, experiments were undertaken to determine the fate of inhaled TDI in sensitized and control strain #2 inbred guinea pigs. For sensitization, animals were exposed to 1 ppm TDI for 3 hours/day on 5 consecutive days. Serum was drawn from these animals on day 21 following initial exposure and found to contain antibodies to TDI. Sensitized and control animals were challenged with an atmosphere of 0.1 ppm 14C-TDI vapor for 3 hr on day 25. During the challenge, blood samples were collected at 30 minute intervals. The animals were sacrificed immediately following challenge and terminal blood samples were collected by cardiac puncture. The results indicated that the label reached the blood stream of the sensitized animal at a rate five times that of the non-sensitized animal. Furthermore, upon separation of the blood into plasma and cell components, the label was found to be associated with the plasma component. The analysis of the blood proteins will be presented to show the form of TDI found in the plasma and the components with which it was associated. NEHS E50532 (MK) and the International Isocynate Institute (WEB).

PULMONARY IRRITATION AND HYPERSENSITIVITY IN GUINEA PIGS EXPOSED TO 4,4'-DIPHENYL METHANE DIISOCYANATE (MDI) AEROSOL. P.S. Thorpe, J.A. Hillebrand and M.H. Karol. Dept. Ind. Env. Hlth. Sci., Univ. of Pittsburgh, Pittsburgh, PA.

Pulmonary irritation was observed in guinea pigs exposed to 0.06-35 ppm MDI aerosol for 3 hr while maintained in body plethysmographs. Breathing patterns were monitored continuously before, during and for 20 hours following exposure. Upon first exposure to MDI, guinea pigs demonstrated a decrease in respiratory frequency (f) typical of pulmonary irritation and an increase in respiratory volume. In animals exposed to concentrations above 1 ppm there was an increase in f above the baseline rate in the 20 hours post-exposure. A concentration-response relationship was apparent for the irritation response. Sensitization to MDI was induced by inhalation of 1.7 ppm, 3 hr/day, for 5 days. Evidence of sensitization included: 1) Respiratory response to inhalation challenge with 0.25 ppm MDI. The response had a delayed onset and was characterized by an increase in respiratory frequency occurring between 5 and 9 hours post-exposure. No such response was observed when naive guinea pigs were similarly challenged. 2) Positive skin responses upon intradermal injection of an MDI-conjugate globulins. The animal model used in this study can be employed to compare potencies of chemicals as industrial allergens. Supported by NEHS E50532.


Exposure to toluene diisocyanate (TDI) has been associated in certain cases with development of sensitization. To detect sensitized individuals, a serologic test was developed which measures TDI-specific IgE antibodies (Karol et al. Am. Ind. Hyg. Assoc. J. 39: 454-458, 1978). Based on an animal model of TDI sensitization (Karol, Am. Rev. Resp. Dis. 122: 965-970, 1980) the possibility was explored that cellular, as well as humoral factors were involved in TDI sensitization. Guinea pigs were injected with TDI and Freund's adjuvant. Two weeks later, mononuclear cells were isolated from the blood. Lymphocytes were evaluated for TDI reactivity by lymphoproliferation and flow cytometry. Proliferation was detected by incorporation of ³H-thymidine in the presence of TDI-guinea pig serum albumin. No proliferation occurred with albumin alone, or with cells from control animals exposed to adjuvant. Flow cytometry confirmed the presence of TDI-reactive cells in peripheral blood. These studies suggest that cellular, as well as humoral, immune components may be of value in early detection of TDI hypersensitivity. Supported by the International Isocynate Institute and NEHS E50532.
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Halogenated aromatic hydrocarbons bind the aromatic hydrocarbon (Ah) receptor to produce a series of immunotoxic effects. The Ah receptor is a product of the Ah gene locus and is expressed in lymphoid and non-lymphoid tissues of Ah responsive (Ah+/Ah+) mice but is expressed poorly in non-responsive Ah-/- mice. To determine its role in lymphoid tissue relative to non-lymphoid tissue in causing immune impairment, bone marrow was used to reconstitute lethally irradiated mice of the same or opposite Ah genotype. 3,3',4,4'-tetrachlorobiphenyl (35 and 350 umol/kg) was given ip 2 days before immunization with sheep erythrocytes. The immune response was determined 5 or 7 days later in normal or chimeric mice, respectively. The antibody responses of Balb/cBy (Ah+/-) and Balb/cBy x DBA/2 hybrids (Ah-/-) were suppressed. The antibody responses of chimeric Balb/cBy, Balb/cBy x DBA/2 mice were also suppressed and thymic atrophy was observed in both cases. Interestingly, the serum antibody titers of DBA/2 mice with chimeric thymus were also decreased but not as severely as in Balb/cBy x DBA/2 mice. Chimeric DBA/2 x DBA/2 mice were not affected. These results indicate that the sensitivity to Ah receptor mediated suppression of the antibody response is determined by the Ah genotype of the lymphoid tissue, including radioreistant thymic epithelium. Supported by NIDRS grant ES02897.

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CELLULAR TARGETS OF DIOXIN-INDUCED HUMORAL IMMUNE SUPPRESSION. N. W. Kerbel and J. A. Brauner. College of Veterinary Medicine and Environmental Health Sciences Center, Oregon State University, Corvallis, OR.

The antibody (Ab) response to sheep erythrocytes (SRBC), a macrophage and T cell dependent (TD) antigen (Ag), is highly sensitive to suppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a major immunotoxic contaminant of pentachlorophenol. The present studies used several approaches to assess the potential cellular defects responsible for TCDD-mediated humoral immune suppression. The effect of Ag dose on the magnitude and kinetics of the Ab response to SRBC was examined to assess the role of Ag availability versus suppressor cell induction as a mechanism for the suppressed Ab response. The sensitivity of T-independent (TI) Ab responses that differ in their requirement for accessory cell help (CNP, TNP, TNP-Ficol) was also examined. The direct role of the thymus and thymus-derived lymphocytes in mediating dioxin effects was assessed in adult thyrectomized (A-Tx) and nude mice. Results indicated that T cells play a significant role in dioxin-induced humoral immune suppression. TI Ab responses were more resistant to TCDD-induced suppression than TI Ab responses and the Ab response of nude mice was more resistant than the Ab response of their nu/+ littermates. Analysis of the Ab response following different Ag dosages suggested that suppression may be due to the induction of suppressor cells. This effect was not mediated by the thymus gland per se since A-Tx mice that did not modify the degree of humoral immune suppression induced by TCDD. Supported by EPA grant R-11157 and NIH grant ES00210.

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IMMUNOTOXICOLOGICAL STUDIES OF HEXACHLORINATED DIOXINS. M.P. Holzschepp, K.L. White, Jr., S.G. Bradley, and A.E. Munson. Medical College of Virginia/VCU, Richmond, VA

Immunosuppression by exposure to the pesticide, Pentachlorophenol, is mediated by its contaminants. We have investigated the effects of one of the major contaminants, 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (HCDD). Female B6C3F1 mice were exposed to either 0.1, 1.0, or 10.0 ug/kg HCDD, or 10.0 ug/kg 7,8-TCDD, or corn oil daily for 14 consecutive days. Parameters reflecting various facets of immunocompetence were measured 24 hr later. In other studies, animals were challenged with various pathogens to assess the host resistance capabilities. Innate immunity was either unaffected--macrophage phagocytosis or NK cell cytotoxicity--or significantly decreased--complement. Cell mediated immunity was also relatively resistant as only the highest dose of HCDD suppressed the DHR to KLH (47%) and the MLR (29%). Humoral immunity, as measured by the antibody response (IgM & IgG) to SRBC, was suppressed in a dose dependent manner. The IgG response was suppressed from 15-64% when measured immediately after exposure and was still suppressed by 35% when measured 50 days after exposure to the highest dose of HCDD. The profile of host resistance was complicated--the resistance to Streptococcus pneumoniae was decreased, while the resistance to Listeria monocytogenes was increased. (Supported in part by NIH ES-1-5001).

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The present studies were undertaken to identify the cellular targets for the observed immunosuppression by DimethylNitrosamine (DMN). The in vivo use of Ab responders and spleenless from B6C3F1 mice exposed to 6 mg/kg DMN for 7 days to the T-independent (T-I) Ag DNP-Ficol (DNP-F) was used for separation-reconstitution studies. The DNP-F response was used since it was suppressed more than the T-dependent response to SRBC and requires macrophage (M0) and B cell function. Spleenocytes were depleted of M0 by adherence and passage through Sephadex G-10. Adherent cells (M0) were obtained by seeding culture wells. Upon culturing vehicle (V) and DNP (D) nonadherent (NA) and adherent (AD) cells, the V-AD + D-NA response was only 15% of the V-AD + V-NA control response, while the D-AD + V-NA response was 6% of the control. The NA population was the primary target confirmed by reversing a 75% suppression by V-AD + D-NA cultures by increasing the % of V-NA cells. M0 function was not markedly affected by DMN since responses of wells seeded with increasing numbers of D-AD cells + 75%NA cells were reduced at most by only 30% of control. The T-independent DNP-F response was demonstrated by anti-thy 1.2 and C treatment of NA cells resulting in control Ab responses but 90% suppressed Con A responsiveness. (Supported by NIH ES03564).
IMMUNOMODULATION OF LOCAL AND SYSTEMIC IMMUNITY AFTER SUBCHRONIC PULMONARY EXPOSURE OF MICE TO BENZ(α)PYRENE. C.T. Schulte-Hillen, A.E. Munson, and R.A. Rhodes. Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN, and Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA.

Inhalation is a major route of exposure to benz(a)pyrene (BaP), yet limited data on the immunotoxicity of inhaled BaP is available. The purpose of this study was to compare the effects of BaP (40 mg/kg), given intratracheally (IT) for 7 days, on a local immune target, the lung-associated lymph nodes (LALN), with the effects on a more distant immune organ, the spleen. B6C3F1 mice were immunized with sheep erythrocytes IT or intraperitoneally (IP) 1 day after the last BaP exposure. When antigen was given IT, the number of antigen-specific antibody-forming cells decreased in the LALN after BaP exposure. In contrast, mice immunized IP after BaP exposure produced an enhanced humoral response in the LALN. In the same animals, there was a suppressed splenic response which correlated with a depressed antigen clearance to the spleen. Antigen clearance to the LALN was not affected by BaP. Thus, immunomodulation was influenced by the route of immunization and by the proximity of the lymphoid tissue to the exposed lung. This work was supported by PHS Grant ES05317-01.

EFFECTS OF ANTIBIOTICS ON NEUTROPHIL CHEMOTAXIS IN THE RAT. P. Tachon, H.I.T., l'Arbresle, France, A. Eyrraud, J.Y. Lombard, J. Descotes, Alexis Carrel Medical Faculty, Lyon, France, A. Laschi-Loquere, C. Veyssoyre, Pasteur Institute, Lyon, France.

The possible deleterious influence of antimicrobial agents on non-specific host defenses is a critical issue to be considered from the immunotoxicological point of view. As antibiotics are used worldwide to treat various infectious diseases, we undertook to study their effects on neutrophil chemotaxis, a relevant aspect of non-specific host defenses, in the Wistar rat. Neutrophil chemotaxis was assessed using a modified Boyden's technique. The effects of erythromycin, josamycin spiramycine and doxycycline were studied either in vivo and in vitro following incubation for 30 min at 37°C. In all cases, the migration of in vivo treated animals was compared to that of control animals. Except for doxycycline, no deleterious influence of any of these three macrolides could be detected following in vivo as well as in vitro treatment as random and activated migrations were not significantly depressed by less than 20% as compared to controls. These results indicate that macrolide derivatives despite high intraneutrophil concentrations are unlikely to impair chemotaxis.


Fisher 344 rats were used to assess the effect of the interferon inducer, poly (I): poly (C) on ozone-impaired alveolar macrophage phagocytosis. Rats were exposed to 1.0 ppm ozone for 4 hrs. and then induced with poly (I): poly (C) at time 0. Alveolar macrophages were obtained by bronchoalveolar lavage at 2, 4, 6, 8, 12, and 24 hrs. after induction, and phagocytosis was quantified by uptake of latex beads. Ozone exposure resulted in impaired phagocytosis that persisted for 24 hrs. Ozone exposure followed by induction with poly (I): poly (C) resulted in decreased phagocytic levels 2 and 4 hrs. after induction; however, levels of phagocytosis greater than nonexposed controls were observed at 6, 8, 12, and 24 hrs. after poly (I): poly (C). This abrogation of ozone-impaired phagocytosis was due to interferon, since administration of rabbit antisera to rat alpha/beta interferon abrogated the poly (I): poly (C) induced enhanced phagocytosis. This abstract does not necessarily reflect EPA policy.

ALTERATION OF HUMORAL AND CELLULAR IMMUNITY IN MANGANESE CHLORIDE TREATED MICE. B. Srisuchar, M.J. Taylor and R.P. Sharma. Center for Environmental Toxicology, Utah State University, Logan, UT 84322.

Male CD-1 mice were injected i.p. daily with MnCl2 (0, 1, 3 and 10 mg/kg) for 6 weeks. No mortality or gross lesions were observed in treated animals. Liver and spleen weights increased in the 10.0 mg/kg MnCl2 exposed group. Packed cell volume decreased in medium and high dose groups. Manganese caused a significant increase in H-thymidine ([3H]-TdR) uptake by splenic cells not simultaneously exposed to mitogens. Phytohemagglutinin, concanavalin A, and pokeweed mitogen responses were significantly greater for splenic cells isolated from treated animals. The mixed lymphocyte response of splenic cells from treated animals co-cultured with mitomycin-C treated allogenic cells was also increased. The production of antibody against the T-dependent sheep red blood cell (SRBC) antigen was markedly abolished by treatment with 10.0 mg/kg MnCl2, treatment. The inhibition was transient as plaque forming cells were observed following SRBC injection into animals that had been removed from MnCl2 treatment. Manganese chloride treatment was immuno-modulatory in male CD-1 mice as evidenced by increased mitogenic responses and decreased antibody production.
Previous work from our laboratory indicated that procainamide induces antinuclear antibodies in beagle dogs (Balazs T., and Robinson C., TAP 71, 299, 1983). In the present study, lymphocytes from 5 adult beagle dogs of both sexes treated with 100-150 mg/procainamide/kg/day and from 5 control animals were studied for changes in resting membrane potential and for their ability to proliferate in the presence of pokeweed mitogen (PWM), concanavalin A and phytohemagglutinin. Dihexylcarbocyanine was used to monitor the resting membrane potential of the lymphocytes. There were no differences between the two groups. Lymphocytes separated on Ficoll hypaque were treated for their proliferative responses (3H-thymidine incorporation). At 2 and 5 months of drug treatment lymphocytes from treated dogs gave significantly lower response to PWM (7159 ± 777 and 4006 ± 871 CPM respectively) than those of control animals (14325 ± 2043 and 7390 ± 708 CPM respectively) whereas the response to phytohemagglutinin and concanavalin A remained unaltered. These findings suggest that the drug induces a selective change in the membrane component responsive to PWM.

The ability of organophosphorus-esters (OPs) to cause long-term neurotoxicities such as organophosphorus-induced delayed neuropathy (OPIDN) after repeated exposures was studied by treating chickens 5 days/week with LD50 levels (i.m.) of paraaxon and agent VX phosphonothioic acid, [N-(3-[Methyl][aminomethyl]-2-[β-Lis[3-Methyl][amino] ethyl] O-ethyl ester with atropine as a protection. Subchronic doses of the neuropathic OP DFP were given at lower levels as a "positive" control. In one run, LD50 levels of paraaxon and VX consistently reduced blood acetylcholinesterase levels to 50% and 60% of normal, respectively. Levels as low as 50µg/kg of DFP resulted in symptoms of OPIDN by the 55th day. In two trials of 102 and 93 days, neither paraaxon or VX caused gross behavioral, biochemical or histopathological symptoms of neuropathy or myopathy. The high dose levels used and the extended time frame of the studies support the idea that the ability to cause OPIDN is a special property of some but not all organophosphorus esters. Supported by USABRMDL (B2PP2816) and NIH (ES00202).

Compounds that effect the immune system often produce both immunomodulation and immunosuppression of a specific immune response depending on the dose administered. Often times suppression of immune function is observed but at doses which are also cytotoxic. Studies were designed in both rats and mice to support the polyphasic dose response curve of potential immune modulators. Animals received i.p. injections of cyclophosphamide (CP) in acute and subchronic dosing regimens. Effects of CP on splenic and peripheral blood lymphocyte response to mitogens were evaluated immediately following treatment and after a period of recovery. Results confirmed the enhancement and suppression of immune function. Further studies are in progress to determine which fraction of an estimated LD50 or MTD will be appropriate to define the high dose in an in vivo immunoxicology study.

Effects of the anticholinesterase compounds, SM, SR and TB on muscarinic receptors of the brain upon subacute exposure were studied in rats. Three different treatment regimens were employed and the binding of 5H-QNB to synaptic membranes was analyzed. In regimen one, 40 injections (28 for TB) of the agents at 30-40% of LD50 were injected s.c. three times a week. The muscarinic receptor density was decreased in the frontal cortex and hippocampus but not in the striatum or N. accumbens-T. of f sutortii by SM, SR and TB were without effect. Acetylcholinesterase (AChE) was inhibited by 74, 47 and 64%, and the mortality rates were 63, 29 and 35% by SM, SR and TB, respectively. In regimens two and three, 10 injections at 50-70% of LD50 of these agents were given at 4- and 7-day intervals, respectively. In regiments one and two, the combined effect of muscarinic receptors was caused by SM in the striatum; SR had no effect and TB was not used. AChE inhibition was 75-85% and the mortalities were 45-70%. In regimen three, no significant receptor changes were seen; AChE inhibition was about 70% and mortalities were 55-95%. The results suggest that the frequency of cholinergic stimulation determines the extent of down-regulation of central muscarinic receptors. (Supported by DAMD17-C-85-5036 from USARICD.)
73 REVERSAL OF IN VIVO PROTEIN SYNTHESIS INHIBITION DURING PROLONGED DFP ADMINISTRATION. R.C. Gupta and W.D. Dettbarn, Department of Pharmacology and Neuromuscular Disease Research Center, Vanderbilt University, Nashville, TN. Sponsor: B.V.R. Sastry

Rats treated daily with DFP (0.5 mg/kg, sc) exhibited the severe symptoms of cholinergic hyperactivity between days 3-5. A significant decrease in the activities of both AChE and BuChE (>80%) occurred in muscles and in brain regions and also hypothermia was evident during early episode of toxicity (days 2-5). During further administration of DFP rats became symptom-free and recovery of AChE and BuChE activities in muscles but not in brain was seen. The rate of in vivo protein synthesis was measured (0.5, 1.0 and 2.0 h after a sc injection of labeled (1-14C)-valine at a dose of 5 μCi/mmol/100 g body wt) 24 h after the 5th and 14th injection. After 5 days DFP treatment, the rate of labeled valine uptake into the free amino acid pool as well as protein bound pool was significantly reduced (>40-50%) in discrete brain regions and skeletal muscles. Following 14 days DFP treatment, protein synthesis in all the skeletal muscles had recovered to the values of controls, but was significantly reduced in brain. The recovery of protein synthesis in skeletal muscle was in part of the mechanism(s) that lead to tolerance development following subacute administration of DFP. This work is supported by Army Grant #DAMD17-83-C-1244.

74 A COMPARISON OF IN VITRO AND IN VIVO NEUROTOXIC ESTERASE INHIBITION BY TRIPHENYL PHOSPHATE (TPP), S. Padilla1, T. Grizzle2, and D. Lyerly2 (US EPA, RTP, NC; 1Northrop Services, RTP, NC). Sponsor: L.W. Reiter

Organophosphorus compounds which inhibit and age neurotoxic esterase (NTE) by >95% within 48 hrs after administration produce a delayed neuropathy characterized by degeneration of large and long central nerve fibers (OPIDN). Recent studies indicated that the organophosphorus neuropathy in adult male Long-Evans rats in a pattern inconsistent with that described in OPIDN-affected rats. With the present s.c. dosing regimen (118.4 mg/kg/ wk, for two wks), both brain and spinal cord NTE were only marginally inhibited (<40%) 4 and 48 hr post-dosing. To expand on these data, TPP was shown in vitro to be a potent (IC50=3.0M) non-reversible and progressive inhibitor of NTE. Preincubation of 10μM TPP in buffer (50 mM Tris, 0.2 mM EDTA, pH 8.0, 37°C), however, resulted in a time-dependent loss of TPP's ability to inhibit NTE (e.g., 0 min preincubation = 100% inhibition; 120 min preincubation = 50% inhibition). Structural analogues of TPP (i.e., triphenyl phosphate, triphenyl phosphate, trimethyl phosphate, phenol) failed to inhibit NTE in vitro at <10μM concentrations. In summary, TPP is a powerful NTE inhibitor in vitro; however, TPP's marginal in vivo NTE inhibition, coupled with the results of the neuropathological survey raised questions as to the causal events mediating TPP-induced neuropathy in the rat.

75 PURIFIED MAMMALIAN ACETYLCHOLINESTERASE AND OXIME TREATMENT OF ORGANOPHOSPHORUS POISONING. D.W. Hanke and M.S. Beckett, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. Sponsor: W.E. Sulien

Oximes with potentially greater potency than the benchmark 2-PAM can be identified and rank ordered by an in vitro assessment of their ability to reactivate acetylcholinesterase (AChE). We reported on this method using eel AChE (SOT, San Diego, 85). However, there is controversy over whether a mammalian AChE should be used in preference to eel AChE (historically selected because of its availability). We report here on the first direct comparative study using highly purified fetal bovine serum (FBS) AChE, a 62 form of true AChE similar to human erythrocyte AChE. Data are presented from the testing of the pyridinium oximes (2 hr, exposure) 2-PAM, TMB-4, HI-6, and toxogonin against sarin (GB), 4-nitrophenyl methylphosphonic acid (MPP), and pyridostigmine. Against GB (0% spontaneous recovery) the rank order was HI-6 (44% ± 4) > 2-PAM (31% ± 4) > toxogonin (27% ± 1) > TMB-4 (24% ± 5). Against MPP (4% ± 0.3 spontaneous recovery) the rank order was HI-6 (95% ± 3) > toxogonin (84% ± 4) > TMB-4 (39% ± 2) > 2-PAM (26% ± 1). Against pyridostigmine (5% ± 0.2 spontaneous recovery) none of the oximes tested significantly accelerated the return of AChE activity. The most effective oxime tested was HI-6. The data indicate that recovery of inhibited FBS AChE differs significantly from recovery of inhibited eel AChE.


The effects of tri-α-toly1 phosphate (TOCP), diisopropylfluorophosphate (DFP), lepto1phos, cyanophos, and salithion were studied in hen brain, spinal cord and sciatic nerve enzyme activity. Adult hens were treated orally or subcutaneously to develop delayed neurotoxicity. Specific radiometric and spectrophotometric techniques were used to measure the activity of 2013-cyclic nucleotide 3'-phosphohydrolase (23-CNase), acid phosphatase (Apase), and acetylcholinesterase (AChE) Activity of 23-CNase activity from sciatic nerve of TOCP (500 mg/kg) and lepto1phos (250 mg/kg) treated hens was significantly inhibited, while the inhibition was less with cyanophos (200 mg/kg) DFP (2 mg/kg), and salithion (130 mg/kg). Changes in Apase activity were observed clearly in the sciatic nerve of TOCP and lepto1phos treated hens. TOCP showed no decrease in the brain and spinal cord AChE of axonic hens. The remarkable decrease of sciatic nerve AChE of TOCP treated hens was mostly associated with nerve degeneration. These results suggest that the criterion of 23-CNase and Apase and its ratio with Apase activity, may serve as a model to investigate the adverse effects of neurophobic and demyelinating agents.
77 EFFECT OF SOMAN ON AMINOPHOSPHOLIPID ORIENTATION IN ELECTROPLAX PLASMA MEMBRANES: USE OF 2,4,6-TRINITROBENZENESULFONIC ACID. T. W. L. St. B. S. Weber and B. B. Rosenberg. Univ. of CT., Sch. of Pharm./Tox., Sch. of Pharm., Storrs, CT.

The membranal effects of organophosphorus compounds that are independent of cholinesterase (ChE) inhibition are not well characterized. TNBS-labeling of phosphatidyethanolamine (PE) and -serine (PS) was used to monitor alterations in membrane organization. Electro eels were exposed in vivo to soman (10 nM) for seven days. Single electroplax were then isolated and exposed to 0.5 mM TNBS under impermeable conditions. The non-entrapped (NI) and entrapped (I) surfaces of electroplax were separated and fractionated into plasma membrane (PM) fractions. TNBS-labeling of PE in the I-PM was decreased from 29±1% to 24±0.3% (p<0.06) after soman exposure. ChE from electroplax, plasma and brain was not inhibited. In vitro, single electroplax were exposed to 0.1 mM soman for 30 min and then to 0.5 mM TNBS. TNBS-labeling of PE was increased from 25±1% to 35±1% (p<0.01) in the I-PM. ChE was inhibited 100% in both I- and NI-PM. The labeling of PE in the NI-PM was not changed by soman. PS was not labeled in control or soman treated electroplax. Soman neither altered permeability to TNBS nor the total amount of PE. These data suggest that soman caused a redistribution of PE between the inner and the outer leaflet of the I-PM. (Supported by U.S. Army Contract DAAD 17-82-C-2096).

79 CONVULSANT AND ANTICONVULSANT INDUCED PERTURBATIONS IN MEMBRANE COMPOSITION AND FUNCTION. M. W. Gill, M. R. Connors and E. A. Schett. Northeastern University, Toxicology Program, Boston, MA 02115.

Epilepsy most likely results from perturbations in electrically excitable or neurosecretory membranes. Indeed, many convulsives agents alter membrane composition and function, while several anticonvulsants act by stabilizing neuronal membranes. In this study, we report the effects of the convulsant methionine sulfoximine (170 mg/kg, 3h before sacrifice) and the anticonvulsant diazepam (5 mg/kg, 1h before sacrifice) on mouse brain phospholipid methyltransferases (PM T1 and II), membrane phospholipid content, and synaptic APase activities. Diazepam (DZ) increased PM T1 (100%) and PM II (50%) activities. Methionine sulfoximine (MSO) increased PM T1 (25%) only. DZ and MSO both significantly decreased membrane phosphatidylcholine content. DZ also increased membrane lysophosphatidylcholine (50%) and sphingomyelin (25%) content. NA-K+ APase was decreased by DZ (25%), increased by MSO (30%) and increased by DZ + MSO (45%). Mg+ APase was decreased by DZ (25%), MSO (17%) and DZ + MSO (22%). These data suggest that the MSO and DZ induced changes in neuronal excitability may, in part, result from alterations in membrane composition and function. Precendent events associated with these alterations are under investigation.

Supported by a grant from the Epilepsy Foundation and NBSR-9462-21.

78 THE ROLE OF PHOSPHOLIPIDS ON THE INTERACTION OF ORGANOPHOSPHATE AND CARBAMATES WITH ACETYLCHOLINE RECEPTOR. K. Mansour, J. V. Vaideeswar, A. Shamo, and Z. Annau. The Johns Hopkins University, U.S. Army Armament, Munitions and Chemical Command, APG, and University of Maryland School of Medicine, Baltimore, MD.

The effects of phospholipids and cholesterol were studied on the functional properties of Torpedo AChR reconstituted into lipid vesicles. Purified AChR were prepared by sucrose gradient and toxin-agerose concanavalin A agarose affinity chromatography and reconstituted into several lipid cholesterol ratios. The magnitude of carbamylcholine-stimulated 22Na influx reached its maximum value at 1 mM carb for the purified vesicles; 0.1 mM for purified vesicles reconstituted into soybean Lα-phosphatidylcholines, and in the range of 1-10 µM for those supplemented with cholesterol (25%). The action was inhibited by the phospholipidic acid derivatives; sarin, soman and isofluorohate and regulated by calcium and the lipid environment. VX, estriophosphate, and the carbamates neostigmine, pyridostigmine and physostigmine inhibited the receptor-channel sites; isofluorohate inhibited carb activation of Lrh phosphatidic binding to the iono channel. The receptor protein position in the membrane reflects an equilibrium state between the interaction of its hydrophobic and hydrophilic parts in the lipid environment. (Supported by U.S. Army Contract DAAD 17-82-C-2096).

80 EFFECT OF MANGANESE EXPOSURE ON REGIONAL BRAIN BIOCHEMICAL AMINES IN MICE. R. Srisuchart and R. P. Sharma. Center for Environmental Toxicology Utah State University, Logan, UT 84322.

Male CD-1 mice were injected i.p. daily with manganese chloride (0, 1.0, 3.0 and 10.0 mg/kg) for 4 weeks. The concentrations of catecholamine and indoleamine neurotransmitters and their major metabolites were determined in six different brain regions. Manganese treatment did not induce mortality, overt signs of toxicity or gross lesions of any organs in the treated animals. High manganese exposure caused a significant reduction of 5-hydroxyindoleacetic acid (5-HIAA) levels in the cerebral cortex and medulla oblongata whereas low manganese exposure produced an opposite effect in the cerebral cortex. A marginal increase in 5-HIAA levels was also observed in the medulla oblongata and midbrain at the low level of manganese exposure. In addition, an increase in the amount of 3,4-dihydroxyphenylacetic acid (DOPAC) was observed both in the medulla oblongata and cerebellum. Concentrations of norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT) were not affected in any brain regions. The results suggested a biphasic dose-effect of manganese on 5-HIAA content in various brain regions. Also, manganese has a more selective effect on the major metabolites of DA (DOPAC), and 5-HT (5-HIAA) than the parent compounds after 4 weeks of exposure.
ALTERATIONS IN REGIONAL BRAIN NEUROTRANSMITTER CONCENTRATIONS INDUCED BY THE ARTIFICIAL SWEETENER ASPARTAME--A TIME STUDY. R.A. Coulombe, Jr., R.P. Sharma and J.M. Hulse, Center for Environmental Toxicology, Utah State University, Logan, UT

We have recently reported that aspartame induces significant increases in regional brain catecholamine neurotransmitter concentrations in CD-1 mice three hours after single oral doses. The present study was conducted to determine the time course of these effects. Male CD-1 mice were dosed orally with 13, 133, and 650 mg/kg aspartame in corn oil. Control animals received corn oil alone. The animals were sampled at 3, 6 and 24 hours after dosing, and the concentrations of norepinephrine (NE), dopamine (DA), serotonin (5-HT), and various metabolites were determined in several brain regions by electrochemical HPLC. Aspartame at 133 and 650 mg/kg induced significant increases in the concentrations of NE, DA and their metabolites in various brain regions, most notably the hypothalamus at 3 hours. In contrast, at 6 and 24 hours after dosing, these effects on regional catecholamine concentrations had disappeared. No significant alterations in regional concentrations of 5-HT and its metabolite were detected at any of the time points. These results indicate that the aspartame-induced increases in regional catecholamine neurotransmitters are short-lived in the CD-1 mouse.


It has been reported that Soman produced convulsion and elevations of cyclic GMP concentrations via alterations in GABA metabolism. We also have shown the relation between convulsions and cyclic nucleotide metabolism. Soman inhibits cyclic GMP phosphodiesterase activity 15 min after treatment. It is known that soluble guanylate cyclase is activated by Ca++. Therefore, the present study was designed to clarify the acute effects of Soman, Sarin and Tabun on Ca++ and Mg++ contents of rat brain using a wet ashing method. Male Sprague-Dawley rats (200-250 g) were used and Ca++ and Mg++ contents were measured by atomic absorption spectrophotometry. Soman induced an increase in Ca++ content 15 min after injection. The increase was due to increases in the cytosolic Ca++ level. Soman and Sarin elevated Mg++ contents 6 hr after treatment. Tabun augmented the Ca++ and Mg++ levels 2 hr after administration. These data suggest that cytosolic calcium influx may play an important role in the onset of increases in cyclic nucleotide levels. (Supported by DAMD17-85-5036 from the USAHRC.I.C.)

EFFECT OF REPEATED DAILY EXPOSURE OF ASPARTAME (NUTRASWEET) ON REGIONAL BRAIN NEUROTRANSMITTERS. R.P. Sharma and R.A. Coulombe, Jr., Center for Environmental Toxicology, Utah State University, Logan, UT

We recently reported that a single oral exposure of mice to aspartane significantly increased norepinephrine and dopamine concentrations in various brain regions. The doses used in that study closely approximated normal as well as abuse levels of the compound. The present study investigated the effect of repeated exposures to aspartame. Male CD-1 mice were given oral doses (0, 13, 133 or 650 mg/kg) once daily for 30 days. One day after the last dose, the animals were decapitated and their brain regions quickly isolated. Analyses of different regions for catecholamine and indoleamine neurotransmitters and their major metabolites by electrochemical HPLC indicated that the increases in adrenergic chemicals observed after a single acute exposure were not apparent after repeated dosing. In contrast, concentration of serotonin and its metabolite 5-hydroxyindoleacetic acid were increased in several regions. The findings are consistent with the homeostatic adaptation of adrenergic pathways in spite of the abundant availability of catecholamine precursor phenylalanine, a component of aspartame. An increased supply of phenylalanine can decrease the brain tryptophan uptake, likely resulting in low regional brain serotonin concentrations.

ALUMINUM DISTRIBUTION AND GLDH ACTIVITY IN PRIMARY HIPPOCAMPAL, ASTROCYTE, AND CEREBELLAR CULTURES TREATED WITH ALGLU·6H2O. G. Klasyb and E. Acosta, Department of Pharmacology/Toxicology, University of Texas, Austin, TX.

Aluminum has been implicated as a toxic agent in several neurological disorders. We have developed an in vitro model for examining the toxic effects of aluminum on CNS neuronal and non-neuronal cells. Cultures were grown for 10-14 days and then treated for 12 hrs-10 days with either 1x10^-5M or 1x10^-4M ALGLU·6H2O (AL). Samples taken from the cell lysate, protein fraction, and culture media were analyzed for aluminum using atomic absorption spectrophotometry. Glutamate dehydrogenase (GLDH) activity, a key enzyme in the synthesis of the neurotransmitter glutamate, was determined in the cultures. Significant increases in aluminum levels over time were seen in both the cell lysate (p<.01) and protein fraction (p<.05) in all cultures. Generally, there was a 10X increase in aluminum in the cell lysate and a 2.5X increase in the protein fraction. The specific activity of GLDH was significantly reduced (p<.01) in cerebellar cultures treated with either 1x10^-5M or 1x10^-4M (AL) over time. Hippocampal and astrocyte GLDH specific activity, however, was unchanged over time. The results suggest a differential toxic effect of aluminum among the three CNS cell types, although they all accumulated aluminum similarly over time.
SUBCHRONIC (13-WK) EVALUATION IN LAYING CHICKEN HENS OF CHLORPYRIFOS-METHYL FOR DELAYED-NEUROTOXICITY. T. Bona-Lloyd, G. C. Jersey, and J. T. Young. HAE, Dow Chemical USA, Freeport, TX 77546. Sponsor: R. J. Kociba

Chlorpyrifos-methyl (0,0-dimethyl-O-[3,5,6-trichloro-2-pyridinyl] phosphorothioate; CP) was assessed for possible induction of delayed-neurotoxicity (DNT). Ten hens, DND, were gavaged with CP at dose levels of 0 (vehicle), 5, 50, or 500 mg/kg/day, 5 days/wk, for 13 weeks. Positive control groups were gavaged with tri-ortho-cresyl phosphate (TOCP) at dose levels of 10 or 30 mg/kg/day. Dosing of the 50 mg/kg/day TOCP hens was stopped in the 7th wk because of marked signs of DNT. Persistent loss of body weight, judged due to acute cholinergic effects, occurred in the 1st wk in the 500 mg/kg/day CP hens; body wt loss, judged to be associated with onset of DNT, occurred after 1 month in the 30 mg/kg/day TOCP group. The CPM-treated hens showed no clinical or histopathological signs of DNT. Clinical signs of DNT occurred in all of the 30 mg/kg/day TOCP group and in 2 of the 10 mg/kg/day TOCP group. Brain and spinal cord lesions were found in the 30 mg/kg/day TOCP group and, to a lesser extent, in the 10 mg/kg/day TOCP group. Peripheral nerve lesions occurred only in the 30 mg/kg/day TOCP group. It was concluded that, under the conditions of the study, CP was not an inducer of DNT.

ORGANOPHOSPHATE INDUCED DELAYED POLYNEUROPATHY (OPIDP) BY CHLORPYRIFOS IN MAN AND HENS. Zelli, B. Bertocci, and A. Moretti. Istituto di Medicina del Lavoro, Università di Padova, Italy.

Cholinergic symptoms developed in a 42y old man after suicide attempt with chlorpyrifos (0,0-diethyl-O-[3,5,6-trichloro- 2-pyridyl] phosphorothioate; 300 mg orally) and disappeared after 3 weeks. On day 50 PNS physiology was normal, but RBC AChE, plasma BuChE and lymphocyte neurotoxity Target Esterase (NTE) were inhibited (56, 90, 60 % respectively). Since NTE was inhibited we predicted the development of OPIDP. On day 40 and thereafter the clinical, physiological and morphological picture was in fact consistent with OPIDP. Chlorpyrifos-exen was incubated for 20 min, pH 8.0, 37C with hen and human brain and with human RBC, plasma and lymphocytes. AChE, BuChE and NTE 150 (µM) were in hen brain 0.005, 0.005, 0.15, in human brain 0.013, 0.004, 0.18, and in human blood 0.007, 0.0007, 0.11 respectively. Chlorpyrifos (150 mg/kg p.o.) caused in hens an increasing inhibition of NTE with 30% (800) on day 4-5. Birds became ataxic on day 20, 37F (1.5 mg/kg s.c.) caused comparable NTE inhibition 6 hours after dosing. Birds became ataxic on day 12. We conclude: a) lymphocyte NTE inhibition predicts OPIDP in men. b) Chlorpyrifos causes OPIDP in man and hens with a longer delay due to a late max effect on NTE. c) the sensitivity of target enzymes is similar in both species.DNR 84-0324,56 & It.Ed.Min. grants.


Variations in salivary cholinesterase (ChE) may serve as the basis for a non-invasive method for the diagnosis of chemical agent exposure. The onset of salivary and blood ChE inhibition following an acute, sublethal dose of DFP, an irreversible ChE inhibitor, was examined. Eight adult male rhesus monkeys, Macaca mulatta, (6-12 kg) were anesthetized with Ketamine (20 mg/kg, im) and control parotid saliva (PS), extraparotid saliva (ES) and blood samples were obtained. A single injection of DFP (0.1 mg/kg, im) was administered; then PS, ES and blood samples were collected at various intervals between 5 and 120 min. Samples were analyzed by the radiometric technique of Johnson & Russell (Anal. Biochem., 14:299, 1971) using a tritiated acetylcholine substrate. ES, RBC, whole blood and plasma ChE were maximally inhibited by 15 min (73%, 63%, 82% and 99%, respectively) while maximal PS ChE inhibition (88%) occurred later (30 min). By 120 min ES ChE was recovering, while PS and blood ChE remained inhibited. In vitro studies with EN-200 crystalline acetyl-ChE inhibitor, and tetraethylpyrophosphoramide, a butyl-ChE inhibitor, demonstrated that PS and ES consisted primarily of acetyl-ChE. The inhibition of acetyl-ChE in saliva demonstrates the feasibility of using salivary ChE for diagnostic screening of anti-ChE compounds.
A sensitive method for determining cholinesterase (ChE) depression due to organophosphate (OP) poisoning is described using a 2-PAM reactivation assay similar to one of Karlog and Poulsen (1963) on the brain of dead and the blood of live animals. Incubation with 10^{-5}M 2-PAM caused significant increases in ChE activity in brain homogenates from cockroaches when lethally poisoned with parathion and isofenphos. Carcasses were left for up to one week at 20°C before reactivation. Control levels of ChE remained stable when measured on a per gram basis, though total activity decreased with brain weight during this time. No reactivation occurred in the controls. Spontaneous reactivation and aging of the phosphorylated enzyme decreased the sensitivity of the assay at one week. Studies of reactivation of RBC ChE in non-lethally poisoned animals are in progress. With this approach, variations in the absolute levels of ChE due to season, time of day, region of brain, age, species and time since death are less likely to interfere with detection of OP poisoning because the inhibition and reactivated activities are determined from the same homogenate. Supported by NIH ES00202.

Three major metabolites of isofenphos were studied for their acute and delayed neurotoxic potentials. ISOs of isofenphos (AChe for I(isofenphos), 10-I-oxon), DN(N-dealkyl I), DN10(N-dealkyl 10) were determined with hen brain, rat brain, and rat RBC by 30 min incubation at 37°C. AChe inhibition was similar in all three systems, ranking in potency as 10-I-oxon < DN10 < DN10. ISOs for DN10 were approximately 10-6M. DN10 was a direct inhibitor of hen brain NTE (neurotoxic esterase) in vitro with an IC50 of 10-6M. N-alkyl I, isofenphos with an additional alkyl group (methyl) on the nitrogen, did not inhibit NTE even when incubated with a liver microsomal activation system. DN1 and DN10 both induced neuropathy at doses lower than 1. At doses of 25 mg/kg, NTE was inhibited 47% (brain), 61% (spinal cord) by DN1 and 60% (brain), 48% (spinal cord) by DN10. N-alkyl I only inhibited NTE 33% (brain), 15% (spinal cord) even at 100 mg/kg. The data suggest that N-dealkylation is an important step in promoting both the acute and the delayed neurotoxicity of isofenphos. (Supported by NIH ES-00202.)

The largest North American epidemic of food borne pesticide poisoning to date occurred during the summer of 1985, and was traced to watermelons grown in Kern Co., CA, contaminated by aldicarb. Aldicarb is a systemic pesticide in the carbamate class of acetylcholinesterase inhibitors, and is the most highly acutely toxic pesticide currently registered in the USA. Cholinergic poisonings were first reported from Oregon on July 3. In California, aldicarb sulfoxide at 2.7 ppm was detected on July 4 in a watermelon purchased in Oakland, CA. Active surveillance, in California, ascertained reports of 618 probable cases and 344 possible cases of poisoning. Another 333 probable and 149 possible cases were reported from other western states and provinces of Canada. Reported illness ranged from mild gastrointestinal upset to severe cholinergic poisoning. Levels of aldicarb sulfoxide in watermelons implicated in human illness ranged from 0.07 ppm to 3 ppm, suggesting that the sulfoxide may be more toxic to humans than the parent compound or that some people may be particularly susceptible. Systemic pesticides with the extreme toxicity of aldicarb can poison crops to toxic levels under certain use conditions, whether intentional or inadvertent.
Cypermethrin is a photosstable, synthetic pyrethroid used to control insects on cotton. Since there is no occupational exposure data on cypermethrin, the present study was designed to assess dermal exposure of, and systemic absorption of an ultra low volume (ULV) by pilots and mixer-loaders during aerial application to cotton of an ultra low volume formulation in oil. "Potential" (protected and exposed skin) exposure was 1.07 (0.26 to 2.65) mg/8 hours for pilots and 10.5 (2.3 to 12.3) mg/8 hours for mixer-loaders. 'Actual' (exposed skin only) exposure was 0.66 (0.08 to 2.08) mg/8 hours for pilots and 2.43 (0.22 to 5.27) mg/8 hours for mixer-loaders. The exposure of pilots was predominantly on the hands, whereas that of mixer-loaders was more uniform. Absorption by mixer-loaders, determined by analysis of urinary metabolites, was 46 to 78 ug cypermethrin equivalents per 3 mix-loads and 12 simulated mix-loads, respectively. This was minimal in relation to their predicted exposure levels, as measured in different mixer-loaders. The study shows that there is minimal toxicological hazard to workers associated with aerial ULV application of cypermethrin.

Proconvulsant actions of pyrethroid insecticides: relationship to the peripheral-type benzodiazepine receptor. T.F. Muro, P. Sotz and L.J. Devaud. College of Pharmacy, Oregon State University, Corvallis, OR. Sponsor: L. J. Weber Pyrethroids are potent pesticides which act as neurotoxins in both insects and mammals. Although the neurotoxic consequences of high-dose exposure are well characterized, no studies have reported on the interaction of low-dose exposure to pyrethroids on seizure susceptibility. The effects of pyrethroids on seizure thresholds in rats were determined by measuring the dose of pentylenetetrazol (PTZ) infused through a tail vein required to elicit a myoclonic jerk. The Type II pyrethroids deltamethrin, fenvalerate and IR, cis-/cis-5 cypermethrin, as well as the Type I pyrethroid permethrin were all found to possess proconvulsant activity. These compounds all produced dose-related reductions in PTZ seizure thresholds. Moreover, the proconvulsant action of cypermethrin displayed stereospecificity in that the IR,cis,-S isomer was the most potent compound tested, while the non-insecticidal isomer, S,cis,-S cypermethrin was devoid of activity. Pretreatment of rats with PK 11195, an antagonist of the peripheral-type benzodiazepine receptor, resulted in a complete reversal of the deltamethrin- and permethrin-induced proconvulsant effects. These results suggest that the observed proconvulsant actions of pyrethroids are mediated via an interaction with the peripheral-type benzodiazepine receptor. (Supported by a grant from OSU Environmental Health Sciences Center)

Comparative metabolism and fate of fenvalerate in Japanese quail and rats. M.M. Mamtaz and R.E. Menzer. Insecticide Toxicology Lab., Dept. of Entomology, U of M., College Park, MD 20742

Adult Japanese quail were administered 100 mg/kg chlorphenyl-[1C]fenvalerate, a-cyano-3-phenoxybenzyl-2-(4-chlorophenyl) isovalerate, for study of its distribution, elimination, and metabolism. Within the first 24 h 90% of the administered dose was eliminated in the excreta. Besides fenvalerate, the following metabolites were present: benzeneacetic acid, 4-chloro-a-(1-methyl-ethyl), cyano-(3-phenoxy-4-hydroxyphenyl) methyl ester [4-OH-fenvalerate]; benzeneacetic acid, 4-chloro-a-(1-methylthyl)-aminocarboxyl, (3-phenoxyphenyl) methyl ester [CONH2-fenvalerate]; 4-chloro-a-(1-methylthyl)-benzeneacetic acid [Cl-V-acid]; and 4-chloro-a-(2-hydroxy-1-methylthyl)-benzeneacetic acid [4-OH-Cl-V acid]. Radioactivity peaked at 3 h (i.w.) by P. Sotz and L.J. Devaud.

We have previously demonstrated that selective chemical modifying reagents and noncompetitive enzyme ligands can decrease the sensitivity of purified human serum ChE to inhibition by organophosphates. The present studies extend these findings to the carbamate neostigmine with peripheral site ligands aprophen and lucanthone, and the arginine-reagent phenylglyoxal. The bimolecular reaction constant (k1), the rate constants for carbamylation (k2) and decarbamyla-
tion (k3) were measured in control, unmodified ChE and compared with enzyme after 30 mins. pre-
treatment with 0.1 x 150 of the modifying reagents. None of the peripheral ligands altered the k2 for neostigmine, but the k1 was decreased to 50% of control by aprophen (2 μM), and to 60%-C by lucanthone (20 nm) and phenylglyoxal (5 μM). It is suggested that peripheral site-reactive drugs may be used to protect the active site of ChE from carbamate insecticides.

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Chemical Modification of Cholinesterase (ChE) Carbamylation Kinetics by Peripheral Site Ligands. J.K. Marquis & C.R. Cyr. Boston University School of Medicine, Boston, MA.
FENVALERATE EFFECTS ON THE Picrotoxin Seizure Threshold and Locomotor Activity in the Rat. K.M. Tolson, W.M. Bourn, and F.O. Risinger. School of Pharmacy, Northeast Louisiana University, Monroe, LA. Sponsor: T.M. Eichholtz.

Lawrence and Casida (Science 221:1399, 1983) have recently reported an interaction of the Type II pyrethroids, in vitro, at or near the picrotoxin binding site on the DABA receptor-ligandophore complex. If fenvalerate (FVN) has an affinity for the picrotoxin receptor, but lower efficacy, one would expect pretreatment with subconvulsive doses of FVN to increase the convulsive threshold for picrotoxin (PTX). The purpose of the present study was to determine the effect of FVN on PTX-produced seizures and locomotor activity. FVN (200 mg/kg and 500 mg/kg dissolved in DMSO) was administered ip to male Sprague-Dawley rats 45 minutes prior to iv infusion of PTX. The threshold to PTX-induced convulsive spiking was significantly increased (48% and 33%) by both doses of FVN. These results indicate a possible blockade of the picrotoxin binding site. In order to verify that these doses of FVN produced a biological effect, animals were tested in an activity cage 45 minutes after ip dosing of FVN or vehicle in similar doses as previously mentioned. Both large and small movements were measured over a 15 minute interval. Both doses produced a decrease (p<0.01) in large and small movements. These data did not cause death in any of the animals. The results of this study indicate subtlethel neurotoxic and behavioral effects of FVN.

BIODEGRADATION OF DDT BY THE WHITE ROT FUNGUS PHANEROCHAETE CHRIOSPORIUM. J.A. Bumpus and S.D. Ault. Department of Biochemistry, Michigan State University, East Lansing, MI.

We have examined the metabolism of DDT by the white rot fungus Phanerochaete chrysosporium. During a thirty day incubation period about 50% of the DDT was metabolized and approximately 8% was mineralized in nutrient nitrogen deficient cultures. Of the remaining 14C-labeled material, 70.3% was extracted into hexane, and 17.8% and 0.5% was extracted by methylene chloride at pH 2.0 or 12.0, respectively. Significantly, at least 11.4% of the labeled material remained in the mycelium and aqueous fraction. Hexane extractable metabolites identified by GC-MS included dicyclo (1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethanol), FW-152 (1,1-Bis(4-chlorophenyl)-2,2-dichloroethanol), and 4,4'-dichlorobenzophenone. The acidic methylene chloride extract contained a metabolite which co-migrated with authentic 4-chlorophenyl acetic acid during HPLC. In nitrogen sufficient cultures, DDT degradation appeared to proceed via a more conventional pathway in which DDD is a major metabolite. As shown previously, mineralization of 14C-DDT was promoted by nutrient nitrogen starvation whereas in nitrogen sufficient cultures, mineralization was suppressed. (EPA C8811444).


Paraquat (PQ) is a weed killer whose recognized toxic effects are characterized by the development of pulmonary lesions which eventually lead to fibrosis. Male Long-Evans adult rats were treated with PQ (30 mg/kg, ip) or vehicle (distilled water) at 4, 8, 12, 24, 48 and 72 hrs after the treatment. While total lipids, phosphatidylcholines (PC) and disaturated PC were only decreased significantly 48 hrs after the treatment, the alkaline phosphatase (AKP) activity of the lung homogenates was significantly reduced as early as 12 hrs. At 48 hrs, the AKP activities in the lung homogenates, lamellar bodies and pulmonary surfactant (SF) were all decreased by 50%. In the lung homogenates, other hydrolases such as the acid phosphatase and β-N-acetyl glucosaminidase, were either respectively less reduced and much later in time, or not at all. The cytoplasmic marker lactate dehydrogenase was not affected by the PQ treatment. Due to the specificity of the toxicity of PQ for the alveolar epithelium, and the close association of an AKP activity with the SF system in the alveolar Type II cells, these results suggest that AKP could be used as an early and sensitive biochemical indicator of pulmonary damage to these cells after PQ poisoning.

MECHANISTIC STUDIES ON THE INHIBITION OF SUPER-OXIDE ANION RADICAL PRODUCTION IN PHAGOCYTIC CELLS BY REACTIVE ALDEHYDES. G. Witz, N.J. Laurie, M.A. Amoroso and B.D. Goldstein. UMDNJ-Rutgers Medical School/Rutgers University, Joint Graduate Program in Toxicology, Piscataway, NJ.

Inhibition of superoxide anion radical production in stimulated phagocytic cells by α,β-unsaturated aldehydes such as acrolein (ACR) and crotonaldehyde (CRO) has been shown in our laboratory to occur in vitro in a dose-related manner. Based on the known reaction of these compounds towards cellular sulfhydryl (SH), the present studies were aimed at investigating changes in cellular SH which might correlate with changes in O_2^- production. Plasma membrane surface SH, measured using carbamoylpyridiniumsulfide, and soluble intracellular SH, measured using 5,5'-dithiobis(2-nitrobenzoox acid), were examined in cells treated with 1-1000 uM aldehyde. In both human polymorphonuclear leukocytes (PMN) and rat alveolar macrophages (AM) there was a dose-related decrease in surface SH and soluble SH after ACR or CRO treatment. At any one aldehyde concentration, the decrease in surface SH was greater than the decrease in soluble SH. In PMN and AM preincubated with 5-40 uM ACR, there was a dose-dependent inhibition in the rate of O_2^- production with no effect on the lag time as measured by cytochrome C reduction. In stimulated PMN there was a dose-related decrease in the rate after addition of 5-40 uM ACR. Supported by NHI grant ES02510.
The toxicity and metabolism of 2-butoxyethyl (2,3,5,6-tetrachloro-2-pyridyl)oxy acetate (triclopyr BEE), was investigated using the slevin and juvenile stages of the salmon. The alevins were more sensitive (96 h LC50 0.26 mg/L) than the juveniles (96 h LC50 1.3 mg/L). The difference in sensitivity may be explained, in part, by a difference in the metabolic or excretory capacity of the two life stages. Analysis of exposure water showed that the t1/2 of the ester in tanks with juveniles is approximately 11 hours while the t1/2 is about 80 hours with an equivalent mass of alevins. Tests with 14C triclopyr BEE show that juveniles rapidly absorbed triclopyr BEE from water (t1/2=1.4 hours) and internally hydrolyzed it to triclopyr acid (t1/2=1.2x10^-4 hours). The acid is rapidly excreted with a t1/2 of 0.5 to 8.7 hours. C-residues in whole fish indicate that triclopyr acid was the main constituent (maximum level 120 mg/kg) with ester 0.1 mg/kg. Several other metabolites were also detected at low levels. Total residue after 59 hours of depuration was <17 of the maximum level at the end of the uptake phase.

The disposition of DEHP in rainbow trout was evaluated by pharmacokinetic analysis of plasma concentration-time data, after acclimation of the fish to 6, 12 or 18 C. Fish were cannulated at the dorsal aorta, then injected with a bolus of 400 μg C-14 DEHP/kg. Serial blood samples were removed for 6 days, and the concentration of unchanged DEHP in plasma was determined by reverse isotope dilution and gas chromatography. A three compartment mammary model, with elimination from the central compartment, best fit the experimental data. Temperature had no effect on the apparent volume of distribution of the central and second shallow compartments, and intercompartmental clearances. Statistical moment theory gave estimates of steady state volume of distribution of 122, 307 and 1163 ml/kg, and estimates of total body clearance of 15, 23 and 35 ml/hr/kg, at 6, 12 and 18 C. Mean residence times of DEHP in rainbow trout at 6, 12 and 18 C were 11, 22 and 33 hours, while the terminal elimination half-life was about 100 hours and was temperature independent. Pharmacokinetic analysis provided a mechanistic evaluation of how the disposition of DEHP was altered in response to environmental temperature.

Analysis of field samples of catfish under active antibiotic treatment revealed consistently high concentrations in the G.I. tract contents, but extremely low tissue levels. Studies in channel catfish compared intravascular (i.v.) and per os (p.o.) administration. The tissues and fluids examined included muscle, liver, bile, head and trunk kidney, spleen, urine and plasma. Following i.v. administration, the concentration in the bile far exceeded that of any other compartment. The next highest concentrations were found in the liver, followed by the kidney. Nearly half of the administered dose was excreted by the urinary route within 48 hrs. The half-life of the drug in the plasma during the initial phase of clearance was approximately 1/2 hr. This antibiotic was bound 70-80% to catfish plasma proteins as determined by ultrafiltration techniques. Following p.o. administration, peak plasma concentrations occurred at 1 hr. with a similar pattern of decline noted by the i.v. route. When the drug was administered in a mixture with feed, the bioavailability was estimated to be less than 5%. The limited bioavailability leads us to question the efficacy of antibiotic use under aquaculture conditions.
Diethyl nitrosamine (DENA) exposure of embryonated trout eggs results in carcinogenicity and lethality. To determine effects of DENA on development of *P. promelas*, we used a microdrop technique to expose embryonated eggs to DENA in dimethyl sulfoxide (DMSO). Concentrations ranged from 2.92 x 10^-7 M (3.58 x 10^-7 g/egg) to 9.72 x 10^-6 M (1.19 x 10^-5 g/egg). Following a single exposure, eggs were rinsed and placed in rearing vessels for 96 hr. Lethality was not a major finding, highest mortality was associated with the highest dose but a LD50 was not reached by 96 hr. Developmental anomalies were seen at 2.92 x 10^-7 M, 2.92 x 10^-6 M, 9.72 x 10^-6 M, 2.92 x 10^-5 M, and 9.72 x 10^-5 M. Defects included: tubular heart - absence of endocardial tube loop formation; enlarged pericardial cavity; hypoplasia of optic cups; and a lack of development of central nervous system tissue as evidenced by midline fusion of optic cups. Results from this study support the usefulness of *P. promelas* embryos in aquatic toxicity testing and demonstrate the teratogenic effect of DENA. Results support previous studies with hydrazine in *P. promelas* where similar pericardial cavity, heart, and microcephalic conditions were observed.

(Supported by USGS Grant No. 14080001G941.)

Organisms are more frequently exposed to complex mixtures than to single chemicals. Using the developing Japanese medaka (*Oryzias latipes*) as a model system, our lab has investigated the lethal and sublethal effects of mixtures containing carbon tetracloride (CCl₄), chloroform (CHCl₃), trichloroethylene (TCE), and tetrachloroethylene (PCE). The nominal LC₅₀'s and the lowest toxic concentration (TC₅₀) for the individual chemicals were determined: CCl₄=96 ppm (55 ppm), CHCl₃=215 ppm (75 ppm), TCE=85 ppm (25 ppm), and PCE=25 ppm (17 ppm). Regulatory agencies currently assume chemicals in a mixture act in a strictly additive manner unless quantitative data suggest otherwise. Therefore, chemical mixtures were examined for additivity using two of the haloegenated hydrocarbons, each at their 1/2 LC₅₀ (equivalent). The mixtures of CCl₄ + TCE (41% death) and CHCl₃ + TCE (57% death) demonstrated dose addition, which is in agreement with the regulatory assumption. However, the mixture of CCl₄ + PCE (0% death) was less than additive, and CCl₄ + CHCl₃ (91% death) exhibited a greater than additive effect. A complex mixture of all four chemicals, each at their 1/4 LC₅₀, exhibited a strictly additive effect, 50% death. (NAES E-01407-4-85)

**THE EFFECTS OF 2,3,7,8-TETRACHLORIDIBENZO-P-DIOXIN (TCDD) ON THE DEVELOPMENT AND SURVIVAL OF THE JAPANESE MEDAKA (ORYZIAS LATIPES). K.R. Cooper, J. Schell, P. KAHN and M. Gallo. Joint Graduate Program in Toxicology, Rutgers/UMDNJ, Piscataway, NJ**

Dioxins readily bioconcentrate in aquatic organisms and are found at high levels within river sediments adjacent to contaminated land sites. Since the river and estuarine systems are often nursery areas for fish, the effects of the 2,3,7,8-TCDD was examined in the developing egg of the Japanese medaka. The eggs were reared in 1.5 ml capped vials and the concentration of TCDD was made up in the rearing solution. The TCDD concentrations tested in this system were 50, 50, 25, 19, 12, and 6 ng/L. In addition, a solvent (nonane) and no treatment control were tested. The eggs were staged for development, death and any gross lesions on a daily basis. Survival post hatch was also examined. No deaths or lesions were observed in the solvent or no treatment controls. The LC₅₀ to hatch equaled 32.0 ng/L (95% confidence 40.0-25.0 ng/L), while the EC₅₀ for gross lesions equaled 19 ng/L (95% confidence 15.0-24.0 ng/L). No deaths or gross lesions were observed until the formation of the liver rudiment (stage 29). TCDD resulted in delayed development and at the highest doses complete cessation of development. There was 100% survival for 3 days post hatch at 19.0 ng/L. (NAES E-01407-4-85)
The structural organization of membranes is dynamically affected by many factors. These include lipid-protein interactions, ionic environments, the nature of component lipids and enzymes of lipid synthesis & modification. We have confirmed previous reports that lipid peroxidation (LP) also affects the physical organization of membrane lipids. The effects of LP are striking in that low level LP among the phospholipids produces substantial decreases in the isotropic motion of membrane lipids. Using the fluorescent probe diphyllythylamine (DPH) with model membranes consisting of various ratios of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), we induced LP by means of two free-radical generating mechanisms: 1. ADP·Fe³⁺ + O₂⁻ and 2. cumene hydroperoxide + Fe. In both cases a progressive increase in LP was measured via formation of thiobarbituric acid reacting products (TBAR) and conjugated dienes. The previously reported effects of PE on the order of PC lamellar structure were noted where increasing proportions of PE produce decreased order. Increased LP (equivalent to TBAR of 0.095, (o2)² = 0.004) were associated with increases in order; an effect similar to that produced by doubling the PC/PE ratio (from 0.45 to 0.90). A consideration of the structural properties of PC/PE membranes suggests that small amounts of peroxidized phospholipids induce a significant membrane reorganization.

111 IMPORTANCE OF THE RATIO OF VITAMIN E (E) TO POLYUNSATURATED FATTY ACIDS (PUFA) IN LIPID PEROXIDATION (LP). M.A. Leedle and S.D. Aust, Center for Environmental Toxicology and the Departments of Pathology and Biochemistry, Michigan State University, East Lansing, MI

The ratio of E to PUFA (mole fraction) was important in the occurrence of LP in rat liver microsomal lipids (NADPH, ADP·Fe³⁺, EDTA·Fe³⁺, and cytochrome P-450 reductase). PUFA were determined with GC (mole fraction) and E with HPLC (mole/uncle lipid PO₄). PUFA were 72% of total fatty acids and E/PUFA ratio was 0.3. Addition of E to achieve ratios of 2, 3.3, and 5.5 resulted in decreased LP with complete inhibition at 5.5, but E decreased at a similar rate regardless of the ratio. In contrast, rat lung microsomal lipids had an E/PUFA ratio of 4.3 with 28% PUFA. LP did not occur and E was not lost during incubation. Addition of E and diesterolophosphatidylcholine to liver lipid to achieve 28% PUFA and a ratio of 4.5 completely inhibited LP. E was lost at a rate 59% less than at 72% PUFA. Lung lipid was added to liver lipid to achieve E/PUFA ratios of 0.62, 0.95, and 3.3 with PUFA of 53%, 46%, and 35%, respectively. LP and initial rates of E loss decreased with increasing amount of lung lipid. (NIH HL3543 and ES07146).

112 ENHANCED GLUTATHIONE BIOSYNTHESIS IN ISOLATED HEPATOCYTES BY VITAMIN E: INHIBITION OF EFUX OF PRECURSOR POOLS. G.A. Pascoe, K. Olafsdottir, and D.J. Reed, Environmental Health Sciences Center, Oregon State University, Corvallis, OR.

Oxidative stress generated in isolated rat hepatocytes by the absence of extracellular calcium depletes intracellular glutathione (GSH) and stimulates its oxidation and efflux. Supplementing the cell incubation media with vitamin E succinate (25μM) prevents this depletion without affecting GSH oxidation or efflux, resulting in a net increase in total cellular GSH. This effect of vitamin E was inhibited by buthionine sulfoximine, but not by diethylmaleate or ethacrynic acid. After prelabelling the pools of sulfur-containing precursors of GSH biosynthesis with 35S-methionine, the absence of extracellular calcium was observed to accelerate their efflux from isolated rat hepatocytes. Vitamin E succinate addition to the incubation media inhibited this efflux, while maintaining cellular GSH levels. These findings were supported by HPLC analyses of cytoplasmic methionine and cysteine contents. From these data we conclude that precursors to GSH biosynthesis via the cystathionine pathway show stimulated efflux during oxidative stress in isolated hepatocytes, resulting in diminished GSH biosynthesis. Vitamin E appears to enhance GSH biosynthesis during oxidative stress by maintaining the sulfur-containing precursor pools. Supported by USPHS ES07060 and ACS-109.
113 ACTIVATION OF LIVER MACROPHAGES MAY CONTRIBUTE TO ACETAMINOPHEN HEPATOTOXICITY. Anne M. Pilaro, Sangchul Lee and Debra L. Leskin. Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

Treatment of rats with acetaminophen (AA, 1.2 g/kg) results in the accumulation of macrophages (MP) in the pericentral regions of the liver. To determine whether AA treatment induces the accumulation and activation of MP, we compared the morphologic and functional properties of MP isolated from livers of control (CMP) and AA-treated (AAMP) rats. MP were isolated 24 hr following AA treatment by collagenase and pronase perfusion, selective digestion and differential centrifugation. We found a 3-5 fold increase in the number of MP isolated from AA-treated rats. These cells were generally larger, rounder and more vacuolated than control cells. Using the Boyden chamber technique, CMP and AAMP were found to display chemotaxis to a variety of stimuli including complement fragments (C5a), synthetic collagens peptides, and 12-O-tetradecanoylphorbol 13-acetate (TPA). AAMP were generally 7-10 times more responsive to chemotactic agents than control cells. MP from AA-treated rats were also found to release 2-4 times more superoxide anion in response to C5a and TPA than control cells. These data demonstrate that AA treatment leads to activation of MP and suggest that these cells may contribute to AA-induced hepatotoxicity. Supported by NIH grants GM34310 and AA5844.

115 CALCIUM TRANSPORT AND HEPATOTOXICITY FOLLOWING N-NITROSODIMETHYLAMINE EXPOSURE IN MICE. F.A. Reitman and H.G. Shertzer. Dept Envir Health, Univ Cincinnati Med Ctr, Cincinnati, OH 45267.

The hepatotoxins N-nitrosodimethylamine (NDMA) or carbon tetrachloride (CCL4) were administered to ICR Swiss mice, and dose/time responses were determined for serum glutamate- pyruvate transaminase activity (SGPT, an index of hepatotoxicity), liver nonprotein sulfhydryl concentration (NPS), and Ca++ uptake activity in liver microsomes and mitochondria. A hepatotoxic NDMA dose (20 mg/kg) did not alter the concentration of NPS or the ability of mouse liver microsomes to accumulate Ca++ until after liver necrosis was apparent. Mitochondrial Ca++ uptake was not affected by NDMA. Administration of CCL4 at doses of 0.05 to 1.0 ml/kg produced high SGPT activities at 24 hr post-dose, and 60% to 80% depression of microsomal Ca++ uptake at 2 hr post-dose. A non-neurogenic CCL4 dose (0.4 ml/kg) did not affect microsomal Ca++ transport. NPS concentrations were similar to control values until 6 hr after administration of 0.2 or 1.0 ml/kg CCL4, then decreased rapidly to 20% of control by 12 hr post-dose. 1.0 ml/kg CCL4 did not inhibit mitochondrial Ca++ uptake prior to the appearance of high SGPT activity. We conclude that early inhibition of microsomal Ca++ uptake is correlated with subsequent hepatotoxicity of CCL4, but not of NDMA.
(Supported by NHLBI grants ES-05373 and CA-28277)

114 INTERACTION OF TRANSITION METALS AND ACTIVE OXYGEN IN DIQUAT CYTOTOXICITY. M.S. Sandy, P. Maldeus, D. Ross, P. Doane and M.T. Smith. School of Public Health, University of California, Berkeley, CA.

Diquat and an isolated hepatocyte system have been used to investigate the mechanism of cytotoxicity induced by active oxygen species formed as a consequence of redox cycling. The glutathione reductase activity of the hepatocytes was selectively inhibited by pretreatment with 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) without altering cellular glutathione levels. Diquat caused a rapid depletion of intracellular GSH with a concomitant increase in GSSG in this compromised hepatocyte system. Toxicity was observed within 60 min, demonstrating the protective role glutathione peroxidase plays in detoxifying the Fenton-type reaction producing H2O2. Several scavengers of active oxygen have been tested for their ability to prevent against GSH loss and toxicity. Only catalase and DMSO were at all effective, supporting a role for H2O2 and the hydroxyl radical (HO·) in producing toxicity. The iron chelator desferrioxamine significantly delayed toxicity whereas the addition of copper ions greatly enhanced the cytotoxic effects of diquat. These results further support the notion that H2O2 and transition metals interact in a Fenton-type reaction to produce diquat cytotoxicity.


Previous studies from this laboratory demonstrated enhanced hepatotoxicity and lethality of bromotrichloromethane in chlorocon pretreated animals. The present study was designed to investigate the role of intracellular calcium homeostasis in the progression of BrCCl3 hepatotoxicity by chlorocon. Since BrCCl3 hepatotoxicity is dependent upon its bioactivation to •Cl2, free radical, its interaction with phenobarbital as a standard inducer of cytochrome P-450 and with meflo as a negative control was also studied. 45Ca uptake by isolated mitochondria and microsomes as well as total calcium levels in these subcellular organelles were measured. Chlorocon pretreatment enhanced the BrCCl3-induced inhibition of mitochondrial and microsomal 45Ca uptake processes. This potentiation of 45Ca uptake inhibition was significantly higher as compared to phenobarbital + BrCCl3 or meflo + BrCCl3 groups. In the animals of all the three pretreatment groups receiving BrCCl3 there was a significant increase in whole liver calcium levels. This increased calcium was distributed mainly in mitochondrial and microsomal fractions. Large increases in cytosolic calcium were observed only in chlorocon + BrCCl3 group of animals. The data emphasizes the role of calcium in the progression of BrCCl3 toxicity.

Carbon tetrachloride (CCl₄) was used as a model toxic agent to investigate alterations in the control of protein phosphorylation in primary rat hepatocyte cultures. Since CCl₄-treatment has been found to increase production of cAMP phosphodiesterase (possibly acting through Ca²⁺-activated phosphatase), we chose to investigate whether other proteins may serve as substrates for Ca²⁺-activated kinases. Therefore, cultured rat hepatocytes were preincubated with [³²P] to label the ATP pool in situ, treated with 0.1 to 2.0 mM CCl₄ for 5 minutes. Cytoplasmic proteins were extracted with digitonin, subjected to polyacrylamide gel electrophoresis, and then analyzed by autoradiography. Following CCl₄-treatment, an increase in the phosphorylation of two protein bands (MW = 93K and 83K) was observed. Both the 93K and 83K M₅ bands exhibited a dose-dependent response to CCl₄-treatment. Phosphorylation of the 93K protein may represent the elevation of phosphatase activity observed after CCl₄ exposure. Although these two phosphoproteins may not be directly involved with the mechanism of CCl₄ hepatotoxicity, further investigation may identify other cellular proteins whose state of phosphorylation is altered following CCl₄ exposure. (Supported by NIH grant 5 RO1 GM 33431.)


Effects of mirex (MX), diethylsulfamide (DEN), p,p'-DDT (DDT), phenobarbital (PB) and phorbol ester (TPA) on cytosolic cAMP-dependent protein kinase (cAMP-PK) activity was studied in vitro. MX and TPA caused significant reduction in the cAMP/cAMP-PK activity ratio (15% and 2%, respectively); TPA was more effective being greater than TPA at all concentrations (5-10³ ng/ml). The effect of DDT on the activity ratio was only marginal. DEN and PB maintained a steady stimulatory effect (13% and 15%, respectively) regardless of the doses. MX and DDT markedly induced the cAMP-PK activity similar to TPA, whereas DEN and PB showed the least effects on the activity. Subject increase in the cAMP-PK activity was observed in all cases. MX, TPA and DDT in combinations with DEN or PB exhibited an enhanced activity ratio (ranging from 15-33% above control). Combinations of MX, TPA and DDT had no stimulatory effects on the ratios. The results may suggest that like TPA, organochlorine pesticides (MX & DDT) may be inhibitory to cAMP-PK activity while DEN and PB stimulate this system. However, MX, DDT and TPA in combination with DEN or PB may potentiate cAMP-PK activity. (Supported by BRSG Grant S07RR05399-24, NIH.)


cAMP-PK activity was measured following neonatal exposure (ip) of MX (1 mg/kg) and DEN (15 mg/kg) 3, 7, 14, & 21 days after birth. Rats were killed at 5, 10, and 20 days of age and total (+cAMP) and basal (-cAMP) PK activities were determined. MX consistently produced an enhancement of both basal (1.2-3.1 fold) and total (1.4-4.8 fold) PK activity; however, -cAMP/+cAMP-PK activity ratios were markedly lower than control (8%-62%). DEN also caused an enhancement of PK activity (1.2-2.5 fold) and total (1.2-2.3 fold) PK activity similar to MX. However, -cAMP/+cAMP-PK activity ratio profile showed a marked increase on Day 5 (15%) which declined to 62% on Day 15, and to 12% at Day 20. The data indicate that an 'epigenetic carcinogen MX caused a significant reduction of cAMP-PK activity throughout the neonatal period. DEN, although initially activated cAMP-PK system, produced a decrease in activity thereafter. Irrespective of the mechanism of action of these carcinogens, neonatal exposure of both MX and DEN seem to cause an overall diminution of cAMP-PK activity. The significance of this cellular response in regard to the neonatal initiation-promotion rat model has to be carefully evaluated. (BRSG-S07RR05399-24).


Metabolic activation by cytochrome P-450 is a necessary step in the initiation of AAF mutagenicity, carcinogeticity and teratogenicity. Thus, it is critical to investigate the onset of cytochrome P-450-mediated AAF metabolism in early mammalian development without maternal metabolic contributions. Mouse embryos were explanted at 4½ days gestation (d.g.), cultured and AAF or aminofluorene (AF) metabolism determined indirectly by measuring sinter chromatid exchanges (CSE). Incubation with AAF for 24 hr resulted in a dose-dependent 2-fold increase in CSE over background. This basal activity was increased an additional 3-fold by preincubation with 5,6-benzoflavone (5,6NF) in outbred mice but not in the genetically Ah-"nonresponsive" AFR/K strain. 7,8NF inhibited 5,6NF induction without affecting uninduced levels. AAF also resulted in increased production of SCE. Basal activity was induced 3-fold by 5,6NF in outbred strains, but not in a strain exhibiting slow acetylation (A/J). These data suggest that N-acetylation is required for AF activation. It is concluded that embryos possess the capacity for cytochrome P-450-mediated metabolism of AAF as early as 7½ d.g., and this activity is regulated by the Ah-receptor. These results suggest an important mechanism leading to the potential toxicity of AAF during embryogenesis.
121 INHIBITION OF HEPATIC UDP-GLUCURONYL TRANSFERASE (UDP-GT) ACTIVITY COINCIDENT WITH ELEVATED PLASMA SEX STEROID CONCENTRATIONS DURING GONADAL MATURATION IN CARR. T.C. S. Stokl, R.A. Tohill, and L.R. Curtis. Oak Creek Laboratory of Biology, Department of Fisheries and Wildlife, Oregon State University, Corvallis, OR.

In vivo glucuronidation of phenolphthalein was inhibited in rainbow trout during onset of gonadal maturation (Comp. Biochem. Physiol. 76C: 107). We further examined this phenomenon in male and female carp by examining hepatic microsomal UDP-GT activity, plasma testosterone and estradiol concentrations, and histological changes in gonadal tissues in time-course studies. Onset of gonadal maturation was modulated by holding fish under different photoperiod/temperature regimes and administration of exogenous gonadotropins. In male and female fish with respective plasma testosterone or estrogen concentrations below 1 ng/ml, UDP-GT activity ranged between 10 and 15 nmol p-nitrophenol conjugated/gm liver/min. Elevations of sex steroids associated with maturational changes in gonads were coincident with 3-fold and greater reductions in UDP-GT activity. Enzyme activity recovered as plasma steroid concentration declined. Since UDP-GT metabolizes sex steroids its inhibition was likely related to maintenance of hormonal concentrations required for gonadal development. (Supported by U.S.A.I.D. Grant DAN-4023-GSS-2074-06).


Assessments of reproductive toxicity risk to human populations should include: 1) determination if the agent is hazardous to normal sexual and reproductive function; 2) characterization of the relationship between dose level and response; and 3) extrapolation between the test species and humans to estimate an allowable exposure level. Steps two and three are seldom possible due to use of inadequate protocols and lack of fundamental knowledge, respectively. Usually, safety factors must be utilized instead. In preparing the guidelines for male reproductive risk assessment at EPA, attention is being focused on such deficiencies. In this presentation, the importance of steps two and three are demonstrated and suggestions are made as to the needed background information and modifications in toxicity testing protocols that could improve the quality of the risk assessments. Particular attention is directed toward the issues of endpoint sensitivity, high to low dose extrapolation and estimation of acceptable lifetime exposures for humans from acute or subchronic toxicity data for the human male reproductive system. (This paper does not reflect the opinion or policy of EPA).

122 EFFECTS OF METHYL MERCURY ON SPERM O2 CONSUMPTION. M.K. Mohamed, T.C. Evans, N.K. Mottet and T.M. Burbacher. Department of Pathology, University of Washington, Seattle, WA.

The current experiments were designed to investigate whether or not the decreased spermatozoal motility, associated with in vitro exposure to methylmercury (MeHg), is related to inhibition of O2 consumption. Oxygen consumption and percent motile spermatozoa were determined for semen samples from healthy male monkeys Macaca fascicularis. MeHg was added to the samples at a concentration of 9 ppm for 15 minutes and then increased to 15 ppm. O2 consumption and percent motility were determined during each period. MeHg addition resulted in decreased sperm motility, but we did not detect any inhibition in the rate of O2 consumption accompanying the decreased motility. On the contrary, the rate of O2 consumption increased at 15 ppm within 15 minutes, while the sperm motility was close to zero. Antimycin inhibited the increased rate of O2 consumption demonstrating the mitochondrial source of this increased rate. Oligomycin also inhibited the increased rate of O2 consumption due to MeHg thus excluding the possibility of uncoupling of mitochondrial oxidative phosphorylation. Interference with mitochondrial energy production does not seem to be the primary mechanism of MeHg induced decreased spermatozoal motility. MeHg interference with the dynein/microtubule sliding assembly now seems to us to be a more teneable hypothesis.

124 EFFECTS OF CHRONIC ORAL ADMINISTRATION OF COBALTIOUS CHLORIDE (CO) ON REPRODUCTION IN MALE MICE. N.G. Pedigo, M.B. Anderson, and W.J. George, Departments of Pharmacology and Anatomy, Tulane University School of Medicine, New Orleans, LA. Sponsor: R.F. Ochillo

Male CD-1 mice received Co (400 ppm) in their drinking water for up to 13 weeks. Evaluation of toxic effects on reproduction was made beginning at week 7, including fertilization studies, testicular histology and weights, and sperm parameters. There were no significant differences between Co-treated and control groups at week 7. Beginning at week 9, testicular weight as a percentage of body weight was significantly decreased from controls (p < .05). Testicular weight continued to decrease to a low value of 0.24% on week 13 as compared with 0.77% for controls. Sperm concentrations showed a significant decline between weeks 9 and 13 (37% of control at week 9, 265 of control at week 11, and 17% of control at week 13). Concurrent measurements of sperm motility and percent motile sperm showed corresponding decreases in Co-treated males as compared with controls. The ability of sperm to fertilize normal ova was evaluated in superovulated females mated with control and Co-treated males. There was a sharp decline in the percent of ova fertilized in the Co-treated group between weeks 11 and 13, with values of 83% and 29%, respectively. Evaluation of the fertilizing ability of sperm has been continued for 12 weeks after termination of Co treatment with no evidence of recovery.
Zonisamide, a new anticonvulsant agent, was evaluated for effects on fertility and general reproduction in a multigeneration study. Prior to mating, mature male and female CD rats (24/group) were given the drug in diet at 0.25, 50, and 100 mg/kg for 60 and 14 days, respectively. Treatment was continued during mating, gestation, parturition and lactation in females. One half of the females in each group was sacrificed at the end of gestation; litter and fetal parameters were evaluated. The remaining animals in the group were allowed to deliver and raise their offspring. Development and physiological parameters of the progeny were evaluated, and F1-derived litters were mated. Suppression of body weight gain and food intake occurred principally at 50 and 100 mg/kg for both sexes of the F1 generation during most of the treatment and postnatal period. Litter parameters of treated animals were comparable to controls. Fetal and neonatal pup weights were reduced at the 50 and 100 mg/kg which persisted until postnatal Day 35 at 100 mg/kg. No behavioral alterations or treatment-related anomalies were found. The fertility rates of F1 and F2 offspring were comparable to controls. From these studies, no adverse effects due to Zonisamide on general reproduction performance would be expected.

EDB-induced sterility in male animals has been reported to be the result of testicular atrophy and/or alterations of sperm morphology and number. The goal of this study was to determine if EDB also induces post-testicular biochemical changes. In particular, to determine the potential for EDB-induced toxicity to the carnitine system, a system believed vital to sperm energy metabolism and motility. EDB doses of 20, 40, and 50 mg/kg significantly reduced carnitine acetyltransferase in male F-344 rats by 5, 10 and 15%, respectively. A dose dependent reduction in endogenous carnitine levels in the epididymal fluid was also observed. Corresponding to these changes in the carnitine system were parallel decreases in 15, 20 and 24% in sperm motility. Significant reductions in caput sperm numbers were also observed at all doses. The ratio of testicular to body weight remained unchanged and there was no evidence of testicular atrophy in these experiments. Therefore, EDB may induce sterility in rats by adversely altering the carnitine system of epididymal spermatozoa in addition to its possible interference with spermatogenesis. [Supported in part by USPHS Grant ES02084].

The metabolism of bis(2-methoxyethyl) ether (diglyme), a testicular toxin, was evaluated in male Sprague-Dawley rats following a single p.o. dose of 5.1 mmol/kg bw. By radioisotope analysis it was shown that 84.5 ± 7.0% of the dose was excreted in the urine by 96 h. The major urinary metabolites isolated by HPLC and characterized by GC-MS were methoxyethoxyacetic acid (MEAA) (67.9 ± 7.3%) and methoxyacetic acid (MAA) (6.2 ± 1.8%). Six other minor unidentified metabolites (< 2%) were observed. The administration of daily consecutive 5.1 mmol/kg bw p.o. doses of diglyme (12 doses) and 2-methoxyethanol (2 doses) both produced histopathologic, gross (testicular wt, atrophy), and enzymatic (LDH-X) evidence of testicular toxicity during serial sacrifices. In contrast, the administration of 20 consecutive daily 5.1 mmol/kg bw doses of MEAA or its precursor metabolite, 2-methoxyethoxyethanol, produced no evidence of gross or histopathologic or enzymatic testicular toxicity. These data indicate diglyme is a testicular toxin by virtue of metabolic cleavage of the central ether linkage and not via O-demethylation at the terminal methoxy groups. These results may have implications relevant to the mammalian toxicity of other polyethylene glycol ethers.

In vitro teratogenicity assays may provide economical alternatives to animal testing as preliminary methods for screening industrial R&D materials. Four assays which predict the potential of a chemical to induce congenital malformations are reviewed. These include the mouse ovariun tumor cell attachment, the human embryonic palatal mesenchyme cell growth inhibition, the mouse embryo limb bud cell culture, and the hydra assays. Based on available data, the sensitivity (the ability to detect teratogens as positives), specificity (the ability to score nonteratogens as negatives), and accuracy (the ratio: number of correct results obtained/number of chemicals tested) of each assay are calculated. Battery selection and predictivity of assays in the battery using Bayes's formula are given. Additional in vitro teratogenicity assays are reviewed and will be considered for inclusion in the test battery selection when validated data are available. A selected test battery appears to be desirable for screening the teratogenic potential of industrial R&D materials.
Nitrpyrin (2-chloro-6-(trichloromethyl)-pyridine), a nitrogen stabilizer, was tested to evaluate its teratogenic potential in pregnant rabbits. Groups of 25-28 Insenized New Zealand White rabbits were administered nitrpyrin in corn oil by gavage on days 6 through 16 of gestation at dose levels of 0, 3, 10 or 30 mg/kg/day and the fetuses examined for evidence of developmental toxicity. Slight maternal toxicity was evident in the 30 mg/kg/day dose group in the form of decreased weight gain during dosing, and increased absolute and relative liver weights at C-section. There were no treatment-related increases in the incidence of malformations in any of the groups when compared to controls. An increased incidence of crooked hyoid bones at 30 mg/kg/day, indicative of only slight fetotoxicity, was the only evidence of any treatment-related effect observed among fetal rabbits. Thus, oral administration of nitrpyrin to rabbits during the period of organogenesis produced evidence of slight fetotoxicity only at a dose level which produced maternal toxicity, with no indication of a teratogenic response.

A standard two-generation reproduction study with CD Sprague-Dawley rats was performed at exposure concentrations of 40, 10, or 0 (control) ppm of NB vapor. No NB-related effects on reproduction were observed at 10 or 1 ppm. At 40 ppm, a decrease in the fertility index of the F₀ and F₁ generations occurred, which was associated with alterations in the male reproductive organs. Specifically, testes and epididymides weights were reduced and seminiferous tubule atrophy, spermatocyte degeneration, and the presence of giant syncytial spermatocytes were observed. The only significant finding in the litters derived from the 40 ppm rats was a decreased body weight of F₁ pups on postnatal day 21. Survival indices were unaltered. To examine the reversibility of this selective effect on male gonads, the 40 ppm F₁ males were allowed a 9-week non-exposure period and mated to naive females. An almost five-fold increase in the fertility index was observed indicating at least partial functional reversibility upon removal from NB exposure. Also the numbers of giant syncytial spermatocytes and degenerated spermatocytes were greatly reduced. Sponsored by the Nitrobenzene Association.
HYPERPROLACTINEMIA AND ANESTRUS IN RATS TREATED WITH AN ANTHYPERTENSIVE AGENT (Losulazine). G.M. Mesfin, M.J. Higgins, D.F. Morris, G.A. Johnson, T.A. Marks. Pathology & Toxicology Research, The Upjohn Co., Kalamazoo, MI 49001

Female Sprague-Dawley rats failed to cycle during treatment with the 4-aminoguanidine antihypertensive agent, losulazine hydrochloride. The mechanism of interrupted estrous cycles was investigated by determining the effect of 10 mg/kg/day of losulazine on hypothalamic catecholamines and serum sex hormones and by evaluating the influence of 16.75 or 6.25 mg/kg/day of bremorcoptine (CB-154), a dopamine receptor agonist, on the reproductive functions of groups of rats treated with losulazine alone or in combination with CB-154. Rats treated with losulazine only were depleted of hypothalamic catecholamines, were hyperprolactinemic and had interrupted estrous cycles. Rats treated with losulazine plus CB-154 or CB-154 alone had low serum prolactin and normal estrous cycles. Groups treated with losulazine alone for 4 weeks had decreased reproductive tract weights and attenuated vaginal mucosa. Rats reverted back to hyperprolactinemia and anestrus shortly after CB-154 withdrawal. These results suggest that hyperprolactinemia, mediated through hypothalamic dopamine depletion, is the mechanism of anestrus in rats treated with losulazine.

PREDICTED NASAL AND TRACHEOBRONCHIAL PARTICLE DEPOSITION IN CHILDREN. R.F. Phalen and M.J. Oltham, Community & Environmental Medicine, University of California, Irvine, CA 92717.

Although considerable information exists on inhaled particle deposition in adults, the influence of body size is relatively poorly understood. In order to obtain estimates of particle deposition efficiencies in the nasal and tracheobronchial regions of newborns, infants, children and adolescents, two mathematical models were utilized. Hounam's relationship between nasal pressure drop and particle collection efficiency and Yeh's equations for tracheobronchial deposition were scaled downward to smaller body dimensions. The analysis indicates that, in many circumstances, smaller individuals will have lower nasal collection efficiencies and greater tracheobronchial efficiencies. Body-size effects lead to predicted differences in dose of a factor of 10 for particle diameters of less than 1 um, for example. Supported by a grant from the Univ. Calif. Toxic Substances Rsh. and Teaching Program.

THE IMPORTANCE OF RAT RESPIRABLE AEROSOLS IN PERFORMING QUANTITATIVE INHALATION TOXICOLOGY.

D.M. Bernstein and A.I. Nikiforov, Battelle, Centre for Toxicology and Biosciences, 7 route de Drize, 1227 Carouge/Geneva, Switzerland.

Rats are one of the most frequently used species in aerosol inhalation toxicology studies. The propriety of the rat as an animal model for man is clearly dependent on the ability to deliver a comparable and quantifiable dose to the rat lung. The differences between man and rat in respiratory tract anatomy, morphometry, and ventilation have a significant bearing on the penetration and deposition of aerosols in each species. Consequences of these differences will be presented with particular emphasis on their influence with regard to total and regional deposition efficiencies. The requirements imposed by species differences necessitate the use of aerosol generation and delivery techniques which produce rat respirable aerosols that have regional deposition patterns proportional to that seen in man. With the delivery of precisely controlled test aerosols and measurements of respiratory frequency and tidal volume, highly accurate quantitative inhalation toxicity studies can be performed.

THE FATE OF PARTICLES DEPOSITED IN THE PLEURAL SPACE.


Various types of particles are known to reach the pleural space within days after deposition in the lung. Little is known, however, about the fate of the particles and particle-cell relationships after particles reach the pleural space compartment (PSC). In the present study, we investigated the fate of particles deposited into the PSC of the Fischer-344 rat using 2.0 um latex microspheres as test-model particles. Under pentobarbital sodium anesthesia, 4 X 10^5 of the microparticles were introduced percutaneously into the PSC and at various times thereafter, the PSC and lungs of the animals were lavaged by three days after instillation of the particles, virtually all of the particles lavaged from the PSC were contained in pleural macrophages and neutrophils. Almost all of these cells contained particles, and 45% of the phagocytes contained 210 microspheres. As of 45 days later, however, only 3% of the pleural phagocytes contained microparticles, and few of these cells contained microparticles. Cells disengaged from the lungs at this time did not contain microparticles, while the endothelial and diaphragmatic lymph nodes were visibly filled with the microparticles. The results of this study demonstrate that particles that reach the PSC can be translocated theretofrom to other sites, perhaps by a phagocyte-mediated process. Light microscopic evidence, however, suggests that least some of the translocated particles move via cellular translocation across endothelial cells lining lymphatic channels. [This work was conducted under the auspices of the DOE].
137 STRAIN DIFFERENCES IN NASAL DEPOSITION OF ACETONE AND ETHANOL VAPORS. J.B. Morris, R.J. Clay and D.G. Cavanagh, School of Pharmacy, University of Connecticut, Storrs, CT.

Previous studies in this laboratory have shown that nasal deposition of acetone and ethanol vapors in the Sprague-Dawley (S-D) rat could be described by a ventilation-perfusion (V-P) model (Morris and Cavanagh, Toxicologist 8: 123, 1985). In the current study, deposition of these vapors was measured in the isolated nasal cavity of the F344 rat under unidirectional, constant velocity flow conditions using flows of 50-300 ml/min. Deposition of both gases attained quasi-steady-state in F344 rats during the 12 minute exposure period. When measured at identical flow rates, quasi-steady-state acetone and ethanol deposition efficiencies were as much as 30% greater in the F344 than in the S-D rat (p<0.005). Deposition of these vapors in the nasal cavity of the F344 rat could not be described by V-P models, but was successfully described by a diffusion limited mass-transfer model. For both gases the overall nasal mass transfer uptake coefficient was constant throughout the entire range of flow rates that were tested. These observations on acetone and ethanol deposition suggest that the nasal deposition process itself may differ in F344 and S-D rats.


A model consisting of "cross-ventilating" Fischer-344 rats has been developed for differential analysis of the pharmacokinetic characteristics (uptake, body distribution, elimination) of inhaled materials deposited in the nasopharyngeal compartment (NC) and the lung compartment (LC). This report describes: (1) surgical and instrumention techniques employed in the model, (2) methods to measure the tidal volumes (Vt), breathing frequencies (f), and flow rates (V) of cross-ventilating animals, and (3) stability of the above parameters, as well as blood gases and blood pH, in animals cross-ventilating. The NC of a donor rat is coupled with the LC of a recipient rat by connecting the donor's trachea to the trachea of the recipient rat under anesthesia. Both animals are implanted with arterial catheters and ventilatory measurements are made on the interfaced animals using a partial body flow plethysmograph. Initial studies have compared blood gas and pH values and ventilatory values of the recipient lung animal with a control (no tracheal preparation) animal 1 h after cross-ventilation began. No significant differences of blood gas and pH values were seen between the NC recipients (pH=7.36±0.04, PaO2=60±1.4 mmHg, PaCO2=38±1.4 mmHg) and intact rats (pH=7.40±0.05, PaO2=65±2.1 mmHg, PaCO2=40±1.4 mmHg). No significant differences were seen in Vt or VE between the NC recipients (Vt=1.4±0.2 ml, VE=150±35 ml/min) and intact animals (Vt=1.4±0.2 ml, VE=142±7 ml/min). (This work was performed under the auspices of the D.O.D.)


The distribution of 10 or 100 microliters of a dye suspension in rat lungs resulting from AI, EN or EI administration was studied. Three 5 μ thick slices were made in a plane approximately perpendicular to the axis of the lobar bronchus of the upper, middle and lower sections of each of the 5 lobes of the lung. The number of bronchi, bronchioles and alveolar ducts with and without dye were counted using the Videoplan Imagine Analyzer. The total area of dye-containing tissue per lung slice was measured with the Magiscan II Imagine Analyzer. The number-percent and the area percent data revealed that after a 4-hour AI, only a very small portion of the calculated 100 μl dye was deposited in the lungs, while the bulk of the dye was deposited oropharyngeally and swallowed. However, the dye which reached the lungs was well distributed down to the alveolar level. In comparison, EN or EI quantitatively delivered a dose of dye into the lung, but the dye was distributed mainly around the lobar bronchi.

AI is the method of choice for studies of pulmonary mechanics and airborne toxicants. EN or EI are useful for pulmonary disposition studies.

140 PULMONARY PARTICULATE RETENTION IN RATS AND GUINEA PIGS CHRONICALLY EXPOSED TO LOW CONCENTRATION OF DIESEL EXHAUST. K. A. Strom, J. T. Johnson and J. B. D'Arcy. Biomedical Science Dept., General Motors Research Labs, Warren, MI. Sponsor: E. W. Lee

In our study of diesel particulate retention and clearance from the lungs, male Fisher 344 rats and male Hartley guinea pigs were exposed to diesel exhaust diluted to 50 μg/m3 of diesel particulate (DP) for 20 hr/day, 7 days/week for 3, 6 and 12 months, with serial sacrifices at 0, 3, 6 and 12 months postexposure. DF increased linearly in the lungs of the guinea pigs to 1.5 mg at 12 months of exposure. Elimination of particulate from the lungs during 12 months postexposure was negligible. The total DF lavaged from the lungs remained constant during the one-year postexposure. The regional lymph nodes received 0.5% of the lungs' DP during the year of exposure and one-year postexposure. Rats accumulated 200 μg DF in 12 months of exposure. In contrast to the guinea pigs, at least 60% of the DP was eliminated from the lungs by three months postexposure. The total DF lavaged from the lungs declined during the postexposure period. As much as 3% of the total pulmonary DP was found in the regional lymph nodes following 12 months of exposure. These experiments indicate that there is ongoing clearance of DF from the lungs of rats, but not from the lungs of guinea pigs.

Fischer-344 rats were exposed to respirable aerosols of antimony trioxide (Sb) at 0, 0.2, 1.0, 5.0, or 25 mg/m³ for 13 weeks. Animals were sacrificed after 1, 2, 4, 8, or 13 weeks of exposure and after recovery periods of 1, 3, 9, 16, or 27 weeks. Growth rate, hematologic, chemical, and histopathology were assessed. In addition, Sb concentrations were measured in the left lung of each animal. Sb caused a reduction in growth rate in animals exposed to 5 and 25 mg/m³; increased lung weights and lung-body weight ratios at most intervals in the animals exposed to 25 mg/m³; and caused histological changes in the lungs of all animals. After 13 weeks, Sb levels in the lungs of male rats exposed to 0.2, 1.0, 5.0, and 25.0 mg/m³ reached 40, 143, 572, and 1830 ug/g respectively. After a 27-week recovery period, the values had dropped to 13, 14, 37, and 70% of the 13-week levels respectively. Similar data were seen in females. These data demonstrate a dose-dependent decrease in the quality of lung clearance which demonstrated that exposure to 0.2 or 1 mg/m³ increased 15% of the Sb in 27 weeks, whereas only 6% or 30% of the Sb is cleared from lungs of rats exposed to 5 or 25 mg/m³. Supported by the Antimony Oxide Industry Association.


Sixty day old Fisher-344 male rats were exposed to 0.2, 0.8, and 1.5 mg/m³ smoke generated from crude petroleum oil. Exposures were for 3.5 hr per day, 4 days per wk for 13 wk. Rats were examined at the end of 13 wk and after a recovery period of 4 wk in clean air. Histopathological evaluation revealed infiltration of alveolar macrophages and polymorphonuclear leukocytes, hyperplasia of type II cells, peribronchiolar lymph node hyperplasia and congestion, diffuse multifocal pneumonia, and formation of multifocal, granulomatous pneumonia. Body weight was decreased, while lung wet and dry weights were increased. Hepatic effects observed were decreased zoxazolamine paralysis time and increased aryl hydrocarbon hydroxylase activity. The pulmonary effects indicate an inflammatory response with possible disease progression. The hepatic effects suggest induction of the P448 system. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

COMPARATIVE EFFECTS OF INHALED IRITANTS ON LUNG CLEARANCE SYSTEMS. R.B. Schlesinger, K.E. Driscoll, J.M. Gearhart and T.A. Vollmuth. Institute of Environmental Medicine, New York University Medical Center, New York, NY.

Clearance is the process whereby the lungs are rid of particles which deposit upon airway surfaces. It is a major component of an integrated defense system which serves to reduce both local damage by and systemic absorption of inhaled toxicants. This paper describes results from studies using rabbits aimed at assessing the relative potencies of inhaled HgS04, NO2 and O3, three important airborne irritants, in affecting clearance of tracer particles both from the bronchial tree and the alveolar region. Exposure levels ranged from 0.1 mg/m³ HgS04, 0.1 ppm O3, and 0.3 ppm NO2; exposures were performed under both acute (2hr/day, 14day) and chronic (2hr/day, 14day) regimes. The results demonstrate that exposure to similar mass concentrations of these materials result in quantitative and/or qualitative differences in response. These type of data are of mechanistic interest since, in these assays, HgS04 acts as a pure acid, O3 as a pure oxidant, and NO2 has oxidant properties but may produce acid upon dissolution in lung fluids.
EFFECT OF COAL ON SILICA AND KAOLIN CYTOTOXICITY


Respirable coal mine dust is a complex mixture of particulates containing coal of different types and minerals derived from rock strata. Coal miners customarily have a mixed dust exposure and epidemiologic studies have shown significant differences in the prevalence of CWP between mines. Since crystalline silica and kaolin are the two major mineral inclusions in the coal mine dust, we have examined the effects of these minerals separately and in combination with coal on in vitro bioassays for cytotoxicity. Sheep blood hemolysis and release of the cytoplasmic enzyme lactate dehydrogenase and lysosomal enzymes β-N-acetylglucosaminidase and β-galactosidase from alveolar macrophages were monitored as indicators of cytotoxicity. Release of alveolar macrophage enzymes and hemolysis were enhanced by mixing silica in varying concentrations with coal whereas, kaolin, potent cytotoxic mineral showed lower than expected cytotoxicity when mixed with coal. The effects were greater than the sum of independent effects of the minerals – indicating synergism. These studies indicate that silica and kaolin may play a role in the cytotoxicity of coal mine dusts.


Recently we showed that inhaled asbestos and glass fibers activate complement in the lungs of exposed animals, generating chemotactic factors at sites of particle deposition. Here we report that inhalation of wollastonite fibers or iron carbonyl powder, particles of different shape and chemistry, generates complement-derived chemoattractants to facilitate macrophage chemotaxis and phagocytosis of particles on alveolar surfaces. Using macrophage chemotaxis as a bioassay for complement activation, we showed that carbonyl iron and wollastonite activated complement in serum (p<0.01) and lavage (p<0.05) compared to controls or Mt. St. Helens ash. Rats were exposed to the aerosolized particle varieties listed above and macrophage accumulation was quantified by scanning electron microscopy. Pulmonary macrophages migrated to sites of wollastonite and carbonyl iron deposition within 24 and 48 hrs (p<0.05), respectively, but did not respond to volcanic ash or room air. Macrophage phagocytosis of inhaled carbonyl iron was evident during the 3 hr exposure and reached a maximal level within 48 hrs. Our results suggest that chemoattractant generation, consequent to complement activation by inhaled dusts, provides the early signal by which macrophages are attracted to and phagocytose particles in the distal lung.

INTERSPECIES COMPARISONS OF LUNG RESPONSES TO INHALED DUSTS. D.B. Warheit, DuPont Haskell Lab., Newark, DE. Sponsor: C.F. Reinhardt.

Chronic exposure to a variety of inhaled dusts has been associated with the development of lung disease. Since toxicological testing requires utilization of several species, we are investigating the pulmonary responses to inhaled particles and fibers, and comparing the rat lung model with other rodents.

Our results demonstrate that inhaled chrysotile asbestos fibers or carbonyl iron particles deposit preferentially at alveolar duct bifurcations in both rats and mice. Pulmonary macrophages (PM) migrate selectively to sites of deposition to phagocytize particles within 24 hrs after exposure (p<0.05). Cell morphology studies showed that a greater percentage of hamster PM were activated and phagocytized iron beads in comparison to rat PM. In addition, increased numbers of eosinophils were recovered by lavage from hamsters, compared to rats or mice. Cell migration studies demonstrated that chemotactic factors for PM were generated by asbestos and zymosan-stimulated rat and mouse serum (p<0.05), although rat PM did not respond to N-formyl peptides. The results of these investigations should provide an in-depth look at the issue of species variations in relation to the simulation of human lung disease, and generate information about the strengths and weaknesses of each animal model.


Several phenylenediamine (PD) dyes with known carcinogenicity in the rodent bioassay were evaluated in the V-79 in vitro metabolic cooperation assay. The inhibition of metabolic cooperation between cells in vitro appears to correlate well with in vivo tumor promotion in mouse skin. For a chemical to be considered as positive in the metabolic cooperation assay, it must show a statistically significant increase in the number of 6-thioguanine resistant colonies over that observed in solvent control cultures. The in vivo data with the phenylenediamine dyes suggest that the degree and position of ring substitution can have a direct effect on the carcinogenic activity. Our data indicate that 4-chloro-o-PD, 4-chloro-m-PD, 2,6-dichloro-o-PD, 2-nitro-o-PD, and o-PD produced positive responses, 4-nitro-o-PD produced a suspect response, while 2-chloro-o-PD sulfate, m-PD, and p-PD produced negative responses in the assay. These data agree with the in vivo bioassay results suggesting that the V-79 metabolic cooperation assay may provide a rapid, quantitative assessment of potential carcinogenic activity for some classes of compounds. (Supported by U.S. E.P.A. contracts 68-02-2566 and 68-02-4032.)
The unique association of azide with uv- and deoxyribonuclease-resistant DNA suggests that azide may serve as a precursor for the study of action and/or induction of these processes. To examine this, racemic azidolamine (AZL), a mutagenic metabolite of azide, and its L-enantiomer were synthesized and tested. Ames assays confirmed AZL as a major mutagenic species and suggested that all activity resided in the L-isomer. The isomer was equimolar with azide. Further, the free amino group was required for full activity; relative potencies of AZL and azidopropionic acid were 7.6 vs 0.05 revertants/mole in TA100. Blocking the carboxyl group had little effect on mutagenic activity. The results reflect enzymatic conversion of AZL to the ultimate mutagen. In another study, plasmid pCP1 was digested with AZL in vivo, isolated, transformed into E. coli strain MC1061 (E. coli strain MC1061 was selected for pCP1 plasmid). Compared to controls (G.1000 mutants/transformant), treated plasmids had a high mutation frequency (2.3%). Results suggest a stable lesion in DNA; they provide hope that it can be isolated and identified.

Gentian violet (GV, hexamethylpararosaniline), a mycosporin used in poultry feed, induces tumors in mice. Several metabolites of GV have been found in tissues and excreta of rats, mice, and chickens, including GV; pentamethylpararosaniline (PMP); N,N,N,N’-tetramethylpararosaniline (DMP); N,N,N,N’-tetramehtylpararosaniline (CP); and leucovirgent violet (LOV), and leucovirgent pentamethylpararosaniline (LPM). The mutagenicity of these metabolites was investigated with S. typhimurium strains TA97, TA98, and TA100 in the Ames plate assay. The dyes GV, PMP, DMP, and LPM were confirmed as toxic, but not mutagenic over the concentration range 50 μg/plate in TA97 and TA98 in the absence of a liver homogenate fraction (S9). In the presence of S9, the dyes induced no mutagenicity response, but S9 did confer protection from toxicity in TA100. However, these same dyes elicited a mutagenic response with E. coli WP2s (trpA vra) when tested for mutagenicity by the phage resistance at a concentration of 5 μM in continuous (chemostat) culture. The responses of no dye, GV, PMP, DMP, and LPM were 0, 2, 36, 31, 89, and 135 mutants/10^8 generation, respectively. Chromatographic evidence indicated that PMP and GV were reduced by the aerobic cultures of E. coli WP2s to LPM and LOV. These leucovirgent derivatives were mutagenic in Salmonella strain TA98 in the presence of S9. Based on the E. coli results, we surmise that d- and LMP are also reduced to mutagenic leucovirgent derivatives that may induce mutations in Salmonella.
POLYAMINE LEVELS AND BIS(CHLOROETHYL)SULFIDE (BCES)-INDUCED DNA DAMAGE. I.T. Mulholland and R.B. Conolly, Toxicology Program, School of Public Health, The U. of Michigan, Ann Arbor, MI 48109

Newborn rat cutaneous epidermal keratinocytes grown with 0.06 mM Ca ("low Ca") form a monolayer while cultures grown with 1.8 mM Ca ++ ("high Ca") are stratified, more closely resembling epidermis in situ. Low Ca cultures have more putrescine, 2.30 vs. 0.27 nmol/mg cells. High Ca cultures have more spermidine (115.5 vs. 30.3 nmol/mg cells) and spermine (662 vs. 111 nmol/mg cells).

Treatment on culture days 3-7 with 20 μM putrescine resulted in putrescine, spermidine and spermine concentrations of 10.1, 13.8 and 23.5 nmol/10^6 cells respectively, in low Ca culture and 11.4, 8.21 and 16.3 nmol/10^6 cells, respectively, in high Ca culture. With control DNA normalized as 100% double-stranded, 5 μM BCES challenge on day 8 resulted in 109.1 ± 2.10 and 91.6 ± 1.58% in normal and putrescine treated high Ca cells, respectively, indicating that putrescine treatment inhibits crossinglinking. However, putrescine treatment did not affect crosslinking in low Ca cells, suggesting that keratinocyte polyamine levels per se do not influence DNA crossinglinking by BCES.

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EFFECT OF PERFLUORINATED CARBOXYLIC ACIDS AND POLYCHLORINATED BIPHENYLS ON INTERCELLULAR COMMUNICATION PROCESSES. A.M. Rogers-Back and J.J. Clarke, Microbiological Associates, Inc. Bethesda, MD

Perfluorinated carboxylic acids of chain length 10 or greater and some polychlorinated aromatic hydrocarbons have similar toxic effects in vitro. The progressive weight loss and general deterioration of the exposed animal suggests that the compounds affect some fundamental cellular processes. We have examined the effects of perfluorooctanoic acid (PFOA), perfluoro-n-decanoic acid (PFDA), 2,2',4,4',5,5' hexachlorobiphenyl and 3,3',4,4',5,5' hexachlorobiphenyl on cell communication between freshly isolated rat hepatocytes and an established rat liver (ARL) cell line. To determine the effect of these compounds on cell communication, the enhanced recovery of 6-thioguanine resistant ARL cells is measured. Enhanced recovery is a measure of the breakdown of gap-junctions between cells. Results indicate that PFOA treatment inhibits cell communication, while treatment with the less toxic PFDA has no effect. The results for the polychlorinated biphenyls are not clear. The less toxic 2,2',4,4',5,5' analogues appear to have more effect on cell communication than the 3,3',4,4',5,5' analogue. However, it is clear that PFDA and 2,2',4,4',5,5' hexachlorobiphenyl have profound inhibitory effects on intercellular communication. Research sponsored by AFOSR contract F49620-84-C-0074.


A structure activity study performed on eight mono-, di-, and trihydrobenzophenones has shown a positive correlation between sister chromatid exchange (SCE) induction and cytotoxicity in Chinese hamster ovary (CHO) cells. CHO cells were exposed to the test agents in the presence and absence of an exogenous metabolic activation system and subsequently examined for cell survival and the induction of SCEs. Hydroxyl substitution at specific positions on the phenyl ring strongly affects SCE induction and cytotoxicity. For cytotoxicity, hydroxylation at the 2 position appears the critical factor. 2-Hydroxybenzophenone (2-HPB) and 2,3-DBPB are up to ten-fold more cytotoxic than other benzophenones tested. Additional hydroxylation at the 4 or 5 position on the phenyl ring reduces the cytotoxicity of the test agent. Of the eight benzophenones tested, only 2,3,4-TRBP, 2,3-DBPB, 3,4-DBPB, and 2-HPB significantly increase the induction of SCEs. The relative potency of the compounds is as listed with the maximal response occurring with 2,3,4-TRBP at three-fold over background.

Chlorodeone (CD) and carbon tetrachloride (CCl₄) combination has been shown to cause greatly potentiated hepatotoxicity in rats. Since inhibition of hepatocyte regeneration was implicated as the underlying mechanism, possible involvement of genotoxic events was investigated. Sprague-Dawley rats (250-300 g) were given an oral dose of 10 mg/kg followed by a single i.p. injection of 100 μl CCl₄/kg on the third day. Hydroxyurea (50 mg/kg) was given immediately after the CCl₄ 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 ADF-riboseyltransferase (ADFT) activity in digitonin-permeabilized hepatocytes. No UDS was detectable in the hepatocytes of CD + CCl₄ treated rats in contrast with CD and CCl₄ (p < 0.05) treatment groups. DNA strand breaks occurred only in CD pretreated rats. ADFT activity was inhibited significantly in animals treated with CD + CCl₄ or with CD alone but was stimulated in CCl₄ treatment group. Our findings indicate that genotoxic mechanisms may be involved in the CD + CCl₄-induced non-renewal and repair in these livers. (Supported by USEPA R-811072 and Fogarty International Center T00336.)


BALB/c 3T3 (3T3) and CM 1071/2 (1071/2) mouse embryo fibroblasts are the most commonly used cell lines for studying in vitro transformation. These assays are designed to allow the expression of morphologically transformed foci of increased cell density and aberrant cell morphology on a confluent monolayer of non-transformed contact-inhibited cells. These systems compare favorably in that the correlation between morphological alteration and tumorigenicity is good and the spontaneous incidence of transformation is low (<7 x 10⁻⁶ for 3T3 and <3 x 10⁻⁶ for 1071/2). Comparative studies were performed by exposing 3T3 or 1071/2 cells for 24 hours without S-9 or for 4 hours in the presence of an Arcoolor-induced rat liver S-9 activation system. Both cell lines gave a strong positive response to selected polycyclic aromatic hydrocarbons. In contrast, only the 3T3 system gave a strong positive response to a variety of other carcinogens (i.e., cinamyl antranilate, aflatoxin B₁, cyclophosphamide, 2-naphthylamine and dimethylnitrosamine), which demonstrates the differences in sensitivity between the two cell lines to various classes of carcinogens. (Supported in part by NCI contract NOI-CR-85617.)

FORMATION OF CARCINOGENIC AND/OR GENOTOXIC ALIPHATIC AZOXY COMPOUNDS FROM THE RESPECTIVE NITROALKANES BY DIRECT MILD CHEMICAL REDUCTION. E.S. Fiala, R. Czernecki, and G.M. Williams. Naylor Dana Institute, American Health Foundation, Valhalla, NY.

One method of synthesizing aliphatic azoxy compounds is by the spontaneous condensation of alkyldihydroxylamines with C-nitroso compounds. This suggested to us the possibility of azoxyalkane formation from the in situ production and condensation of the N-hydroxy and C-nitroso intermediates of nitroalkane reduction. In fact, we found that mild reduction of nitroethane, nitroethene, 1-nitropropane (1-NP) or 2-nitropropane (2-NP) with Zn=NH₂Cl leads to the formation of azoxyethane (AOE), azoxyethane (AOE), 1-azoxypropane (1-AP) or 2-azoxypropane (2-AP), respectively, in yields as high as 24%. Three of the four azoxy compounds, as well as 1-NP and 2-NP were found to be strongly genotoxic in the Williams hepatocyte UDS assay. AOM and AOE are known to be strong carcinogens in rodents. Bioassays for the carcinogenicity of 1-AP and 2-AP as well as their precursors, 1-NP and 2-NP are currently underway in this laboratory. The case of formation of carcinogenic and/or genotoxic aliphatic azoxy compounds by mild reduction of nitroalkanes as well as from the simple oxidation of aliphatic amines (Carcinogenesis 1:97, 1980) suggests that, under certain conditions, similar reactions could also occur in the environment.


A widely used end-point in assessing genotoxicity of test chemicals is the induction of sister chromatid exchanges (SCEs). Although the genetic significance of SCEs is still unclear, several factors, including cell cycle kinetics, may influence the frequencies of SCEs in cultured mammalian cells in vitro. Spleen, bone marrow, lymph node and spermatogonial cells in vivo have been shown to have different average cell generation times (AGT) for these somatic and germ cells from male Sprague-Dawley rats (N=6) in vivo to determine any tissue specificity for spontaneous SCE rate and if a relationship exists between SCE frequency and AGT in vivo. AGTs and SCE were determined following in vivo incorporation of 5-bromo-2-deoxyuridine (1-1g/kg) into chromosomal DNA. The SCE frequencies of the somatic cells were not different (5.8-6.6 SCE/cell). The SCE rate of the germ cells was 20% higher. Both significantly less than that of the somatic cells. The AGT's of the cell types examined varied as spermatogonial > spleen > bone marrow > lymph node. The markedly long AGT or other inherent properties of the spermatogonial cells might have influenced the SCE rate in these cells.
2,3,7,8 TETRACHLORODIBENZO-P-DIOXIN ALTERS CONTRACTION FORCE IN ISOLATED GUINEA PIG ATRIA. D.W. Hewitt and F. Matsunaga. Pesticide Research Center, Michigan State University, E. Lansing, MI.

Guinea pig atria were isolated 10 and 20 days after administration of TCDD (1ug/kg) or aceate; corn oil. The inotropic and chronotropic responses to isoproterenol were then measured using a GRASS S9 stimulator and a physiograph recorder. Atria from 20 day TCDD treated animals displayed basal contraction forces 1.6 and of that of pair-fed and obvious differences were noted in the inotropic response but not the chronotropic. When corrected for size differences, the % of maximum developed tension of the atria as a function of dose in muscle from pair-fed animals, mirrored that of young ad lib while that from TCDD treated animals followed a response similar to old ad lib control. 2.7 and 3.1x more isoproterenol was needed to obtain 50% maximum contractility in preparations from 10 and 20 day animals respectively, TCDD compared to pair-fed. The ED50's were similar between 1)20 day TCDD and old ad lib control, and 2)pair-fed control and young ad lib animals. If prolonged exposure to TCDD causes the entire heart to be depressed in its response to endogenous catecholamines, this effect coupled with other toxic lesions (i.e. hyperlipidemia) could contribute to lethality. (Supported by NIH Research Grant ES0 1963).

161 MUTAGENICITY OF UNSTABLE AGENTS IN SALMONELLA. Stephen M. DiZio and John G. Rieder. Wyeth Laboratories, Paoli, Pa 19301. Sponsor: Mark Hite

In these experiments, 4-oxo-4(3-(2-quinolinoxyphenoxy)phenyllaminobutanoic acid, methyl ester (Wy-45,723) was screened for mutagenicity in Salmonella and found to be active, producing a dose-response, in strain TA 1537 in the absence of metabolic activation. The mutagenicity of Wy-45,723 was initially attributed to its quinoline moiety. However, Wy-45,723 was later shown to rapidly (1-2 hrs) form an unstable cyclic derivative at 37°C in the media, either in the presence or absence of Salmonella. Addition of Wy-45,723 at various times to incubating test plates of TA 1537 revealed that, at the time of exponential growth, neither Wy-45,723 nor its cyclic derivative was mutagenic, although maximal reactivity of TA 1537 to the reference compound acridine was demonstrated. These results suggest that an intermediate formed in the transformation of Wy-45,723 is responsible for the activity of the test compound. They further demonstrate that predictions based upon structure vs. genetic activity which do not take into account compound stability may fail to identify a key feature necessary to the construction of a non-genotoxic chemical.

162 UROPHYRIN (UR0) ACCUMULATION IN CULTURED CHICK EMBRYO LIVER CELLS (CELC) TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD). R.W. Lambrecht, W.J. Bement, P.R. Sinclair, J.P. Sinclair, and H.L. Becton. White River Junction, VT and Dartmouth Medical School, Hanover, NH. Sponsor: R.P. Smith.

Primary cultures of CELC were used to study the mechanism by which URO accumulates after exposure to TCDD or to 3,4,5,6-tetrachlorobiphenyl (TCB). Increasing concentrations of either of these two compounds alone (up to 100µg compound per ml media) caused similar levels of URO to accumulate. When 5-aminolevulinic acid (a porphyrin precursor) was also given to treated cells (ALA-loading), TCDD caused URO accumulation at lower concentrations than did TCB. However, in cells pretreated with 3-methylcholanthrene and ALA-loaded, TCB was as potent as TCDD in causing URO accumulation. URO accumulation in cells treated with either TCDD or TCB (with or without ALA-loading) was reversed by the addition of piperonyl butoxide or ellipticine (both inhibitors of cytochrome P-448), but not by SKF-525A (an inhibitor of cytochrome P-450). Both TCDD and TCB appear to cause URO accumulation in CELC by a common mechanism involving cytochrome P-448. URO accumulation does not appear to be due to covalent binding of metabolites of TCDD or TCB to uroporphyrinogen decarboxylase, because this accumulation was not rapidly reversed by inhibitors of cytochrome P-448.

163 A STUDY OF THYROID STATUS AFTER A NONANOREXICENIC DOSE OF TCDD. W. Roth, P.A. Bank and S.D. Aust, Department of Biochemistry and Center for Environmental Toxicology, Michigan State University, East Lansing, MI.

Several biochemical and histological indicators of thyroid status were measured in tissues of euthyroid and thyroidectomized Sprague-Dawley rats at various times after a single, nonanorexicenic dose (5µg/kg) of TCDD. Malic enzyme activity in hepatic cytosol of euthyroid rats treated with TCDD was 150% that in controls. The activity of malic enzyme in cytosol of TCDD-treated, thyroidectomized rats was slightly higher than that of thyroidectomized controls (25%), but was still less than 10% of that found in euthyroid animals. 3,5,3'-Triodothyronine (T3) levels in serum were elevated despite a 75% decrease in thyroxine (T4) levels. Measurements of glucagon and muscle myopathy were also suggestive of a hyperthyroid state. (NIH ES05985).
TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes increased protein kinase C activity in the hepatic plasma membrane of the rat and guinea pig. D.W. Bombick, B.V. Madhukar, D.W. Brewster, and F. Matsunaga. Pesticide Research Center, Michigan State University, East Lansing, MI.

Protein kinase activity (both cAMP dependent and independent) was increased in hepatic plasma membranes from TCDD-treated rats. In addition, gel electrophorograms illustrated increased substrate phosphorylation of hepatic plasma membranes from rats and guinea pigs ten days after LD50 doses were administered. Protein kinases that could regulate EGF receptor activity were considered important since this laboratory has shown the loss of EGF binding to be a sensitive and early indicator of TCDD toxicity. Protein kinase C (PKC) activity was examined since TPA (12-0-tetradecanoyl phorbol-13-acetate) affects increased PKC activity and resulting decreased EGF binding. PKC was elevated two fold in hepatic plasma membrane isolated from rats and guinea pigs ten days after an LD50 dose of TCDD was given. Other kinases stimulated by TCDD may also be important to TCDD’s action on EGF receptor activity as well as other toxic manifestations, however, PKC and its substrates may be a critical factor. (Supported by NEHS Research Grant ESO 1962).


EPIDERMAL HYPERPLASIA AND ALTERATIONS IN KERATINIZATION IN THE HAIRLESS MOUSE INDUCED BY TCDD. C.J. Molloy, M.A. Cello, and J.D. Laekin. UMDNJ-Rutgers Medical School, Piscataway, NJ.

TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin) has been shown to be a skin tumor promoter in the hairless mouse strain HR2/J, hr/hr. We have found that topical treatment of these mice, as well as their hairless littermates (hr+)/+, with TCDD (1-10 nmol in 0.2ml acetone) leads to epidermal thickening within 24-48 hours. This response is similar to hyperplasia induced by 12-0-tetradecanoyl phorbol-13-acetate (TPA). Histologic examination of TCDD treated skin revealed an increase in the size of the nucleated cell layer in both the follicular and interfollicular epidermis. Patterns of keratinization in treated skin were assessed by polycrylamide gel electrophoresis. Several keratins in the 45-70 kd MW range were found to be synthesized only in TCDD treated epidermis. These proteins were present in both hr/hr and hr/+ strains indicating that their appearance was independent of hr locus. These data demonstrate that TCDD induces specific phenotypic changes in epidermal cells that may be important in the mechanism of TCDD dermatotoxicity.

EFFECTS OF 2,3,7,8-TCDD ON THE ESTROGEN RECEPTOR IN IMMATURE FEMALE LONG EVANS RATS. M. Horn, P. S. Klaunig, J. Figge, and S. Safe: Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas 77843-4457

2,3,7,8-TCDD was administered to immatures (21 day or 60 day old) female Long Evans rats and levels of cytosol estrogen receptor (ER) were measured in the uterus using both the sucrose density gradient and hydroxylapatite assays. ER levels in the liver were determined by the charcoal-coated dextran method and the hydroxylapatite assay. In both 21 and 60 day otd rats, 2,3,7,8-TCDD caused a dose-response decrease in estrogen receptor level in both the uterus and liver. At a dose level of 50 ug/kg, there was a time-dependent decrease in uterine and hepatic ER levels in the 21 and 60 day old rats. Preliminary studies with structurally diverse polychlorinated dibenzo-p-dioxin congener suggests that down regulation of ER levels is dependent on the 2,3,7,8-TCDD receptor and the significance of these results is discussed. (Supported by the Texas Agricultural Experiment Station).

DEPRESSION OF RAT TESTICULAR 17-HYDROXYLASE AND 17,20-LYASE AFTER ADMINISTRATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD). C.A. Mebus and W.M. Piper. Dept. of Pharmacology, Univ. of Nebr. Med. Ctr., Omaha, NE 68103

Toxic effects of TCDD such as chloracne, hirsutism, and skin hyperpigmentation suggest endocrine involvement. It is known that TCDD can cause decreases in testicular heme, testicular microsomal cytochrome P-450, and serum testosterone in the rat. Therefore, this study was designed to examine the activities of the testicular microsomal cytochrome P-450 mediated enzymes 17-hydroxylase and 17,20-lyase. Male Sprague-Dawley rats (220-240g) were given a single, oral dose of TCDD (50 ug/kg). 17-hydroxylase and 17,20-lyase activities were determined seven and fourteen days after TCDD administration. Seven day 17-hydroxylase activity decreased to 73% and 40% of control values, respectively. Fourteen day 17-hydroxylase and 17,20-lyase activities were decreased to 23% and 19% of control values respectively. Testicular microsomal cytochrome P-450 and serum testosterone were also determined at seven and fourteen days and decreased to 35% and 38%, and 23% and 36% of control values respectively. These results indicate that decreased testosterone production following treatment with TCDD could be due to decreased activities of the testicular microsomal enzymes 17-hydroxylase and 17,20-lyase. (Supported by NIH Grant ES-0243).

A significant decrease in serum PRL within 6 h of TCDD (50 mg/kg) suggests TCDD toxicity may reflect an ongoing cascade of hormonal alteration. Serum PRL was 45±7 in controls compared to 1443 ng/ml in the TCDD-treated rats (p<0.01). A significant decrease in serum PRL was also detected at 8 h (p<0.05) following depression of corticosterone at 8 h. This time sequence suggests that the PRL decrease in serum may be involved in the later alterations in corticosterone and T4. By 7 days, the TCDD-treated rats have significantly higher serum PRL. PRL-stimulated ODC decreased in thymus and spleen (70-90% of pair-fed controls) within 2 days of TCDD administration. Tissue sensitivity at 2 days post-TCDD was thymus>adrenal> spleen>heart>kidney>liver. However, 7 days post-TCDD liver ODC activity in response to PRL was only 12% that detectable in pair-fed controls (253 vs 199±32 pmol/30 min/mg protein). Spleen, on the other hand, was maximally decreased in terms of ODC responsiveness within 2 days. Earlier reports of decreased ODC induction in the liver of TCDD-treated rats in response to dexamethasone and aminophylline (Potter et al., Biochem. Pharmacol. 31:367, 1962) point to the generality of decreased receptor mediated activity of diverse hormones in response to TCDD administration. Others have reported decreased EGF receptor binding in TCDD-treated mice and a human cell line (Hadhukar et al., PNAS 81:7407, 1994 and Hudson et al., Tox. Appl. Pharmacol. 77:251, 1985). In summary, major alterations in PRL levels and PRL receptor mediated activity occur in response to TCDD and may be a critical part of TCDD toxicity.

THE EFFECTS OF RECEPTOR MODULATION ON THE BIOLOGIC AND TOXIC ACTIONS OF 2,3,7,8-TCDD. A. Bannister, M. Kelley and S. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas 77843.

Treatment of immature C57BL/6J mice with 2,2',4,4',5,5'-hexachlorobiphenyl (HCB, 1000-1000 umol/kg) resulted in a 44-220% increase in levels of the 2,3,7,8-TCDD hepatic cytokine receptor protein for up to 14 days. The increased binding was not due to an alteration of the affinity of 2,3,7,8-TCDD for the receptor as shown by Scatchard analysis. Mice were pretreated with 2,2',4,4',5,5'-HCB (500 umol/kg) and after 7 days 2,3,7,8-TCDD (2.5 mg/kg) was administered by ip injection; 14 days later the hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) activities were 587 and 1383 pmol/min/mg respectively. In contrast these activities were 380 and 437 pmol/min/mg respectively in animals treated with only 2,3,7,8-TCDD. These synergistic effects were only seen in mice treated with submaximal enzyme-inducing doses of 2,3,7,8-TCDD. Receptor modulation did not alter the toxicity of 2,3,7,8-TCDD (Supported by the Texas Agricultural Experiment Station).

LACK OF COOPERATIVITY IN THE BINDING OF 2,3,7,8-TCDD TO THE Ah RECEPTOR: K. Farrell and S. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843.

Positive cooperativity is a subtle mechanism of receptor control whereby small increments in ligand concentration facilitates further ligand-receptor interactions and results in an increased biologic response. The role of positive cooperativity for the binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to its hepatic cytosolic receptor protein has been investigated in our laboratory. Under equilibrium conditions the noncovalent interaction of 2,3,7,8-TCDD and its receptor protein exhibit linear Scatchard plots and the Hill plot coefficients are approximately one. These data do not support positive cooperativity and indicate that the receptor binding of 2,3,7,8-TCDD is primarily dependent on the affinity of the ligand for its receptor (Supported by the Texas Agricultural Experiment Station).

THE BIOLOGIC AND TOXIC EFFECTS OF 2,3,7,8-TCDD METABOLITES: G. Mason and S. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843.

2-Hydroxy-3,7,8-trichlorodibenzo-p-dioxin (2- OH-3,7,8-TrCDD) and 2-hydroxy-1,3,7,8- tetrachlorodibenzo-p-dioxin (2-OH-1,3,7,8-TCDD) are two major urinary metabolites of 2,3,7,8-TCDD. Both metabolites have been synthesized and administered at dose levels of 100, 1000 and 5000 ug/kg to immature male Wistar rats and their effects on body and thymus weights and hepatic microsomal aryl hydrocarbon hydroxylase (AHH) induction were determined. The two metabolites did not cause significant thymic atrophy or body weight loss and were at least 1000 times less toxic than 2,3,7,8-TCDD. The EDBO values for hepatic microsomal AHH and ethoxyresorufin O-deethylase (EROD) activities were 2,800 and 2,300 ug/kg respectively for 2- OH-3,7,8-TrCDD and > 5000 ug/kg for 2-OH- 1,3,7,8-TCDD; both compounds were greater than 1000 times less active than 2,3,7,8-TCDD. These results are consistent with the lower receptor binding affinities of the metabolites coupled with their expected rapid metabolic clearance. (Supported by the National Institutes of Health and the Texas Agricultural Experiment Station).
INTERACTIVE EFFECTS OF Di(2-ethylhexyl)phthalate (DEHP) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) ON LIPID METABOLISM IN F344 RATS. K. Tomaszewski, F. Harrington, A. Greenwell, C. Hahn, J. Noon, T. L. Birnbaum and R. Melnick. NTP,NIH, Research Triangle Park, NC.

Male F344 rats were treated with DEHP (a hypolipidemic agent) and TCDD (a hypolipidemic agent) and examined for interactions. Treatment groups (p.o.) included: A) corn oil control; B) DEHP control (2g/kg daily); C) TCDD control (160 ug/kg single dose); D) 7-day DEHP treatment+ single dose of TCDD+ daily dosing with DEHP; E) single dose of TCDD+ daily dosing with DEHP. Weight loss was observed in C, D, and E after dosing with TCDD; the loss in group D was minimal after 14 days. Liver weights were increased by both DEHP and TCDD. Thymus weights were decreased in all groups (C, D, E) treated with TCDD. Serum triglyceride levels were increased 2-5 fold in C, decreased 50% in D, maintained at slightly higher levels in D than in A, and initially decreased in E but elevated after 14 days. There was no effect of any treatment on mitochondrial fatty acid oxidation. DEHP caused a 6-fold increase in peroxisomal palmitoyl CoA oxidase (PCO) activity, while TCDD had no effect on PCO. Activity of PCO was lower in D and E than in B. These results indicate that 1) an increase in serum lipid (group E) does not induce peroxisomal proliferation, 2) enhanced peroxisomal fatty acid oxidation is effective in decreasing TCDD-induced hyperlipidemia 3) the decrease in body weight caused by TCDD is not due to impairment in lipid metabolism.

HUMAN EXPOSURE TO PCBs AND PCDFs OCCURRED IN A 1979 ACCIDENTAL FOOD POISONING EPISODE IN TAIWAN. Our objective is to evaluate the role of individual congeners in the production of biochemical effects detected in abdominal fat of exposed individuals. Benzo[a]pyrene BaP hydroxylase was measured in placentas. Ah receptor was characterized in placental cytosolic and nuclear fractions by a variety of receptor binding techniques using 3H-TCDD as the ligand. Microsomal BaP hydroxylase was dramatically elevated in placentas of exposed subjects (compared to those of nonexposed, nonsmoking control subjects). Concentrations of Ah receptor in placental preparations from exposed or control subjects were uniformly low; approximately 1.0 pmol/mg cytosolic protein. Several PCBs and PCDFs were detected in samples from exposed subjects. Both 2,3,4,7,8-penta CDF and 1,2,3,4,7,8-hexa CDF were found in a concentration range of 100-500 ppt. These PCDFs bind the Ah receptor nearly as well as TCDD. The majority of PCBs found were comprised of penta, hexa and hepta congeners; total PCBs averaged approximately 25 ppb. These studies indicate that potent PCDFs are present in human tissues five years after a PCB poisoning episode.


NON human primates are sensitive to the toxic effects of polychlorinated biphenyls (PCB). Aroclor 1016, in contrast to Aroclor 1254, contains greatly reduced concentrations of "toxic isomers and dibenzofuran contaminants. For 300 days three adult female cynomolgus monkeys were given Aroclor 1016 3 times a week at a daily dose equivalent of 5.0mg/kg. Three other monkeys were given vehicle only. None of the treated monkeys displayed clinical signs of PCB intoxication noted with Aroclor 1254. Pathological changes were minimal in comparison to Aroclor 1254, but thyroid alterations were noted. Serum enzymes, hematology and immunology were altered slightly in treated animals. Estrus cycles appeared normal although blood PCB levels exceeded 1500 ppb. Hepatic cytochrome P450 levels and monooxygenase activities were increased. Aroclor 1254 is toxic to monkeys than Aroclor 1254. This is consistent with rodent studies linking toxicity to planar PCB isomers that vary in concentration within different PCB formulations.


Subchronic toxicity assessments were performed with B6C3FI mice and Fischer 344 rats preliminary to chronic toxicity/carcinogenicity studies. Animals were administered each antihistamine (doxylamine, pyrilamine, triprolidine, tripelennamine, thymolidamine, and methapyrilene) admixed in the feed and clinical signs of toxicity, body weight and food and water consumption were measured weekly. Animals were necropsied after 90 days and organ weights taken. Microscopic pathology was performed in all major organs in the high dose and control groups or the next lowest dose where lesions were noted, and on all lesions observed grossly. Methapyrilene was by far the most toxic antihistamine showing effects in the liver (bile duct hyperplasia, hepatic necrosis) at dose levels as low as 100 ppm in rats and 2000 ppm in mice. Cholangiofibrosis was evident at 800 ppm in male and female rats. The lower antihistamines exhibited varying degrees of liver (fatty metamorphosis, cytoplasmic vacuolization, necrosis, cytoemaly, and karyomegaly) and salivary gland (cytomegalic and necrosis) alterations that were compound-related. Estimated maximum tolerated doses established from these studies were doxylamine: R=5000 ppm, M=250 ppm; pyrilamine: R=3000 ppm, M=1500 ppm; triprolidine: R=4000 ppm, M=2000 ppm; tripelennamine: R=2000 ppm, M=4000 ppm; thymolidamine: R=1000 ppm, M=2000 ppm and methapyrilene: R=800 ppm, M=2000 ppm. All compounds except thymolidamine have entered chronic studies.


Subchronic toxicity assessments were performed with B6C3FI mice and Fischer 344 rats preliminary to chronic toxicity/carcinogenicity studies. Animals were administered each antihistamine (doxylamine, pyril
TOXICOLOGY AND EXPERIMENTAL THERAPEUTICS OF TETRAPLATIN (NSC 363812) INFUSIONS IN MICE.
S.D. Harrison, Jr., D.J. Dykes, H.D. Giles, J. Flookman,* C.L. Litterst,* and D.P. Griesold, Jr. Southern Research Institute, Birmingham, AL and *Div. of Cancer Treatment, NCI, Bethesda, MD

Tetraplatin (TP) is a cyclohexanediamine platinum (Pt) analog recently selected for development by the NCI. Schedule dependence suggested infusion of TP might optimize therapy. CDFl female mice were implanted ip with 10^3 L1210 leukemia cells. In 3 expts, groups of 6-10 mice received a single sc TP bolus (24, 18, 12, 6, 3, 1.5 mg/kg) or TP infusion (5.7, 3.8, 2.6 mg/kg/day sc, Alzet cos-
motic pumps; 96, 72, 48, or 24 hr). Nontumored mice provided urinalyses, organ wts, GI and renal histopathology, and plasma for Pt determinations. The maximum tolerated bolus dose of TP, 18 mg/kg, produced a 6-log leukemia cell kill. The MTD for infusion, 3.8 mg/kg/day for 72 hr, produced a 3-
log cell kill. Delineation of a plasma Pt thresh-
hold for toxicity was confounded because infused Pt was highly protein bound. However, the total infusion dose threshold for toxicity, as reflected by nonleukemic deaths among tumor-bearing mice, was about 11 mg/kg. Urinalyses, kidney/body wt ratios, and histopathology revealed minimal nephrotoxicity in infused mice, but the effects observed were consistent with the 11 mg/kg total dose threshold. Infusion seemed to reduce the therapeutic index of TP, possibly reflecting an altered distribution of cytotoxic Pt. Supported by Contract NO1-CM-77580, DCT, NCI and Grant RO1 RO5676, NIH.

CHRONIC ORAL SAFETY STUDY OF CELPIROLOL IN RATS.

This study was conducted to define the toxico-
logical potential of Celriprolol in rats after one year of treatment. Groups of 20 male & 20 female Sprague-Dawley rats received Celriprolol mixed in diet at dosage levels of 0, 100, 400 or 1600 mg/kg/day for one year. Transient hyper-
activity was observed in Celriprolol-treated rats during weeks 11-25 in a dose related fash-
ion. Similar dose related lower body weight gain & food consumption were also noted in Cel-
riprolol treated animals. There were no consist-
tent treatment related changes in hematologic & clinical chemistries with the exception of lower serum total protein, albumin, cholesterol & bilirubin levels in the high dose animals as compared with controls. Histopathological changes included an increase in macrophages in the lungs & decreases in the incidence of chronic focal nephritis & prostatitis in treated animals. Increase in pulmonary macrophages could be due to the non-specific stress syn-
drome that could result from decreased food intake. Decreased incidences in nephritis & prostatitis could be due to its vasodilatory effect, thus improved blood flow through organ systems.

AGE-RELATED SUSCEPTIBILITY TO DRUG-INDUCED ARTHROPATHY IN DOGS AND RABBITS. T.A. Barron, B.A. Mayes, C. Rosero-Ferret, and J. Legros. Sterling-Winthrop Research Institute, Rensselaer, NY and Dijon Research Centre, Dijon, France.

Naphthyridine/quinoline drugs are known to induce arthropathy in immature animals. Rosoxacin, a quinoline antibacterial, was admin-
istered orally by stomach tube to groups of immature dogs in single daily doses of 35 and 135 mg/kg, and to mature dogs at 135 mg/kg for two weeks. Rosoxacin was also given to groups of immature rabbits at 62.5, 100, and 250 mg/kg, and mature rabbits at 15, 60, and 120 mg/kg for two weeks. Groups of immature and mature animals given vehicle served as controls. No abnor-
malities in posture or gait were seen in any animal. At necropsy, arthropathy was observed in only immature animals; dogs at 135 mg/kg, and rabbits at 100 and 250 mg/kg. The arthropathy consisted of focal circumsorbed white areas and/or irregular erosions which were observed in several different joints, most notably the humeral and femoral heads, and femoral condyles. Microscopic examination revealed a cleft-like loss of matrix in the intermediate articular cartilage with islands of regenerating chondrocytes and fribi-
llation at the margins. In some cases, the edges of the cleft extended to the surface cartilage which eventually sloughed.

INTERSPECIES COMPARISON OF TIME COURSE, DOSE RESPONSE, AND MEDIATORS OF CHEMICALLY INDUCED SKIN IRRITATION. E. Patrick and H.I. Melbarch, Department of Dermatology, University of California, San Francisco, CA.

Following topical application to mice methyl sulfoxide, croton oil, and ethyl phenylpropiolate (EPP) produce irritant inflammation by different mechanisms. To determine if chemicals produce irritant inflammation by the same mechan-
isms in different species, the time course, dose response and mediator involvement in responses to methyl sulfoxide, croton oil, and EPP of mice, rats, and rabbits were compared. Solu-
tions of the irritants in acetone were applied to one ear of ICR mice and Sprague-Dawley rats and to the shaved back of New Zealand White rabbits. Ear thickness measurements in mice and rats were used to quantitate the degree of inflammation; reactions in rabbits were evaluated visually for erythema and edema. Both qualitative and quantitative differences in response were observed. Rats were less reactive to methyl sulfoxide and EPP than mice or rabbits. EPP induced inflammation was maxi-
mum at 8 hours after application in mice and rabbits and at 1-2 hours in rats. The degree of suppression of inflammation pro-
duced by the antagonists, synthesis inhibitors, and depleting agents used to study mediator involvement varied by species. The varied effects of the pharmacologic interventions were generally reflective of the relative sensitivity of that species to the putative mediator. These results suggest that differences in potency of irritants in different species are due in part to amplification of differences in mechanisms of inflammation.
CGS 7525A, a potential antidepressant agent, was administered orally via gavage as a suspension in 3% cornstarch to groups of CD-1 mice for 78 weeks and to groups of Sprague-Dawley CD rats for 104 weeks at daily doses of 6, 20, or 60 mg/kg. Additional groups of mice and rats received equivalent volumes of 3% cornstarch and served as controls. The stated objective of the studies was to determine the carcinogenic potential of the test compound. The compound was non-carcinogenic in both species. However, microscopic examination of tissues from CGS 7525A-treated mice and rats of both sexes revealed brown, yellow, or orange-red pigment in the neurons of the brain, follicular epithelium of the thyroid, myofibers of the heart, tubular cells of the kidney, and epithelium of the urinary bladder. Pigment was also observed in the sinusoidal lining cells of the liver in mice and in the zona reticularis of the adrenals in rats. The incidence and degree of pigmentation were generally dose-related in both species; however, the presence of the pigment did not cause microscopic evidence of cellular damage. These results indicate that long-term oral administration of CGS 7525A to CD-1 mice and Sprague-Dawley rats elicits a dose-related accumulation of intracellular pigment in a variety of tissue sites.

This work was supported, in part, by grant 23037 from Hoffmann-La Roche, Inc.

CGS 7525A was administered orally via gavage to Sprague-Dawley CD rats at daily doses of 15, 45, or 135 mg/kg for 52 weeks, to beagle dogs at daily doses of 5, 10, or 30 mg/kg for 52 weeks, and to CD-1 mice and Sprague-Dawley CD rats at daily doses of 6, 20, or 60 mg/kg for 78 and 104 weeks, respectively. The compound elicited reductions in body weight parameters at the highest dose in each study. Dark thyroids were observed grossly in rats at doses > 60 mg/kg. Microscopically-observed lipoforesin-like pigmentation was apparent in the brain, liver, kidney, or gall-bladder of dogs at all doses. Likewise, pigmentation was evident in the brain, thyroid, heart, liver, kidney, and urinary bladder in each rodent study. The incidence and degree of pigmentation were generally dose-related in each study; however, the presence of the pigment did not cause microscopic evidence of cellular damage. The pigment was still observed in rats and dogs following a four-week recovery period. The accumulation of pigment at various tissue sites in three different animals with relatively low doses of CGS 7525A is considered to be a significant risk factor in the relative safety assessment of the compound.

Ascorbic acid or a placebo (5g/day) was given to healthy male volunteers for two weeks prior to alcohol consumption. During this period, their diets were restricted as to ascorbic acid intake. The dose of alcohol was 1.0 g/kg. Alcohol was consumed over a 2.5 hour period. One-half hour after alcohol consumption various tests were administered to measure motor coordination, intellectual function and color vision. Blood samples were taken hourly up to 10 hours to measure indices of acute alcohol toxicity such as serum enzymes, triglycerides, and blood lactate/pyruvate ratios. Blood ethanol clearance was also determined. With ascorbic acid pretreatment there was, in general, an average 13% reduction in alcohol's impairment of motor coordination, a 16% reduction in impairment of intellectual function, and an average 40% reduction in impairment of color vision. Ascorbic acid pretreatment also resulted, in general, in an average 20% reduction in serum triglycerides and a 12% increase in the rate of blood ethanol clearance.

This chronic toxicity of bromovinyleoxyuridine in the beagle dog, C.P. Chengellis, C. Port, F. Kotsonis and B.C. Dickie, G.D. Searle Research & Development, Skokie, IL, and Harlestone Laboratories America, Madison, Wt.

Bromovinyleoxyuridine, an antiviral agent, was given at dosages of 0, 5, 12 and 30 mg/kg/day (PO) to beagle dogs (six/sext/dosage) for 1 year. The males at 30 mg/kg gained significantly less weight than the control males. There were no effects on feed consumption, respiration, body temperature or heart rates. In males at 12 mg/kg and in both sexes at 30 mg/kg, there were slight but statistically significant decreases in mean corpuscular volume, but no significant changes in RBC count, hemaglobin or hemoglobin. During the latter part of the study, mean partial thromboplastin time, serum alanine aminotransferase and serum alkaline phosphatase increased, and serum cholesterol decreased in males at 30 mg/kg. Histopathologically, bile duct proliferation and hyperplasia of the gall bladder epithelium were present in both sexes at 12 and 30 mg/kg. Additionally, bone marrow hypocellularity and testicular tubular cell atrophy were present in males at 30 mg/kg. Thus, the liver, testes and bone marrow are the major target organs.
ORAL TOXICOLOGIC EVALUATION OF THE NOOTROPIC,
Path. Exp. Tox. Dept., Warner-Lambert/Parke-
Davis Pharm. Res., Knessassua, ON and
Ann Arbor, MI.

CI-911, known chemically as dihydro-1H-
pyrrololine-3,5(2H,6H)dione, is a nootropic
agent effective in animal models of cognitive
deficits. The acute and subacute oral toxicity
was evaluated in monkeys, dogs and Cynomolgus
monkeys: subchronic toxicity was evaluated in
rats and monkeys. No target organ toxicity was
demonstrated in rats except for alterations in
blood biochemistry following 2 and 13 weeks
administration. The maximum tolerated dose was
200 mg/kg based on body weight gain suppression.
Target organ toxicity in dogs was manifested by
increased creatinine phosphokinase and skeletal
muscle changes at dose levels of 500 mg/kg and
higher. No toxicity was observed in monkeys
after 2 weeks; after 13 weeks, 500 mg/kg caused
hepaticcellular necrosis and elevated plasma
transaminase levels. No toxic effects were
observed on repeated administration to dogs and
monkeys at 250 mg/kg for 2 and 13 weeks,
respectively, providing a wide margin of safety
for human clinical trials.

EXAMINATION OF THE MECHANISM OF THYROID HYPER-
PLASIA IN RATS CAUSED BY A LEUKOTRIENE ANTAGONIST
(L-649,923). D.A. Eigenberg, J.E. Sanders, L.J.
Bracht, L.S. Argenbright, W.R. Wang, and G.T.
Mina. Dept. of Safety Assessment and Animal Drug
Metabolism, Merck Sharp & Dohme Research Labora-
tories, West Point, PA and Rahway, NJ. Sponsor:
R.T. Robertson

Thyroid hyperplasia and/or heptomegaly were ob-
served in a 14-week oral toxicity study with L-
649,923, a leukotriene antagonist at doses of 50
and 150 mg/kg/day. In a 16-day study L-649,923
caused an increase in plasma TSH and hepatic en-
zyme induction, but did not affect plasma T3
and T4 levels. Light microscopy and ultrastructural
examination of the liver and thyroid showed chan-
ges indicative of hepatic enzyme induction and
increased stimulation of the thyroid by TSH. Be-
cause other hepatic enzyme inducers cause thyroid
hypertrophy by increasing the turnover of plasma
T3 and T4 it was hypothesized that L-649,923-in-
duced thyroid hyperplasia might be occurring by
the same mechanism. To examine this theory, rats
were treated p.o. with 300 mg/kg/day of L-649,923
for 17 days. On day 15, all rats were treated i.v.
with L-thyroxine (33 uCi/rat). At various times after
treatment, blood was collected and plasma levels
of L-thyroxine were determined. The clearance and
elimination rate constant were significantly lar-
ger than the control group (P<0.01). This work
proves that L-649,923 increases the plasma
turnover of thyroxine which results in a stimula-
tion of TSH and thyroid hyperplasia.

OXIDANT EFFECTS OF FLUOSOL DA-20® AND OXYGEN IN
THE RAT LUNG. A.J. Delucia, B.G. Jones, B.V.
Acuff, and J.R. Dunbar, III. East Tennessee St.
University, Johnson City, TN 37614.

Fluosol DA-20® (Fluosol), a blood substitute
containing perfluorodecalin and perfluorotri-
propylamine, can be used to enhance O2-delivery
in a variety of patients. While much is known
about the chemistry of Fluosol, little is known
of its adverse side effects, which include a
possible association with O2 to cause pulmonary
injury similar to the adult respiratory distress
syndrome (ARDS). We perfused lungs of adult
Sprague-Dawley rats for 2 hrs. under the
following conditions: a.) control perfusate,
normoxia (C) or hyperoxia (OC); 50% Fluosol,
normoxia (F) or hyperoxia (OF). Lungs were
homogenized for assay of oxidized (GSSG), total
glutathione (TGL) and GSSG/TGL ratio (R). Cytosol
was assayed for enzymatic activity of the
glutathione peroxidase pathway. A 19% decrease in
TGL (F<0.01) was significant to Fluosol
treatment; however there was no corresponding
effect of Fluosol on GSSG level (6% increase) or
R (12% increase). The largest differences
resulted from an O2-Fluosol interaction, i.e., a
21% decrease in TGL, a 13% increase in GSSG, and
a 35% increase in R (F<0.01 interaction), as seen
by comparison of Group C. Enzymatic activities were insignificantly altered by theour treatments. We hypothesize that pulmonary
oxidant injury may occur as a direct result of
the combined use of O2 and Fluosol in patients.

EFFECT OF DIETARY BUTYLATED HYDROXYANISOLE
(BHA) ON GLUTATHIONE-S-TRANSFERASE AND EPOXIDE
HYDRODASE IN BEAGLE DOGS. P.P. Saptenza, J.
Stewart, G.J. Reeds and J.J. Peggins, FDA, CFSAW,
Division of Toxicology, Washington, D.C. Sponsor:
E. Miller

In conjunction with a study to evaluate the
effect of BHA on stomach tissue of male and
female dogs after daily administration in the
diet (0, 1.0 and 3.3%) for 6 mos. (20 animals
per treatment group), the hepatic enzyme activ-
ities of glutathione-S-transferase (GS) and
epoxide hydrolase (EH) were examined. These
enzyme systems function in the metabolism or
detoxification of both endogenous and exogenous
substances. Enzyme analysis of hepatic tissue
showed a significant increase (p<0.05) in GS
and EH activity in both sexes of the BHA-
treated dogs compared to the controls. Mean
values for GS (nmol/mn/mg protein) were 851
(F) and 743 (M) for controls; 1209 (F) and
1273 (M) for 1.0% BHA diet; and 1245 (F) and
1246 (M) for 3.3% BHA diet. Mean values for EH
(nmol diol/mn/mg protein) were 27 (F) and 28
(M) for controls; 40.5 (F) and 36.1 (M) for
1.0% BHA diet; and 40.5 (F) and 40.3 (M) for
1.3% diet. These results show that prolonged
administration of BHA, a widely used anti-
oxidant in foods, can cause hepatic enzyme
induction in Beagle dogs.

In conjunction with a study to evaluate the effect of BHA on stomach tissue of beagle dogs after dietary administration of 0, 1, and 1.3% BHA for 6 mos. (10 males, 10 females/treatment), hepatic microsomes were isolated and the following assays were performed: protein, androsterone-demethylase (AP), aniline hydroxylase (AH), cytochrome P-450 (P-450), cytochrome b5 (B5), cytochrome-c reductase (Cyt-c), and glucuronyl-transferase (GT). No sex effects were observed for any parameters. BHA treatment at both dose levels significantly increased activities of all enzymes measured except Cyt-c, which increased significantly (3%) only in the 1.3% group, and AH, which was not significantly different from control. GT increased 61 and 62% in the 1 and 1.3% groups, resp. AP increased 30% in the 1% and 41% in the 1.3% groups. The microsomal content of P-450 and B5 increased 24 and 48%, resp. in the 1% and 35 and 40% in the 1.3% groups. No differences were observed in microsomal protein content. Liver weights of the 1 and 1.3% groups significantly increased over control. These results indicate that BHA increases most of the MFO parameters measured after long-term administration.


The analysis of the adverse effects at low blood lead levels (PbBs) was undertaken to establish a scientific basis for the protection of a lead-free world in drinking water. At the cellular level very low concentrations of lead are toxic. These toxic effects are manifested differently by each organ system. There are some toxic effects of lead that do not appear to have a threshold. The lack of thresholds was shown for central nervous system effects, vitamin D metabolism effects, and various enzyme reactions. The lead literature is very extensive and relates the observed human effects to measured PbBs. There are many studies on children, neonates and fetuses that suggest that the effects of lead are expressed at lower levels the younger the child. Blood lead levels between 15 and 20 μg/dl appear to be at the level when many adverse effects appear. The combination of these effects at low PbBs was used as the basis for the proposed lead drinking water regulation. These subclinical toxic effects need to be incalculated in the pediatric medical field. Blood pressure effects in the general population that occur at 8 μg/dl PbBs are also discussed in relation to other low level effects. The potential for decreasing stroke, myocardial infarction, and death is proposed.


Lead-poisoned children receive 5 day courses of 1000 mg CaEDTA/m² surface area/day. Urinary excretion of Pb, Zn and Cu was monitored during treatment. CaEDTA treatment increased daily urinary output of Pb about 20-fold and of Zn about 17-fold. Urinary Cu loss was unchanged. Concentrations of Pb in blood and of Zn in plasma fell during CaEDTA treatment. After 96 hrs. of treatment, blood Pb concentration declined to 50 percent of pretreatment value and plasma Zn concentration to 65 percent of pretreatment value. After therapy, plasma Zn concentration rebounded to its pretreatment value; blood Pb concentration did not rise within 48 hrs. after treatment. In a pilot study, oral supplementation with 15, 30 or 45 mg of Zn and 1.5 mg of Cu/day during CaEDTA treatment did not alter the reduction of blood Pb concentration but decreased the fall in plasma Zn concentration. Urinary loss of Pb and Zn was unaffected by Zn and Cu supplementation. This suggests that loss of Zn is an important effect of CaEDTA therapy. Oral Zn and Cu supplementation may partly compensate for loss of Zn. (Supported by grants NCH 240458 and NIH MO-RR 00052).

THE CELLULAR METABOLISM OF LEAD. EFFECTS OF CALCIOTROPIC HORMONES AND PHOSPHODIESTERASE INHIBITORS. J. F. Rosen and J. P. Founde, Albert Einstein College of Medicine, Bronx, NY, and Brookhaven National Laboratory, Upton, NY.

Kinetic analysis of Ca in cultured osteoclastic bone cells (OC) resolved three intracellular pools of Ca, extracellular fluid (ECF), and a Ca pool (ICF), a peptide that inhibits OC activity, increased the size of the slowest exchanging Ca pool, which includes mitochondrial Ca, while parathyroid hormone-like agents, phosphodiesterase inhibitors (theophylline-TH50 and 3-isobutyl-1-methylxanthine-IBMX), produced a rise in Ca in the same intracellular pool. This study was undertaken to define and compare the effects of CT, TH50 and IBMX on the steady-state kinetic distribution of 210Pb. Bone cells, derived from mouse calvariae, were enriched for OC by a sequential collagenase digestion and maintained in primary culture for 1 week. OC were then labeled for 3 hr in 5 μM Pb (1 μCi/μl) in the presence of CT, TH50 or IBMX, and the kinetic parameters were obtained by analysis of 210Pb washout curves. TH50 and IBMX had very similar effects on lead metabolism, decreasing the size of S2 and S3, the more slowly exchanging pools. In contrast, CT primarily altered the size of S2 and the associated transfer functions. These results indicate that CT, TH50 and IBMX modulate cellular Pb metabolism, though TH50 and IBMX produce differential effects on Ca compared to Pb.
A PHYSIOLOGICAL MODEL OF LEAD KINETICS IN RATS.  
E.J. O'Flaherty. University of Cincinnati College of Medicine, Cincinnati, OH.

Standards for exposure to lead in workplace air and in ambient air are based on descriptions of the relationship between lead in air and lead in blood. Many mathematical analyses of this curvilinear relationship have been published. However, none of these descriptive models has any predictive power across age or species. The purpose of this study is to establish a physiological toxicokinetic model, whose component compartments and organ perfusion rates are based on anatomic and physiological values, for oral lead exposure in the rat. Information required to develop such a model includes: (1) the gastrointestinal absorption rate and whether it is dose-dependent, (2) the relative magnitudes of urinary and fecal excretion and whether these are dose-dependent, and (3) the tissue-to-blood concentration ratios for physiologically distinct tissue groups. The results of experiments providing this information are presented. Special attention is paid to the dependence of rates of change of hormone-blood and kidney-to-blood concentration ratios on dose rate, and to the dependence of the equilibrium kidney-to-blood concentration ratio on dose rate. Implications of these nonlinearities, as well as those of the nonlinear relationship of blood lead to air lead, to design of a physiological model are discussed. (Supported by ES-00159).

EFFECT OF ORAL LEAD ON THE VITAMIN D ENDOCRINE SYSTEM OF RABBITS. D. P. Peterson and M. H. Bhattacharyya, Argonne National Laboratory, Argonne, IL. Sponsor: M. G. Cherian

Children with excessive environmental lead (Pb) exposure were reported to have strikingly reduced circulating levels of plasma 1,25(OH)2D3, the hormonal form of vitamin D (Rosen et al., New Engl. J. Med., 302, 1128, 1980). To study the effect of Pb on the vitamin D endocrine system, we fed weanling New Zealand White female rabbits diets containing either 0, 200, or 400 ppm Pb for 17 weeks. During this period, blood lead (Pb-B), free erythrocyte porphyrins (FEP), plasma calcium and phosphorus, and plasma 1,25(OH)2D were measured. By three weeks, Pb-B values reached a steady state of < 5, 48, and 52 μg Pb/dl blood for the 0, 200, and 400 ppm Pb groups, respectively. FEP levels of the Pb-exposed rabbits increased steadily and at 17 weeks for the 200 and 400 ppm Pb groups were 3.6- and 6.0-fold greater, respectively, than the O-Pb control value. No decrease in plasma 1,25(OH)2D was observed in response to Pb exposure: plasma 1,25(OH)2D levels were 24-27 pg/mL at 1 week and 12-15 pg/mL at 17 weeks independent of dietary Pb. Pb-exposed weanling rabbits thus showed increased FEP levels similar to those reported in Pb-exposed children but no corresponding decreases in 1,25(OH)2D, vitamin D. Work supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38.
To study the pulmonary inflammatory response induced by cadmium deposited in the lung and its correlation to lung epithelial permeability, 30 μg Cd were instilled intratracheally into male Long Evans rats as CdO dust (diam. particle size 0.5 μm), CdCl₂ and Cd-metallothionein (CdMT) in 0.2 ml of saline. Control rats received 0.2 ml of saline intratracheally. Rats were exsanguinated under anesthesia for lung lavage at 2, 6, 24, and 48 hr after instillation. Changes in lung epithelial permeability were determined by measuring activity of i.p. injected 59mTc-DTPA in lung lavage fluid. Cell viability was significantly decreased at all time points after CdCl₂ and CdO administration, whereas in the CdMT group viability was decreased at 24 and 48 hr only. Significant effects occurred earlier after CdCl₂ than after CdO administration as demonstrated by the appearance of PMN's and peroxidase positive macrophages and by increase in permeability. CdMT induced an early (2 hr) influx of PMN's which peaked at 24 hr (3 orders of magnitude greater than control). No changes of epithelial permeability at any time point after CdMT were seen. Thus, PMN’s migrating into the alveoli did not necessarily cause an increase in permeability. (Supported by NIH Grants ES01247 and ES01248.)

The effects of cadmium chloride (CdCl₂) and methyl merccur chloride (CH₃HgCl) on rat brain K⁺-Paranitrophenyl Phosphatase (KPNPase), Na⁺, K⁺-ATPase and H⁻-Ouabain binding were studied in vitro. Both the heavy metals significantly inhibited all the three parameters at all the concentrations studied. The inhibition by highest concentration of CdCl₂ was found to be 88%, 78% and 94% of KPNPase, Na⁺, K⁺-ATPase and H⁻-Ouabain binding respectively. CdCl₂ also inhibited KPNPase (86%) and Na⁺, K⁺-ATPase (76%). H⁻-Ouabain binding was unaffected by CdCl₂. The SH reagents such as dithiothreitol, glutathione and cysteine (1-100 μM), reversed the inhibition produced by the heavy metals. For complete reversal of the inhibited enzyme the heavy metal and chlors compound ratio was 1:10 for CH₃HgCl and 1:5 for CdCl₂ suggesting greater effectiveness of CH₃HgCl over CdCl₂. These studies suggest that the chlors compounds may be of therapeutic value in the heavy metal toxicity.

Rats were exposed to 30 μg cadmium in the form of cadmium chloride, cadmium oxide and cadmium metallothionein. Serum proteases in lavage were assayed using synthetic substrates selective for elastase and chymotrypsin-like protease. The activity of both proteases in the lavage pellet increased with time following exposure. The ratios of chymotrypsin-like activity at 48 versus 2 hours postexposure were 26, 19, and 35 for cadmium chloride, cadmium oxide and cadmium metallothionein respectively. A similar pattern was seen for elastase with 48 hour levels exceeding those at 2 hours postexposure by 19, 21, and 29 fold respectively. Both proteases were also detectable in the lavage supernatant. Chymotrypsin activity was higher at 48 hours postexposure than 2 hours for each of the cadmium compounds tested with ratios of 2.5, 2.5 and 1.4 for the chloride, oxide and metallothionein compounds respectively. There was no consistent pattern of elevation of supernatant elastase postexposure. Supernatant chymotrypsin-like activity correlated with permeability in cadmium chloride and cadmium oxide exposed rats, r=0.85 & r=0.87 respectively. This relationship was not apparent in cadmium metallothionein exposed rats.

Our previous studies indicated a marked hypertrophy of the adrenal glands in both rats and mice following trimethyltin exposure. We will investigate the influence of corticosterone on lesion production by TMT in the hippocampus. Young, male CD-1 mice (intact or adrenalectomized) were used. The adrenalectomized mice were further divided into 4 groups: Group I - no hormone supplementation, Group II to Group IV, implanted (s.c.) with pellets of corticosterone at the dose of 0.15 mg, 1.5 mg, and 7.5 mg respectively. All animals were injected (i.p.) with 3.0 mg TMT/kg b.w., five days after the hormone implantation and were sacrificed 48 hours later. At sacrifice, blood samples were obtained for corticosterone determination. Brains were also removed for pathological examination. It was found that TMT-treated, adrenalectomized animals (Group I) showed even more severe lesions in the dentate granule cells than the TMT-treated intact mice. While mice implanted with 0.15 mg corticosterone pellet still showed pathology in the dentate granule cells, treated in animals implanted with 1.5 or 7.5 mg corticosterone pellets. Radiolimnmunossay indicated a depletion of corticosterone in the adrenalectomized animals with corresponding increase in the plasma corticosterone level following hormone pellet implantation. The result strongly suggest that corticosterone, which has an inhibitory property to hippocampal neurons, may mediate protection towards these neurons against TMT toxicity by reducing hyperexcitability in these neurons.

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201 MORPHOLOGICAL CHANGES IN THE MOUSE ADRENAL AND HIPPOCAMPUS FOLLOWING TRIMETHYLTHIONE (TM) EXPOSURE. D.W. Cockerill, L.K. Chang, F.G. Rivens, and A. Hough. Department of Pathology, Univ. of Arkansas for Medical Sciences, Little Rock, AR.

The purpose of the present investigation is to compare and contrast the cumulative effects of acute toxic experiences on the mouse adrenal histology and its relationship with neuropathology occurrence. Young, male CD-1 mice were divided into three groups: Group I - injected with 1.0 mg TM/kg b.w.; Group II - injected with 2 consecutive days with 1.5 mg TM/kg b.w.; Group III - injected with a single acute dose of 3.0 mg TM/kg b.w. Control animals were injected with saline solution. The brain and adrenal glands were subjected to light microscopic examination. Although all animals received the same total amount of TM, pathological changes in the granule cells of the fascia dentata appeared to be: Group III > Group II > Group I, suggesting that acute exposures produced a more severe damage to the fascia dentata neurons. Likewise, the adrenal weights of the animals were: Group III > Group II > Group I > control. Significant proliferation and enlargement of the inner eosinophilic or the "X-zone" were observed in the TM-treated, particularly Group II and III, animals, suggesting an increased demand for corticosterone production. In view of the drastic change in the TM cells, they are likely to be affected by TM via hypercorticism, as suggested in our previous investigations, and the inhibitory function of corticosterone on the hippocampal neurons, it was possible that the changes in the adrenal glands observed may represent a feedback response to the hypercortisolemic state of the hippocampal neurons under the influence of TM.


Methylmercury (MM) is a neurotoxic environmental contaminant. Evidence suggests that it is also immunotoxic. We hypothesized that MM affects the immune system by damaging the microtubular (MT) system of mature lymphocytes. We examined the effects of MM upon lymphocyte MTs following in vivo or in vitro exposure using immunofluorescent staining and electron microscopy. Adult male BALB/c mice were given 10 mg MM/kg ip, and sacrificed 48 hr later. Lymphocytes were cultured from spleens by standard methods. For in vitro studies unstimulated and mitogen stimulated lymphocytes were exposed to 2.5 x 10^{-2} M MM for various times. MTs from MM-treated animals displayed an unusual segmental fragmentation, giving the MT a "dashed line" appearance. MT changes were more pronounced in unstimulated cells than stimulated cells. In vitro breakage of MTs followed a reproducible time-course, beginning with fragmentation and ending in complete loss of MTs by 40 min. Ultrastructurally, small MT fragments were identified in the centrosomal region only. MTs showed little recovery after removal of MM from medium. MM may cause immunotoxicity by disturbing MTs; essential for normal lymphocyte functions. (Supported by NSERC.)

203 INFLUENCE OF METAL AND STRESS PRETREATMENT ON ACUTE CADMIUM TOXICITY. K.S. Baer and W.H. Benson. Division of Pharmacology, Toxicology and Nuclear Pharmacy, School of Pharmacy, Northeast Louisiana University, Monroe, LA.

Previous investigations have demonstrated that metallothionein (MT) is induced not only by certain heavy metals, but also by a variety of other factors, including stress. While MT synthesis has been observed in body metals exposed to stress, these studies have also explored the resulting protective effect of these stressors on the acute toxicity of cadmium. Mortalities of 80% and 10% were observed for mice orally administered challenge doses of 100 mg Cd/kg and 150 mg Cd/kg, respectively. To determine a protective cadmium pretreatment dose, animals were administered 2.5, 5, 10, 20, 25 and 50 mg Cd/kg 24 hr prior to cadmium challenge. In animals pretreated with 10 mg Cd/kg, mortalities of 2% and 75% were observed with the respective challenge doses. Following cold stress (4°C, 12 hr), mortalities of 40% and 75% were observed with cadmium challenge doses of 100 and 150 mg/kg, respectively. The correlation of hepatic MT concentration with cadmium and cold pretreatments also was examined. Results of this study indicate that stressors, such as cold, influence the acute toxicity of cadmium to the same magnitude as metal pretreatment. This induced tolerance to cadmium was attenuated, in part, to the induction of MT synthesis.


The Fischer-344 rat is a popular animal model for investigating the pulmonary effects of fibrotic and toxic agents. Endpoints assessed in these studies often include lung weight/body weight ratio, and lung dry weight/wet weight ratio. In one component (I) of this study, we examined how lung weight (W_L) relate to the body weight (W_B) of male Fischer-344 rats over an age span of 10-160 days. We also compared individual lobe weights to total lung weights, and lung dry weight/wet weight ratios. In a second component (II) of the study, we assessed the variability among different treatment groups of age-matched rats (80 day old) in terms of lung wet weights, and lung weight/body weight ratios. In one component studies, W_L was related to W_B by the following expression: W_L = 0.599 x W_B + 1.912, r = 0.95. The percentage contribution of the individual lobe weights to W_L remained constant as the animals grew. The dry weights of the lobes consistently represented 82% of the weights. In component II studies, the average lung wet weights of animals from different treatment groups varied by 10%; lung weight/body weight ratios varied by 25%. In addition to providing a fundamental data base on normal lung growth in the Fischer-344 rat, as indexed by lung weight, the results of these studies are useful for the design of toxicologic investigations in which lung wet and dry weight changes are endpoints, and aging and/or different treatment groups of animals are studied. (This work was supported by the D.O.R. and D.O.B.)
STEREOLICAL DESCRIPTION OF ALVEOLOCAPILLARY LESIONS AFTER EXPERIMENTAL INJURY IN MURINE LUNG. P.R. Filion, P.A. Coulombe, M.G. Côté. Department of Pharmacology, Université de Montréal, Montréal, Qc., Canada.

The capillary network of pulmonary alveolar septa presents a serious challenge to the investigative methods of quantitative microscopy. The minute endothelial covering of this vessel, its close apposition to the alveolar epithelium and its extensive permeation of all septa will require the precise descriptive tools of stereology. The experimental toxic model Butylated Hydroxytoluene (BHT) is used, in mice, to initiate a set sequence of degenerative and reparative epithelial events, culminating in the renewal of 30% of the alveolar pneumocytic cover. The capillary endothelium follows the same temporal and quantitative pattern of response to BHT. The simplest and most easily obtained stereological parameters are used to qualify these reactions, in the endothelial cell as well as in the capillary lumen. Of greatest interest are the surface density measure, indicating the disappearance of cells from the luminal face; and the mean thickness of the cell, which can be further divided into subclasses of cells according to size, and which documents the lesion, destruction, and renewal of the endothelium. Vascular events are similarly described. Endothelial and epithelial events appear closely coordinated within the alveolo-capillary barrier. Supported by MRC Canada.


Porcine pancreatic elastase (PPE) instilled into the lungs of experimental animals has been used widely to induce panlobular emphysema. Our objective was to determine a dose of intratracheally instilled PPE which would produce a minimally detectable lesion. This model would be valuable for assessing the impact of air pollutants on the progression of lung disease. Male F-344 rats 12 wks old, were instilled with 0, 300, 450, 600, or 750 IU PPE/Kg diluted in 0.5 ml 0.9% saline; actual PPE activity was assayed by the method of Bliedt et al. (1973). At 4 wks post-exposure, lung volumes (LW), CO diffusing capacity (DL), respiratory system compliance (Cr), and distribution of ventilation (DV) were evaluated. The lungs of each rat were then fixed in glutaraldehyde at 25 cm Hg0 pressure for morphological study. Significant increases in Cr and LV and decreases in DL and DV were consistent with mild emphysematous lesions at all dose levels. Although a dose-related effect was observed, the between-dose differences could not be distinguished statistically. These functional data will be correlated with morphometrical determinations. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

RESPIRATORY EFFECTS OF HYDROGEN CHLORIDE IN THE BABOON. H.L. Kaplan, A. Anzueo, W.S. Switzer, R.K. Hinderman. Southwest Research Institute, San Antonio, TX; UT Health Science Center, SA, TX; B.F. Goodrich, Akron, OH.

Although studies of combustion gases indicate that rodents are a reasonable model for evaluating hypoxia-producing agents, the suitability of rodents as a model for the effects of irritant gases in man has not been established. In a study of nonhuman primates, 4 groups of 3 anesthetized baboons were exposed in a head box for 15 minutes to air or one of three nominal HCl concentrations, 500, 5,000 or 10,000 ppm. Respiratory parameters (f, Vt, MV) were measured by inductive plethysmography. Pulmonary function tests (PFTs) and CO2 challenge were conducted at 3 days and 3 months post exposure. In contrast to rodent, in which the typical response is a decrease in f, the primates exhibited variable increases in f and consistent, large increases in Vt. At the higher concentrations, blood pH and pO2 were reduced and pCO2 was elevated. At 3 days and 3-months post exposure, blood gases, PFTs and CO2 challenge response of 500 and 5000 ppm exposed animals did not differ from controls. In the 10,000 ppm exposed animals, PFTs and response to CO2 were decreased at 3 days; 3-month tests are in progress. The threshold concentration of HCl for 3-day post exposure changes in respiratory response and PFTs appears to be between 5000 and 10,000 ppm. (Sponsored by The Vinyl Institute.)

CHANGES IN THE MINUTE VENTILATION OF RATS EXPOSED TO THE VAPOR PHASE OF DILUTED CIGARETTE SMOKE. C.B.E. Coggins and R. Ventron. Battelle, Centre for Toxicology and Biosciences, 7 route de Drize, CH-1227 Carouge-Geneva, Switzerland.

Cigarette smoke is composed of particulate matter, vapors/gases and semi-volatiles, that fraction of the whole smoke which passes through a Cambridge filter being defined as "vapor phase". Exposures of rats to dilute cigarette smoke can result in substantial changes in breathing pattern, but it is not known which of the above components of whole smoke are responsible for these changes. Rats were exposed nose-only to diluted cigarette smoke, filtered in one group to remove the particulate matter. Comparisons were made with unfiltered smoke. Using whole-body plethysmography and a pneumotachograph, estimates were made of breathing frequency and tidal volume during exposure to smoke and during air breaks before and after smoke exposure. Similar depressions (up to 37% when compared with air values) were noted in both filtered and unfiltered smoke groups, indicating that the particulate phase of the smoke is not involved in the response. Suggestions are made on the likely causation of the response, and reference is made to the importance of minute ventilation in inhalation studies with laboratory animals.

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A series of experiments was conducted with 8 guinea pigs receiving different levels of CO₂ (from 3 to 20%) while maintaining 20% O₂ in inspired air to assess the capacity of CO₂ to increase tidal volume (VT) and respiratory frequency (f) to a maximum and the resulting arterial blood changes. Each animal was fitted with a carotid cannula for blood sampling (surgically implanted under anesthesia 2 days prior to testing) and placed in a whole body plethysmograph to indirectly obtain VT by measuring the pressure change (ΔP) created with each breath. ΔP and to a lesser extent f increased from air breathing at 3 and 6% CO₂ and reached a maximum at 10% CO₂. Arterial blood O₂ and CO₂ were (X and C.V.) 70.1 (7.7) and 27.7 (9.6) respectively during air and 93.0 (11.3) and 34.7 (5.6) during 10% CO₂. The hydrogen ion activity (in moles/l) were 30.3 (10.1) and 50.8 (4.7) under air and 10% CO₂ respectively. It appears that normal guinea pigs have ventilation-perfusion inequality during air breathing which is substantially diminished during 10% CO₂ and that this concentration is probably the most appropriate for challenging guinea pigs to evaluate the performance of their respiratory system. Supported by NIEHS Grant # RO1-ES02747.

Six groups of male English short-hair guinea pigs were exposed to a paraquat aerosol for four hours. Concentrations of the paraquat aerosols ranged from 0.83 to 2.07 mg/m³ with a mass median diameter + geometric standard deviation of 0.12 ± 0.05 at a middle concentration. Respiratory parameters of each animal were assessed prior to exposure, immediately following exposure, and one day following exposure. The respiratory parameters were monitored during air and 10% CO₂ breathing using a whole body plethysmograph fitted with a head chamber. A concentration related decrease in tidal volume and increase in respiratory frequency were found one day following exposure when breathing air and the CO₂ mixture. The slopes of the concentration response curves obtained from these respiratory parameters were very steep. Inspection of the flow-volume (V-VT) loops showed abnormal rectangular shapes one day following exposure with the degree of severity also varying with the concentration. In conclusion, this study provides quantitative information on the respiratory toxicity following a paraquat inhalation exposure. Supported under Grant No. RO1-ES02747 from the National Institute of Environmental Health Sciences.

Several non-invasive methods have been recently described for assessing pulmonary performance in guinea pigs using 10% CO₂ challenges. Wong and Alarie, TAP, 1982; Schaper et al., TAP, 1985. They have been successfully used in acute and chronic exposures to numerous airborne chemicals. The tested chemicals induced two types of effects, obstruction or reflex restriction, with mixed effects seen in some cases (Schaper, et al., 1985). Characteristic V-VT loops were associated with obstruction and reflex restriction distinct from those obtained normally in air or CO₂. Many correlations have been found between our animal responses and those of humans. First, V-VT loops for normal guinea pigs during air and CO₂ challenge correspond to partial-expiration V-VT loops in normal humans. Secondly, two abnormal ventilatory patterns have been recognized in man, like abnormal patterns induced in guinea pigs. Finally, there are striking similarities between V-VT loops for guinea pigs and humans breathing abnormally. These data suggest that the animal model will permit prediction of responses in man. Supported by NIH Grant No. 2 RO1 ES02747.

INTERIM RESULTS OF CHRONIC INHALATION TOXICITY OF METHYL BROMIDE IN B6C3F1 MICE. S.B. Haber, 1 B.T. Drew, 1 and R.S.H. Yang. 2 Medical Dept., Brookhaven National Laboratory, Upton, NY and 2 National Toxicology Program, NIEHS, RTP, NC.

Earlier subchronic and acute inhalation studies with methyl bromide indicated a steep dose-response curve. Potential target organs included the nervous system and kidneys. To assess the chronic inhalation toxicity and carcinogenicity of methyl bromide, male and female B6C3F1 mice are being exposed to 0, 10, 33 or 100 ppm methyl bromide, 6 hr/day, 5 days/week for two years, terminating 9/96. Endpoints include body weight, clinical signs and mortality, and at 6, 15 and 24 months animals are sacrificed for organ weights, histology and histopathology. In addition, a subgroup of animals in each dosage group is being monitored for neurobehavioral and neuropathological changes. After only 20 weeks of exposure, 48% of the males and 12% of the females in the 100 ppm group died. Exposures were terminated in that group and the surviving mice are being observed for the duration of the study. Neurological signs, similar to those observed in previous studies, were seen in the early death animals of the 100 ppm group. Hematicologic and organ weight data from the 6 month interim sacrifice did not show any significant effects in the 0, 10 or 33 ppm dosage groups. Neurobehavioral changes are largely qualitative, but more indicative of impending toxicity.

Sixty day-old male CD rats were exposed (nose-only) to C-Methyl bromide (50ppm, 1400ppm/cc) for three minutes. Immediately following exposure some of the animals were killed, blood was drawn and tissues removed. The remainder were held in metabolism cages for up to 32 hours, during which time urine, feces, and expired air were collected. The data indicated that the liver, lung, and kidney were the major organs of C deposition immediately following exposure. Thirty-two hours after exposure, the major routes of excretion were pulmonary and renal with approximately 43% and 21% of the total radiolabel being eliminated respectively. A clearance model fit to the data from the various organs showed the kidney exhibiting the shortest T2/2 clearance of 0.60 hrs, while the brain had the longest T2/2 of 6.5 hrs. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

PATHOLOGY OF METHYL BROMIDE TOXICITY. S.L. Eustis, S.B. Haber, R.T. Drew, and R.H. Yang. National Toxicology Program, NIEHS, RTP, NC; Medical Department, Brookhaven National Laboratory, Upton, NY.

As part of our methyl bromide toxicity program, a special 6-week study was conducted in which male and female Fischer 344 rats and B6C3F1 mice were exposed by inhalation to 160 ppm methyl bromide or air 6 hrs/day, 5 days/week. The animals were scheduled for sacrifice either after 3, 10, or 30 exposures, or when 50% mortality was observed in any group. There were clear species and sex-related differences in susceptibility of specific organs and tissues to methyl bromide toxicity. Necrosis of neurons occurred primarily in the cerebral cortex, hippocampus, and thalamus of the brain of treated rats whereas neuronal necrosis in the internal granular layer of the cerebellar folia was more frequent in mice. Nephrosis occurred in all treated groups and was likely a major cause of morbidity and death. Necrosis of the olfactory epithelium was more severe and extensive in rats than mice. Myocardial degeneration occurred in male and female rats and to a lesser degree in male mice. There was atrophy of the "x-zone" of the adrenal cortex in female mice and cytoplasmic vacuolation of the adrenal cortex in rats. Testicular degeneration occurred in male rats and mice.

FORMALDEHYDE-INDUCED NEOPLASIA, ACUTE TOXIC RESPONSES AND CELL TURNOVER IN THE NASAL PASSAGES OF F-344 RATS. E.A. Groes, J.A. Swenberg, and K.T. Morgan. CIIT, Research Triangle Park, NC.

Inhalation exposure of F-344 rats to formaldehyde gas causes defective mucociliary clearance, acute cytotoxicity, increased cell proliferation and squamous cell carcinomas. Histologic sections and autoradiograms from previously reported acute and chronic inhalation studies of formaldehyde were re-examined to determine and compare the sites of each of these responses. Three areas were selected for study on the basis of tumor distribution: 1.) anterior-lateral nasoturbinate and adjacent lateral wall; 2.) mid-ventral septum; 3.) medial maxilloturbinate. Inhibition of mucociliary function and acute cytotoxicity were observed in all 3 regions, while region 1 had the highest number of squamous cell carcinomas (97%) and the highest rate of cell proliferation (2.37%). Region 3 had considerable inhibition of mucociliary function and acute cytotoxicity, but was not a site of origin of any neoplasms and had normal cell turnover (0.21%). Thus cell proliferation correlated better with tumor distribution than did mucociliary function or acute cytotoxicity. This finding supports the proposed role of cell turnover in the carcinogenic process.

PATHOLOGY OF METHYL BROMIDE TOXICITY. S.L. Eustis, S.B. Haber, R.T. Drew, and R.H. Yang. National Toxicology Program, NIEHS, RTP, NC; Medical Department, Brookhaven National Laboratory, Upton, NY.

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Previous studies have shown a good correlation between areas of defective mucociliary function and morphologic lesions in rat nasal mucosa following whole-body exposure to formaldehyde gas. This study was undertaken to investigate the possible reversibility of functional lesions during the delay associated with removal of animals from a large inhalation chamber. A head only exposure chamber was developed and male F-344 rats were exposed for 10, 20, 45 or 90 min, or 6 hr, to 2 or 15 ppm formaldehyde, with recovery groups examined 1 hr after the end of selected exposures. Formaldehyde-induced inhibition of mucociliary function was progressive, both concentration and time dependent and occurred primarily on the anterior-medial maxilloturbinate, septum, lateral nasoturbinate and lateral wall. Rats examined 1 hr after cessation of formaldehyde exposure showed less extensive inhibition of mucociliary function indicating recovery in the more posterior regions of the nose. This study shows that the effects of formaldehyde on mucociliary function were more extensive than previously reported but may be rapidly reversed in some areas of the nose.

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Formaldehyde (HCHO) is a nasal carcinogen in rats following chronic inhalation exposure to 15 ppm; however, no information is available on retention of HCHO by the rat nose. Therefore, we determined the percentage of HCHO gas retained in the nasal passages of anesthetized male F-344 rats exposed in a nose-only system to 2, 6, 15 or 50 ppm C-14 labeled HCHO. This gas was pulled continuously through the nose for 30 min at 15 ml/min (estimated minute volume) via a nasopharyngeal cannula and then bubbled through a sodium bicarbonate trapping solution. The amount of HCHO in the solution was determined by liquid scintillation counting. At the end of the exposure nasal washings were collected, solubilized and counted to determine the amount of C-14 retained in nasal secretions. Results indicated that >99% of the HCHO was retained by the nose, regardless of airborne concentration. A linear relationship between C-14 in the nasal washings and administered HCHO concentration indicated that HCHO retention in nasal secretions was concentration-related. These results demonstrate the high efficiency of HCHO removal by rat nasal passages.

THE INFLUENCE OF INHALED FORMALDEHYDE ON LUNG CYTOCHROME P-450. P. Badeaux, C.E. Dallas, J.C. Theiss, and E.J. Fairchild. The University of Texas Health Science Center at Houston, School of Public Health, Houston, TX.

The effect of formaldehyde exposure on cytochrome P-450 levels in lungs of Sprague-Dawley rats was assessed following 0.5, 3 or 15 ppm, or 0 ppm (control). Whole body exposures were conducted in dynamic, monitored exposure systems for 6 hrs/day and 5 days/wk; for periods of exposure of either 1 wk, 3 months or 6 months, but some for 1 day only. Lung microsomal fractions were prepared by standard procedure and total protein and P-450 were determined for 3 rats from each exposure group for each time point. Two separate sets of exposure studies (with controls) were conducted, thus duplicating all parameters. P-450 at 18 hrs post-exposure decreased to non-detectable levels (compared to control = 0.03 nmole/mg protein) for all formaldehyde doses, while total protein was unchanged. After 1 week, there was a highly significant and reproducible dose-dependent increase in P-450 levels, e.g., 0.5 ppm = 962% control, 3 ppm = 725%, and 15 ppm = 1008%. P-450 levels remained significantly elevated at all formaldehyde concentrations after 3 and 6 months, but the magnitude of the elevation decreased with increased duration of formaldehyde exposure. e.g., 15 ppm = 1008% at 1 week, 302% at 3 months, and 150% at 6 months. Thus, formaldehyde altered P-450, but the direction of change was dependent upon the sequence of exposure. The findings are discussed.
221 TOXICOLOGIC EFFECTS OF A 4-DAY INHALATION EXPOSURE OF RATS AND MICE TO BROMOBENZENE. J.H. Roycroft**, R.A. Miller**, H.A. Ragan**, W.M. Kluwe*, *NIH, National Toxicology Program, RTP, NC; **Battelle Pacific Northwest Laboratories, Richland, WA. Sponsor: J. Hennear

Male and female Fischer 344/N rats and B6C3F1 mice (10/sex/species/exposure concentration) were exposed by inhalation to 0, 10, 30, 100, 300 or 900 ppm bromobenzene for 6 hrs/day for 4 days. Compound-induced mortality was observed in mice (10/10 males at 300 and 900 ppm; 9/10 females at 900 ppm) and male rats (4/10 at 900 ppm). A reduction in weight gain was observed primarily in the 900 ppm groups of both sexes and species. Dose-related clinical signs included restlessness, facial grooming movements, closed eyelids, ocular discharge, listlessness, somnolence, ataxia and/or tremors. Centrilobular necrosis of the liver, cortical necrosis of the kidneys and inflammatory cells in the lung with mixed inflammatory cells were identified in rats (900 ppm). In mice, the liver and kidney necrosis (accompanied by cortical degeneration) was more prevalent and dose-related. Cytomegaly of the liver hepatocytes was also more pronounced and increased hemosiderosis pigmentation of the kidneys were observed in exposed mice. Unlike exposed rats, luncs of exposed mice were unremarkable when compared to control animals. Treatment-related effects were more pronounced in mice than rats and in males than in females.


Fischer-344 rats were exposed to 1, 5, and 10 mg EDMP/m^3 for 13 weeks (6 hr/day, 5 day/week). RBC acetylcholinesterase (AChE) activity was determined at weeks 2, 5, 6, and 13, and at 2 and 4 weeks postexposure. Male and female rats had dose-related AChE depression and females had larger reductions than males. After 2 weeks of exposure to 5 mg EDMP/m^3, male and female rats had RBC-AChE of, respectively, 50% and 20% of normal. RBC-AChE returned to normal by 4 weeks postexposure. A kinetic model was developed for RBC-AChE inhibition. The components of the model were (1) loss of AChE due to reaction with EDMP, k, (2) zero-order replacement of new red blood cells, k, (3) loss of AChE due to RBC destruction, k, and (4) regeneration of AChE from EDMP inhibited enzyme, k. Values for k, k, k, and k were 2.0% of basal AChE day^-1, 0.02 day^-1, and 0.05 day^-2, respectively, in both sexes. Values for k were 0.13 (mg/m^3) day^-1 for females and 0.04 (mg/m^3) day^-1 for males. The model was successfully used to predict inhibition from a single 6 hr exposure to 57 mg EDMP/m^3.

223 SPECIES DIFFERENCES IN THE DISPOSITION AND METABOLISM OF INHALED BUTADIENE. J.A. Bond, A.R. Dahl, R.F. Henderson, J.S. Dutcher, J.L. Maederly, and L.S. Birnbaum*. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; *NIH, Research Triangle Park, NC

The purpose of this investigation was to determine if there were differences in the disposition of inhaled 1,3-butadiene (BD) between rats and mice and if these differences were consistent with the greater tumorigenic response of mice to inhaled BD than of rats. Male Sprague Dawley rats and B6C3F1 mice were exposed nose-only to concentrations ranging from 14 to 13,000 µg BD/L air (0.08-7100 ppm; 25°C; 620 Torr) for 6 hr. At the end of the 6-hr exposure and at all concentrations of BD tested, mice retained about 4 to 7 times the "dose" (µmol/kg body weight) of BD and metabolites than did rats. In both species and at all BD concentrations tested, both urine and expired air combined accounted for about 75% to 85% of the total 14C eliminated after termination of exposure. Analysis of rat and mouse blood samples indicated the presence of both 1,2-epoxy-3-butene (EB) and diepoxybutane. Blood from mice contained two to five times the concentration of EB than did the blood of rats. Thus, species difference in the disposition of inhaled BD that may explain, in part, the species differences in sensitivity to BD. (Research supported under U.S. DOE Contract DE-AC04-76EV01013 through IAA 222-Y04-ES-0092 with NIH.)


Azodicarbonamide (ADA) is used as a blowing agent in plastics, as an aging agent in flour, and as a bread dough conditioner. Male F344/N rats were administered ADA by gavage, intratracheal instillation, or by inhalation exposure to determine the disposition. After gavage, 30% of the administered ADA was absorbed whereas after intratracheal instillation, absorption was 90%. Rats exposed by inhalation to achieve body burdens of 24 or 1230 µg ADA did not have significantly different modes of excretion of 14C-ADA equivalents. ADA was converted to biurea under physiological conditions and only biurea was present in excreta. 14C-ADA equivalents were present in all tissues immediately after inhalation exposure and cleared rapidly. No storage depot was observed. The rate of buildup of 14C-ADA equivalents in blood was linearly related to the lung content. A lung content of 99% of the body burden was cleared with a half-life of 0.6 days and 1% was retained with a half-life of 46 days. Results indicate that inhaled ADA is rapidly converted to biurea which is eliminated rapidly from the body predominately via urine. (Research supported by NIH through Interagency Agreement 222-Y01-ES-20092 under U.S. DOE Contract No. DE-AC04-76EV01013.)
STUDIES OF THE FATE OF INHALED ISOPRENE (2-METHYL-1,3-BUTADIENE) IN RATS MAY PREDICT ITS TOXICITY. A.R. Dahl, J.A. Bond, and R.F. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; and L.S. Birnbaum, NIEMS/NTP, Research Triangle Park, NC

The inhalation toxicity of isoprene, a major industrial chemical, needs to be addressed. Before long-term inhalation studies begin, it is important to obtain toxicokinetic data. Male, 11-12-week-old, F344 rats were exposed nose-only in groups of 30 to 6 hours to concentrations of 4-I4C-isoprene ranging from 8,000 ppm. Urinary and fecal metabolites, exhaled isoprene and volatile metabolites were determined. Time dependence of blood levels of volatile and non-volatile metabolites and isoprene was determined for each exposure concentration. Results indicated that promutagenic 3,4-epoxy-2-methyl-1-butene and mutagenic isoprene diepoxydioxide were formed and circulated throughout the system in blood. Rates of total metabolite and isoprene diepoxydioxide production appeared to be exponentially related to air concentrations of isoprene. Total amount of metabolites produced per unit of exposure concentration decreased as the air concentration of isoprene increased. These observations, together with similar studies on the known carcinogenic properties of butadiene, indicate that isoprene may have toxic properties similar to butadiene.

(Research supported by U.S. DOE Contract DE-AC04-76EV01013 through IAA 222-Y04-ES-0092 with NIEMS.)

DISPOSITION OF 14C-DIBENZO(c,g)CARBAZOLE AEROSOLS IN RATS AFTER INHALATION. P.H. Ayres, J.A. Bond, M.A. Medinsky, Y.S. Cheng, D. Hirshfield, and R.O. McClements. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Dibenzo(c,g)carbazole (DCC) is a nitrogen-containing polycyclic aromatic hydrocarbon that has been detected in tobacco tars, industrial oils, and diesel engine exhaust fumes. The purpose of this research was to determine disposition of DCC in rats after inhalation. Rats were exposed nose-only to 1.1 or 13 mg 14C-DCC/L air for 60 min. The fractional deposition for two exposures ranged from 13% to 16%. The dominant route of excretion of 14C following exposure to either concentration of DCC was the feces, accounting for about 95% of the total 14C eliminated. Radioactivity was widely distributed to all tissues examined with the respiratory tract, upper gastrointestinal tract, liver, and adrenals containing the highest concentration of 14C within 1 hr after exposure. Approximately 60% to 98% of the initial tissue burden was cleared with half-times ranging from 1.5 to 14 days. Several metabolites were detected in urine and feces. The results from this study indicate that DCC was rapidly absorbed from the lungs, translocated to many tissues, and extensively metabolized prior to elimination primarily in the feces.

(Research supported by U.S. DOE Contract DE-AC04-76EV01013.)

SUBCHRONIC INHALATION STUDY OF A VOLATILE GASOLINE HYDROCARBON MIXTURE. C. Aranyi, W. O'Shea, B.J. Cockrell*, C.E. Holdsworth*, and S.C. Lewis*. IIT Research Institute, Chicago, IL; Experimental Pathology Laboratories, VA, American Petroleum Institute,* Washington, DC and Exxon Corporation*, East Millstone, NJ

Male rats appear to be particularly prone to kidney damage following exposures to a variety of petroleum-derived hydrocarbons, including wholly vaporized unleaded gasoline. Unleaded gasoline, however, varies substantially in composition from vapors encountered occupationally. A study was conducted using a distillation cut of gasoline boiling at or below 145°F that approximates the character of vaporous hydrocarbon mixtures measured under actual occupational conditions. Male and female Fisher-344 rats inhaled vapors of this mixture at 1,200 or 5,200 ppm (v/v) 6 hours/day, 5 days/week, for 13 weeks. Body weights were not affected by the exposures, nor were any gross lesions observed at necropsy. In particular, no adverse kidney effects were observed in the male rats. Increased relative kidney weights were statistically significant in female rats. However, histologic examination of the kidneys showed no evidence of hydrocarbon-associated toxic effects. These findings support the conclusion that lower-boiling hydrocarbons of gasoline are non-nephrotoxic in the very sensitive male Fisher-344 rat. (Conducted at IITRI and EPI on funds provided by the API.)


The toxicity of HCCPD (a chemical intermediate used in the manufacture of resins, flame retardants, and pesticides) was studied by exposing groups of 10 rats and 10 mice of each sex to 0, 0.04, 0.15, and 1.0 mg/L air for 6 hours/day, 5 days/week for 13 weeks. HCCPD at ≥ 1 ppm caused 100% mortality in rats and mice at 0.04 ppm, 50% of male and 20% female mice died prior to termination. Body weight gains of male rats at ≥ 0.04 and of both sexes of mice at 0.4 ppm were more than 10% lower than those of the controls. Relative weights of heart, kidney, lung, and testis of male rats and relative weights of liver and lung of male mice at 0.4 ppm were increased. No compound-related changes in hematology or clinical chemistry were noted in either rats or mice. Dose-related histopathological alterations in the respiratory tract epithelium were noted in rats at ≥ 0.4 ppm and in mice at ≥ 0.15 ppm. These included, necrosis and acute inflammation in rats and mice and hyperplasia and metaplasia in mice. The target organ for the toxicity of HCCPD in both rats and mice is the respiratory tract. The non-observable-effect level was estimated to be 0.15 ppm for rats and 0.04 ppm for mice.

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Monomethylamine (MMA) is a flammable gas used as an intermediate in organic synthesis. To evaluate the subchronic inhalation toxicity of MMA, groups of 10 CD rats were exposed nose-only, 6 hr/d, 5 d/wk, for 2 weeks to 75, 250 or 750 ppm MMA in air. After the 10th exposure, specimens for hematologic, clinical chemical and urine analyses were collected and 5 rats/group were sacrificed for histopathological evaluation. After a 14-day recovery period, the remaining rats were subjected to the same battery of analyses. At the 750 ppm exposure level, 5/10 rats died or were killed in extremis during the study. While no significant histopathologic changes were observed after the 10th exposure to 750 ppm, rats from this group had clinical and hematologic evidence of hepatic injury.

Although the nasal turbinate mucosa was eroded and ulcerated in rats from the 250 ppm exposure group, nasal lesions were not observed after a 14-day recovery period. No adverse effects were caused by repeated exposure to 75 ppm MMA.

A subchronic toxicity study of 1,2-epoxybutane (CAS NO. 106-88-7; butylene oxide) was conducted in male and female Fischer 344/N rats and B6C3F1 mice. This chemical is primarily used as a corrosion inhibitor (acid scavenger) in chlorinated solvents such as methyl chloroform and trichloroethylene, at levels of 3-8%. The chemical was administered as a vapor for six hours per day, five days per week, for 12 weeks at concentrations of 0, 50, 100, 200, 400, and 800 ppm to groups of 10 animals/species/sex/dose. No dose-related mortality was seen in rats; in mice, there was 100% mortality at 800 ppm. Histopathologic evaluation of rats showed chronic inflammation of the nasal mucosa in both male and females at 800 ppm. Nasal mucosal inflammation was seen in male and female mice at 200, 400, 800 ppm and was more severe than that seen in rats. Some mice at 800 ppm also showed squamous metaplasia of the nasal mucosa. Other dose-related lesions seen in mice at 800 ppm included necrosis of the kidney, spleen, and thymus; lesions in these organs were not diagnosed in rats. Subchronic exposure of 1,2-epoxybutane caused more severe effects in mice than in rats.

This study was conducted to evaluate the potential toxic hazard of PAA in the workplace. PAA is used as a solvent and vapor inhalation is anticipated to be a major source of exposure. To assess the hazard of single exposures, Wistar rats were exposed to vapor concentrations of 976 ppm (dynamic generation) for 4 hours, or 3628 ppm (static conditions) for 6 hours. A transient weight loss was seen at both exposure concentrations, with signs of CNS depression and sensory irritation at the higher. F-344 rats, 10/sex/group, received 9, 6-hr exposures to mean vapor concentrations of 0, 290, 593 or 1198 ppm over an 11-day period. A slight increase in kidney weights occurred in 1198 ppm males. No histological changes were noted in selected tissues, including kidneys. Four groups of 10 F-344 rats/group were exposed to PAA vapor concentrations of 0, 101, 303 or 595 ppm for 6 hr/d, 5 d/wk, for 14 weeks. Half of the animals were killed at the end of the exposure regimen, the remaining sacrificed after a 4-week recovery period. No exposure-related effects were observed on the parameters monitored for this study. Within the constraints of the experimental conditions, these findings indicate PAA has a low potential to produce adverse effects in the workplace.
Hydrogen cyanide (HCN) is a rapidly acting toxic compound. Fischer 344 rats were exposed head-only to preset atmospheres of HCN mixed with air such that oxygen deprivation was not a factor. LC50 values (including 24 hr deaths) determined for exposures ranging from 1-60 min were 3000-86 ppm, respectively. The incapacitating levels were approximately 65% of the lethal levels. Many incapacitated animals exhibited convulsions during recovery. Blood pressures initially increased, then decreased before returning to normal. Inevitable death was signaled by a substantial drop in blood pressure. Heart rates decreased 65-84% in all exposures. Time-weighted average blood cyanide levels increased with increasing average atmospheric HCN concentrations. With the exception of some animals exposed for 1 min, those with time-weighted average blood cyanide levels above 2.0 ug/ml died. HCN also produced immediate increases in the arterial blood plasma histamine levels. Most deaths occurred during the exposures or within 24 hours. Some animals, however, did not recover immediately but continued to lose considerable weight (up to 56%) over many days (up to 38) before dying.

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The aerosol formulation of procaterol hydrochloride hemihydrate, a new beta-agonist bronchodilating agent, was evaluated for potential long-term toxicity in rats by nose-only exposure. Groups of 20 Sprague-Dawley rats/sex were exposed to aerosols at estimated 10, 50 and 100 fold multiples of the human clinical dose of 60 ug/day. Additional groups received placebo aerosol or room air. Exposure were 1 hour/day, 7 days/week for 6 months. Body weight and food consumption were recorded weekly. Clinical pathology determinations were at 3 months of exposure (10/sex/group) and termination (all survivors). All animals underwent complete gross and microscopic pathology examinations at termination. Six deaths occurred, none were drug related. No pharmacotoxic signs were noted in any group. Statistically significant increases in absolute heart weights, occurred in high dose males (11%) and females (8%) and in mid-dose females (13%). There were no other drug-related changes. There were no findings of drug-related cardiotoxicity or of mesovarian leiomyoma induction in this study. Procaterol aerosol appeared well tolerated by rats under the conditions of this study, since recognized lesions seen with this drug class were not observed.
FACTORS CONTROLLING NITROSAMINE FORMATION IN THE LUNG: A UNIQUE AMINE UPTAKE SYSTEM. C.R. Shoaf and D.B. Menzel. Departments of Pharmacology and Medicine, Comprehensive Cancer Center, Duke University Medical Center, Durham, NC.

This study seeks to characterize the uptake of hydroxyproline (HP) into the rat lung from the vascular system using the isolated ventilated perfused lung (IVPL) and further to determine whether this amine is trapped sufficiently to act as a reactant with NO₂ in the production of N-nitrosohydroxyproline (NHOP). HP was found to be absorbed by the IVPL with a Vₐₕₗ of 968 nmoles/min/µg dry weight tissue and a Kₘ of 217 µM at a perfusion rate of 2.2 ml/min. Similar to the 5-hydroxryptamine (5HT) uptake system, the uptake of HP was inhibited (56%) in a sodium-free medium. However, 5HT (and putrescine) did not inhibit uptake of HP. Uptake of HP was not sensitive to sodium cyanide or carbonyl cyanide 4-(trifluoromethoxy)-phenylhydrazine. While it has been shown here that the lung preferentially sequesters HP, published nitrosation rates of morpholine by NO₂ suggest that only about 0.0001% HP would be converted to NHOP in this IVPL system. This uptake system is unlike the 5-HT or diamine pulmonary uptake systems previously described. Finally, a rate of nitrosation sufficient to monitor by reaction of NO₂ with radiolabeled HP is unlikely to be obtained. (Supported by EPA Cooperative Agreement CR809713 and NIH Grants CA 14536 and RO1693.)

ACUTE AND SUBCHRONIC INHALATION TOXICITY OF CRASOL NAVY BLUE (ONB). B. Ballantyne, Applied Toxicology Department, Union Carbide Corporation, Danbury, CT.

ONB, a water-insoluble nitroaromatic dye, may be ground during its manufacture and thus dispersed in the atmosphere. Therefore, the potential for lung injury by single and repeated exposure was investigated. Male rats and guinea pigs acutely exposed to 173 mg/cu m for 30 min did not show any signs of toxicity or respiratory tract histopathology at various sacrifice periods from 6 hr to 28 days postexposure. For repeated exposure, rats, mice, and guinea pigs were exposed 6 hr/day, for 20 or 100 days, to 2 mg ONB/cu m (particle size, 447 < 10 µm). Except for decreased guinea pig body weight during exposure, no adverse effects were noted over a one-year period from the first exposure, at the end of which time they were sacrificed. No respiratory tract histopathology was present in mice and guinea pigs, but rats had alveolar and interalveolar foci with cytologic and cytochemical features characteristic of alveolar proteinosis. Since morphologically similar, but fewer and smaller, lesions were seen in control (unexposed) rats, it is probable that exposure to respirable ONB particles may enhance, rather than initiate, the pulmonary alveolar proteinosis in this species. Thus, although ONB is not a primary respiratory tract irritant, marked exposure may enhance the development of pulmonary alveolar proteinosis in the rat, a species particularly sensitive to the development of this lesion by inhalation of inert nonfibrogenic particles.


EGHE is a alkyglycol ether, the low molecular weight homolog of which have been shown to produce hematologic and reproductive system toxicity by inhalation. The low vapor pressure of EGHE allows for a maximum vapor concentration near 85 ppm (22°C). An acute 4-hr exposure of Vistar rats to 83 ppm EGHE produced no toxic effects. F344 rats were exposed for 9 days (6 hr/d) to 0, 19, 41, or 84 ppm EGHE; depressed body weight gains and increased liver/body weight values were observed at 84 ppm EGHE. In a 13-week study, rats were exposed to mean EGHE concentrations of 0, 20, 41, or 71 ppm (6 hr/d, 5 d/wk). EGHE-related effects included: perinatal deaths in females at 20, 41, and 71 ppm and males at 71 ppm; depressed body weight gains in both sexes at 71 ppm and females at 41 ppm; increased absolute and/or relative liver and kidney weights in both sexes at 71 ppm, and to a lesser extent, at 41 ppm; and alteration of several serum enzymes in females at 71 ppm. No gross or histopathologic lesions were found. Only the increased liver weights (71 ppm group) persisted through a 1-mo recovery period. The changes observed at 41 ppm and below were not considered to be biologically significant. Hematologic abnormalities and testicular atrophy found with some shorter chain alkyglycol ethers were not observed with EGHE.


The present studies were designed to investigate whether lungs can utilize 5-hydroxryptophan (5-HTP), the first tryptophan metabolite, formed elsewhere and transported for the synthesis of 5-HT. The perfusate concentrations of 5-HIAA increased significantly during rabbit lung perfusion with 10 µM ¹⁴C-5-HTP and they were not different when the lung was pre-perfused with 0.5 mM CP and later perfused with 10 µM 5-HTP. 5-HT, but not 5-HIAA, was detected in the perfusate and increased with time of perfusion when the rat lung was perfused either with 10 µM 5-HTP or with 0.5 mM CP and 10 µM 5-HTP. Lung contents of 5-HT and 5-HIAA were significantly higher in the rat lungs and only 5-HIAA increased in the rabbit lungs after 60 min perfusion with 10 µM 5-HTP. Pre-perfusion with 0.5 mM CP increased the greater increase in the 5-HT content of both rabbit and rat lungs. These results were further confirmed by doing in vitro incubation studies. These results provided evidence that lung can synthesize 5-HT from the circulating 5-HTP. However, pulmonary uptake and metabolism of 5-HTP is much less when compared to the metabolite, 5-HT. Hence, while pulmonary contribution of 5-HT to the circulating levels is possible, its pharmacodynamic significance remains to be investigated. (Supported by MS Heart Assoc. and HL-20622.)
Polyamines have been implicated as important for cell growth and proliferation. Therefore, they could be important for recovery of lungs damaged due to toxicity of chemicals and oxidant gases. The objective of the present studies was to characterize the uptake, and metabolism of polyamines in isolated, perfused, ventilated rabbit lung preparations. Lungs were perfused using Krebs' buffer with albumin in which these compounds were included at an initial concentration of 1, 5, 10 and 20 μM. Perfusates samples were withdrawn at various times points for analyses. After perfusion for 1 hr, the lungs were also analyzed. It was observed that there was active uptake of polyamines by isolated perfused rabbit lungs. Even at the highest concentration used, the lungs were viable, and showed no signs of edema. The uptake of polyamines by lungs seemed to increase linearly for the concentrations of polyamines studied. Analysis of the perfusate and lung samples for metabolites using HPLC-post column derivatization by o-phthalaldehyde reagent did not show any unknown metabolic products. (Supported by Mississippi Lung Association.)

The pneumotoxic properties of NMB are well established (Toxicol. Appl. Pharmacol. 23:85). Impairment of pulmonary 5HT clearance has been implicated in the response to some pneumotoxins. We therefore investigated a potential role for 5HT in NMB-induced pulmonary edema and hydrothorax. Depletion of 5HT stores with reserpine, 3 mg/kg, 24 hours prior to ip dosing with 0.12 mmols/kg NMB significantly reduced the incorporation of 14C-2-thymidine into mouse pulmonary DNA. Pretreatment of mice with the same dose of reserpine had no effect on time to death and mortality induced by a lethal or supralethal dose of NMB (0.24 and 0.6 mmols/kg, respectively). Using the isolated perfused rat lung, we demonstrated that lungs obtained 30 min prior to dosing with 0.12 mmols/kg NMB exhibited impaired 5HT removal (as measured by HPLC-EZ) 12 hours after dosing. This was accompanied by elevated lung wet weight/body weight ratios, five hours after administration of the same NMB dose, rats exhibited elevated lung wet weight/heart weight ratios and normal pulmonary 5HT uptake. The above evidence suggests that 5HT is not a primary mediator in the expression of pneumotoxicity induced by NMB. (Supported by Grant ES-02335.)

The objective was to investigate whether isolated perfused rabbit lungs can utilize external glutathione (GSH). GSH rapidly disappeared from perfusate containing bovine serum albumin (BSA) during circulation or incubation without lungs. Only 50% of added GSH was recovered as oxidized GSH (GSSG). Replacing BSA with dextran in the perfusate (DP) increased the half-life of GSH 10-fold. Inclusion of GSH in either BSA or DP did not increase pulmonary GSH in control perfused lungs. However, small but significant increase of pulmonary GSH occurred in diethylmaleate (DEM)-pretreated (GSH depleted) lungs perfused with BSA. This inefficient utilization of external GSH was unrelated to the rapid GSH oxidation in the presence of BSA since GSH depleted lungs perfused with DP utilized external GSH even less efficiently. The t½ of GSH in BSA was decreased in the presence of lung while in DP it was unaffected, indicating that the lungs did not catabolize external GSH or GSSG effectively. DEM-pretreatment resulted in decreased GSH and GSSG and increased cystine release from the lungs. In conclusion, GSH was rapidly oxidized in the presence of BSA and, although some utilization of external GSH was observed in the lungs, the mechanism(s) is rather inefficient. (Supported by HL-20622.)

Imbalances in pulmonary proteinase/anti-proteinase levels have been implicated in the development of destructive lung disease, particularly in the known to be chronic in these antienzymes. This investigation is an attempt to develop a rat model of a-1-Pi deficiency for the study of this risk factor in air pollutant-induced lung disease. Male rats treated 2X daily with the liver toxicant Eth (50 mg/kg ip) for 3 wks show a 40% depression of serum a-1-Pi (as trypsin inhibitory capacity, TIC). No mortality was observed and body weight decreased less than 10%. To obviate the need for rigorous adherence to daily injection schedules, Eth was incorporated into standard rat chow at 0.0, 0.1, or 0.3% and fed to groups of 15 male F-344 rats. A persistent dose dependent decrease in food consumption and body weight was observed. Through the last completed observation at 4 wks, the 0.3% group showed a 35% decrease in serum a-1-Pi activity. At 6 wks of treatment, pulmonary function tests will be performed, serum and lavage fluid will be assayed for a-1-Pi concentration and activity, and major organs will be evaluated for histopathologic changes. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
Normal and castrated male mice were exposed to inhalation at 150 ppm trichloroethylene (TCE) for one month. The increase in plasma butyrylcholinesterase (BuChE) activity after the exposure was of the same magnitude as the increase seen after castration. Administration of testosterone with osmotic minipumps almost restored the normal testosterone and BuChE levels in castrates. The effect of TCE exposure on BuChE activity in these animals was the same as on normal males. Testosterone levels were not influenced by the TCE exposure in normal males or in castrates given testosterone. The BuChE activity changes induced through exposure are thus not due to effects on testosterone. The results from these animal experiments do not support the epidemiological findings of decreased testosterone levels in solvent exposed humans.

The LC50 (µg/t-2/L air) for a 10-min exposure of mice to an aerosol of a saline suspension of T-2 is 35. The corresponding LC50 of an aerosol of an ethanol solution of T-2 is 325. Both aerosols have a median aerodynamic diameter of 0.6 µm. In an attempt to explain the difference in LC50's between the two aerosols, experiments were designed to measure total body (TB), lower respiratory tract (LRT), and nasal turbinate (NT) retention of T-2 in mice exposed to either an aerosol of a T-2 suspension or solution. At LC50 aerosol mass concentrations, TB retention of T-2 in mice exposed to an aerosol of a T-2 solution was twice that retained in mice exposed to an aerosol of a T-2 suspension. In contrast, a similar amount of T-2 was retained in the LRT and NT of mice exposed to either aerosol. In separate dose-response studies, we found that % lethality was similar among mice exposed to either aerosol if % lethality was correlated with LRT and NT retention rather than with TB retention or aerosol mass concentration. Thus we conclude that toxicity to the respiratory tract is the major element in acute inhalation toxicity of T-2 toxin and that ethanol aids in translocation of T-2 from the respiratory tract.
MCTP produces pulmonary endothelial cell injury and elevated pulmonary arterial pressure in rats. Platelets release vasoactive substances when they encounter an injured endothelium, and may be involved in MCTP-induced pulmonary hypertension by this mechanism. One such substance is thromboxane A2 (TXA2). To study this interaction, TXA2 release was examined in isolated, blood-perfused lungs. Seven or 14 days after treatment with MCTP (4 mg/kg) or its vehicle dimethylformamide (DMF), rats were killed and their lungs were isolated and perfused (8 ml/min, single pass) with citrated-blood collected from a separate group of untreated donor rats. The concentrations of stable metabolites of TXA2, and prostacyclin (PGI2) were determined in the plasma obtained from the collected effluent. Seven days after MCTP treatment, approximately the time when pulmonary hypertension is beginning to develop, the concentration of TXB2 in the plasma effluent was not significantly different in lungs from MCTP- and DMF-treated rats. By Day 14, however, when pulmonary hypertension is established, release of TXB2 was significantly greater in lungs from MCTP-treated rats. PGI2 release was not different between the two groups at either time measured. These results indicate that pulmonary hypertension in MCTP-treated rats is associated with increased release of TXA2 in isolated lungs perfused with a platelet-containing medium. (Supported by NIH grant ES02561.)

Lung damage caused by ozone (O3) exposure in rats has been shown by various assays to be synergized by concurrent exposure to acid aerosols. We determined whether dimethylthiourea (DMTU), a potential scavenger of hydroxyl radicals in vivo, protected rats from the pulmonary damage caused by exposure to O3 or to O3 combined with a respirable aerosol of sulfuric acid. Rats were given daily intraperitoneal injections of DMTU during exposure to 0.20 or 0.64 ppm of O3 with or without concurrent exposure to 1 mg/m3 of sulfuric acid aerosol. DMTU prevented increases in whole lung protein content and lung lavage protein content and enzyme activities from groups of rats exposed to 0.64 ppm of O3 alone or with sulfuric acid aerosol. Daily treatment with DMTU also prevented increases in lung collagen synthesis rates from groups of rats exposed to 0.20 or 0.64 ppm of O3 alone or with sulfuric acid aerosol. We conclude that: 1) daily treatment with DMTU protected rats from lung damage induced by exposure to O3 or to O3 + acid aerosol, 2) both O3- and O3 + acid aerosol-mediated lung damage may progress through a mechanism involving the participation of hydroxyl radicals.


Mature female rabbits (2.5-3.5yr) were randomly exposed to 0, 0.1, 0.2, 0.4, and 0.6 ppm O3 for 3h with a week rest between each exposure. Plasma concns. of Vit A, C and E were determined by HPLC 2 and 5 h following discontinuation of the O3. Plasma Vit A ranged from 57 to 95ug/dl (Mean 78.2 ± 9.3 S.D.), Vit C from 0.510 to 0.966 mg/dl (Mean 0.725 ± 0.119 S.D.) and Vit E from 0.597 to 0.986 mg/dl (Mean 0.776 ± 0.092 S.D.).

Statistical analysis by ANOVA for repeated measures showed no significant differences between the vitamin concns. related either to the ozone dose or the time the sample was taken. We conclude that the inhalation of O3 by the animals used in this study had no effect on the plasma concns. of Vit A, C or E.

Analysis of recovered fibers from lung tissue is essential for the assessment of their biological activity in regards to retention and damage. These parameters have been studied in rats and humans exposed nose-only for 6 hrs/day, 5 days/week for up to 26 months to 0.45 μm, 3.1 μm, 5.4 μm and 6.1 μm mean dia. glass fibers, 1.8 μm mean dia. ceramic fiber, 2.7 μm mean dia. mineral wool and 150 cromudolite monodispersed aerosols ranging from 3 mg/m3 for 0.45 μm to 15 mg/m3 for the 3.1 μm mean dia. glass fibers. Fibers were recovered from tissue by sodium hypochlorite digestion and examined by electron microscopy. Animal variation occurred, but the fiber the dust, the greater the amount of dust recovered. Fiber numbers from the coarse fiber exposed groups were larger in comparison to the finer dusts when the aerosol fiber concentrations were normalized indicating differential dust retention. Microscopy of recovered fibers showed that cromuolite and ceramic fibers were unaffected by lung residence while mineral wool and glass fibers showed evidence of etching. The aerosols and recovered dusts contained many non-fibrous particles. These need to be taken into account when assessing the biological effect of dusts as should the considerable size dependent fiber loss that was identified to be inherent in tissue digestion methods.
253 TOXIC DAMAGE TO THE TRACHEA ASSESSED BY MULTIPARAMETER FLOW CYTOMETRY. N.F. Johnson, and J.S. Wilson. Los Alamos National Laboratory, Los Alamos, NM. Sponsor: E.F. Henderson

The upper respiratory tract is a major target for toxicant damage leading to neoplastic and non-neoplastic lesions. Fischer-344 rats have been exposed to ozone (15ppm) for 30 minutes and left to recover for periods of up to 30 days with comparable non-exposed rats. The tracheal cells were isolated by biochemical mechanical means, stained with Hoescht 33342, and assessed by flow cytometry (forward light scatter, ssG2M phases of the cell cycle) was determined for the controls. Ozone modified the populations with the corrected cells becoming more and the ciliated cells less prominent and a 90% increase occurred in the number of cycling cells. The changes mirrored those seen with light microscopy. Flow cytometry can be used to rapidly monitor tissue changes, cell proliferation and also provides a means to sort cell populations for mechanistic studies of toxic damage to the lung.

254 CYSTEAMINE-PRETREATMENT PROTECTION FROM OXYGEN TOXICITY IN THE RAT: THE ROLE OF SULFHYDRL OXIDATION. D.T. Kirkpatrick and R.D. Mavis, Division of Toxicology, University of Rochester Medical Center, Rochester, NY, 14642.

Inactivation of critical sulphydryl-dependent enzymes may play an important part in the mechanism of oxygen toxicity. Previously, we found decreases in lung protein sulphydryl content and sulphydryl-dependent enzyme activities during the first 48 hr of exposure to pure oxygen. In this study rats were pretreated with cysteamine during the 30 hr period preceding oxygen exposure. A 48 hr oxygen exposure results in pulmonary injury as indicated by increases in lung wet weight to dry weight ratio and lavage protein, lactate dehydrogenase, and aryl sulfotransferase. Pretreatment with cysteamine protected rats from injury, with nearly complete blockade of these increases. The cysteamine-pretreatment protocol resulted in a >50 percent increase in lung glutathione (GSH) at the start of the exposure. Following the exposure, cysteamine-pretreated animals did not have the nearly 30 percent loss of protein sulphydryls that was seen in saline pretreated controls. The sulphydryl-dependent enzymes, succinate-cytochrome c reductase, and membrane aldehyde dehydrogenase were similarly protected. These results are consistent with an important role for sulphydryl oxidation in the mechanism of pulmonary oxygen toxicity. (Supported by US DOE Contract No. DE-FG02- B5ER0282 and NIH Training Grant No. 5 732-HL07026.)

255 CORRELATION OF HISTOPATHOLOGIC LESIONS WITH FUNCTIONAL IMMUNE RESPONSE EFFECTS. J. Cavagnaro, C. Slabik, R. Alsaker and F. Reno, Hazleton Laboratories Corporation, Vienna, VA.

Proper assessment of immunotoxicity includes measurements of body weight changes, clinical observations and physical measurements, organ weights and organ/body weight ratios, clinical laboratory studies and histopathology combined with specific measurements of immune function. Studies were designed to correlate the sensitivity of findings from a histopathologic assessment of immune organs i.e. thymus, spleen and lymph nodes, to the data obtained from immune function assays i.e. lymphocyte blastogenesis response to mitogens and mixed lymphocyte response. Results showed a good correlation when the compound suppressed the immune response. However, when the compound enhanced the immune response histological changes in animals were less readily detected and quantified. In cases of compounds that stimulate the immune system, immune function assays and/or immunohistochemical techniques should be more sensitive indicators of immune system toxicity than routine histological evaluation.


The potential immunomodulating and immunotoxic effects of drugs and chemicals are subjects of growing interest. The mononuclear phagocyte system (MPS) is a key target for the pharmacologic and toxicologic actions of immunomodulatory drugs. We have adapted to the dog techniques for assessing peripheral blood mononocyte (PBMC) function in order to monitor this system, when indicated, during routine toxicity studies. Techniques for isolating PBMs were compared; a method employing hypotonic Ficoll-Hypaque centrifugation was adapted, which resulted in >90% purity and a greater yield of PBMs than obtained with other established methods. Methods for measuring antibody-dependent cytotoxicity, and the production and release of II-1, PGE-2 and superoxide were adapted to the dog. Assays for PGE-2 and superoxide have not been reported previously for the dog. A novel assay for IL-1, based on its ability in the presence of the calcium ionophore A23187 to induce IL-2 production in EL4 mouse thymoma cells, was found to be far more sensitive than the conventional C3H/HeJ mouse thymocyte assay for measuring this interleukin in the dog. Together, these assays should allow us to better define drug and chemical effects on the MPS of the dog.
MACROPHAGE FUNCTIONAL ACTIVITIES VERSUS CELLULAR PARAMETERS UPON SUBLETHAL PESTICIDE EXPOSURE.

Toxicity of selected organochlorine, organophosphate and carbamate pesticides on the functions and cellular parameters and soluble factors of peritoneal macrophages was examined in inbred A/J, C57Bl/6 and (C57Bl/6 x A/J)F1 mouse strains. Single, sublethal doses of organochlorine pesticide, dieldrin suppressed phagocytosis, bactericidal activity, antigen processing and decreased cellular intrinsic resistance to in vitro virus-induced cytolyis. Cell viability, generation of superoxide anion by macrophages, and activation of cells by the vehicle, increase in cell number in peritoneal cavity after chemical elicitation or immunological activation, however, appeared to be only slightly affected or unchanged by i.p. sublethal dieldrin exposure. The data obtained for other selected pesticides also correlated with a general phenomenon of pesticide-induced inhibition of macrophage functional activities without major effects on cellular parameters, such as cell viability, adherence to plastic, and others. Resistance of the O2−-generating system to exposure of the pesticides is discussed in terms of a lack of direct relationship between macrophage phagocytosis and the activity of the NAD(P)H-dependent, O2−-generating system. Supported by NSERC.

IMMUNOPOTENTIATION BY VERRUCARIN A IN CD-1 MICE. B.J. Hughes, M.J. Taylor, R.P. Sharma, Toxicology Program, Utah State University, Logan, UT

Verrucarin A (Ver A), a macrocyclic trichothe-cene mycotoxin, was examined for its immunomodulatory effects. The dose, prepared in 1% DMSO in physiological saline, was administered i.p at 1/2 the LD50 (0.35 mg/kg). Lymphocyte proliferation was studied on days 0, 2, 4, and 7 after dosing. On day 2 no difference in "H-thymidine ("H-TdR) incorporation was observed using Con A, LPS, PHA, or PWM. On day 4, treated animals showed significant increases in "H-TdR uptake when stimulated with Con A, PHA, or PWM (p<.05). On day 7, PHA was increased (p<.001) above controls while Con A, PWM and LPS were not significantly different. Evaluation of antibody production by the hemolytic plaque assay was done administering SRBC as controls, SRBC and Ver A simultaneously and Ver A 2 days after SRBC challenge. Plaque forming cells and cells per spleen were increased when Ver A was administered 2 days after SRBC challenge. Spleen weights increased when Ver A was administered simultaneously with SRBC and significantly increased when toxin was given 2 days after antigen. The indications of this experiment were that Ver A exerted an effect, particularly on T-lymphocytes, was immunostimulatory in vivo, and its effects on α-SRBC production were time dependent. Supported in part by USPHS ES 07997.

EFFECT OF CARRAGEenan ON THE IMMUNOREGULATORY CAPACITY OF MACROPHAGES. S. Nicklin, K. Baker and K. Miller. BIBRA, Garshalton, Surrey UK. Sponsor D.M. Conning

Small quantities of orally administered carrageenan accumulate in intestinal lymphoid tissues of rats prior to macrophage transport into the periphery. As macrophages are pivotal cells within the immune system, we have investigated the effect of carrageenan on the immunoregulatory functions of these cells. RNA and protein synthesis was induced in cultured macrophages shortly after exposure to microgram quantities of carrageenan. Whereas levels of carrageenan in excess of 10 μg/ml eventually proved toxic, analysis of supernatants obtained from treated cultures revealed a significant level of the immunostimulatory agent interleukin-1 (IL-1). The addition of carrageenan to a thymocyte proliferative assay however resulted in reduced blastogenesis. These results indicate that μg quantities of carrageenan can trigger the release of both stimulatory and inhibitory mediators from macrophages. The former was identified as IL-1, the latter awaits characterisation but is believed to be of prostaglandin origin. These results indicate that carrageenan associated changes in immune competence may stem from altered internal regulation rather than direct toxicity. (Supported by UK Ministry of Agriculture, Fisheries and Food)

LACK OF IMMUNOSUPPRESSIVE EFFECTS OF ACUTE AND SUBACUTE ADMINISTRATION OF MALATHION ON MURINE IMMUNE RESPONSES. R.E. Rodgers, N. Leung, C.F. Ware, B.H. Devens and T. Immura, Division of Biomedical Sciences and Toxicology, University of California, Riverside, CA 92521-0121.

Malathion has been previously shown to cause allergic responses and suppress the generation of a humoral immune response in vivo. In this study, the effect of in vivo administration of malathion on cellular, humoral, and mitogenic responses was examined. Acute (50% LD50) or subacute (10% LD50 per day for fourteen days) treatment with malathion in vivo did not affect the in vitro generation of specific antibody secreting cells to sheep red blood cells (SRBC) or cytotoxic T lymphocytes (CTL) to allogeneic tumor. However, five days following acute administration of malathion, there was a slight increase in humoral immune responsiveness. Acute treatment with 50% LD50 purified malathion did not affect body weight, splenic cell number or thymus size. However, mitogenic responses to Concanavalin A (ConA) and lipopolysaccharide (LPS) was significantly enhanced on all days tested following acute administration of malathion. In contrast, subacute treatment with malathion did not affect mitogenic response to ConA or LPS, but led to a significant decrease in thymic cell number. Supported by PHS ES03105.

Our purpose was to determine the relationship between murine cytomegalovirus (MCMV) induced depression of cytochrome P-450 activity and the interferon response following MCMV infection. Infection of both CD-1 mice (high interferon producers) and BALB/c mice (low interferon producers) with virulent MCMV resulted in enhanced pentobarbital-induced sleeping time and depressed P-450 levels 3 and 6 days post infection. Administration of anti-interferon antibodies prior to infection with MCMV resulted in increased virus titers in the liver but did not alter the depression of P-450 levels seen in infected animals. Inoculation of mice with poly (I) : poly (C) resulted in similar changes in sleeping time and P-450 levels but not to the degree seen in virus-infected animals. CD-1 mice infected with attenuated MCMV, a strain of virus which induces interferon but does not infect the liver, did not show these effects. While previous work in this and other laboratories has suggested that interferon and interferon inducers depress cytochrome P-450 levels, evidence from this study suggests that the effects of MCMV on metabolism were not related to the interferon response. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

EFFECTS ON ALVEOLAR MACROPHAGES OF RATS INHALING RED PHOSPHORUS BUTYL RUBBER (RP/B) COMBUSTION PRODUCTS. Catherine Aranyi, Jeannie N. Bradolf, and Robert L. Sherwood. IIT Research Institute, Chicago, IL 60616

Sprague Dawley male rats were exposed to RP/B aerosol mass concentrations ranging from 0.3 to 1.2 mg/L. Initially rats received single 3.5-hr exposures to 1.0 mg/L of RP/B. In subsequent studies the animals inhaled the aerosol for 2.25 hr/day on 4 consecutive days/week for 4- and 13-weeks. Biological endpoints were examined within 1 hr after the last exposure and for selected groups from the 4- or 13-week studies also after 2 or 8 weeks of recovery, respectively. Pulmonary bactericidal activity to inhaled 35S-K. pneumoniae was depressed after the acute and the 13-week exposures. Pulmonary free cells collected by lavage (90-99% macrophages) generally showed decreasing trends in total numbers, increased ATII levels, and decreased exoezyme activity for 5 nucleotidase. Changes in other exoezyme activities were not consistent. In vitro phagocytosis of 35CRBCs was decreased following a single exposure only. Terminal broncholar fibrosis was observed in all rats after 4- and 13-week exposures to >0.75 mg/L of RP/B. The severity of the lesions increased with the severity of the exposure conditions. Except for the fibrosis, most changes were reversible. (Supported by the U.S. Army Medical Research and Development Command, Contract No. DAMID-82-C-2121.)

STUDIES ON MACROPHAGE TRANSFERRIN RECEPTOR EXPRESSION AND TUMORSTATIC activity C.S. Dickens, M.R. Myers, and L.B. Schook. Medical College of Virginia Richmond VA (Sponsor: F.H. Dick)

Macrophages (Mph) are involved in antigen presentation, the destruction of intracellular parasites and tumor cells. Our laboratory has focused on the origin of such Mph diversity. We have related stages of Mph cytostatic activity to a melanoma tumor to the expression of the transferrin binding receptor on Mph which have low levels of Tfr have high cytostatic activity. Treatment of animals with dimethylnitrosamine (DMN) increased the resistance to challenge with the melanoma in vivo and in vitro. DMN treatment also resulted in decreased Tfr binding of natural Mph obtained following i.p. administration of thioglycollate and Con A or adherence of splenic cells. Changes in Tfr number after DMN exposure resulted from changes in Mph differentiation from bone marrow cells. Decreased numbers of Tfr also reflected changes in the kinetics of Mph differentiation. These results aid our understanding of the origins of Mph phenotypes and regulation of various effector and effector functions. Supported by PHS grants ES 03468 and CA 09210.

IMMUNOLOGICAL RESPONSES OF GUINEA PIGS TO SUBCHRONIC INHALATION OF DETERGENT DUSTS CONTAINING VARIOUS LEVELS OF ENZYME ANTIGENS. D.A. McNeill, R.I. Ritz, B.L.B. Evans, R. Deskin, J.L. Russell, and G.L. Fisher. Battelle Columbus Laboratories, Columbus, OH and The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH.

Groups of 30 female Hartley guinea pigs were exposed six hours per day, four days per week for ten weeks to 1 mg/m^2 of an aerosolized, powdered detergent containing either 3.5, 1.1, 0.35 or 0.11% of a proteolytic enzyme, Subtilisin A. A fifth group was exposed to filtered room air. Weekly serum samples were obtained for subsequent passive cutaneous anaphylaxis (PCA) assays for the detection of allergic antibodies and microimmunodiffusion (MID) assays for the detection of precipitating antibodies. Sensitization to the enzyme antigen was demonstrated to be both enzyme concentration dependent and time dependent with the PCA and MID responses increasing with dose level and length of exposure. During Weeks 4 through 10 animals showed respiratory symptoms during exposure. The initial appearance of these symptoms correlated with the initial appearance of measurable serum allergic antibodies.
DETECTION OF HALOTHANE INDUCED ANTIGEN USING SPECIFIC ANTI-HALOTHANE METABOLITE ANTIBODIES. T.P. Roth, A.H. Callis, and A.J. Gandolfi, Dept. of Anesthesiology, Univ. of AZ, Tucson, AZ.

Multiple halothane exposures of rabbits induces an antibody which is cross reactive with trifluoroacetylated rabbit serum albumin (TFA-RSA). In addition, antibodies were generated against TFA-RSA by TFA-RSA bound to the antibody matrix (68.5%) whereas no RSA bound. More liver protein from rabbits exposed repeatedly to halothane bound to the antibody matrix (30.7%) than from control animals. Blocking studies revealed that liver proteins present only in treated animals were responsible for this enhanced binding. A portion of these halothane-treated liver proteins appeared to be cross reactive with TFA-RSA. These studies suggest that halothane exposures induce proteins that bind with halothane metabolites; proteins which may serve in the initiation of a halothane-induced immune response. (NIM (94) 3478R)

EXPERIMENTAL CONTACT-SENSITIZATION BY ISOCYANATES. P.S. Thorne, J.A. Hillebrand, G.R. Lewis, and M.H. Karol, Dept. Ind. Env. Health, Univ. of Pittsburgh, Pittsburgh, PA 15261

The sensitizing potencies of four industrial disocyanates were determined in Balb/cByJ mice by topical application of increasing doses of isocyanate, followed five days later by topical ear challenge with the highest non-irritating dose of isocyanate. Ear thickness increase was determined from measurements taken prior to application of the challenge dose and 24 hours post-challenge. The response was calculated as both the average increase in ear thickness for each group and as the percent of responders. Each isocyanate induced dose-dependent sensitization. However, at high doses there was reduced response. The order of potency of the disocyanates was as follows: hexamethylene diisocyanate (HDI) > 1,4-cyclohexylmethylene diisocyanate (MDI) > diphenylmethane diisocyanate (MDI) > toluene diisocyanate (TDI). Cross-reactivity of isocyanates was explored. Mice sensitized with HDI, MDI, MDI and TDI were challenged with either the homologous diisocyanate, or with other mono- or di-functional isocyanates. Cross-reactions were noted between and among aryl and alkyl isocyanates. Use of this animal model demonstrated striking differences in sensitizing potencies of isocyanates. Supported by NIEHS ES01532.


Dose response curves of the delayed contact sensitization potential and/or partial response data were developed for fifty-two sensitizers so that a concentration of each agent which produced a response in the region of 50% of a test population could be estimated. Potencies of all 52 materials were then compared by graphical methods on both log and mol equivalent bases. Dose response information from both guinea pig and human sensitization test systems was also developed and/or collected and compared to the MEST data, revealing a good rank-order correlation between test systems for predicting relative potencies of agents in this endpoint.

CHARACTERIZATION OF ETHYLENEDIAMINE-INDUCED HYPERSENSITIVITY IN THE GUINEA PIG. C. Babik, K. Hastings, and J. Dean. CIBIT, RTP, NC

Ethylene diamine (EDA) is reported to be a contact sensitizer. A guinea pig model was developed to characterize the induced cellular and humoral immune responses involved in EDA hypersensitivity. Animals were exposed to 10%, 20%, 30%, or 40% EDA in an ethanol vehicle, once a week for three weeks, by an occlusive patch application. Fourteen days after the last exposure, the guinea pigs were challenged dermally with 2% EDA (a non-irritating concentration) and the resulting erythema was scored at 24 and 48 hr. Severity grades for the reactions ranged from 0.8 (on a scale of 0-3.0) for 10% EDA to 2.5 for 40% EDA. The animals were then bled by cardiac puncture and their lymphocytes cultured in vitro; blastogenic responses were determined by stimulation with EDA-conjugated to guinea pig serum albumin (GSA) or with the carrier molecule GSA alone. Lymphocyte proliferation in culture was observed to be EDA-GSA dose-dependent. The stimulation indices (H-thymidine incorporation by stimulated cells/control cells) for lymphocytes originating from animals treated with 10%, 20%, 30%, or 40% EDA were 2.6, 3.1, 6.6, and 9.1, respectively. An ELISA assay was used to monitor antibody production. However, taken together, the data indicate that dermally applied EDA induces a predominantly delayed hypersensitivity response.
270 Allergic T-cell Responses in C57BL/6J Mice Following Sub-Chronic Benzene Inhalation. G. J. Rosenthal and C.A. Snyder. New York Univ. Med. Ctr., Institute of Environmental Medicine, NY, NY.

Previous studies performed in this laboratory have demonstrated immunotoxic effects of benzene using inhalation as the route of exposure. The objective of this study was to investigate the effects of inhaled benzene on cell mediated immune responses in male C57BL/6J mice. The DNA virus induced polyoma PYB6 was used to assess T-cell mediated tumor resistance. Exposure to 100 ppm benzene induced lethal tumor growth in 90% of the mice inoculated with 10⁴ PYB6 tumor cells. Only 30% of the air controls succumbed to this tumor inoculum. Animals exposed to 10 ppm or 30 ppm benzene exhibited tumor growth similar to controls. Cell mediated cytotoxicity was assessed in an ⁵¹Cr-release study utilizing the allogeneic P815 mastocytoma target cells. Results of this study demonstrated a benzene induced depression in the lytic ability of cytotoxic T lymphocytes taken from mice exposed to 100 ppm benzene but not 30 ppm or 10 ppm benzene (20 exposures, 5 d/wk, 6 hr/d).

In vitro studies were performed using spleen cells from benzene exposed mice. A delay in peak mixed lymphocyte response (MLR) was observed following 20 exposures (5 d/wk, 6 hr/d) to either 100 or 10 ppm benzene. This delay was therefore not concentration dependent. Co-culture experiments done within the context of MLR indicated that benzene was not inducing suppressor cell activity. In addition, benzene inhalation did not alter the % of B cells, T cells or subsets of T cells in spleen cell suspensions.

Phagocytosis is an important immune function to quantify in order to assess a number of responses including exposure to pollutants. Inhalation exposure to toxic chemicals may affect baseline alveolar or peritoneal macrophage phagocytosis or interferon-stimulated phagocytosis. A model was developed to assess alveolar and peritoneal macrophage phagocytosis of latex beads in the same rat. Levels of circulating interferon can also be quantified in the same animal. Poly (1): poly (c) at a dose of 400 μg per rat was used for interferon induction. Circulating levels of serum interferon reached a titer of 640 units 4 hrs. post injection. The kinetics of stimulated phagocytosis were similar for both alveolar and peritoneal macrophages. Administration of rabbit antiserum to rat alpha/beta interferon abrogated the enhanced phagocytic levels. Baseline levels of non-induced alveolar macrophage phagocytosis were higher than poly (1): poly (c) stimulated peritoneal macrophage phagocytosis in the same animal. This abstract does not necessarily reflect EPA policy.

SUPPRESSION OF NK CELLS BY ENDOSTENOUS OPIATES (END-OP) IN VITRO. J.M. Exon. University of Idaho, Moscow, ID 83843.

A good deal of experimental evidence has been accumulated to indicate the existence of regulatory interaction between the central nervous, endocrine and immune systems. For instance, some classical neurotransmitter hormones and neurotransmitters have been reported to have immunomodulating activity (e.g. glucocorticoids, sex hormones, endorphine, enkephaline, growth hormone and thyroid stimulating hormone). Conversely, some immune cytokine hormones have been shown to have effects on the central nervous system and the function of endocrine glands (e.g. interferon, interleukin 1, histamines and prostaglandins). Specific high affinity receptors for hormones and neurotransmitters are present on immunocytes. Also, immune dysfunction occurs in CNS-affected as well as non-CNS-affected animals and this immunotoxicity and the CNS-mediated immune dysfunction may be identical to classical neuroendocrine hormones (e.g. ACTH, endorphin, growth hormone, TSH, choriclin gonadotropin).

The endorphine (END) and enkephaline (ENK) are neurotransmitters in the endorphine superfamiliy and are produced in the pituitary and adrenal, respectively. These peptides are thought to act as endogenous opiates since they bind to opiate receptors in the brain and produce analgesic and other effects. Specific nonopiate receptors for END and ENK are present in virtually all populations of lymphocytes and phagocytes. Both END and ENK have been shown previously to have immunomodulating potential. In this study we examined the in vitro effects of physiological levels of these enzymes on PGE2, both END and ENK on T cell proliferation and T cell mediated lysis of immunocytes. Both END and ENK significantly suppressed PGE2. Endogenous PGE2 was stimulated by ENK. Further experimentation is required to clarify the effects of these endogenous opiates on immune function. These neuropeptides could represent an important class of natural therapeutic agents based on their general pharmacological activity as analogues, antidepresants, anticonvulsants, anti-inflammatory agents and possible immunomodulators.

COPPER DEFICIENCY INDUCES IMMUNE DYSFUNCTION IN RATS. J.R. Williams, J.M. Arrensen, L.D. Koller and J.H. Exon. Veterinary Medicine, University of Idaho, Moscow, ID.

The effects of dietary copper deficiency on multiple immune functions were analyzed in male Sprague-Dawley rats. Eight week old rats were maintained on diets adequate (6 ppm), marginally deficient (2 ppm), or depleted (0 ppm) in Cu from the time of parturition. Haptic Cu concentrations and serum ceruloplasmin levels were analyzed to confirm Cu deficiency. Body and organ weights were recorded and multiple immune responses, including antibody titers, delayed-type hypersensitivity (DTH), and natural killer (NK) cell cytotoxicity, were measured in each animal. Although body weight was unaffected by the Cu deficiency, the liver, heart, and thymus weights were, expressed as a percent of body weight, were depressed. The thymic medulla in 0 ppm and 2 ppm treated animals was small, indistinct and frequently infiltrated by cortical thymocytes. Antibody titers and NK cell cytotoxicity were markedly suppressed in animals fed a Cu depleted diet (0 ppm). Male rats given 2 ppm showed a significant increase in DTH responsiveness. These studies suggest that certain components of the immune system are copper dependent.


Centamicin and the long acting oxytetracycline, l规矩ugin (LA 200), are two of the widely used broad-spectrum veterinary antibiotics. Gentamicin (GEN), like other aminoglycosides, has potential nephrotoxicity and ototoxicity which precludes its use in these situations. Oxytetracycline, on the other hand, is considered relatively safe for prolonged therapy or prophylaxis. However, it is not known whether, or another antibiotic, is capable of compromising the immune system. An antibiotic-induced immunodeficit may lessen its anti-microbial activity. The immunotoxicity of GEN and LA 200 was measured in 8 week old male Sprague Dawley rats after 12 days of therapy. Animals received either GEN given at twice daily or LA 200 administered in every third day. Gentamicin was given at the therapeutic level (3 mg/kg) and at doses 10 and 50 fold greater than therapeutic. LA 200 was given at the therapeutic dose (4 mg/kg) and 10 and 100 fold greater concentrations. Immune responses assessed in each animal consisted of delayed-type hypersensitivity (DTH), natural killer (NK) cell cytotoxicity, synthesis of interleukin 2 (IL-2), and antibody production to keyhole limpet hemocyanin (KLH). Immune cell numbers, organ weights and histopathology were also assessed. A significant decrease in the DTH or IL-2 was measured in GEN-treated rats at the 10 (p < .05) and 50 (p < .005) fold doses and for LA 200 at the 10 (p < .005) and 100 (p < .001) fold doses. NK cell activity was significantly depressed in all groups of GEN-treated rats. Neither antibiotic altered synthesis of IL-2 or antibody production. Oxytetracycline, evident by body weight loss (p < .05) and nephrotoxicity (p < .005), was present for only the highest dose of gentamicin and LA 200. These results indicate GEN compromises NK cell and T cell functions at therapeutic doses (NK) or levels below which signs of nephrotoxicity occur (DTH). LA 200 significantly suppressed T cell function at a dose 10 fold greater than therapeutic. It is possible that these antibiotics act via a non-specific mechanism that affects multiple immune functions. These results suggest that these antibiotics specifically alter specific and non-specific cell-mediated immune responses in laboratory animals and may have similar effects in veterinary species and humans.
**277** RESPONSE OF T CELL DEFICIENT MICE TO OZONE EXPOSURE. D. Dziedzic and H. J. White. Biomedical Science Dept., General Motors Research Labs, Warren, MI. Sponsor: E. W. Lee

Ozone inhalation produces hyperplasia and functional alterations of T cells of mediastinal lymph nodes of mice. In the present work we determined how animals which lack T cells respond to ozone inhalation. Using the athymic nude mouse as a model of T cell depletion, we compared the response of homozygous (nu/nu) T cell deficient mice with heterozygous (nu/+ ) mice which possess normal T cell function. We divided each of the two types of mouse into control or ozone exposed (0.75 ppm, 20 hrs/day for up to 14 days) groups. By 7 days, the mediastinal lymph node hyperplastic response was markedly reduced in nu/nu mice (0.78 ± 0.05 x 10^8 cells in exposed nu/nu vs. 0.60 ± 0.02 x 10^8 in control nu/nu compared to 7.33 ± 1.39 x 10^8 in exposed nu/+ animals vs. 1.16 ± 0.26 x 10^8 in control nu/+). By 14 days post exposure, the lungs showed increased damage as revealed by increased lung weight (0.39 ± 0.13 g in exposed nu/nu vs. 0.18 ± 0.006 g in controls nu/nu compared to 0.23 ± 0.14 g in exposed nu/+ vs. 0.15 ± 0.005 g in control nu/+). An increase of lung lesion volume was determined morphometrically (6.37 ± 0.19% in nu/nu animals vs. 2.48 ± 0.29% in nu/+ animals). These results indicate that T lymphocytes are the principal lymphocyte that responds to ozone and that lung damage from ozone is exacerbated in animals lacking T cells.

**279** HOST RESISTANCE TO PLASMODIUM YOELII IN IMMUNOTOXICOLOGY STUDIES. M.M. Fournet, S.G. Bradley and K.L. White, Jr. Depts. of Microbiology and Immunology, and Biostatistics. Medical College of Virginia/Virginia Commonwealth University, Richmond, VA

*Plasmodium yoelii*, a murine malaria producing a self-limiting infection, has been selected as one of the infectious models in the NTP Immunotoxicology Panel. An automated procedure capable of measuring parasitemia and reticulocyte cell numbers from the same blood sample has been developed using the EPICS V flow cytometer. This procedure utilizes acridine orange to stain the nucleic acids present in both parasites and reticulocytes. Automated counts (100,000 cells) show excellent correlation with manual counts (100 cells). Lithium carbonate (lith), a commonly prescribed antidepressant, has been assessed for potential immunotoxic effects. In female B6CF1 mice exposed by oral gavage to lith at doses up to 400 mg/kg for 14 days and challenged i.v. with *P. yoelii* 24 hours after the last treatment, no differences were observed in parasitemia and reticulocyte numbers, as compared to vehicle controls. Cyclophosphamide was included as a positive control. Although no effects were observed with lithium treatment, this automated procedure has potential for rapid evaluation of parasitemia and reticulocytosis in this protozoan model. (Supported by NIH ES 55094)


Mice infected with CMV show increased sensitivity to parathion poisoning and lower serum cholinesterase levels following parathion treatment than uninfected controls. Studies were designed to determine whether this could be directly related to 1) virus induction of interferon which is known to affect some metabolic processes, or 2) virus infection of the liver. No correlation between virus titers in the liver and enhanced parathion sensitivity was observed. Enhanced sensitivity was seen in mice given doses of virus too low to produce detectable liver infection. The virus affected parathion sensitivity in both CD-1 and BALB/c mice. CD-1 mice produced high levels of interferon while BALB/c produced low levels of interferon in response to CMV. Mice given a regimen of poly I:C sufficient to induce interferon and depress cytochrome P450 levels were not more sensitive to parathion than untreated controls. The data suggest that increased sensitivity to parathion in CMV infected mice cannot be directly related to either virus infection of the liver or virus induced interferon. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.


Phorbol myristate acetate (PMA), a potent tumor promoter, has a variety of effects on the immune system, causing either membrane alteration or modulating patterns of cell differentiation. In murine T cells, PMA potentiates activation, while in B cells, it blocks terminal differentiation. Recent studies have demonstrated that PMA induces resting B cells (G0) to proceed only to the early stages of activation (G1). Here we report evidence that the PMA-induced suppression of differentiation is a consequence of increased expression of IL2 receptors, which results in the dependence of IL2 for normal B cell differentiation. While PMA inhibited LPS or P(ab')2 induced DNA synthesis(s), it had no effect on the ability of cells to enter S, as measured by increased RNA synthesis and Ia expression. Supernatants from a T cell lymphoma (EL4) containing multiple B cell growth factors, as well as purified IL2 overcame PMA induced suppression. However, supernatants from an IL2 deficient T cell line (LSF20) failed to provide a stimulus for differentiation. The above studies indicate that in the presence of PMA, IL2 is an obligate growth factor for B cell differentiation. These data also indicate that like PMA-induced activation of cell differentiation, PMA-induced inhibition of cell differentiation is regulated by changes in receptor expression.

Ethylene dichloride (EDC), one of the largest volume chemicals produced and used in the world, is a mutagen and suspected carcinogen. Ethylene dichloride was evaluated with respect to its effect on basic immunological function using a standard lymphocyte blastogenesis (LCB) assay. Balb/C mice were injected, i.p., with either 50, 100 or 200 mg/kg of EDC in corn oil. Cell suspensions from spleens removed 24, 48, or 72 hours after administration of EDC were incubated with concanavalin A (con A), a T cell mitogen, or pokeweed mitogen (PWM), a B and T cell mitogen. Spleen cells removed 48 and 72 hours after exposure to EDC exhibited a dose-dependent suppression of LCB in response to con A. When PWM was used, a dose-dependent stimulation of blastogenesis was observed with EDC at 48 hours after exposure. Animals pretreated with pheno- barbital (80 mg/kg/day for 3 days) appeared to exhibit a greater suppression of con A induced LCB following EDC exposure than did the animals given EDC alone. These data suggest that a metabolite of EDC may alter this immune response.

Pseudomonas Immune Globulin (FIG) has a potential therapeutic use to improve the host defense mechanism in patients with severe burns. Studies were conducted in different animal species to ensure the safety of human FIG, a 5% solution. In acute studies, dose levels of 200, 600 or 1000 mg/kg were administered iv to 5 rats/sex/group or 3 rabbits/sex/group. Control animals received placebo vehicle. A single dose administration of FIG produced no local irritation of the injection site or systemic toxicity in both species. Repeated daily iv infusion of these dose levels for five days produced no adverse effects in rats, whereas, in rabbits at 200, 400 or 600 mg/kg/day doses caused mortality, anemia, mottled liver with centrolobular necrosis, congestion of lungs and or myocardial necrosis. However, a dose level of 600 mg/kg/day for 5 days produced no clinical, histological or organ histomorphological changes in cynomolgus monkeys. It is concluded that the rat and cynomolgus monkey can well tolerate heterologous FIG, whereas, the rabbit as a species is sensitive to this foreign protein and exhibited hemolysis with resulting sequelae.


CI-922, a mediator release inhibitor, was considered nonmutagenic in standard Salmonella bacterial assays for reverse point and frameshift mutation. Median lethal doses in acute toxicity studies were 2400 mg/kg in BEC37 mice and greater than 750 mg/kg in Wistar rats. In dogs, escalating doses of 5-640 mg/kg were administered, and emesis, reduced food consumption, and yellow colored stool were noted after a dose of 640 mg/kg. In rats, CI-922 was well tolerated as a diet admixture at dose levels of 50, 150, 300, 750 and 1500 mg/kg, and when administered intraduodenally for two weeks at dose levels of 100, 300 and 750 mg/kg. CI-922 was administered for two weeks in dogs at dose levels of 125, 250 and 500 mg/kg, with emesis and weight loss noted at doses of 250 and 500 mg/kg, and fecal impaction at 500 mg/kg. These preliminary studies established that CI-922 was well tolerated up to doses of 1500 mg/kg in the rat, and up to doses of 250 mg/kg in the dog, indicating an adequate margin of safety for clinical trials in man.


We have previously shown that thermal injury to the skin of anesthetized rats results in systemic complement activation, generation of neutrophil-derived oxidants, acute lung injury, and appearance of lipid peroxidation products. Another consistent finding in this animal model is early development (within 15-20 min postburn) of intravascular hemolysis. In order to investigate the mechanism and mediator(s) involved in the pathogenesis of thermal-injury-induced intravascular hemolysis, the role of complement and neutrophils was evaluated and the effect of antioxidants determined. Our studies show that hemoglobinemia was markedly attenuated in thermally injured rats previously depleted of complement or blood neutrophils. Pretreatment of thermally injured rats with catalase, superoxide dismutase or scavengers of hydroxyl radical result in significant protection from intravascular hemolysis. In vitro analysis of red cells obtained from thermally injured rats failed to show evidence of uptake of complement components (C3, C7, C8) suggesting that complement-mediated lysis is not involved in the mechanism of red cell injury. These observations suggest that acute intravascular hemolysis after skin burns is mainly mediated by oxygen radicals.
Previous in vivo and in vitro electrophysiological and biochemical experiments in our laboratory have demonstrated that low-level developmental Pb exposure (postnatal days 0-21; 0.27 Pb acetate/kg/day) produces a rod selective deficit at 90 days of age (d.o.a.). To further examine this rod selective deficit and to characterize its time course the relationship of rhodopsin content per eye (Rh) and b-wave visual threshold (sensitivity) was examined in control (C) and developmentally Pb-exposed rats at 30, 60, and 90 d.o.a. Rod ERGs were recorded in dark-adapted, anesthetized C and Pb rats to determine the b-wave sensitivity. Rh in dark-adapted C and Pb rats were determined using a modification of the Fulton and Baker (1964) procedure. In 30 d.o.a. C and Pb Rh was 70% and threshold was 20%; in 60 d.o.a. C and Pb Rh was 50% and threshold was 50%; in 90 d.o.a. C and Pb Rh was 30% and threshold was 100%. These changes in Rh and b-wave sensitivity in Pb rats are similar to those in adult C during dark-adaptation. These biochemical and electrophysiological results demonstrate that low-level developmental Pb exposure produces an early and permanent loss of Rh with a corresponding loss of rod retinal sensitivity. In addition, these findings are consistent with our quantitative retinal morphometric data showing a 20-25% loss of rods following developmental Pb exposure (Neurosci. Abs., 1985). Supported by NIH grants ES 01283 and ES 01282 (D.AF).

Macaque monkeys were given 2,5-hexanedione orally (75 mg/kg/day) 5 days/wk. Reduced visual sensitivity and altered visuomotor performance were seen after approximately 12 weeks of dosing. Exposure was terminated while signs of neuropathy were minimal but there was further progression of toxicity (especially motor weakness) for approximately 5 weeks. Contrary to our previous results in cats, visual acuity (and contrast sensitivity) was disrupted while flicker resolution was spared. Preliminary morphological results from these monkeys also differed from those seen in cats. These findings suggest a different pattern of vulnerability in visual neurons of cats and monkeys to hexacarbon intoxication. Supported by grants EPA 812402010, ES 01885 and ES 01247.

Our preliminary electrophysiological studies in the retinocollateral pathway of hooded rats exposed to 2,5-hexanedione (2,5-HO) demonstrated that middle diameter, medium-conducting (12) optic tract (OT) axons were affected before, and more severely, than large diameter, fast-conducting (11) OT axons (Toxicologist, 1985). To further examine this we have now conducted electrophysiological studies in 24 control and 2,5-HO rats. The aim was to determine the most sensitive, selective, and graded functional measure(s) that best correlated with our early preferential functional effects in 24 axons. Exposure parameters examined were: gmt/g/day, days on 2,5-HO, total 2,5-HO, and rate of intake. Functional characteristics of simultaneously recorded OT presynaptic (11,12) and postsynaptic superior colliculus (SC C2) field potentials examined were: OT conduction velocity (CV), absolute and relative refractory periods (ARP and RRP), X hyperexcitability (XH), XCC D2 depression (XD) and synaptic delay (SD). The following changes in functional measures were observed. 11/12 OTs were affected. The first excitability changes were seen in SC C2, which does not occur in controls, and a loss of SC C2 XD, RRP increased in 11, 12, and SC C2 with the latter two showing earlier, graded, and larger changes. One functional measure that correlated with daily dose, days on 2,5-HO or total 2,5-HO was C2 RRP, C3, and/or 11/12 CV, respect. Rate of intake correlated with 11/12 RRP and 12 XH. In conclusion, SC C2 RRP and C3 XH were sensitive, selective, and graded while SC C2 XD was sensitive and selective. These studies reveal the importance of examining several exposure parameters and functional measures in a neurotoxicological/neuropsychological study to fully understand the functional effects. Supported by NIH grants ES 01283 and ES 01282 (D.AF).

Alternations of evoked potentials (EP) and axonal transport (AT) are possible consequences of exposure to neurotoxic compounds, but the relationship between the two is uncertain. To investigate this issue, Long-Evans rats were surgically equipped with chronic electrodes for electrically stimulating the optic chiasm and recording EPs in the superior colliculus. Intracranial injections were made of vehicle or of 5% tunicamycin (THM), an antibiotic which blocks the glycosylation of proteins. Rapid AT (<12 hrs) was eliminated in an all-or-none fashion, with some rats (67%, 4 of 6) showing virtually no transport and others being equal to controls. ED recordings were normal 4 days post-THM. By 7 days, a selective loss of a postsynaptic component with input from large diameter axons was seen in 75% (6 of 8) of individual rats. A postsynaptic component arising from the input of small diameter axons was unaffected 7 days post-THM. The results indicate that THM-induced blockage of rapid AT precedes EP alterations by a period of several days.
METHYLMERCURY (MeHg): IT'S EFFECTS ON PROTEIN SYNTHESIS AND AXONAL TRANSPORT IN THE RAT VISUAL SYSTEM. M. Aschner, P. M. Rodier, and J. N. Finklestein. Departments of Anatomy and Pediatrics, University of Rochester, Rochester, N.Y. Sponsor: T. W. Clarkson.

Axonal transport of proteins was quantified by following an intracranial injection of [3H]proline along the visual pathway by autoradiography. When MeHg was injected directly into the vitreous body simultaneously with labeled proline, retinal protein synthesis and the rate of fast axonal transport were significantly reduced compared to vehicle controls injected with 5 mM Na2CO3. I.P. injection of cycloheximide depressed protein bound radioactivity (PBB) in the optic nerve to a lesser degree than in the retina. Conversely, MeHg reduced PBB in the axon more so than in the retina. Therefore, MeHg's affect on transport is not solely dependent on its affect on synthesis. In systemically-exposed rats (1 mg/kg/day, 4-6 or 12 days) the rate of protein synthesis and axonal transport were significantly accelerated compared to vehicle controls. It is suggested that this type of response may represent a compensatory mechanism during the early stages of systemic MeHg intoxication.

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DOSE-EFFECT RELATIONSHIP AND DISTRIBUTION OF METHYLMERCURY (MeHg) IN THE RAT VISUAL SYSTEM. M. Aschner, P. M. Rodier, and J. N. Finklestein. Departments of Anatomy and Pediatrics, University of Rochester, Rochester, N.Y. Sponsor: T. W. Clarkson.

Hg was detected in the visual pathway both under local and systemic exposure, however its distribution and concentration were different. Systemic exposure (4 mg/kg/day, 4-6 or 12 days) produced an evenly distributed concentration of Hg in the visual system. High doses of topically injected MeHg (0.04-36.16 μg) resulted in Hg detection only in the retina and proximal axon. The concentration of Hg in the retina was greater than that observed in systemically-treated rats. These were the same doses which inhibited the fast axonal transport. Thus, it is likely that the failure of Hg to distribute along the axon is related to the toxic effect of MeHg at high doses. When local doses were reduced (0.13-0.9 μg) to produce equal retinal concentrations in the two paradigms, the local application effect on protein synthesis and fast axonal transport was reduced and approached control values.

Further, the distribution of Hg came to resemble that observed in systemically-exposed animals. However, no increase in transport or synthesis occurred at any dose. It is therefore concluded that MeHg in the two treatment paradigms produces two different dose-effect curves.

Supported in part by NIH-ES 01247 and NIH-ES 01248.


We previously have demonstrated a preferential loss in the visual evoked response (VER) to low spatial frequency stimulation following diisopropyl fluorophosphate (DFP), and recovery of the VER within 10 to 20 hours without significant recovery of AChE activity. Here we sought evidence through HPLC analysis of retina and cortex for putative neurotransmitters other than ACh that might be altered following DFP. Anesthetized and paralyzed adult cats were held in a headholder and VERs were recorded from stainless steel bone screws. Following removal of one eye and a small sample of visual cortex, DFP was given. After a variable period of survival, the other eye and additional visual cortex were removed and samples prepared for HPLC. There was a consistent decrease in retinal dopamine (DA) and an increase in the cortical DOPAC/DA ratio at all time points, although changes in other putative neurotransmitters or their metabolites were not consistent. Other changes were consistent only within a particular period of time. Cortical GABA was decreased early on, but increased above baseline by six hours. Retinal GABA was just the opposite. It appears that both the cortical and retinal DA and GABA systems are altered following DFP and may be involved in visual recovery.


Triadimefon (TDF) is a systemic fungicide with an LD50 of about 800 mg/kg in rats. Little is known about the potential neurotoxicity of this compound. We administered TDF p.o. in corn oil (1 mg/kg) to male Long-Evans hooded rats at dosages of 0, 50, 100, and 200 mg/kg, and evaluated the effects on body temperature, pentyleneetrazol (PTZ) seizure susceptibility, paired flash evoked potentials (PFPs) and pattern reversal evoked potentials (PREPs). Body temperature was altered only by the 200 mg/kg dosage, which, at an ambient temperature of 21.5°C produced maximal hyperthermia (2°C above controls) 1.5 hr after administration. Subsequent studies were performed in a warm room (30°C) to minimize the influence of hyperthermia on other endpoints. Only the 200mg/kg dosage was tested in the PTZ study, where, 1 hr after treatment, it decreased latency and increased duration of the seizure. In the FEP study, TDF produced a small increase in latency of the second response to the paired stimuli, particularly in peak N3. PREP latencies were not altered, but the amplitude of PREP peak PIN2 was depressed at all dosages 1 hr after treatment. The data indicate that at 1/4 the oral LD50, triadimefon increases brain excitability and at 1/8 the LD50 produces a selective effect upon a measure of pattern vision.
EFFECT OF CONCURRENT EXPOSURE TO NOISE AND TOLUENE ON HEARING IN SPRAGUE-DAWLEY RATS. M.J. Sullivan and R.B. Connolly, Kresge Hearing Research Institute, Toxicology Program, School of Public Health, The University of Michigan, Ann Arbor, MI 48109

Sprague-Dawley rats exposed to toluene show inner ear changes that resemble those caused by noise. We have tested the hypothesis that toluene potentiates noise-induced hearing loss. Eight rats/group were exposed to white noise at 0, 85, 95, 100 and 110 dB above background for 6 hr/day, 5 days/week for 4 wk. Concurrently, 8 rats/group were dosed by gavage with 103.4 or 617.8 mg toluene/kg, then exposed to white noise at 0, 85 or 100 dB for 6 hr/day, 5 days/week for 4 wk. Thirty days after exposure ended, brainstem auditory evoked response (BAER) thresholds were recorded at 0.5, 1, 2, 4, 8, 16 and 32 kHz. Rats were then sacrificed and cochleograms prepared. Significant changes in hair cell loss and BAER thresholds were seen in the 100 dB only and 617.8 mg toluene only exposures. Concurrent exposure to toluene and noise had an additive effect on hair cell loss, indicating that toluene does not potentiate noise-induced inner ear damage. (Supported by the American Petroleum Institute)

SUBCHRONIC NEUROTOXICITY IN RATS OF THE STRUCTURAL FUMIGANT, SULFURYL FLUORIDE. J.L. Mattson, R.R. Albee, D.L. Eisenbrandt, L.K. Chang. Dow Chemical U.S.A., Mammalian and Environmental Toxicology Research Laboratory, 1803 Building, Midland, MI, and University of Arkansas, College of Medicine, Little Rock, AR.

Male and female Fischer 344 rats were exposed to sulfuryl fluoride vapors at 300, 100, 30, or 0 ppm for 6 hrs/day, 5 days/week, for 3 months. No treatment effects were detected at 30 ppm. All animals appeared clinically normal at the end of exposure, although 300 ppm caused a decrease in body weight gain. Grip strength was unaffected. Significant slowing of visual (VER), auditory brainstem (ABR) and somatosensory (SER) evoked responses and a decrease in flicker fusion was detected at 300 ppm. Also, there was some evidence of slowing of ABR's and SER's at 100 ppm. The ABR of all rats exposed to 300 ppm was monitored for 2 months post-exposure; the latencies of the ABR returned to normal during recovery. The slowing of evoked potentials was mostly in the early components of all waveforms, which suggests that myelin was diffusely affected. Histopathologic examination revealed that rats exposed to 300 ppm sulfuryl fluoride had a slight amount of vacuolation in the cuneate-putamen which was more evident in the white tracts than in the gray substance.

METHODOLOGICAL CONSIDERATIONS FOR THE RAPID ASSESSMENT OF SENSORY DYSFUNCTION BY REFLEX MODIFICATION. J.R. Wecker, J.A. Foss, and J.R. Ison, Department of Psychology, University of Rochester, Rochester, NY. Sponsor: R.V. Wood

Reflex modification consists of the elicitation of a reflex and the modulation of that reflex by the prior presentation of an auditory, visual, or tactile stimulus. Although the methodology is simple, efficient, and amenable to automation, extraneous conditions can affect the results. Stereotyped movements, such as face washing and grooming, decreased both evocation and modifi- cation of the acoustic startle reflex in rats, however, other concurrent activities had little effect. Power amplifiers used for stimulus generation produced background noise which affected the size of the startle response and masked the test stimulus. Relays eliminated that noise, but produced low frequency transients when tones were used as startle stimuli. We recommend filtered noise for both the test and startle stimuli to eliminate the transients and provide a rapid assessment of hearing loss. Quantitative assessment of loss in dB can be determined by varying the intensity of the test stimulus and calculating that intensity which produces a criterion effect (e.g. 20%). Delivering at least ten trials per condition and using randomized presentation minimizes the effect of activity. The effectiveness of this procedure was validated in rats exposed to kanamycin or high intensity sound. (Supported by EPA contract CR-811229.


This study examined the ontogeny of the rat's auditory startle response (ASR). Both peak amplitude (Amp) and latency to onset (Lat) of the response were measured in pups tested daily from postnatal days (PND) 12-21. Lat decreased steadily over days. In contrast, Amp increased non-monotonically. With low background noise levels (45-50 dB) Amp decreased from PND 16-18 and at higher levels (75-80 dB) remained constant from PND 16-19. The development of sensitization to background noise (45-50 dB) was also examined. Sensitization developed by PND 17, as indicated by a shorter Lat and higher Amp with increasing noise levels. The role of serotonin (5-HT) in ASR development was evaluated by testing rats on PND 13, 17 and 21. p-Chlorophenylalanine (PCPA), which depletes 5-HT, had no effect on ASR on PND 13 but produced a modest increase (30%) in Amp on PND 17 and 21. Repletion of 5-HT using 5-hydroxytryptophan did not antagonize this effect of PCPA. The 5-HT agonist 5-methoxy-N,N-dimethyltryptamine also did not alter ASR on PND 13 but then markedly increased (125-350%) Amp on PND 17 and 21; this effect was blocked by the 5-HT antagonist cyproheptadine. None of the treatments affected sensitization. These data indicate that 5-HT begins to exert its known modulatory effect on ASR between PND 13 and 17. (*Supported by a NRC Research Associateship.)
The means by which noise exposure damages hearing is uncertain; one possible mechanism is that under conditions of noise exposure, the metabolic demands of the cochlea exceed the available supply of oxygen. Such a view finds support in the amelioration of noise damage when exposure occurs in an oxygen-rich environment. It also leads one to predict that the cochlea will be sensitive to direct hypoxic insult. We have examined the effects on hearing of Carbon Monoxide (CO), alone and in combination with noise.

Neither moderate subchronic (300 ppm, up to 8 weeks) nor acute high-level (1200 ppm, 3.5 hours) CO exposures produced permanent changes in auditory detection thresholds. In contrast, acute CO exposures markedly potentiate hearing loss produced by exposure to a 110 dBa broadband noise, and also lead to a shift in the frequency at which maximum impairment is observed.

The potentiation of noise-induced hearing loss by CO supports a role of cochlear hypoxia in noise-induced hearing loss. The failure of CO alone to produce auditory effects may reflect the influence of compensatory mechanisms such as increased cochlear blood flow. (Supported by NIHES grants R01C0800125 and ES02852).


Recent work has demonstrated the ability to differentiate the in vivo effects of low dosages of two pyrethroids, cismethrin and deltamethrin. Two behavioral tests, motor activity and the acoustic startle response (ASR), were utilized to further characterize the differential behavioral effects of a variety of Type I and II pyrethroids. Dosage-effect functions for various compounds of both types were determined for both figure-8-maze activity and the ASR. All compounds produced dosage dependent decreases in motor activity. The Type I compound permethrin, and DDT increased amplitude, decreased sensitization, and had no effect on latency of the ASR. In contrast, the Type II pyrethroids cypermethrin, cyfluthrin and flucythrinate, decreased amplitude, decreased sensitization, and increased latency of the ASR. Fenvalerate increased amplitude, had no effect on latency, and, unlike the other compounds tested, increased ASR sensitization. Fluvinate had no effect on any measure of the ASR. These data extend our previous findings of differential effects of Type I and II pyrethroids on the ASR. Structure-activity relationships are discussed.

Prenatal N2O Exposure Alters Startle Reflex in Adult Mice. S.A. Rice, Departments of Anesthesia, Stanford University School of Medicine and VA Medical Center, Palo Alto, CA.

Auditory and tactile startle reflex reactivities were measured to determine if prenatal N2O exposure produces enduring neurobehavioral dysfunction in mice. Pregnant 5W mice were exposed either to air or to 5%, 15% or 35% N2O for 4 hr/day, days 6-15 of pregnancy. Male and female offspring from 10 litters per exposure group were tested on 60 and 95 days of age in an SPG startle response system. Six acoustic startle stimuli (10 msec, 110 dB noise) and six tactile stimulii (20 psi air burst) were delivered automatically in each trial. Counts related to the force of the startle reflex from each stimulus were averaged and analyzed by ANOVA. N2O exposed groups were hyporeactive compared to the air exposed group for both startle reflexes (P<0.005); N2O counts ranged from 29-70% of air counts on day 60 and from 3-47% on day 95. No sex-related treatment effects were observed. These significant hyporeactivities reveal that prenatal N2O exposure induces enduring neurobehavioral dysfunction in mice which, acknowledging the limitations of across species extrapolation, suggest the potential persistent effects of in utero N2O exposure in other species, including humans. (Supported by Departments of Anesthesia, Stanford University and VA Medical Center, CA).


Inhalation exposure and generation facilities were modified to facilitate the containment and monitoring of highly reactive and toxic methyl isocyanate (MIC) during acute and repeated dose toxicity studies. Single and multiple exposures were conducted using both sexes of F344 rats and CD-1 and B6C3F1 mice to 0-60 ppm MIC for 2- and 6-hrs duration. Two-stage dilution of MIC vapor was performed using N2 as a carrier from stainless steel cylinders. The delivery and exposure systems used stainless steel, teflon (PTFE), and borosilicate glass materials. MIC chamber concentrations were maintained within ±10% of set point using a computerized feedback control system. MIC was monitored to 1 ppm with infrared spectroscopy (Miran 90) and to 20 ppb with high performance liquid chromatography (HPLC).

Effluent exhausts from all operations were scrubbed of MIC using whetlerized activated carbon. All hazardous wastes were disposed of via established NIHES protocols.

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Anecdotal reports have suggested sodium thiosulfate administration improved the condition of selected survivors of the MIC release in Bhopal, India. To determine if cyanide was involved in the toxicity of MIC, the blood of male Fischer 344 rats was assayed for cyanide following 2-hour inhalation exposures to concentrations of MIC up to 60 ppm. Also, mortality was compared in rats given no antidote, or given 1 or multiple doses of NaNO2 (25 mg/kg, sc) and Na2S2O3 (0.25 g/kg, ip), doses shown effective to prevent mortality from 10 mg/kg KCN given by gavage. No difference in cyanide blood level was observed between controls and animals exposed to MIC either immediately or 48 hours after exposure. The minimum quantitative level for cyanide was 50 ng/g blood. Literature indicates Sprague-Dawley rats receiving a lethal dose of KCN had blood levels at time of death of 950 ng/g. Cyanide was detected in the blood of rats given sublethal doses (2.5 mg/kg) of KCN by gavage. Cyanide antidote treatment did not prevent mortality of male rats exposed to MIC.

Methyl isocyanate (MIC) is a volatile, toxic gas which has caused acute pulmonary disease in exposed individuals. To study the initial cellular responses in delineated airways exposed to MIC, 344 rats were exposed in inhalation chambers to 1 or 10 ppm for 2 hrs. Immediately after exposure to both doses, a few red cells were seen in the airways. By 24 hrs. post-exposure to 10 ppm, numerous acute and chronic inflammatory cells had migrated into the alveolar lumina and were associated with red blood cells, proteinaceous exudates and cell debris as well as a marked degree of epithelial necrosis and sloughing. At 72 hrs. after the 10 ppm dose, there was an apparent increase in the acute inflammation and sloughing of large sheets of alveolar epithelium. By 7 days post-exposure, the inflammatory events had subsided and large areas of airway surface exhibited rapid proliferation of squamous, undifferentiated epithelial cells. Between 7 days and one month post-exposure to 10 ppm MIC, there was an ongoing process of intrabronchial fibrogenesis which resulted in bands of connective tissue protruding into numerous alveolar lumina and distorting the broncholar and bronchiolar walls. However, a relatively normal well-differentiated airway epithelium was present by one month after exposure. A slight increase in the number of alveolar macrophages was the only change noted in the lung parenchyma. No evidence was noted that 10 ppm but not 3 ppm MIC induces a rapid inflammatory response which is accompanied by a loss of airway epithelium and a repair phase which results in endobronchial fibrosis in the lungs of rats.


The incident in Bhopal, India has raised new concern over MIC as a pulmonary toxicant. To ascertain the functional sequelae of a single MIC exposure to the mammalian lung, pulmonary function was assessed in male F-344 rats 1, 2, 4, 7 and 13 wk after a 2 hr exposure to 0, 3, 10 or 30 ppm MIC. No significant changes were observed in the 3 ppm rats through 13 wks. At 1 wk, diffusing capacity (DL), compliance (C) and the homogeneity of ventilation (HV) were depressed in the 10 and 30 ppm rats. Since no 30 ppm rats survived beyond 1 wk, only the 0, 3 and 10 ppm rats were assessed at later times. By 13 wks, the 10 ppm rats exhibited dramatic increases in lung volumes while DL was only mildly depressed. No specific of compliance was significantly decreased, expiratory times were increased, and HV was severely impaired in this group. These results suggest the development and likely progression of obstructive airway lesion with associated severe gas trapping. A steep concentration response relationship appears to exist between 3 and 10 ppm MIC. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
There is a controversy about a causal relationship between the reported increase in still births, abortions, infant mortality, low birth weight babies, menstrual disturbances and the decrease in libido in the Bhopal population and the MIC leak from the Union Carbide plant on December 3, 1984. We, therefore, studied the reproductive toxicity of MIC in mice. Exposure of pregnant mice on day 8 of gestation to 9 or 15 ppm MIC for 3 h caused a decrease in maternal body weight gain, death of 14% dams and complete resorption in 75% animals; placental weights, fetal body weights and skeleton sizes were decreased but there was no significant increase in external or internal organ abnormalities. MIC was more toxic to dams on day 14 (64% mortality) than on day 8 (14% mortality) of gestation. These effects of MIC were much less pronounced at 2 and 6 ppm. MIC (9 ppm for 3 h) adversely affected the estrus cycle and the fertility of both male and female mice. The exact mechanism of the reproductive toxicity of MIC remains to be established. Supported in part by the Faculty of Medicine, McGill University.

The effects of inhaled methyl isocyanate (MIC) on reproduction and late-pregnancy development were studied in CD-1 mice exposed 6 hr/day for 4 days to concentrations of 0.1, or 3 ppm MIC. (I) Growth and survival were observed among offspring from pregnant mice exposed on gestation days 14 through 17. (II) Mating trials were conducted on male and female mice during weeks 1, 8 and 17 after exposure. (III) Exposed male mice mated to groups of unexposed females for eight weekly intervals were used for a dominant lethal study. The exposure regimen used in these studies for MIC caused significant pathologic alterations, primarily of respiratory tract tissues, in similarly exposed B6CF1 mice and F344 rats. Treatment-related effects observed in study I included decreased live litter size at birth and decreased neonatal survival throughout lactation. Maternal survival and maternal and neonatal body weights were unaffected. Exposure of adults prior to mating (study II) had no effect on fertility or neonatal growth and development. No evidence of a dominant lethal effect (study III) was observed in unexposed females mated to MIC-exposed males.

To study pathologic changes caused by MIC inhalation, tissues were evaluated from male and female rats killed immediately and through day 91, following a single 2-hour exposure to 0, 3, 10 or 30 ppm MIC. Gross pathology in high dose rats included lung consolidation, hemorrhage and failure to deflate. Early microscopic changes in the nasopharynx included marked erosion of olfactory and respiratory epithelium, and accumulation of fibrinous and serous fluid. On day 1, acute inflammation and fibrinopurulent exudate partially blocked nasal passages. Epithelial cells were sloughed from the trachea, bronchi and bronchioles; granulomatous lesions were seen in these areas by day 3. Small airways were occasionally blocked by exfoliated epithelial cells, exudate, and fibrinous or mucous plugs. On days 7 and 14, regenerating fibrinous tissue was observed protruding into air- ways. Epithelial growth over this tissue partially contributed to airway obstruction. Damages to the lung parenchyma was limited to moderate inflammation. Respiratory lesions appeared reversible in low dose rats, but persistent and fatal in high dose rats. Pathology in non-respiratory tissues appeared secondary to pulmonary injury.

The release of methyl isocyanate in Bhopal, India caused temporary blindness and other eye injuries in many of the exposed people. MIC is known to be corrosive and to irritate intact skin and mucous membranes, but little is known about the extent of ocular damage incurred during exposure to its vapors. To examine this, the eyes of male and female F344/N rats were evaluated immediately after exposing animals to 0, 3, 10, or 30 ppm for 2 hours, and periodically during a 91-day recovery period. Eyes were examined with sodium fluorescein and ultraviolet light, and by histopathologic examination of sections (including lids, cornea, lens, retina, optic nerve and hardierian gland). During exposures to > 10 ppm, rats kept their eyes partially closed, and copious lacrimation was evident. The ocular lining fluoresced gross or microscopic evidence of epithelial erosion or ulceration of the cornea, or of surrounding tissues immediately after, or at any time following exposures. No skin irritation was noted. Two-hour exposures to concentrations of > 10 ppm MIC resulted in severe respiratory injury, and death but it would appear that natural protective mechanisms of the eye were adequate to prevent ocular damage at these exposure levels.
TOXICITY OF INHALED METHYL ISOcyanate (MIC) IN F344/N RATS AND B6C3F1 MICE. J.R. Bucher, B.N. Gupta, B.A. Schwetz, M. Thompson, B. Adkins, Jr., NIAMS/NTP, and Northrop Services, Inc., Research Triangle Park, NC

To help predict long term health effects in survivors of the MIC release in Bhopal, India, the NTP examined rats and mice following short MIC inhalation exposures. Animals exposed for 2 hrs. to concentrations up to 30 ppm were evaluated immediately after, and in the ensuing 3 months for mortality, clinical signs, and changes in clinical chemistries (BUN, creatinine, ALT, AP, CK, SDH and blood and brain cholesterol), hematology (CBC and methemoglobin), and body and organ weights (brain, lung, liver, kidney, thymus, spleen, testis). Deaths of rats occurred after exposures to >10 ppm, and in mice exposed to 30 ppm. Mortality began 12-24 hours following exposures, and continued through day 78, in two phases; male rats and mice exposed to 20 or 30 ppm died primarily within 4 days, but deaths of male rats exposed to 10 ppm and female rats and mice exposed to 10-30 ppm generally occurred after 8-9 days. Deaths were preceded by marked respiratory distress. Surviving animals lost weight following exposures, but later gained at the control rate. Lung weight increased up to 2x in exposed animals versus controls throughout the 3 month period. Clinical chemistry and hematology results were unremarkable. Results are consistent with the respiratory system as the primary target of MIC toxicity, and suggest persistent pulmonary symptoms.

IMMUNOTOXICITY OF METHYL ISOcyanate IN MICE. N. Silver, D. Gernolec, S. Vore, A. Tucker, and M. Luster, NIEHS, Research Triangle Park, NC

The purpose of this study was to determine the effects of inhaled methyl isocyanate (MIC) on immune function and host resistance in mice. Female B6C3F1 mice were exposed to 0.1, or 3 ppm MIC 6 hr/day for 4 consecutive days. The antibody response to sheep red blood cells was not affected by MIC. The response of splenic lymphocytes to Con A was slightly suppressed, and the response to allogeneic leukocytes (mixed leukocyte culture) was suppressed in a dose-related fashion. The ability of mice to resist challenge with pathogenic organisms or transplantable tumor cells was examined following administration of either the mouse malaria parasite Plasmodium yoelii, B16F10 tumor cells, Listeria monocytogenes, or influenza virus. There were no statistically significant increases in host susceptibility in any of the models as a result of chemical exposure, although there was a trend toward increased mortality in chemically exposed mice challenged intranasally with influenza. In summary, cellular immunity was slightly compromised by MIC exposure, but not severely enough to alter host resistance. The changes detected in immune function could reflect the response of the animal to trauma which can occur following exposure to toxic doses of chemicals.


As a result of the industrial accident in which thousands of people were exposed to methyl isocyanate (MIC), concern was directed toward possible long-term health effects. The well-recognized immunologic consequences of isocyanate exposure prompted investigation of antibody production to MIC. Using procedures developed in this laboratory, guinea pigs were exposed to MIC in reactive form. Three weeks later, blood was drawn and serum evaluated for antibodies to MIC using ELISA. To detect antibodies, an antigen was prepared by reaction of MIC with guinea pig serum albumin. Analysis revealed that it contained approximately 50 moles MIC/mole protein. Antibodies were detected in each of the animals injected with MIC, with titers reaching 1:25600. Inhibition assays revealed specific MIC conjugated. Serum from patients exposed to MIC during the spill in December 1984 were similarly evaluated for antibody. Several of the 173 sera examined demonstrated antibodies reactive with MIC. Specificity was confirmed by inhibition assays. In addition, total IgG levels appeared to be elevated following MIC exposure. The persistence and health consequences of the antibody response remains of prime concern. Supported by NIH grant ES01537.


The genetic toxicity of MIC was investigated in a variety of test systems ranging from bacteria to mice. Negative results were obtained in the Salmonella histidine reversion assay using five strains in a preincubation protocol. Positive results were obtained for three endpoints in cultured mammalian cells. Reproducible, dose-related increases were observed for trifluorothymidine resistant colonies of L5178Y cells and for both sister-chromatid exchanges (SCE) and chromosomal aberrations (CA) in CHO cells. Effects on all three endpoints were evident in the absence of rat liver S9. Drosophila sex-linked recessive lethal tests conducted by feeding, inhalation, and injection were uniformly negative. CA, SCE, micronucleus, and cell cycle kinetics determinations were made in B6C3F1 mice following single and multiple inhalation exposures. Cell cycle time was increased in proliferating bone marrow cells, and there was evidence of small, exposure-related increases in both SCE and aberration frequencies. A mutagenic agent, MIC is electrophilic and can interact with DNA components as well as with proteins associated with eukaryotic chromosomes which may account for the genotoxic effects observed in these studies.

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AVOIDANCE OF ETHYL PARATHION-CONTAMINATED FEED BY 14-DAY OLD MALLARDS AND NORTHERN BOBWHITE.

Assessment of the behavioral response of 14-day old mallards and bobwhite to feed treated with ethyl parathion was performed. Prior to conducting the feed avoidance tests, given dietary LC50 tests were performed to determine a baseline against which the food avoidance data could be compared. For the food avoidance tests, the birds were given free choice between treated and untreated feed. The dietary concentration at which mallards and bobwhite began to avoid ethyl parathion in their food supply was determined by calculating a food avoidance concentration 50 value (FA50). A given contaminant, when present in the food source at concentrations exceeding its FA50 for a given species will elicit an avoidance response by that species. The animal will discriminate against the treated diet and consume greater quantities of the untreated feed, if available. An effective avoidance index (EAI), which is conceptionally similar to a margin of safety, can be calculated by the ratio LC50/FA50; the greater the EAI, the greater the margin of safety. Test values for ethyl parathion are as follows: mallard LC50 = 152 ppm, FA50 = 10.3 ppm, EAI = 14.8; bobwhite LC50 = 132 ppm, FA50 = 29 ppm, EAI = 4.6.


The influence of dietary HCHOL on the toxicity of DZ was examined in male mice. HCHOL was induced with a diet containing 1% cholesterol and 0.5% cholic acid, which were replaced with 1.5% casein for the controls. After 8 weeks on the diets, animals were injected IP with single doses of DZ ranging from 0-125 mg/kg. Effects of HCHOL pre-treatment included: decreases in serum fatty acids, triglycerides, and HDL; and increases in serum cholesterol and LDL, whole blood and hepatic acetylcholinesterase activity (AchE), hepatic microsomal cytochrome P-450, cytochrome P-450 reductase, and ethylmorphine N-demethylase (EMO) activities, and liver weight that were associated with gross and microscopic evidence of fatty infiltration. Effects of DZ administration alone included: salivation, excessive lacrimation, and diarrhea; decreases in whole blood and hepatic AchE activity; and increases in EMO activity. DZ was administered to HCHOL animals, the LD50 was 64 mg/kg as compared to 84 mg/kg for the controls. Mean survival times for the HCHOL mice at 60-80 mg/kg were significantly reduced when compared to controls. Haptic DZ levels were also higher in the HCHOL animals. The results suggest that HCHOL may induce modifications in lipid metabolism and an increase in susceptibility to DZ-induced toxicity.


This study was undertaken to compare organophosphate detoxication in tissues of cows and rats. Liver activation of DYPONATE (DYP), paraoxon activation (PAR) and malathion activation (MAL) and liver and plasma inactivation of DYPONATE-oxon (DX), paraoxon (PX) and malathion in vitro in tissues from male and female rats. Liver microsomal suspensions from cows and rats activated DYP, PAR and MAL to about the same extent (0.5 to 3 nmoles oxon formed/mg protein/30 min). Liver inactivation of the oxons was 3 to 8 times greater in cows than rats. Plasma oxon inactivation was 39, 39 and 2000 for rats and 0, 19 and 0 for cows (nmoles/10ul/5min) for DX, PX and DX, respectively. Cow plasma had little cholinesterase and no detectable carbamoylase activity. Thus, in contrast to rats, cows were deficient in plasma esterase-dependent organophosphate detoxication. Any oxon leaving the liver would less likely be inactivated in plasma and would more likely inhibit critical acetylcholinesterase and cause toxicity. Since most organophosphates are readily detoxified by mammalian plasma esterases, these results suggest that cows may be unusually sensitive to most organophosphate insecticides.

SIMILARITY OF ISOZYME PATTERN OF SERUM LACTATE DEHYDROGENASE (LDH) FOLLOWING TREATMENTS WITH 0,2'-TRIMETHYL PHOSPHOROTHIOATE (OMS-TMP) AND PARAQUAT IN RATS. A. Kozumi, Y. Tada, L. Hasegawa and T. Inamura, Division of Toxicology and Physiology, University of California, Riverside, CA.

The purpose of the present study was to determine whether LDH activity and its isozyme pattern were capable of detecting lung injury and possible extracellular damage caused by OMS-TMP, Carbontetrachloride (CC14), (ml/kg, l.P., 24h) and paraquat (25 mg/kg, i.p., 48h) were selected as standard chemicals for liver, lung and kidney injuries, respectively. The activity of LDH increased 4 times the control level following treatment with OMS-TMP (20 mg/kg, p.o., 72 h). Treatment with CC14 increased activity to 8 times while paraquat increased activity only slightly. Isoelectricfophoresis of serum samples showed that OMS-TMP increased activities of isozymes LDH 1 and LDH 2. This isozyme pattern was similar to the pattern of paraquat, but quite different from the pattern in animals treated with CC14 or in control animals where LDH 5 was most predominant. By histological examination, cloudy swelling in the proximal tubuli in the kidney was observed. Thus, the isozyme pattern strongly suggests that OMS-TMP causes similar organ damage, i.e., lung and kidney, as evoked by paraquat.

The rate of collagen synthesis by minced lung tissue from O, O,S-trimethyl phosphorothioate (OOS-TMP) treated and control rats was evaluated in vitro by measuring the rate of synthesis of acid-insoluble [3H]-hydroxyproline from [3H]-proline. Rats treated with 40 mg/kg of OOS-TMP were analyzed at 1, 3, 7, and 14 days after treatment. Collagen synthesis increased significantly by day 1 and remained elevated throughout the observation period. The content of total lung hydroxyproline increased significantly by day 7 and remained elevated for 14 days following treatment. A dose-response study at 20, 40, and 60 mg/kg of OOS-TMP was carried out on day 7 following treatment. There was a linear relationship between the rate of in vitro collagen synthesis and doses of OOS-TMP up to 40 mg/kg. Structural analysis of lung tissue by immunofluorescent staining revealed that lung collagen revealed gross disruption of the basement membrane and septa along the entire time course. These data suggest that OOS-TMP is capable of inducing an acute fibrotic response in the lung.


Toxicity of methyl parathion (MP) is largely dependent upon its metabolic fate, which is determined by multiple biotransformation pathways. As other insecticides may potentiate the toxicity of MP and other organophosphates by altering biotransformation, analysis of each metabolite of MP is essential for such studies on pesticide interactions. A RP-HPLC technique was developed which allows resolution of MP, MP-oxon (MPO), desmethyl-MP, desmethyl-MPO, p-nitrophenol (PNP), PNP-glucuronide and PNP-sulfate in a single chromatographic system. A 15 cm C-18 RP column was utilized. Water and 80% acetonitrile, each containing 0.5 mM tetrabutylammonium phosphate (TBAp; ion-pairing reagent) at pH 3.0, were utilized as mobile phases. Peak quantification of all metabolites was linear between 1 and 10 nmol, and detection limits were less than 1 pmol. Coefficients of variation for the individual metabolites ranged between 1 and 20%. This method was successfully utilized to determine metabolite profiles of MP in isolated hepatocytes and various subcellular fractions. Because this method is both simple and rapid, it may be useful for clinical determination of MP metabolites in human tissues. (Supported by NIH Grant ES-03424).

CHOLINESTERASE ACTIVITIES IN BEAGLE DOGS USING AN AUTOMATED ENZYME ANALYZER. B. J. Quinn, T. E. Shellenberger, and A. S. Tegeris. Tegeris Laboratories, Inc., Laurel, MD.

Acquisition of a Baker Model 600 Centrifichem for the determination of plasma, erythrocyte (RBC) and brain cholinesterase (ChE) activity in beagle dogs in acute, subchronic and chronic toxicity studies with organophosphate pesticides necessitated a reevaluation of baseline ChE activity for historical control purposes as well as of storage conditions for brain tissue after collection prior to analysis.

Acute, subchronic and chronic studies with nearly 100 male and 100 female beagle dogs have been initiated and/or completed in the last 18 months. Plasma ChE activity determined on all animals during the pretest period and then on controls during each study was readily reproducible and stable in both males and females. RBC ChE activity, however, was somewhat more variable and less reproducible. Brain ChE activities in controls at termination of a study were higher in the cerebellum than in the cerebrum; activities tended to be variable which probably reflects sampling problems from various sections of the brain tissue. Analysis of brain tissue from multiple brain sections revealed ChE activities that declined in the following order: pons > cerebellum > basal medulla > cerebral / gray matter > white matter. ChE activity in brain samples stored frozen were variable but remained stable for 2 to 4 weeks. ChE activity of brain tissue homogenates, however, decreased approximately 30% when stored frozen for 1 week.

EFFECTS OF THREE ORGANOPHOSPHATE INSECTICIDES ON NTE, ACHE, AND GAIT IN CHICKS. M. F. El-Awar, R. M. Francis. Institute for Environmental Studies, University of Illinois, Urbana, IL.

Correlations between NTE and AChE inhibition and alterations in gait were examined for the delayed neurotoxin desbromo-leptophos, the non-neurotoxin fenitrothion, and fenthion, a suspect neurotoxin, in chicks below the age of sensitivity to OPIDN. Desbromo-leptophos, 75 mg/kg po, caused 94% NTE inhibition and 65% AChE inhibition 24 hours after treatment, with gradual return to normal values by 20 days after treatment for NTE, by 30 days for AChE. Fenitrothion, 100 mg/kg po, caused 56% AChE inhibition, but no NTE inhibition, 24 hours after treatment. At 10 days post-treatment, AChE inhibition had decreased to 25%, inhibition of NTE had reached 25%. Fenthion, an avicide, was given in 7 percutaneous doses, 5 mg/kg/day. AChE inhibition reached 99% 24 hours after the 3rd dose; NTE inhibition never exceeded 25% during or after treatment. Desbromo-leptophos and fenthion significantly increased the size of the angle of stride in treated chicks; the non-OPIDN-inducing fenitrothion did not. Fenthion and fenitrothion, but not desbromo-leptophos, decreased weight gain in exposed chicks. Fenthion-treated chicks became ataxic at the normal age for onset of sensitivity to OPIDN. Minimal NTE inhibition, long latency, and immaturity of the chicks at treatment distinguish fenthion-induced ataxia from classical OPIDN. The effect of these insecticides in vivo was also evaluated.
BLOOD CHOLINESTERASE AND RESPIRATORY SINUS ARRHYTHMIA (RSA) RESPONSES TO PENTHION AND ATROPINE SULFATE IN DOGS. M.S. Mostrom and J.A. Declair., University of Illinois, Department of Veterinary Biosciences, Urbana, IL

Blood cholinesterase (CHE) was assessed after subacute exposures to a 20% fenthion formulation. Fifteen male random source dogs were dermally exposed at 0, 8 (14 day interval for 2 treatments), and 33 mg/kg (7 day interval for 4 treatments) followed by a 14 day observation period. Erythrocyte CHE activity exhibited a slight downward trend after the 33 mg/kg with a maximum inhibition of 68% of normal activity occurring 9 days after the fourth treatment. Plasma CHE inhibition was dose related with peak depression of 68% and 78% of normal occurring 4 days following the final treatment of 8 and 33 mg/kg, respectively. No clinical signs of toxicity were observed. Subsequent to the fenthion treatment and the observation period, all 15 dogs were challenged with atropine sulfate 0.02 mg/kg, administered subcutaneously. As a noninvasive index of cardiac vagal tone, the estimate of RSA amplitude (V) was obtained using a 15 min electrocardiogram. Measurement of RSA data at 0, 25, 75, and 100 min post-atropine demonstrated the sensitivity of V to the vagolytic action of atropine with a significant decrease of V at 25 min. The atropine effect differentiated the groups according to fenthion treatment (i.e., dogs treated with 33 mg/kg of fenthion did not respond to atropine as much as the control dogs).

PULMONARY LESIONS AND DELAYED MORTALITY CAUSED BY 0,0,5-TRIMETHYL PHOSPHOROTHIOATE (005-TP) IN COMPLEMENT C5 SUFFICIENT (C5+=B10.D2/nSn) AND DEFICIENT (C5-=B10.D2/nSn) MICE. A. Kolzumi, D. O. Elliston, K. Hashigawa, I. K. Thomas and I. Inamura. Division of Toxicology and Physiology, University of California, Riverside, CA

005-TP was given to C5+ and C5- mice at 30 mg/kg o.p. By three days, labored breathing, stridor and body weight loss became prominent. By histological examination, disappearance of the apical bulges of Clara cells and destruction of the alveolar walls were observed. In C5+ mice, additional changes due to inflammatory responses were detected. In another experiment, mortality pattern and body weight decrease after treatment with 005-TP were compared in C5+, C5-, and Swiss Webster (SW) mice. C5- mice showed the typical pattern of delayed death. C5+ mice lost weight for three days following treatment, while C5- mice continued to lose weight for 7 days. LD50 decreased as a function of time. Although at 3 days LD50s were essentially the same in these mice (120 to 145 mg/kg), the LD50 at 14 days in C5- mice (305 mg/kg) was much lower than in C5+ (101 mg/kg) or SW (132 mg/kg). The present study demonstrated that symptoms of delayed toxicity in mice was due to pulmonary lesions and suggests that incompetence in the immune surveillance system due to lack of C5 might play a role in the typical delayed mortality.

ENHANCED LEVELS OF LIPID PEROXIDATION AND XANTHINE OXIDASE (XO) ACTIVITY IN THE LUNG IN MALE SPRAGUE-DAWLEY RATS FOLLOWING AFTER TREATMENT WITH 0,0,5-TRIMETHYL PHOSPHOROTHIOATE (005). L. Hasegawa, A. Kolzumi, and T. Imamura. Division of Toxicology and Physiology, University of California, Riverside, CA

The purpose of the study is to investigate lipid peroxidation and XO activity after treatment of 005 and/or 0,0,5-trimethyl phosphorothioate (005), which is an 005 antagonist. Animals (4 per group) were dosed with 005 at 40 mg/kg and sacrificed 24, 72 and 168 hr after the treatment. In comparison with control levels, 005 increased lipid peroxidation in lung and liver at all time points. However, XO activity was increased in lung at 24 hr while no significant increases were observed in any time points in liver. In lung, lipid peroxidation and XO activity increased in a dose-dependent manner. In contrast, although lipid peroxidation in liver increased in a dose-dependent manner, XO activity in liver did not change. The enhancement of lipid peroxidation both in liver and lung were cancelled completely by co-administered 005. In accordance with this trend, 005 inhibited the increase of XO activity in lung. Thus, it was concluded that 1) 005 increased XO activity in lung but not in the liver; 2) 005 increased the lipid peroxidation in lung moderately and in liver extensively; 3) these effects of 005 on lipid peroxidation and XO activity were abolished by 005.

INHIBITION OF L-C DOUGING DURING SKELETAL MUSCLE CONTRACTIONS BY THE FORMAMIDINE INSECTICIDE METABOLITE U-46481. Y.C. Ravikumar, J.A. Rieger and Y.S. Reddy. Departments of Pharmacodynamics & Toxicology and Physiology, University of Oklahoma, Oklahoma City, OK 73190. 1Present Address: School of Pharmacy, Northeast Louisiana University, Monroe Louisiana 71209-0470.

The mechanism by which the amidraz metabolite R'-(4,4-xyl)-N-methylformanidene (U-40481) decreased skeletal muscle contractility was investigated. Rectus abdominis and sartorius muscles, from Rana pipiens, pre-treated with U-40481 (the HCl salt, 5 mM for 30 min) produced lesser maximal tension and took longer to do so in response to a bolus addition of caffeine (5 mM); also, the area under caffeine-induced contracture over 60 min was decreased only during the "falling/relaxation" phase of the contracture. In electrically stimulated sartorius muscles, U-40481 (2 mM) increased the tetanic tension/twitch tension ratio and in lower concentrations (0.2-1.0 mM) it potentiated the inhibition of maximal twitches caused by proclaine. U-40481, however, did not significantly decrease ATPase activity of myofilaments isolated from rat diaphragm muscles.

These results strongly suggest that U-40481 decreased active state duration during skeletal muscle contractions by an action on the excitation-contraction coupling mechanism; possibly by increasing the rate of calcium ion resequestration from the myofilbrillar region by intracellular storage sites.
325 EFFECT OF TOXAPHENE (TOX) AND PARATHION (PA) MIXTURE ON THE MIXED FUNCTION OXIDOREDUCTASES (MFO) D.J. Knuth, N.G.S. Rao, and A.K. Chaturvedi, Dept. of Pharm. Sci./Tox., Coll. of Pharmacy, N.D. State Univ., Fargo, ND

Though the effects of individual agricultural chemicals (ACs) are known, the interactions of the multitude of ACs are lacking. More information is needed for the interaction of such mixtures to assess their toxicity. The mixture of heavily used insecticides, TOX (50 mg/kg) and PA (5 mg/kg), had been studied. Its daily exposure to ICR male mice (21-24 g) for 7 days in corn oil by oral intubation had been found to lower the pentobarbital (PET)-induced sleep. Therefore, the effect of PA, TOX and their mixture on the hepatic MFO was studied. TOX, and TOX & PA pretreatment induced the metabolism of amiodiure (−37%), aniline (−45%), phenacetin (−242%) and benzopyrene (−140%), and increased (59%) the hepatic P-450 contents. The percent change was the same in both groups. Similarly, the in vitro rate of metabolism of PET was higher (43%) in both TOX, and TOX & PA groups. The metabolism of these agents and the P-450 level in the PA group were not different than the control. These data suggest that the presence of PA in the mixture does not alter the ability of TOX to induce the MFO. However, TOX has a potential to lower the toxicity of PA, presumably by altering its metabolism.


Male and female Sprague-Dawley rats were used to study the effects of endrin, endrin aldehyde and endrin ketone on hepatobiliary function and the propensity of these compounds to potentiate CC14-induced hepatotoxicity. The study was prompted by previous work demonstrating potentiation of CC14 hepatotoxicity by chlordecone, also a ketoclorocarbon. Dietary endrin (5 ppm and 10 ppm, 15 days) failed to induce significant hepatotoxicity as determined by serum enzyme (SGPT, SGOT, ICD, OCT) levels, while dietary treatment with the endrin derivatives slightly elevated some enzyme levels. Alteration in the excretion of an anionic model compound, phenolphthalein glucuronide (PG), by male rats receiving 10 ppm endrin implied a reduction in the biliary excretory and/or transport functions independent of changes in bile flow. The same treatment produced a cholestatic effect in female rats, resulting in an increase in PG excretion. The endrin derivatives did not alter PG excretion or bile flow. Both dose levels of endrin, endrin aldehyde (10 ppm) and endrin ketone (5 ppm) failed to potentiate the effects of a single dose of CC14 (100 µl/kg). Female rats exhibited a greater sensitivity to CC14 and endrin/CC14 treatments than did similarly treated male rats. (Supported by R-811072 and ES-07045.)

327 LINDANE-INDUCED ALVEOLAR BRONCHOIALIZATION AND ALVEOLAR CELL TUMORS IN YELLOW AND PSEUDOAGOUTI AVY/a MICE. R.L. Morrissey* and G.L. Wolff., *Pathology Associates Inc., Liamsville, MD and National Center for Toxicological Research, Jefferson, AR

Female (Y.S x VY) F1 hybrid mice were grouped by color pattern into obese yellow, lean pseudoagouti and lean black groups. Half of each group received 160 ppm lindane in feed for 24 months. Lindane treatment resulted in 68.4, 74.5 and 82.3% incidence of alveolar bronchioization respectively in yellow, pseudoagouti and black mice, compared with 14.7, 10.5 and 10.4% in the color matched controls. Ultrastructurally the cells lining peribronchiolar alveoli were Clara cells. The incidence of alveolar cell tumors was significantly increased by lindane treatment in the genetically identical (AVY/a) yellow (18.9%) and pseudoagouti (13.8%) but not in the black a/a (3.1%) mice, compared with 4.2, 6.3 and 2.1% respectively in the color matched controls. Thus, hyperplasia of Clara cells occurred independently of the AVY gene in this F-1 hybrid in response to lindane but tumor induction was dependent on the AVY genotype. Lindane induction of lung Testons in the AVY/a mice and the absence of such lesions in earlier carcinogenicity tests of lindane in other mouse stocks indicates that this F1 hybrid may be a more sensitive test model for carcinogenicity.
Amitraz (AMZ) has behavioral and neurochemical actions more prolonged than those of chloroform (CDM), another major commercial formamide pesticide (Moser and MacPhail, SOT abstract, 1986). A dose of 40 mg/kg CDM produced large changes in rat pattern reversal visual evoked potentials (PREPs) which lasted less than 24 hrs (Boyes and Dyer, Exp. Neurol., 89:391, 1985). Previous experiments found an acute dose of 100mg/kg AMZ to be roughly equivalent to 40 mg/kg CDM 2 hrs after treatment. In the present study, bi-phasic changes were observed in PREPs measured 2hrs-3days after an acute dose of 100 mg/kg AMZ. Pronounced increases in PREP N1P1 and P2N3 amplitudes were present 2 hrs after AMZ treatment, but attenuated by 24 hrs. Subsequently, P2N3 amplitude was significantly reduced by AMZ. This second phase of changes returned to control values 3 days post-treatment; a duration of action similar to behavioral and neurochemical changes. Whether the prolonged actions of AMZ result from persistence of the compound or active metabolites, or from some residual physiological changes is presently unknown. VCM was supported by a NRC Research Associateship.

Picoloram (4-amino-3,5,6-trichloropicolinic acid) is a herbicide used in the control of broad-leaved weeds and woody plants. Seventy Fischer 344 rats/sex/dose level were fed diets containing 0, 20, 60, or 200 mg picoram/kg body weight/day for up to two years. Ten rats/sex/dose level were terminated at 6 and 12 months for a battery of clinical pathologic, gross and histopathologic examinations. Picoram is actively excreted by the kidney and there was an equi-vocal increase in renal weight in rats given 200 mg/kg/day. However, there were no changes in urinalysis parameters, serum urea nitrogen or renal histopathologic changes suggestive of a toxic effect due to picoram ingestion for up to two years. The only effects attributed to treatment were restricted to the liver and consisted of increased liver weight and minimal histopathological changes of the centrilobular hepatocytes. These effects were present in rats of both sexes given 200 mg/kg/day and, to a lesser extent, in rats given 60 mg/kg/day. The hepatic effects did not progress with prolonged treatment. There were no increases in tumor incidence related to picoram ingestion.

Amitraz (ANZ), a formamide pesticide often compared to chloroform, has been shown in mice to have effects lasting on the order of days (Moser and MacPhail, 1985). In this experiment we determined the time course of AMZ effects on motor activity and monoamine oxidase (MAO) inhibition in male Long Evans hooded rats. Rats were tested daily in photocell activity measurement devices. AMZ was administered i.p. no more than twice a week. Motor activity decreased within 20 min after doses as low as 3 mg/kg. Activity slowly recovered to control levels over 4-5 days after dosing with 100-200 mg/kg. The inhibition of MAO was measured in whole brain at various times after dosing with ANZ (10-300 mg/kg). Prolonged effects were again observed at doses >700 mg/kg, for as long as 7-10 days after dosing. In addition, some animals died 2-5 days after a 300 mg/kg dose. Whether these persistent effects are due to slow elimination of AMZ, the formation of an active metabolite(s), or an irreversible change such as enzyme inhibition, is still unclear. These prolonged effects, not seen with other formamides, suggest that the neurobehavioral and toxicokinetic effects of AMZ deserve more study. VCM supported by a NRC Research Associateship.

The purpose of this study was to assess chronic toxicity and oncogenic potential of tridiphane herbicide (2-(3,5-dichlorophenyl)-2(2,2,2-trichloroethyl) oxirane). Mice received up to 35 mg/kg/day and rats received up to 50 mg/kg/day in the diet through 2 years. Ten rodents/sex/dose were sacrificed at 6 months and 12 months, and all survivors were terminated at 24 months. Parameters evaluated included body weights, food consumption, clinical observations, palpable masses, clinical chemistry, hematology, organ weights, gross pathology and histopathology. Body weights of high-dose male and female rats generally were decreased throughout the 2-year study. Increased mortality was present in high-dose female mice during the last 6 months. High-dose male and female rats and mice had microscopic hepatocellular hypertrophy with concomitant increases in liver weights. High-dose female mice had an increased incidence of benign liver tumors. Male mice as well as male and female rats had no tumorigenic response. The NOEL for mice was 10 mg/kg/day; the NOEL for male rats was 3 mg/kg/day and for female rats 5 mg/kg/day.

In order to investigate the mechanisms involved in chlordecone (CLD)-induced hypothermia, we examined colonic (Tc) and tail skin (Ts) temperatures, evaporative water loss, metabolic rate, and preferred ambient temperature (pTa) following CLD exposure. Single ip dosages (0, 50 and 75 mg/kg) in corn oil were administered to Fischer-344 rats. Only 75 mg/kg resulted in reduced Tc, which occurred 2, 3, 4 and 6 hr post-exposure (maximum, 0.7°C at 4 hr). Tc was increased after both 50 and 75 mg/kg at 24 hr. Tc was elevated 6 and 24 hr after 75 mg/kg and 24 hr after 50 mg/kg. Since cremor developed ~3 hr post-dosing and intensified with time, increases in Tc and Tg occurred 6 and 24 hr post-dosing may be due to increased muscular activity. Evaporative water loss was significantly decreased (10-25%) 3, 4 and 24 hr after 50 mg/kg and 24 hr after 75 mg/kg, although metabolic rate was not changed. These data suggest that peripheral heat dissipating mechanisms may not explain the hypothermia produced by CLD. Both 50 and 75 mg/kg significantly decreased (3°C) pTa 1.6 to 9 hr post-dosing. The decrease in pTa along with a decrease in Tc indicate that CLD invokes a form of regulated hypothermia, e.g., a decrease in set point.


The subchronic toxicity of DIFOLATAN Technical (Captanfol) was determined in SD rats and guinea pigs (GP). Groups of 10 rats and GP of each sex were exposed 6 hrs/day, 5 days/wk to average concentrations of 0, 0.1, 0.5 and 1.5 mg/m³. Five additional rats and GP of each sex in the 0 and 1.5 mg/m³ groups were held for a 6-week recovery period. Dust was generated with a single Wright Dust Feed Mechanism and delivered via a dilution system to the chambers. Concentrations were monitored by filter samples which were analyzed for Captanfol. Mass median aerodynamic diameters were 2.0 to 2.3 μm. There were no overt signs of toxicity or effects on survival or body weight. Effects which may have been treatment-related were decreased leukocyte counts in 1.5 mg/m³ male rats, and decreased serum globulin in 1.5 mg/m³ male and female rats. The only treatment-related histopathologic effect was a subtle scalloping of the anterior nasal mucosa in 0.5 mg/m³ male and 1.5 mg/m³ male and female rats. This change was less prominent after 6 weeks recovery and may have been due to local irritation from deposited Captanfol. There were no histopathologic effects observed in GP.


The effect of paraquat and diquat on microsomal mixed function oxidation (MFO) was determined by estimation of ethylmorphine and benzphetamine N-demethylase activities. Both activities were examined in vitro in the absence and presence of paraquat or diquat (0.5-10.0 μM) using lung, liver, and kidney microsomal preparations. Benzphetamine metabolism in lung microsomes was inhibited by paraquat and diquat in a concentration-dependent manner; at the highest concentration (10.0 μM) inhibition was 42% and 48%, respectively. Ethylmorphine metabolism was unaffected in lung preparations. However, in liver microsomes, both benzphetamine and ethylmorphine N-demethylase activities were inhibited. At highest concentration of paraquat and diquat, benzphetamine metabolism was decreased 55% and 59%, and ethylmorphine 60% and 61%, respectively. Benzphetamine was without effect on MFO in kidney microsomes. The effects of paraquat and diquat on MFO activities are dependent upon sub-strate and the source of microsomal preparations, and cannot be predicted. Supported by NIH Grant ES02846 and VA Research Funds.

CHARACTERIZATION OF THE INDUCTION OF HEPATIC MICROsomal METABOLISM PRODUCED BY DICOFOL IN RATS. B.A. Narloch, M.P. LaVont, D.E. Moody, B.D. Hamnock, and L.R. Shull. Departments of Environmental Toxicology and of Entomology, University of California, Davis, CA.

In a comparative study, the induction effects of dicofol, technical Keltthane and DDT on hepatic microsomal and cytosolic enzyme activities in rats were compared to those effects produced by phenobarbital (PB) and B-naphthoflavone (BNF). Male rats (ca. 250 G) were injected (ip) for four consecutive days with 1.0 ml of vehicle containing either dicofol (1.5, 15.0, 29.5 or 59.0 mg), technical Keltthane (dicofol content equal to 29.5 or 59.0 μM), DDT (59.0 μM), PB (59.0 μM) or BNF (36.7 μM). Liver weights, microsomal protein and cytochrome P450 concentrations, and microsomal and cytosolic enzyme specific activities were measured. Dicofol produced dose-related increases in all of the parameters measured, except liver weight and cytosolic epoxide hydrolase activity. At a concentration of 59.0 μM, dicofol increased the concentrations of microsomal protein (1.7-fold) and cytochrome P450 (2.9-fold), and the specific activities of cytochrome c reductase (1.6-fold), ethoxyconumarin 0-deethylase (2.3-fold), amiprine N-demethylase (3.0-fold), microsomal epoxide hydrolase (2.6-fold) and glutathione S-transferase (2.0-fold). The induction potency of dicofol was equivalent to technical Keltthane, DDT and PB at equinsolar (59.0 μM) concentrations of chemical.

Dinocap technical was tested for developmental toxicity in New Zealand white rabbits in 3 experiments. After oral exposure to 3, 12, 48 and 64 mg/kg (0-7-19 of gestation) hydrocephalus and/or malformations of the neural tube and skull were observed at all doses. Maternal weight gain was depressed at 48 and 64 mg/kg and reduced stool volume was apparent at 12 mg/kg. Embryofetal toxicity was present as increased post implantation losses at 12, 48 and 64 mg/kg and delayed fetal maturation at 48 and 64 mg/kg. A second oral study in rabbits at doses of 0.1, 0.5 and 48 mg/kg produced no treatment related effects at 0.1 or 0.5 mg/kg and no hydrocephalic fetuses at 48 mg/kg. Reduced maternal weight gain and post implantation embryonic losses were observed at 48 mg/kg. These data failed to confirm the teratogenicity of dinocap, but were not inconsistent with the first study and a NOEL of 0.5 mg/kg was demonstrated. A third study was conducted at maternal doses of 25, 50 and 100 mg/kg (highest dose permissible). All doses were dermal irritants; at 100 mg/kg maternal weight gain and feed consumption were reduced, but no dose produced developmental toxicity. It was concluded that dinocap was not embryofetotoxic or teratogenic by the dermal route in rabbits.

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OSAR OF 2-BENZOYL-1,3-INDANDIONE ANTICOAGULANT RODENTICIDES: SYNTHESIS, CHROMATOGRAPHY AND SPECTROSCOPIC PROPERTIES. K. Opong-Mensah and W. R. Porter. School of Pharmacy, University of Wisconsin, Madison, WI.

Selected 2-benzoyl-1,3-indandiones with substituents in the phenyl ring were prepared by the condensation of dimethyl phthalate with appropriately substituted acetoephones under controlled conditions in the presence of sodium methoxide to give (substituent, m.p. °C, yield) H, 105-107, 29%; 4'-OCH₃, 122-123, 33%; 3'-CH₃, 98-99, 37%; 4'-CH₃, 120-121, 32%; and 4'-CH(CH₃)₂, d. 180.17%. Similarly, diphenacine was prepared (m.p. 146-147, 43% yield). Yields were about twice that reported for those compounds that were not new. The compounds were separated on silica gel TLC using cyclohexane: dichloroethane:acetic acid::50:50:0.6 and visualized with phosphomolybdic acid with Rf 0.12, 0.10, 0.17, 0.13, 0.24, and 0.18. They were separated by reverse-phase HPLC using 15% methanol in water with retention times of 5.5, 6.7, 6.0, 6.4, 7.0, and 12 minutes. Their methoxide derivatives were separable by GC on 3% OV-17 at 200°C with retention times 12.5, 13.3, 12.8, 13.0, 18.5, and 19.1 minutes. Their mass spectra were consistent with the assigned structures. IR and NMR data indicated the compounds exist in solution as the enol. Epoxidation of the benzoyl group was preferred.

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OSAR OF 2-BENZOYL-1,3-INDANDIONE ANTICOAGULANT RODENTICIDES: PHYSICO-CHEMICAL PROPERTIES AND LETHALITY TO MICE. K. Opong-Mensah and W. R. Porter. School of Pharmacy, University of Wisconsin, Madison, WI.

Selected 2-benzoyl-1,3-indandiones with substituents in the phenyl ring were studied [R = H, 4'-OCH₃, 3'-CH₃, 4'-CH₃, and 4'-CH(CH₃)₂] and compared to diphenacine. Their log₁₀ n-octanol/ water partition coefficients were found to be 1.14±0.03, 1.31±0.02, 1.24±0.04, 1.31±0.02, 1.43±0.03, and 2.47±0.03. They had pKₐ 3.08±0.02, 3.91±0.01, 2.63±0.01, 2.93±0.02, 3.91±0.01, and 3.31±0.01. Their LD₅₀ following i.p. injection in male mice was found to be (median m/kg, 95% confidence limits) 0.398 (0.030-0.749), 0.266 (0.021-0.497), 0.083 (0.065-0.105), 0.077 (0.060-0.098), 0.096 (0.071-0.129), and 0.048 (0.035-0.064). Correlation of the log₁₀ LD₅₀ with log P, pKₐ, IR carbonyl stretching, amino acid content, Hammett sigma, Taft steric parameter, molar refractivity, highest occupied molecular orbital energy, and lowest unoccupied molecular orbital energy was poor. Partial least-squares methods revealed a strong correlation with the average carbon bond C13-NMR chemical shift and pKₐ (r = -0.84). A plot of log₁₀ LD₅₀ versus the average chemical shift had intercept 4.73±0.68 and slope -0.0196 ±0.0038. The log₁₀ LD₅₀ values predicted by this model agreed closely with those experimentally determined (r = 0.98 excluding diphenacine, r = 0.96 including diphenacine).
NON-PROTEIN SULFHYDRYL CONTENT IN RATS FOLLOWING ACUTE INHALATION EXPOSURE TO 1,3-DICHLOROPROPENE. G.D. Fisher and W.W. Kilgore. Department of Environmental Toxicology, University of California, Davis, CA, 95616.

1,3-Dichloropropene (DCP) is a volatile, commonly used agricultural nematocide. It is principally excreted as the mercapturic acid by orally exposed rats. Consistent with this, tissue non-protein sulfhydryl (NPS) content was determined in rats following acute inhalation exposure. A dynamic, nose-only system was designed, constructed and used in this experiment. Male Sprague-Dawley rats were exposed for one hour to 0, 10, 100 or 700 ppm of a commercial formulation of DCP. Blood was removed by cardiac puncture at the time of sacrifice, two hours after exposure, and analyzed by gas chromatography for cis- and trans-DCP isomers. Neither isomer was detected although both were present in a preliminary study using 100 mg/kg given by oral gavage. NPS content was measured in hearts, kidneys, livers and lungs. Dose-dependent depletion of NPS of up to 50-75% was found. The most severe decrease occurred in the lungs, with kidneys also being affected.

SULFAGALAZINE-MEDIATED ALTERATIONS IN RAT TESTICULAR HEME AND CYTOCHROME P-450. J.K. West and W.N. Piper. Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE.

Clinical observations have shown that prolonged sulfagalazine (SZ) administration in ulcerative colitis treatment may result in male infertility. Studies suggest a possible effect on testosterone synthesis and spermatogenesis. Various sulfonamides are known to impair hepatic heme synthesis and cytochrome P-450 levels by the inhibition of uroporphyrinogen I synthetase (URO-S). Since P-450 mediates essential steroidogenic reactions, the effects on testicular heme synthesis by SZ and its metabolites were examined. SZ was analyzed for its effects on microsomal heme and P-450 in rat testes. SZ was given 1 p i. at 500 mg/kg/day for five consecutive days. SZ demonstrated significant decreases in heme (31%) & P-450 (32%). SZ and the metabolites, sulfapyridine (SF) and S-aminoacylacetate (5-ASA) were examined for their effect on the heme pathway enzyme, URO-S. Testicular cytosolic URO-S fractions were assayed with SZ, SF and 5-ASA (10^{-4}M). In vitro enzymatic activity was inhibited 66% by SF, 67% by SP and was unchanged by 5-ASA. These findings indicate that SZ and SF may mediate decreases of heme and cytochrome P-450 by URO-S inhibition. These changes may reduce heme protein steroiogenic reactions and be related to infertility in prolonged sulfagalazine treatment. (Supported by March of Dimes Grant # 15-44).


Disorders of the adrenal cortex are known to result in disturbances in testicular function. Treatment with high doses of glucocorticoids has been reported to decrease plasma testosterone in rats and humans. The present study was initiated to examine the effects of dexamethasone, a synthetic adrenocortical steroid on levels of testicular microsomal heme, cytochrome P-450 and cytochrome P-450 mediated 17-hydroxylase (17-OH) and 17,20-lyase activities. Male, Sprague-Dawley rats (150-180 g) were given 5 mg/kg body weight of dexamethasone subcutaneously for three days. Heme and cytochrome P-450 activities were determined 16 hours after the final dose. Testicular heme and cytochrome P-450 were decreased to 58 and 60 percent of control values; activities of the microsomal cytochrome P-450 mediated enzymes 17-OH and 17,20-lyase were decreased to 66 and 60 percent of control values respectively. Serum testosterone was decreased to 35 percent of control. These results indicate that decreased testosterone production following treatment with glucocorticoids is a result of decreased activities of testicular microsomal P-450 and its mediated enzymes 17-hydroxylase and 17,20-lyase. (Supported by NIH grant #ES-02423).


Xenobiotic changes in serum testosterone(T) are difficult to assign to extragonadal endocrine alterations or to a direct effect on the Leydig cell. Some mechanisms responsible for T production. Several metals cations(Met+) have been shown to alter both in vivo T and pituitary hormones in lab animals. The studies reported here, using a primary testicular cell culture technique, are designed to evaluate LC T biosynthesis in the presence of several Met+. To determine the site of toxic action, the LC are stimulated to produce T by using exogenous hormones or substrates. For receptor (R) stimulation human chorionic gonadotropin (hCG) is used, and for post R stimulation db cAMP is used. The substrate 20 a-hydroxycholesterol (CHOL) was used for mitochondrial (MT) stimulation and pregnenolone (PG) for post-MT stimulation. Testicular cells were incubated with the Met+ (10^{-4} to 5x10^{-2} M) for 3 hr in the presence of either hCG, db cAMP, CHOL or PREG and T was determined by RIA. A dose response depression in both hCG and db-cAMP stimulated T production was seen with some Met+ treatment but not all. Met+ caused an increase in the CHOL and PREG stimulated T production indicating an increase in either enzyme activity or substrate availability.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
INFLUENCE OF DIET ON METABOLISM AND ELIMINATION OF ESTRADIOL IN MICE. F.R. Fullerton and D.L. Greeman, National Center for Toxicological Research, Jefferson, AR. Sponsor: W.T. Allaben

The effects of NIH-07 (NIH) and AIN-76A (AIN) diets on metabolism and elimination of (6,7,8) estradiol (E) were compared in female B6CF1 mice. Mice were fed NIH or AIN diet for eight weeks prior to treatment with E. In the first phase of the study, E was injected into each mouse (i.v.); in the second phase, the same mice were gavaged (p.o.) with E. Following each E administration, samples of blood were obtained over a 24 hour period and urine and feces were obtained over a 48 hour period. Total radioactivity was determined for each sample. Urine and plasma were analyzed by HPLC and the radioactively labeled E metabolites tentatively identified and quantified. During the first 48 hours more total radioactivity was excreted in the urine of AIN-fed mice than in NIH-fed mice after i.v. or p.o. administration. However, total excretion (urine + fecal) during this period was not significantly higher, and total radioactivity in the plasma was slightly lower after 48 hours in NIH-fed mice than in AIN-fed mice. There were no apparent differences in the patterns of urinary metabolites regardless of diet or route of administration. However, there were dietary and metabolic administration-related differences in the plasma metabolite patterns. Data from the study suggested that the primary route of E excretion and the E metabolites formed in mice are different from primates.

ROLE OF THYROXINE (T4) IN PERFLUORO-o-DECANOIC ACID (PFDA)-INDUCED HYPOPHAGIA, BODY WEIGHT LOSS, AND HYPOTHERMIA. D.M. Gutshall, G.D. Pilcher* and A.E. Langley, Dept. of Pharm. and Tox., Wright State Univ., Dayton, OH and *School of Pharmacy, Univ. Wisconsin, Madison, WI. Spon: R.L. Koerker

Previous studies with rats have demonstrated that PFDA (75mg/kg) reduces serum thyroid hormones and causes hypophagia with body weight loss, and hypothermia over a delayed timecourse. The relationship between the hypophagic, weight loss and hypothemic effects to the thyroid state of the animal was examined over a 14 day time course by giving PFDA-treated rats daily injections of T4 (0.2mg/kg). Rats were sacrificed on day 14 for quantification of serum T4. T4 supplementation reversed the hypophagia seen in PFDA-treated rats but not the body weight loss nor hypothermia. PFDA/T4-treated rats exhibited a decrease (t35%) in total serum T4 while rats supplemented with T4 had elevated total serum T4 (+50%) compared to pair-fed controls. On the other hand, the inability of T4 to prevent or reverse PFDA-induced body weight loss and hypothermia suggest that other mechanisms are responsible for these effects. In addition, the data suggest that PFDA reduces serum T4 by altering serum transport capability.

(Supported by AFOSR grant #82-0264.)


Female rats (Sprague-Dawley, 250-300 g) were treated with ethanol (4 g/kg) via gastric intubation for 1, 2, 4 and 6 hrs. The liver and ovaries were removed and homogenized to measure peroxidase activity, tissue ascorbate and calcium contents using published procedures. The peroxidase activity measured 0, 1, 2, 4 and 6 hrs after ethanol was 2.5, 8.0, 8.5 and 15.5 units/g in the liver and 12.7, 1.7, 0.4, 2.9 and 10 units/g in the ovaries. The tissue ascorbate content measured at the same time points was 0.41, 0.38, 0.34, 0.29 and 0.27 mg/g in the liver and 1.21, 1.42, 1.55, 1.70 and 1.60 mg/g in the ovaries; and the tissue Ca++ level was 32, 34, 36, 45 and 47 g/g in the liver and 121, 117, 96, 77 and 64 g/g in the ovaries. The peroxidase activities correlated negatively with the tissue ascorbate contents and positively with the tissue Ca++ levels. In hypophysectomized rats, acute alcohol treatment caused relatively little changes in peroxidase activity in both organs. These results indicate that acute alcohol treatment alters the release of pituitary hormones regulating the infiltration of polymorphonuclear leukocytes into these organs. (Supported by AAS848.)

EFFECTS OF PERINATAL EXPOSURE TO ETHYLENETHIOUREA ON THE THYROID OF RATS AND MICE EXPOSED AS ADULTS: 9- AND 24-MONTH SACRIFICES. C.E. Wilkinson, T.R. Kurta, and R.S. Chhabra, Battelle Columbus Labs, Columbus, OH, and National Toxicology Program, NIEHS, Research Triangle Park, NC

The influence of perinatal pre-exposure on the effects of chronic dietary exposure to ethylene-thiourea (ETU) was examined in the 9 and 24-month periods of adult exposure. Rat and mouse dams received ETU in feed during breeding, gestation, and lactation. Pup exposure continued at maternal dose levels until assignment to chronic dosing groups at 8 weeks of age. Decreases in serum thyroxine levels (mice and rats) at 9 months were maintained at 24 months, while serum triiodothyronine levels were comparable to controls at both sacrifices. Elevations in serum thyroid-stimulating hormone levels of both species were greater at study termination than at 9 months demonstrating increasing pituitary activation with extended ETU-induced hypothyroidism. The influence of perinatal exposure on thyroid hormone status correlated with increased thyroid weights of rats. In addition, ETU effects were more severe in female rats pre-exposed during early development. Thus, while adult exposure was necessary to elicit thyroid toxicity, perinatal exposure made these alterations more severe at both the 9- and 24-month sacrifices. (Supported by NIEHS Contract No. N01-ES-8-2151.)
Twenty-five male Sprague-Dawley rats (208 ± 2 g) were administered i.p. 0, 1, 5, 25 or 125 mg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in corn oil. On days 1, 2, 4, 8, 16 and 32 after dosing, blood was collected from the tail vein and thyroid stimulating hormone (TSH), total thyroxine (T₄), total triiodothyronine (T₃), free thyroxine (FT₄) and reverse triiodothyronine (RT₃) were determined using radioimmunoassays. Data were analyzed by two-way analysis of variance for repeated measures followed by Duncan's multiple comparison test. TSH and T₄ were not affected by any dose at any time point of measurement. T₄ and FT₄ were decreased (p < 0.01) at each dose level to about the same extent by day 2 to 4 after dosing. However, return of T₄ and FT₄ to normal values occurred in a dose-dependent manner, except in rats that died. RT₃ was also decreased at each dose level early on and returned to normal levels in a dose-dependent fashion. These responses are not compatible with changes expected for animals in a state of reduced feed intake. Therefore, it is concluded that in terms of thyroid hormone homeostasis TCDD-treated rats are "unaware" of the fact that they are wasting away.

A practical in vivo method of determining both congenital and drug induced alterations in ¹²⁵I-uptake and organification was developed. Control and propylthiouracil (PTU, an inhibitor of iodide organification) pretreated rats were given carrier-free radiiodide (¹³¹I) in sterile saline i.v. After 3 hours the skin and muscles of the ventral cervical region of anesthetized rats were reflected exposing the thyroid gland. The ¹³¹I content of the thyroid was measured externally using a specially designed 2 cm NaI scintillation detector connected to a discriminator/pulse height analyser. An i.v. injection of NaClO₃ was given to release any non-covalently bound ¹³¹I from the thyroid gland. One hour later, another gland count was performed and the animal sacrificed. The thyroid gland was removed and the carcass was again counted in order to determine the contribution of non-thyroidal ¹³¹I in the gland count. After NaClO₃ administration a small amount of ¹³¹I was lost from the thyroid of control rats, whereas in PTU treated rats essentially all of ¹³¹I in the gland was released. This is a sensitive method to determine inhibition of ¹²⁵I organification. (This abstract does not necessarily reflect EPA policy.)
DEVELOPMENTAL TOXICITY OF ESTROGENS AND ANTI-ESTROGENS IN THE RAT UTERUS. W.S. Branham, M.L. Leamons, D.A. Zehr, J.J. Chen, and P.W. Sheehan. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR, and Dept. of Biology, Univ. of Central Arkansas, Conway, AR

Rat uterine gland genesis occurs between postnatal days 9-15 and is increased by 15th increasing uterine weight and decreasing luminal epithelium (LE) height. Tamoxifen (TAM) inhibits gland genesis when given prior to (neonatal; PND 1-5) or during (infantile; PND 10-14) gland genesis. 17β-estradiol (E2) reduces gland number when given to neonatal rats and delays gland genesis when given to infantile rats. We now report that the antiestrogen clomiphene citrate (CC) inhibited uterine gland genesis by 90% and the synthetic estrogens diethylstilbestrol (DES) and ethynylestradiol (EE) reduced gland number by 25-54% on PND 26 after treatment of neonatal or infantile rats. Morphometric analyses of uterine cell populations on PND 26 after neonatal treatment showed that all test compounds reduced LE cross-sectional area by approximately 40% compared to controls and stromal area was reduced after TAM, E2, DES, or EE. In infantile rats only E2 and EE reduced LE area. While TAM and CC increased stromal area after infantile exposure, estrogens had no effect. These data indicate that uterine gland genesis inhibition by the antiestrogens TAM and CC occurs independently of quantitative changes in uterine LE or stroma.


An anti-tumor agent, the cationic dye rhodamine 123 (Rh 123), localizes in mitochondria and inhibits ATP synthesis. To test for selective embryotoxicity, pregnant mice were injected i.p. with up to 15 mg/kg Rh 123 on gestation days 7-10 (plug = day 1). Others were given saline or 2-deoxyglucose (2-DOG), an inhibitor of nonmitochondrial ATP production, alone or with Rh 123. Prenatal death was increased by Rh 123 when combined with 500 mg/kg 2-DOG (mortalities of 36, 43, or 59% for Rh 123 doses of 8, 12, or 15 mg/kg), while Rh 123 or 2-DOG alone had little or no effect compared to controls (9% and 19% vs 8%, respectively). Combined treatments decreased fetal weights and increased gross malformations (20% to 33% malformed vs 0% to 3% for controls or either inhibitor given alone). Skeletal malformations were seen, up to 72% at the high combined dose. In subsequent tests, another cationic rhodamine (6C) was maternally lethal at 6 mg/kg/day while two neutral rhodamines (B and 116) had little effect (given with 2-DOG) at doses eqimolar to those for Rh 123. Such results suggest that rhodamine dyes may be useful to test the role of altered mitochondrial function in teratogenesis. (Supported by grant #1238, University of Alabama Research Grants Committee)

Timed pregnant rats were exposed to NB vapor at 0, 1, 10, or 40 ppm (mean analytical values of 0.0, 1.06, 9.8, and 39.4 ppm, respectively) on gestational days (gd) 6 through 15, 6 hr/day. At sacrifice on gd 21, fetuses were evaluated for external, visceral, and skeletal malformations and variations. Maternal toxicity was observed: reduced weight gain during the exposure period at 40 ppm, and elevated absolute and relative spleen weight at 10 and 40 ppm. There was no effect of treatment on maternal liver, kidney, or gravid uterine weights, on pre- or postimplantation loss including resorptions or dead fetuses, on sex ratio of live fetuses, or on fetal body weights per litter. There were also no treatment-related effects on the incidence of fetal malformations or variations. In summary, there was no embryofetal toxicity (including teratogenicity) at exposure concentrations of NB during organogenesis in CD® rats which produced some maternal toxicity (10 and 40 ppm) or at an exposure concentration which produced no observable maternal toxicity (1 ppm). Research was sponsored by the Nitrobenzene Association.


Previous studies have shown that pregnant rats exposed to 50 or 100 ppm ACR during gestation and lactation produce litters which exhibit reduced postnatal growth (Zenick et al., 1985). The current research was designed to distinguish the pre- and postnatal components of this response utilizing a cross-fostering design. In the initial study, pregnant rats receiving 0 or 75 ppm ACR, were hyperactive (treated) and responded very poorly to cross-fostering with subsequent pup loss. In a second study, pregnant rats received 0 or 50 ppm ACR with five groups examined: Group A—pups born to and reared by control dams; Group B—pups born to ACR rats, reared by control dams; Group C—pups born to control rats, reared by ACR dams; D—pups born to and reared by ACR rats; and Group E pups reared by dams exposed to ACR only postnatally. Preliminary analysis revealed that prenatal exposure only had no effect on pup weight gain (Group B); however, postnatal exposure only (Groups C and E) was sufficient to depress growth. Interestingly, Group C showed a greater degree of growth retardation than Group D, suggesting that prenatal exposure may induce mechanisms of detoxification in the fetus. This abstract does not reflect EPA policy or opinion.


HCTZ, a thiazide diuretic, was evaluated for developmental toxicity in CD rats (0, 100, 300, or 1000 mg/kg/day) and CD-1 mice (0, 300, 1000, or 3000 mg/kg/day) dosed daily by gavage on gestational days (gd) 6-15. Timed-pregnant dams (>20/group) were sacrificed (gd 20, rats: gd 17, mice) and all fetuses were examined externally, visceral, and skeletally. In rats, maternal wt. gain during treatment was reduced at the high dose. HCTZ produced no dose-related fetal toxicity, and did not increase the incidence of malformations in CD rat fetuses. In mice, no significant signs of maternal toxicity were observed at any dose level. HCTZ did not affect any measure of embryo or fetotoxicity. A marginal increase in the incidence of malformations was observed, but was accounted for by two malformed fetuses in the high dose group, and no malformed fetuses in any other dose group. Thus, HCTZ treatment during organogenesis had no effect on prenatal survival, and produced no significant dose-related embryo or fetotoxicity. [Supported by NCTR/NTP Contract No. 222-80-2031(C)].


MAA, the proposed teratogenic metabolite of 2-methoxyethanol (ME), is equipotent to ME when given by gavage. Competitive inhibition of alcohol dehydrogenase (ADH) with ethanol (EtOH) or 4-methylpyrazole attenuates ME teratogenicity. Unexpectedly paw dysmorphogenesis induced by oral MAA is also altered by EtOH. Although ME teratogenicity depends primarily on ADH-mediated metabolism, subsequent metabolism and/or other biological processes might be influential. MAA teratogenesis and disposition (14C) in maternal and conceptus compartments were examined after gavage or tail vein (iv) injection. Mice received 2.9 or 3.8 mmole MAA/kg on gestation day 11. A reduction in paw malformations occurred after iv MAA compared to that produced following gavage. However, changes in the route of administration either influenced elimination phase kinetics of 14C from maternal blood nor altered embryonal accumulation of radioactivity (embryo/whole blood 14C ratio = 1.6). Our observations coupled with other findings that rat embryos accumulate MAA after ME dosing in vivo indicate that ME teratogenicity does not solely depend on embryonal MAA accumulation but involves other processes, e.g. further metabolism or tissue-specific disposition of MAA.

The teratogenic potential of the fungicide dinocap was tested in CD-1 mice. Pregnant mice were dosed by intubation with dinocap in corn oil on gestation days 7-16. Doses were used 0, 5, 10, 20, 40 and 80 mg/kg/day, based on d 6 weight. Dams were killed on d 18, at which time fetuses were counted, weighed, and preserved for necropsy or skeletal examination. Twenty-six of 90 dams in the 80 mg/kg group died during dosing. There was no dose-related maternal mortality at lower doses. Net maternal weight gain (wt gain minus gravid uterus weight) was affected at 80 mg/kg. The number of live fetuses per litter was decreased and resorptions increased at 80 mg/kg. There were dose-related decreases in gravid uterus weight and fetal weight, which were significant at 10 mg/kg and above. Cleft palate was found in fetuses at 5(1/234; 0.4%), 20(46/195; 23.6%), 40(140/185; 75.7%), and 80(63/83; 75.1%) mg/kg. There was a dose-related increase in supernumerary ribs, and a low frequency of exencephaly at the highest dose. This study shows that dinocap is teratogenic in the CD-1 mouse at doses well below those causing maternal toxicity. We have previously found that dinocap also interferes with otolith formation (Gray et al., TCM in press), and data on effects on fetal otoliths will be presented.


Studies indicate that azo dyes containing benzidine (B), dimethylnbenzidine (DMB), or dimethoxybenzidine (DMOB) can be reduced by the intestinal microflora to their parent compounds, which are in turn responsible for producing tumors in a variety of organs and tissues. Congo Red (CR), a B-based dye produces testicular agenesis in male mice exposed in utero to days 6-12 of gestation (Gray et al., 1984). This study was undertaken to determine if other dyes could produce similar teratogenic effects. Pregnant mice were dosed orally on days 6-12 of gestation at the rate of 1 g/kg/d with one of the following dyes: Trypan Blue, Chlorazol Black (CBE), Evans Blue, Benzoipurpurin 4B, Naphthol Blue Black or Sudan III. The growth and viability of the pups was monitored and the results were checked for prepuetal separation as an index of puberty. The males were necropsied at 49 days of age and the reproductive organs were weighed. The target organ in this study, the testes, were smaller in the males exposed in utero to CR or CBE. Histological examination of the testes of these animals revealed seminiferous tubular atrophy accompanied by apparent interstitial cell hyperplasia surrounding these atrophic tubules. Results of the present study demonstrate that B-based (CR and CBE) but not DMB or DMOB-based dyes causes testicular agenesis in the fetal male mouse.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
Oxocetyl 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 constituent. It is a solvent developed primarily for use in surface coatings. The teratogenic and fetotoxic potential of oxocetyl acetate was evaluated in Sprague-Dawley rats treated from Day 6 to Day 15 of gestation (GD). The test material was administered via oral gavage at doses of 0, 100, 500, and 1,000 mg/kg. Rates were sacrificed on GD 20 and the fetuses were examined for external, visceral, and skeletal malformations. The high dose (1,000 mg/kg) resulted in significant maternal toxicity as evidenced by reductions in body weight gain and food consumption. A slight, but not statistically significant increase in resorptions was observed in these animals. Signs suggestive of embryo/fetotoxicity were not observed in the mid or low dose animals. The mean number of malformed fetuses per litter was not statistically different between the control and any dose group. The number of litters with at least one malformed fetus was statistically elevated in the high dose group. However, this finding cannot be dissociated from the maternal toxicity which was produced. No teratogenic effects were observed at maternally nontoxic doses.

The teratogenic potential and developmental effects of acetonitrile were evaluated in rats. Aqueous solutions of acetonitrile were administered by gavage on days 6 through 18 of gestation at doses of 0, 2.0, 15.0, and 30.0 mg/kg/day. Doses were sacrificed on day 29 and the number of corpora lutea, implantations, early/late resorptions, and live/dead fetuses were recorded. Fetuses were weighed and examined for skeletal malformations and developmental variations. Maternal toxicity was expressed in the high dosage group as increased incidence of maternal death, abortion, anorexia and suppressed weight gain. Agent-related suppression of body weight gain was evident in the dams of the middle dosage group. No evidence of maternal toxicity was observed in the low dosage group. At the high dose, a significant decrease occurred in the average number of live-born fetuses. No agent-related embryo or fetal effects were observed at the middle or low doses. On the basis of these data, acetonitrile is not considered a developmental toxicant in rabbits at doses below those producing maternal toxicity.

Female New Zealand White albino rabbits (Langshaw Farms) were bred, using natural breeding techniques and orally dosed with 3 mg/kg of 6-amino-5-nicotinamide on either gestation day 9 (Group I) or gestation day 11 (Group II). The day breeding was confirmed was designated as gestation day 0. The does were observed daily for mortality, morbidity, overt toxic signs, and signs of abortion. All surviving does were sacrificed on gestation day 28, uterine contents were examined and findings recorded, and viable fetuses were examined for external development, weighed, and sacrificed. Each fetus was examined for visceral anomalies, sexed, and eviscerated. The heads were removed from approximately ½ of the fetuses/litters, retained in Bouin's fixative, and examined for internal anomalies; the remaining carcasses and fetuses were retained in alcohol and subsequently processed and examined for skeletal findings. Maternal toxicity (2 Group II does died and 2 aborted; 1 Group I doe died and 1 aborted) and fetotoxicity (82% of the Group I fetuses and 45% of the Group II fetuses resorbed) were noted. External, skeletal, and internal head anomalies were noted for 92 to 100 percent of the viable fetuses obtained.

Atrazine Technical was evaluated for its embryotoxic, fetotoxic, and teratogenic potential in rats and rabbits. The compound was orally administered at 0, 10, 70, or 700 mg/kg/day (MKD) to groups of rats on gestational days 6-15, while rabbits were administered dose of 0, 1, 5, or 75 MKD on gestational days 7-19. Maternal toxicity was observed at doses ≥ 70 MKD in rats, and at doses ≥ 5 MKD in rabbits. Fetotoxicity, resulting from maternal toxicity, was observed in rats at doses ≥ 70 MKD. In rabbits, embryotoxic and fetotoxic effects were observed at the maternally toxic, 75 MKD dose level. There were no adverse maternal or fetal effects in either rats or rabbits at the low dose levels. These findings indicate that Atrazine Technical is not teratogenic in these species.

Bis(2-dimethylaminoethyl)ether (NIA® Catalyst A-99, CAS No. 3303-62-3) has wide use in the manufacture of polyurethane foams. Timed-pregnant does were exposed to A-99 by daily dermal application on gestational days (gd) 6 through 18, 6 hr/day, at concentrations of 1.0 ml of 0, 1, 5 or 10% (v/v) in water. An untreated control group was also employed. At scheduled sacrifice on gd 29, fetuses were evaluated for external, visceral and skeletal malformations and variations. Maternal toxicity was indicated by decreased weight gain during the exposure period at 10%, severe skin erythema and edema at the application site at 5% and 10% with some recovery (slight skin irritation was observed at 1% with rapid total recovery), increased relative kidney weight at 10%, and histopathologic changes in kidneys at 5 and 10% (vacuolar swelling in collecting ducts). No treatment-related effects were seen on pre- or postimplantation losses. Fetotoxicity, expressed as reduced fetal weight/litter, was observed at 10% A-99. There were no effects of treatment on the incidence of fetal malformations or variations. The NOEL was 1% for does and 5% for conceptuses after dermal exposure to A-99 during organogenesis. Research was sponsored by UCC, Silicones and Urethane Intermediates Division.


The aerosol formulation of procateral hydrochloride hemihydrate, a new beta-agonist bronchodilating agent, was evaluated for teratogenic potential in rats by nose-only exposure. Groups of 25 pregnant Sprague-Dawley rats were exposed to filtered room air (negative control), placebo aerosol (vehicle control), or active aerosol at 10, 50 and 100 fold multiples of the estimated human clinical dose of 60 μg/day. Exposures were 1 hour/day on gestation Days 6 through 17. All groups were observed daily for clinical signs of toxicity. Body weight and food consumption were recorded on gestation days 0, 6, and 18 through 20. Sacrifice and cesarean section examinations were performed on Day 20. Fetuses were weighed individually and examined grossly for external malformations. Approximately one-half of the fetuses were subjected to soft-tissue examination while the remaining fetuses were cleared for skeletal evaluation. Animals died during the study and there were no drug-related changes. Thus, procateral aerosol did not evoke a teratogenic response under the conditions of this study in this species.

TERATOGENICITY OF ALCIDE ALLEY® IN RABBITS. M. Abdel-Brahim, S. Gerges, G. Skowronski, R. Turkall, and S. Von Hagen, Pharmacology Dept., NJ Medical School, Newark, NJ.

Alcide Alley is a highly effective germicidal compound. Alley was evaluated for teratogenic potential in rabbits. Pregnant rabbits were administered 2.0 g/kg Alley gel (containing 1.2% or 0.3% g NaClO as active ingredient) or placebo topically on days 6 through 18 of gestation. Maximum erythema was recorded in the high dose group on day 11 (Draize score = 3.5). By day 18, all rabbits' skin in the high dose treatment appeared normal. Maternal toxicity as evidenced by decreased body weight gain was observed in the low dose and placebo gel groups. Fetal weights and lengths were significantly reduced in all gel groups. There was some incidence of skeletal anomalies in all gel treated groups, however, these incidences were not statistically different from control. The commonly occurring skeletal defects were incomplete ossification of skull bones, small or missing sternebrae and extra ribs. The incidence of visceral abnormalities in all gel groups was not statistically different than control animals. Visceral anomalies included heart displacement, fusion or serratation of the liver, and kidney displacement. Alcide Alley gel at the concentrations tested was non-teratogenic to rabbit fetuses.

AN INHALATION TERATOLOGY STUDY IN THE RABBIT WITH NITROBENZENE. K.E. Schroeder, J.B. Terrill, J.P. Lyon, A.M. Kaplan, and G. Kummel.

This inhalation teratology study was conducted to evaluate maternal toxicity, as well as embryotoxic, fetotoxic and/or teratogenic potential of nitrobenzene (NB) in the rabbit. In a pilot study, NB administered to pregnant rabbits (12/group) at targeted dose levels of 0, 10, 40 and 80 ppm from Days 7 to 19 of gestation produced a statistically significant increase in methemoglobin values at 40 ppm on Day 20 and at 80 ppm on Days 13, 19 and 20. In the teratology study, NB was administered to pregnant rabbits (22/group) at target exposure levels of 0, 10, 40 and 100 ppm for 6 hrs/day from Days 7-19 of gestation. Females were sacrificed at gestation Day 30 and fetuses evaluated for external, visceral and skeletal malformations. Cumulative mean analytical concentrations were 9.9, 41 and 101 ppm NB. No adverse effects of treatment were evident at the 9.9 ppm level. At the 41 and 101 ppm levels, liver weights were slightly higher than control liver weights and methemoglobin values (Day 30) were significantly increased as compared to control animals. At 101 ppm a slight increase in fetal resorption was seen. No teratogenicity was evident at any levels evaluated.
Because of its incorporation into a wide range of commercial products, the potential exists for dermal exposure to ethylene glycol monomethyl ether (EGME) during pregnancy. The purpose of this study was to assess the teratogenic potential of various EGME doses when applied dermally to the rat. Three groups of timed mated F344 rats were dermally exposed to EGME on days 7 through 16 of pregnancy at doses of 1.0, 0.5, and 0.25 g/kg applied in 5 divided doses over an 8 hr. period. Maternal toxicity was evaluated using hematological parameters and weight gain. C-sections were performed after 20 days of gestation; half the fetuses were evaluated for soft tissue malformations while the other half were examined for skeletal defects. No signs of maternal toxicity were noted. Dams receiving 2 ml/kg EGME differed significantly (p < .01) from control and other treatment females in number of live fetuses per dam, number of resorptions per dam, fetal body weight, and fetal crown-rump length. Teratogenic effects included unfused centra, delayed sternebral ossification, right aorta, and dilatation of the renal pelvis. The total malformations observed in each of the treatment groups were dose related and significantly greater (p < .01) than seen in the control group.

4-NPI is an aromatic molecule with a displaceable nitro group and a reactive phthalimide function. Studies in rabbits were conducted to determine the teratogenic potential of 4-NPI and the similarity of any fetal response to that induced by thalidomide. 4-NPI was administered to New Zealand White rabbits by gavage in carboxymethyl cellulose at dosage levels of 10, 50, and 200 mg/kg/day on days 6-19 of gestation. Thalidomide was administered at 150 mg/kg/day for the same period. Fetuses from thalidomide treated does exhibited the malformations and mortality typically induced by this drug. At 200 mg/kg/day, 4-NPI administration led to maternal toxicity as evidenced by anorexia and body weight loss during treatment, with a dramatic "catch-up" after treatment. Correspondingly, fetal death was increased in this group, although the incidence was not as remarkable as in the thalidomide group. An increase in fetal malformations was evident in the high-dose 4-NPI group; however, these malformations were neither as frequent nor of the same type as those induced by thalidomide. Administration of 4-NPI at 10 mg/kg/day produced no evidence of embryotoxicity or teratogenicity.
ALTERATION OF RETINOIC ACID MOLECULAR STRUCTURE AND TERATOGENICITY IN HAMSTERS. W.B. Howard, C.C. Willhite, and R.P. Sharma. Center for Environmental Toxicology, Utah State University, Logan, UT

Retinoids are used in treatment of cancers and dermatologic disease. Certain congeners of all-trans-retinoic acid (RA) are human teratogens. The teratogenicity of ethyl all-trans-9-(4-methoxy-2, 3, 6-trimethylphenyl)-3, 7-dimethyl-2,4, 6, 8-nonatetraethylamine (Motretinid; MR), ethyl all-trans-9-4-hydroxy-2, 3, 6-trimethylphenyl)-3, 7-dimethyl-2, 4, 6, 8-nonatetraenoate (Roli-476b; Roli) and all-trans-9-(2-acetyl-5, 5-dimethyl-1-cyclopenten-1-yl)-3, 7-dimethyl-2, 4, 6, 8-nonatetraenoic acid (R06-7699; R06) were evaluated in hamsters and compared to RA. Pregnant hamsters were dosed orally with retinoids at 10 AM on day 8 and fetuses obtained on day 14 by laparotomy. The ethylamide congener (MR) of etretinate, a potent teratogen in hamsters (FAAT 4;977, 1984), had no teratologic effects upon maternally toxic levels (330 mg/kg). Other congeners induced malformations similar to those induced by RA. The effective dose for R08 was similar to that of RA, whereas R06 had similar effects at doses 6 times higher than RA or R08. The results suggest that a hydroxyl residue at carbon 3 reduced teratologic effects compared to its methoxy congener, etretinate. Replacement of the ring in RA by a cyclopentyl structure as in R08 failed to alter its teratogenicity. (Supported in part by HD 21399. Retinoids were gifts of Hoffmann-LaRoche, Inc.)

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BIOCHEMICAL STUDIES ON THE ROLE OF BIOTRANSFORMATION IN MYCOTOXIN-INDUCED EMBRYOTOXICITY IN VITRO. F. Geissler, D. Eaton and E. Faustman-Watts. Department of Environmental Health, University of Washington, Seattle WA

The objective of this investigation was to examine the embryotoxicity of aflatoxin B1 (AFB1) using an in vitro culture system and to define biochemically the role of biotransformation in this toxic process. We have observed that AF6B1 is both teratogenic and embryolethal in cultured day ten rat embryos. The spectrum of teratogenic effects by AF6B1 includes decreased embryonic size, decreased macromolecular content, neural tube abnormalities, and areas of tissue necrosis dorsal to the mandibular arches. The addition of hepatic S-9 fractions and cofactors (complete monooxygenase system) from adult male rats preincubated with either phenobarbital or 3-methylcholanthrene to the embryo cultures substantially increased the embryolethal but not the teratogenic potency of AF6B1. Furthermore, no qualitative change in the types of malformations produced by culture condition was noted (AF6B1 alone or with a complete monooxygenase system). Studies conducted in our laboratory using radiolabeled AF6B1 and high pressure liquid chromatographic techniques have allowed us to examine specific metabolites generated in our embryo culture system under these embryolethal and teratogenic conditions. These studies were supported by NIH grants ES-03157 and ES-07032.

BIOCHEMICAL INDICATORS OF THYROID HORMONE ACTIVITY IN RAT MATERNAL AND FETAL TISSUES FOLLOWING EXPOSURE TO NITROFEN. C.L. Tellone and J.M. Mansson, Department of Reproductive and Developmental Toxicology, Smith Kline and French Laboratories, Philadelphia, PA

Previous studies have suggested that nitrofen may be teratogenic via alterations in maternal and fetal thyroid hormone status and is metabolized to a T₃-active intermediate. To further evaluate the thymic activity of nitrofen, pregnant SD rats were exposed to nitrofen at 50 mg/kg on days 6-15 of gestation. Maternal and fetal T₃ and T₄ serum levels, malic enzyme activity in liver and lung, and fetal lung surfactant content, biochemical markers of thyroxine exposure, were evaluated on days 15-21. Maternal serum T₃ levels were significantly elevated reflecting an increase in endogenous T₃ or the detection of nitrofen/metabolites as T₃. Maternal serum T₄ levels initially depressed, rose to exceed control values in an apparent negative feedback to the elevated T₃ levels. Alterations in fetal parameters included the precocious appearance and elevation in lung phospholipid (surfactant), a decrease in fetal T₄ levels and an elevation in malic enzyme activity in the fetal lung indicating that a thymic response has been evoked in nitrofen-treated fetuses. These data support further investigation of the relationship between nitrofen's thymic properties and perturbed fetal development.
381 ESTRODIOL (E2), ESTRIOL (E3) AND TAMOXIFEN (TAM)
INDUCTION OF AN ORNITHINE DECARBOXYLASE (ODC)
ACTIVITY REFRACTORY PERIOD IN THE RAT UTERUS.
W.A. Smith, H. L. Medlock, W.S. Branhman, and D.M.
Sheehan, Division of Reproductive and Developmental
Toxicology, National Center for Toxicological
Research, Jefferson, AR and University of Arkansas.
For Medical Sciences, Little Rock, AR

Estrogens are uterine developmental toxicants.
In the immature rat uterus, DNA synthesis is re-
fractory to multiple E2 doses. E2 is known to
induce a 6-hr peak of uterine ODC activity and
subsequently, DNA synthesis in 5-day-old rats.
We tested the hypothesis that E2, as well as the
partial agonist E3 and the agonist/antagonist
TAM, induce a refractory period for uterine ODC
activity. Rats (5 day, ~14 gm) were injected sc
at both 10 and 18 hr with E2 or E3 (10 ug/10 ul),
TAM (100 ug/10 ul) or vehicle (10 ul sesame oil)
and sacrificed at 24 hr. ODC activity in 0 plus
18 hr controls (vehicle) was 1.0 + 0.17 U (mean
+ SD; U = mmol CO2/hr/mg protein). Maximal ODC
activity was found after 0 hr vehicle plus 18 hr
E2 or E3 (6.25 + 0.80 U; 6.49 + 0.23 U, respec-
tively). E2 at 0 hr followed by E2 or E3 at 18
hr decreased ODC activity (3.26 + 0.08 U; 3.89 +
0.70, respectively). E3 or TAM at 0 hr plus 18
hr E3 decreased ODC activity to the same extent
(4.19 + 0.69 U; 4.09 + 0.35, respectively).
Thus, E2, E3 and TAM induce a refractory period
in the rat uterus, as measured by ODC activity,
suggesting that they may work by similar
mechanisms.

382 INHIBITION OF UTERINE GROWTH, ORNITHINE DECAR-
BOXYLASE (ODC) ACTIVITY, AND DNA ACCUMULATION IN THE
IMMATURE RAT. W.S. Branhman, H.L. Medlock, M.L.
Leamons, P.J. Webb, and D.M. Sheehan, Division of
Reproductive and Developmental Toxicology, Na-
tional Center for Toxicological Research, Jeff-
erson, AR

Tamoxifen (TAM) is a developmental toxicant as
demonstrated by its inhibition of uterine gland
growth in the postnatal rat. We examined estro-
gen agonist and antagonist effects of increasing
doses of 17b-estradiol (E2), TAM, and mono
hydroxylamino
tamoxifen (OH-T) on uterine weight, ODC activity,
and DNA accumulation in ovariectomized rats. TAM
and OH-T elicited dose-related ODC activity compar-
able in magnitude to the 6- and 18-hr E2-induced
ODC activity; however, peak activity is delayed
at lower doses. Dose-response curves at 6 hrs
after E2, TAM, or OH-T indicate correspondence
between ODC activity and uterine growth in-
creases were also dose-related (E2OH-T) TAM) 48
hrs after administration; however, TAM and OH-T
elicit only 50% of the maximal E2-induced DNA
response. In rats pretreated for 48 hrs with doses
of E2, TAM, or OH-T and followed by a single dose
of E2 (10 ug/rat), both 6- and 18-hr E2-induced
ODC activity as well as uterine weight and DNA accumulation were inhibited.
These data indicate that the long-term (48-hr)
inhibitory effects of E2, TAM, or OH-T on E2-
induced uterine responses are related to short-
term ODC activity inhibition and may result from
refractoriness to further stimulation.

383 DETECTION OF DNA DAMAGE IN RODENT EMBRYOS EX-
POSED TO ALKYLATING AGENTS IN VITRO: CORRELATIONS WITH
DEVELOPMENTAL TOXICITY. E. Faustman-Watts, S. Little,
Z. Kirby and P. Mirkes, Deps. of Environmental Health and Pediatrics,
Univ. of Washington, Seattle, WA.

The objective of this investigation was to de-
termine the extent of DNA single-strand breaks
in embryos exposed in vitro to alkylating agents
that produce developmental toxicity. A series
of methylating and ethylating agents were used
at concentrations that produced significant in-
creases in embryo malformations. These studies
are one of a group of investigations in our
laboratory that are aimed at identifying bio-
chemical correlates of alkylating-induced de-
velopmental toxicity. The observations dis-
cussed in this abstract were made on embryos cul-
tured in a modified whole rodent embryo culture
system. Day 10 Sprague-Dawley rat embryos were
exposed for 2 hours, then removed from culture
and trypsinized to produce a cell suspension.
These cells were then analyzed by alkaline elu-
tion techniques to detect the presence of
single-strand DNA breaks. Significant levels of
DNA damage were detected at or below those
concentrations producing developmental toxicity.
Relationship of embryo viability, malformation
and DNA damage dose-response curves was highly
agent-dependent. These studies were supported by
NIH grants ES-03157 and HD-12647.

384 IN VITRO CULTURE OF POST-IMPLANTATION HAMSTER
EMBRYOS - A SECOND APPROACH. M.T. Ebbon, P.E.
Beyer, L.A. Oglesby, R.J. Kavlock, U.S. EPA, and
Northrop Services, Inc., Research Triangle Park,
NC. Sponsor: R.W. Chadwick

In vitro culture of intact rat and mouse embryos
has been described extensively, but information
on the culture of whole hamster embryos is sparse.
The present studies examine the culture require-
ments of early somite stage hamster embryos and
assess the embryotoxic effects of sodium salicy-
late on these embryos. Hamster embryos explanted
GD-6.0 and 9.0 were cultured in Weymouth's
Embryo-Hepatocyte Co-Cultivation Medium (EHCM),
70% McCoy's-30% male rat serum (MRS) or 100% male
rat serum (MRS) for 24 hrs under 4% O2, 5% CO2,
5% N2. In a second study Sodium salicylate was
added to MRS in concentrations of 250, 500, and
400 ug/ml. Embryos cultured GD-9 to 10 grew best in
MRS. Hamster embryos GD-8, explanted at the same
somite stage as mouse embryos, completed the major
phases of organogenesis within 24 hrs compared to
36-48 hrs in the mouse. Embryos exposed to sodi-
um salicylate in MRS died of embryotoxicity.
CNS defects, lack of hind limb buds and lack of
axial rotation were the major anomalies observed.
Thus, post-implantation hamster embryos develop
normally in culture and the sodium salicylate
embryotoxicity seen in hamsters in vivo compares
well with that seen in hamsters in vivo.
Nitrosoureas (NF) produces abnormal axial rotation in day 10 rat embryos cultured for 24 hr in vitro. At initial concentrations of 25 μM, NF caused a 50% incidence of incomplete axial rotation and abnormal tail morphology when these criteria were used as a single endpoint. Addition of 1 mM L-buthionine-S,R-methionine (L-BSO), an inhibitor of glutathione (GSH) synthesis, at the beginning of the culture period potentiated the embryotoxic effects of NF resulting in a 80% malformation rate. L-BSO alone caused no perceptible decrease in embryonic GSH but reduced levels in the yolk sac by 50%. When NF and L-BSO were both added to the culture medium, the levels of GSH were significantly reduced in both the embryo (45%) and the yolk sac (70%). Addition of 5 mM 2-oxoacetyl-L-seleno-cysteine (OAC) to culture medium containing NF prevented a 20% malformation rate, indicating that OAC provides protection against the effects of NF. Although OAC supplies additional cysteine for GSH synthesis, no changes in GSH levels were observed after 24 hr in embryos or yolk sacs when OAC alone was added to the culture medium. (Supported by NIH grants HD-01281 and RR-07032)

The distribution of [14C]acrylamide was studied by whole-body autoradiography in male and pregnant mice. The V3.5- and 17.5-day pregnant Swiss Webster mice. Mice were killed at 0.5, 2, 6, 12, and 24 hr. The pregnant mice were frozen at 3 and 24 hr. The major sites of radioactivity in the male were GI contents, epithelium of oral cavity, esophagus and bronchi, liver, pancreas, testes, brain and gallbladders. By 3 hr absorption from the stomach was virtually complete and most of the renal and hepatic elimination was complete by 1 day. There was prolonged elimination of radioactivity from the testis through the epididymis; the only radioactivity remaining at 9 days was in the tail of the epididymis and the crypts of the epithelium of the penis. The pharmacodynamics appear to be similar in the male and pregnant mice. Fetal tissues were fairly uniformly labeled in the 13.5-day mouse, but selective uptake was seen in the 17.5-day fetus. The most remarkable site was the fetal skin. This study reveals that in mice acrylamide is efficiently absorbed from the stomach and eliminated by the liver, kidney and possibly the pancreas. The mechanisms of localization in fetal skin and the epididymis deserve further study. (Supported by Pharmacol Research Foundation, Inc.)

The ability of aluminum, administered prenatally, to cross the placental barrier and to accumulate in fetal tissue was tested in mice using both intraperitoneal (ip) and oral routes of exposure. Pregnant BALB/c mice were dosed with 100 or 150 mg/kg/day AlCl3 saline ip on days 7 through 15 of gestation and with 200 or 300 mg/kg/day of the same preparation orally over the same period of gestation. Control animals were similarly treated with saline. Individual fetuses, their placental sacs, and maternal livers were assayed for aluminum content by electrothermal atomic absorption spectroscopy on oven-dried samples. There was a three-fold increase in total body aluminum content in fetuses administered 100 mg/kg/day compared to controls (592 ± 178 vs. 208 ± 705 μg/g dry weight; p < 0.001) At a dosage of 150 mg/kg/day (ip), the increase was not as marked, but still significant (1276 ± 576 μg/g, p < 0.05). In animals dosed at 100 mg/kg/day (ip), the placenta had an aluminum content over ten fold greater than controls (2750 ± 6046 vs. 2677 ± 1804 μg/g; p < 0.001). Oral dosing did not increase placental aluminum to the same extent (200 mg/kg/day - 5909 ± 2323, p < 0.005; 300 mg/kg/day - 3068 ± 2274 mg/g NS). Maternal liver aluminum content was increased only in the ip-dosed animals. It is apparent that the body burden of aluminum in fetal mice is significantly increased following both ip and oral dosing of AlCl3 and that intraperitoneal dosing at 100 mg/kg/day resulted in maximum fetal and placental aluminum content.

MATERNAL / PLACENTAL / FETAL DISTRIBUTION OF ALUMINUM IN MICE FOLLOWING IN UTERO EXPOSURE. Donald J. Cannon, Joan M. Cramer*, Letha Smith and J. Dale Wilkins*. Departments of Biochemistry and Pediatrics*, University of Arkansas for Medical Sciences and V.A. Medical Center, Little Rock, AR.

The majority of research on male reproductive toxicity has examined single agent exposures. The purpose of this study was to examine reproductive risks in workers with multiple chemical waste exposures. Two strategies were employed: 1) a retrospective study examining the relationship between work and reproductive histories and 2) evaluations of semen. The exposures in this population are multidimensional, varying in chronicity, periodicity, dose, and number of agents. A number of exposure models have been developed and applied to the data. The reproductive history was obtained from the wives of the 231 employees, 141 exposed and 90 unexposed. Of the 231 women interviewed a total of 464 pregnancies were generated.

Initial analyses, assessing the worker's exposure 4 months prior to the wife's pregnancy and potential fetal loss have revealed no significant trends. The results of these analyses will guide the selection of the most appropriate model(s) to apply to the semen data. (Supported by March of Dimes 15-50)


This study assessed the possible relationship between methyl chloride (MeCl) induced epididymal inflammation and the formation of dominant lethal mutations in sperm of F-344 rats. Groups of 40 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 postexposure), previously shown to inhibit MeCl-induced epididymal inflammation. Each male was caged with one female weekly for 3 weeks; females were killed 13-18 days after mating. MeCl caused significant increases in postimplantation loss at postexposure week 1 (0.84/female vs. 0.29 control) and in dead implants/total implants at both week 1 (0.10 vs. 0.04 control) and week 2 (0.24 vs. 0.06 control). These increases were not observed in females bred to males treated with BW755C during MeCl exposure. Co-administration of BW755C to males along with MeCl reduced the percentage of mated females with two or more postimplantation losses from 31% to 6% (week 1) and 50% to 12% (week 2). Thus, the dominant lethal mutations induced by MeCl appear to be secondary to its coincident induction of inflammation in the epididymis. This study demonstrates the potential genotoxicity of in vivo inflammatory processes.


Methyl chloride (MeCl), a known testicular and epididymal toxicant in F-344 rats, induced a significant increase in pre-implantation (PI) loss when assessed in a dominant lethal test (DLT). This study examined the possibility that these losses were due to failure of fertilization and not to pre-implantation embryonic death. Groups of males were exposed to 3000 ppm MeCl 6 hr/day for 5 days or to triethylenemelamine (TEM, 0.2 mg/kg i.p. on day 5) and bred to 2 females weekly for up to 8 weeks. Females were killed 12 hr post-mating, embryos and ova isolated and scored as fertilized or unfertilized based on the presence or absence of sperm heads within the egg. The percentage of unfertilized ova in the MeCl group ranged from 33 to 93% over 8 weeks and equaled or exceeded the frequency of PI loss recorded in the DLT during each week (14 to 94%). In contrast, although the known genotoxic TEM induced 71% PI loss in the DLT during week 3, only 22% of the TEM group were unfertilized at this time point. Failure of fertilization in the MeCl group likely was due to the sperm damage resulting from MeCl exposure. The data suggest that the PI losses induced by MeCl are due to the cytotoxic, not genotoxic, effects of the exposure.

IMPRINTING OF HEPATIC MONOOXYGENASE ACTIVITY BY NEONATAL POLYCHLORINATED BIPHENYLS (AROCLOL 1254) EXPOSURE. E.M.K. Lui. Department of Pharmacology and Toxicology, University of Western Ontario, London, Ontario, Canada.

Hepatic arylhydrocarbon hydroxylase (AHH), ethylmorphine N-demethylase (EM) and estrogen 2-hydroxylase (E2OH) activities were higher in adult male than in female rats. The administration of Arochlor 1254 (A) (20 or 40 mg/kg, s.c.) to male and female rats on alternate days during the first 20 days of age resulted in 2-3 fold increases in hepatic AHH and EM as determined at 70-80 days of age. Hepatic E2OH of adult males was, however, reduced by such treatment. Similar treatment effects were observed when the animals were examined at 150 days of age. Since A is known to be an enzyme inducer, the effect of exposure to this agent (40 mg/kg, for 3 consecutive days) in adult males was also studied. Marked induction of AHH and EM was observed during the first post-treatment week; however, the effect was greatly reduced when examined 2 weeks later. Although E2OH was inducible by A, the effect was relatively small. These data suggest that the observed neonatal effect was probably not due to residual enzyme induction effect of A but rather reflect its effect on imprinting. This speculation was supported by the finding that treatment of male rats with A between day 10-30 postpartum failed to alter these hepatic parameters when examined at 90 days of age.
Environmental pollutants such as DDT and poly- 
chlorinated biphenyls are potent inducers of 
liver mixed function oxidase (MFO) enzymes. 
Because of their high lipid solubility, these 
compounds are mobilized into milk during 
lactation. Lactational exposure of neonates to 
enzyme inducing agents may increase their 
susceptibility to genotoxic agents which are 
activated by MFO enzymes. In order to test 
this hypothesis, maternal rats were treated 
with Aroclor 1254 on days 7-10 postpartum, 
following which induction of DNA repair by 15 
genotoxic chemicals was evaluated in primary 
neonatal hepatocytes. DNA repair responses 
to several aromatic amines and polycyclic 
aromatic hydrocarbons were dramatically 
increased by lactational exposure to Aroclor. 
Similarly, administration of DDE to female 
rats for 5 weeks prior to mating, throughout 
gestation and up to postpartum day 11-12 
resulted in a large increase in the neonatal 
DNA repair response to dichlofluanide. 
These findings suggest potential synergisms 
between exposure of neonates to DNA-reactive 
chemicals and enzyme-inducing agents received 
via the milk.

A SCANNING ELECTRON MICROSCOPIC STUDY OF CRANIOPACIAL DYSMORPHIA INDUCED BY ISOFETINOL. D.W. 
Irving and C.C. Willhite, WRRC, USDA, Berkeley, 
CA and Hazard Evaluation Section, Dept. Health 
Services, State of California, Berkeley, CA

ORL administration of 40-80 mg/day of 13-cis- 
retinoic acid (isoretinoin) during the first 
trimester of human pregnancy induces severe 
congenital malformations: rudimentary external 
eyes, stenotic auditory canals, cleft palate, 
triangular microcephalic skull, micrognathia, 
depressed maxilla and brain and heart defects. 
A similar malformation pattern occurs in fetal 
hamsters treated with isoretinoin. Pregnant 
hamsters were given a single oral dose of 50 mg/ 
kg isoretinoin or the vehicle at 10:00 A.M. on 
day 8 of gestation. The embryos or fetuses were 
collected at 4, 8, 12, 24, 48 and 72 hr after 
treatment, fixed in 2.5% glutaraldehyde, post- 
fixed in 1% OsO₄, sputter-coated with gold and 
viewed in a Hitachi S-530 scanning electron 
microscope. Craniofacial changes were evident 
in within 8 hr of treatment and progressed in 
severity with increasing gestational age. 
Hypoplasia of the mandibular process of the first 
branchial arch, a reduced branchial pouch, 
rudimentary second branchial arch and collapse of 
the prosencephalon were observed. Hypoplasia of 
the components of the first branchial arch, 
perhaps a result of a deficiency in the neural 
crest, can account for retinoic-induced 
malformations of the face, jaw and ears. 
(Supported by NIH Grant NC 23395.)

DISRUPTION OF THE REPRODUCTIVE PROCESS IN 
FEMALE RATS RESULTING FROM CONSTANT EXPOSURE TO 
LIGHT. L. S. Tutak# and A. T. Arthur, 
CIBA-GEIGY Pharmaceuticals, Summit, NJ. 
#Present Address: Stuart Pharmaceuticals, 
Wilmington, DE. Sponsor: L. E. Conger.

An acclimation colony of 119 virgin female 
Sprague-Dawley rats had promating vaginal 
washings performed in preparation for a Segment 
III reproductive toxicology study. The 
washings indicated that 96% of these animals 
had irregular estrous cycles. Most were in 
constant estrous. It was subsequently 
discovered that the automatic lighting system 
for their animal room had malfunctioned leaving 
them in constant light for an undetermined 
period of time (an estimated 7 weeks). After 
the animals were moved to a room with a 
properly functioning light/dark cycle (14/10 
hours), some recovery of estrous cycling was 
observed. After 4 weeks of proper lighting, 
39% had normal estrous cycles; after 7 weeks, 
47% were apparently normal. Ophthalmic 
examinations indicated no remarkable changes. 
Upon mating, only 41 of 119 (34.5%) achieved 
pregnancy through a 11-day cohabitation 
period. Normally, at least 90% would have 
have become pregnant. Therefore, exposure to 
constant light for approximately 7 weeks may 
have irreversibly disrupted these animals' 
reproductive processes.

K. Robinson and G.L. Mendoza. 
"Teratology of HI-6 in the Rat" 

HI-6 is an experimental chemical that is 
therapeutically effective against soman poisoning 
in laboratory animals. It is a promising 
chemical that may be used to replace toxogonin, 
which is ineffective against soman. As a part of 
the safety-in-use data, its teratogenic potential 
was studied in the rat.

Groups of mated female CD Sprague-Dawley rats 
were treated by the intramuscular route with HI-6 
at dosages of 0, 40, 120 or 240 mg/kg/day from 
day 6 to day 15 of gestation inclusive, and a 
third group were treated by the same route 
at dosages of 360 mg/kg/day from days 6 to 9, 9 
to 12, or 12 to 15 of gestation (D 6-9, D 9-12 
and D 12-15). Maternal toxicity was observed at 
the 240 mg/kg/day level and in the 3 groups 
treated at 360 mg/kg. The rats were sacrificed 
on day 20 of gestation, 2 fetuses per litter were 
examined histologically and the remaining fetuses 
were examined for external, visceral and skeletal 
abnormalities. Embryolethality was evidenced by 
significantly elevated postimplantation losses in 
the 360 mg/kg/D 6-9 and D 9-12 dosage groups. 
The incidence of fetuses with major malformations 
was significantly increased in the 240 mg/kg/day, 
360 mg/kg/D 6-9 and 360 mg/kg/D 9-12 groups. 
HI-6 was found to be teratogenic and fetotoxic 
when given at 240 mg/kg/day throughout major 
organogenesis, and was also embryolethal at 360 
mg/kg/D 6-9 and D 9-12.
Cytochromes P-450b and P-450c are highly homologous proteins which represent the major hepatic P-450s in the phenobarbital (PB) pretreated rat. Previous research in our laboratory has demonstrated that cytochrome P-450b and P-450c mRNAs are readily detectable late in fetal rat liver development after transplacental PB treatment. (Glachelli, C.M. and Omiecinski, C.J., JBC, 1986, in press). In the present study, hybridization techniques were utilized to determine the precise onset of PB-inducibility in fetal rats. Hepatic P-450b and P-450c mRNAs were first detected on gestational day 21. Parallel increases in PB-induced levels of P-450b and P-450c mRNA were observed with increasing age. Twenty-four days after birth a clear sex-specific expression of P-450b and P-450c mRNA was observed. In PB-induced male rats, both mRNAs levels were 2-3X greater than the corresponding adult levels. However, in female rats, equal or slightly lower than adult levels were noted. Studies utilizing polycymal RNA preparations indicated that P-450b and P-450c mRNAs were always associated with the polynucleotides, indicating active cellular utilization. Supported by NIN grant GM-32821 and Training Grant EH-07032.


Tb Pb is a toxicant which shares the neurotoxic properties of other organolements such as trimethyltin and triethyllead. We have previously demonstrated that after a single oral dosage, TMB is rapidly taken up into the blood, reaches a peak six hours post-dosing, and remains in the original species form. Differences in the clearance of TMB from blood at differing dosages may suggest a role for distribution in the neurotoxicity caused by the compound. The following study was designed to detect possible differences in the T1/2 and clearance of TMB from blood due to dosage. TMB (5,15,30 or 40mg/kg) was administered orally to Long Evans male (70 day old) rats and blood was drawn from the tail vein of each rat at 1, 7, 14, 21, 28, 35, 42, and 56 days post-dosing. The concentration of blood lead was determined by flameless AA. At a neurotoxic dosage of 40 mg/kg, Pb levels rose from 1 day post-dosing to 7 day post dosing. At a 30 mg/kg dosage Pb levels remained constant through the first 7 days post-dosing and then decreased up to 56 days post-dosing. At the lower dosages of 5 and 15 mg/kg the levels decreased from 1 day post-dosing to 56 day post-dosing. We demonstrated a dose-related difference in the pattern of Pb concentration in the blood over time. (Pol ES01104, T32 ES07126 and T32 ES07017)
The initial intracellular ligands for lead (in kidney of rats not pretreated with metals to induce metallothionein) have been shown to be 10,000 and 63,000 dalton cytosolic proteins which facilitate the intracellular transport of lead into rat kidney nuclei (Mistry et al., J. P. E. T. 232: 462-469, 1985). Attempts to isolate the 10,000 dalton receptor-complex have been hampered by dissociation of lead from the protein during anion exchange (DEAE) and reverse phase high performance liquid chromatography. Hydrophobic interaction chromatography has been used successfully to resolve the 10,000 dalton PbBP complex with retention times identical to metallothionein standards. The PbBP is also heat stable (85°C for 10 minutes) and as shown by Mistry et al., Biochem. Pharmacol. (in press), the ability of metal cations to displace lead from the complex parallels their increasing affinity for metallothionein (Pb > Zn > Cd > Cu). An endogenous 10,000 dalton renal protein was labeled with 65Zn and purified by the standard procedures for metallothionein (gel filtration and DEAE anion exchange chromatography). These data are consistent with the hypothesis that the endogenous renal 10,000 dalton PbZn-binding protein is metallothionein. (Supported by NRSA 5T32 ES-07126).

A low molecular weight high-affinity PbBP in kidney appears to account for the relative insensitivity of renal ALAD to Pb inhibition. A PbBP also exists in brain cytosol but is not detectable in liver. This study was undertaken to examine the relative sensitivity of brain and liver ALAD to Pb inhibition and to determine if inhibition of hepatic ALAD by Pb could be reversed by addition of partially-purified brain PbBP to liver cytosol. Determination of IC50 values showed that brain ALAD was 2-times more resistant to Pb inhibition compared to liver. A concentration-dependent reversal of Pb-induced inhibition of hepatic ALAD activity was observed for both brain and kidney PbBP. Inhibition of ALAD activity was partially reversed by addition of brain PbBP over a concentration range of 0.1-1.6 μM Pb. Incubation of 65Zn-labeled brain and kidney PbBP fractions with purified bovine liver ALAD demonstrated that the PbBP donate Zn to ALAD. Thus, brain PbBP confers resistance to Pb inhibition of liver ALAD in vitro, and may account for the relative insensitivity of brain ALAD to Pb inhibition. The mechanism for the attenuation of Pb inhibition of ALAD by brain and kidney PbBP appears to involve chelation of Pb and donation of Zn to the enzyme. (Supported by NRSA 1F32 ES-03107-01A1).
A previous study has shown that rising medium concentrations of lead (Pb) enhanced $^{45}$Ca uptake by osteoclastic bone cells (OC) in a dose-dependent manner (T.A.P. 71-031, 1971). These and other results in hepatocytes suggest that perturbations in cellular Ca metabolism induced by Pb may have the potential of disturbing multiple cell functions that depend upon Ca as a second messenger. This study was undertaken to characterize the steady state kinetic distribution of $^{45}$Ca as a function of medium Pb levels. Bone cells, derived from mouse calvariae, were enriched for OC by a sequential collagenase digestion and maintained in primary culture for 1 week. OC were then incubated for 20 hr in medium containing 0, 10 or 50 pM Pb and $^{45}$Ca; and the kinetic parameters were obtained by analysis of $^{45}$Ca washout curves. Three kinetic pools of intracellular Ca were resolved; and rising medium Pb concentrations (0 - 50 pM) produced a marked increase in total cellular Ca (16.64 + 3.18 nmol/ng cell protein). The largest increase (>300%) was found in a slowly exchanging pool characterized as mitochondrial Ca. These results indicate that Pb perturbs Ca homeostasis in OC and emphasize further similar intracellular pathways for Pb and Ca in OC and other cells.

The biosynthesis of heme is very sensitive to lead and can be used as an indicator of lead exposure. An understanding of the relationship between exposure pattern and the status of heme precursors and enzymes is required for this indicator system to be useful as a diagnostic tool.

Compartmental models have been developed for the distribution of lead in man, and for the formation of heme in bone marrow. These models are connected by the inhibition of heme biosynthetic enzymes caused by lead in bone marrow. The models provide a good fit for kinetic data on blood lead, d-amino levulinic acid dehydratase (ALAD), and erythrocyte protoporphyrin (EP) in lead-exposed experimental subjects. The same models also provide a good description of EP and ALAD vs. blood lead in exposed populations, assuming equilibrium conditions. This allows estimates of differences in some toxicokinetic parameters among subpopulations e.g. black vs. white children. A parameter related to the lead-binding capacity of the erythrocyte increases with iron status (transferrin saturation).

**BLOOD LEAD LEVELS AND STATURE IN THE NHANES II Survey.** C.R. Angle, J. Schwartz, and H. Pitcher. Dept. of Pediatrics, Univ. of Neb Med Center, Omaha, NE; U.S. Environmental Protection Agency, Washington, DC.

The second National Health and Nutrition Examination Survey (NHANES II) incorporated comprehensive medical anthropometric and dietary assessment. Blood leads (PbB) were 5-35 pg/gl. In multiple weighted linear regressions of adjusted data from 2695 children 6 mos - 7 yrs, 91% of the variance in height, 72% of the variance in weight and 58% of the variance in chest circumference were explained by 5 variables: age (yrs), race, sex, total dietary protein and hematoctrit or transferrin saturation. The coefficients were stable after correction for collinearity. Variables that did not significantly improve the models included family income, degree of urbanization, serum albumin, copper, iron and zinc, dietary carbohydrate, fat, calcium, phosphate, phosphorus, Vitamin A, nicin, riboflavin and thiamine. Correlation does not imply causality, but the highly significant regression of stature on PbB merits investigation of these observations in other surveys and consideration of the multiple biologic mechanisms by which low level PbB could modify growth.


Several aspects of the cellular metabolism of calcium and lead are quite similar. The calmodulin inhibitor W-13 was used to investigate the possible dependence of cell calcium and lead homeostasis on the intracellular calcium binding protein calmodulin (CaM). The effect of W-13 on the metabolism of 210-Pb and 45-Ca in rat hepatocytes was studied by labeling cultures for 3 hrs in medium containing 45-Ca or 210-Pb (3 uM) with 60 uM W-13 or no treatment. 45-Ca and 210-Pb efflux from the cultures were measured following the 3 hr labeling period. Data obtained from the efflux experiments were used to calculate the kinetic parameters associated with the cellular metabolism of lead and calcium. Hepatocyte lead and calcium metabolism was defined by 3 intracellular pools, S1, S2, and S3. W-13 treatment caused a shift in intracellular calcium from the largest pool, S1, to the less rapidly exchanging pools, S2 and S3. W-13 treatment did not increase total cell calcium. Cell lead metabolism was affected little by W-13 treatment. These results indicate that CaM plays a role in the metabolism of calcium in the resting cell. In contrast, cell lead metabolism does not appear to be dependent on CaM.

Mature and immature astroglia in culture take up and concentrate lead (Pb) from the medium. In vivo studies have shown a relationship between Pb toxicity to the central nervous system and cellular Zn levels. We studied the effects of Zn on Pb uptake by immature rat astroglia in culture. Astroglia prepared from 1-2 day old rat cerebrocortical cultures were cultured in Waymouth's 752/1 medium supplemented with 10% fetal bovine serum, FeSO₄, CuSO₄, MgCl₂, MnSO₄, folate acid and ZnSO₄. The normal Zn concentration of the complete medium was 0.008 μg/ml. Prior to Pb treatment the cultures were switched to media with normal, high, or low concentrations of Zn. The cultures were then treated for 1-3 days with medium containing 0.1 mM Pb acetate and one of the three Zn levels. The intracellular Pb concentrations were measured by atomic absorption spectroscopy at 3 time points after treatment. At all time-points tested Pb uptake by Zn-deficient cultures was significantly greater than uptake by the other two Pb treated groups. A significant decrease in cell number was also noted in the Zn deficient Pb treated group. Funded by Formula Animal Health Funding, Project #6852, BRSG-4-84, and EPA Grant #R811500-01-0.

EFFECT OF ZINC ON ACETAMINOPHEN BIOTRANSFORMATION IN MALE RATS. A.M. Pour, M.H. Davies, A. Blacker, S.A. Weil, and R.C. Schmael, Dept. Pharmacodynamics-Toxicology, Univ Neb Med Ctr, Omaha, NE 68105.

Previous studies have shown that acute pretreatment with zinc (acetate) can ameliorate acetaminophen (AAP) hepatotoxicity in rats. These studies were undertaken to examine the effect of zinc on AAP biotransformation as a possible mechanism for its protective effect. In animals pretreated with zinc (6 mg Zn/Kg, ip) at 48 and 24 hr prior hepatic cytochrome P-450 content was reduced by 20%. Urinary excretion of AAP + metabolites over 72 hr was increased by 25% in Zn treated rats, with the excretions of AAP, AAP-glucuronide, and AAP-sulfate being significantly increased, with that of AAP-gluthathione being decreased, and that of AAP-mercapurate being unchanged in Zn treated animals. Urinary excretion of AAP + metabolites over 4 hr was decreased in Zn treated rats (18.6% vs. 25.8%) while the pattern of metabolites was unchanged. Urinary excretion quantity and pattern were the same as before. Plasma decline of AAP in Zn-treated rats was increased with the half-life in Zn-treated rats 120 min vs 154 min in controls. These studies provide evidence that Zn may protect against AAP hepatotoxicity by altering both Phase I and II biotransformation of AAP. Supported by Burroughs-Wellcome Toxicology Scholar Award.


One approach to improving cancer chemotherapy could involve the use of trace elements to induce a response in normal, vital cells that could protect these cells against the cytotoxic effects of alkylating agents which are used in cancer chemotherapy. Research in our laboratory has demonstrated that pretreatment of normal, human fibroblasts, but not human tumor cell lines, for 12 hours with 100 μM ZnCl₂, resulted in a 7-9 fold increase in cell survival following exposure to melphalan (Cancer Res. 1985. 45:2567-2571). In vitro studies with cell cultures have been extended to work in vivo with mice. Mice (N=20) which are maintained on the Teklad 1-485 diet are pretreated with zinc gluconate by oral gavage (12 mg/kg) at 24 and 6 hours prior to an intraperitoneal injection of nitrogen mustard (4 mg/kg). Control groups (N=20) are pretreated with an oral gavage of physiological saline. The zinc pretreated group have a 3-fold increase in survival as compared to the saline pretreated group. This work suggests that the results obtained in vitro with cell cultures can be extended to in vivo work with mice and further applications are targeted to cancer chemotherapy.

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Male Sprague-Dawley rats were treated with cadmium chloride 1.5 mg/kg for seven days by daily intraperitoneal injections. Liver, kidney and testes were removed and homogenized. Activities of enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were measured. In vitro breakdown of ribose-5-phosphate was also determined. Male newborn rats were injected subcutaneously at 2.5 mg/kg every two days for thirty days. At the end of the treatment liver, kidney and testes were removed and enzyme activities were measured as mentioned above. Cadmium significantly inhibited the activities of all three enzymes in testes of adult rats but no significant change was observed in the testicular tissue of newborn rats.
420 CADMIUM CARDIOTOXICITY: EFFECTS ON CYTOPLASMIC AND MITOCHONDRIAL ANTIOXIDANT DEFENSE SYSTEMS IN PRESENCE OF HIGH DIETARY COPPER. J.J. Sprowls and J.S. Jamoll Toxicology Program, St. John's University, NY

Weanling Sprague-Dawley rats were fed a low-selenium (Se) diet or this diet supplemented with 0.0, 0.1 and 0.5 ppm Se (as sodium selenite). The feed of all rats contained 50 ppm copper (Cu), as copper sulfate. On day 28 of the study, rats were treated via osmotic minipumps (Alzet 2002) with 5 mg cadmium (as CdCl₂). Cd treatment resulted in a significant reduction in cytosolic glutathione peroxidase (GSHPx) and a marked increase in lipid peroxidation only in rats fed the low-Se diet. Cytosolic superoxide dismutase (SOD) was unaffected. Cd treatment caused significant reductions in GSHPx and SOD in the mitochondria of all treated rats. Cd levels increased with dietary Se. Cd treatment also resulted in significant reductions in heart Se concentrations in rats fed the low-Se diet.

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422 INDUCTION OF METALLOTHIONEIN (MT) FOLLOWING ADMINISTRATION OF URETHANE. E.A. Brzezinka, L.D. Lehman, and C.D. Klaassen. Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Med. Ctr., Kansas City, KS

Induction of hepatic MT by urethane (ethyl carbamate) has been characterized. Male C57BL/6 mice were treated with urethane (0, 0.5, 1.0, 1.5 and 2 g/kg; ip) and 18 hours later hepatic MT concentrations were determined with the Cd-hemoglobin radioassay. Urethane (1 g/kg and higher) significantly increased hepatic MT levels resulting in a 14-fold increase after 2 g/kg. Gel filtration and anion-exchange chromatography confirmed induction of MT by urethane. Pretreatment with actinomycin-D prevented induction of MT by urethane. Time-course experiments indicated that MT increased significantly at 6 hr after administration of urethane (1.5 g/kg) and reached a maximum between 12 and 24 hours. Administration of equimolar dosages (20 mmol/kg) of urethane, N-hydroxyurethane and methyl carbamate indicated that some carbamates (urethane and N-hydroxyurethane) induce MT but others (methyl carbamate) do not. MT induction was also observed with other commonly used anesthetics (pentobarbital and phenobarbital). In conclusion, urethane induces hepatic MT but this effect is not related to its anesthetic action, nor is it a common property of all carbamates. (Supported by USPHS grants ES-01142 and ES-07079 and a Proctor and Gamble fellowship).


This study was performed to compare induction of metallothionein (MT) by dexamethasone (Dx) to that of Cd and Zn and to survey the inducing potential of a variety of steroids in rat hepatocyte cultures. MT was quantitated by the Cd-hemoglobin radioassay. Dx was a more potent inducer than Cd or Zn, achieving maximal induction at 0.03 μM whereas maximal induction by the metals occurred at concentrations of 3 μM or greater. However, metals produced a 2-4 times greater increase in MT than did Dx. Over a 3-day culture period, Cd elicited a continuous increase in MT, Zn produced a transient peak at 24 hr, and Dx produced a plateau concentration at 24 hr. Of 45 steroids examined, 7 were full agonists, 5 partial agonists and 2 were inactive for MT induction. For the full agonists, the lowest concentration yielding maximal MT induction was 0.001 μM. The concentration of steroids that induced tyrosine aminotransferase, a glucocorticoid specific effect, correlated with those that induced MT. In conclusion, in rat hepatocytes, steroids: (1) are more potent inducers of MT than Cd or Zn, (2) differ from metals in the extent and time course of induction, and (3) apparently induce MT by interaction with the glucocorticoid receptor. (Supported by USPHS grants ES-01142 and ES-07079)

423 SEPARATION AND QUANTITATION OF METALLOTHIONEINS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) COUPLED WITH ATOMIC ABSORPTION SPECTROPHOTOMETRY (AAS). L.D. Lehman and C.D. Klaassen. Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS

A rapid, reproducible and sensitive HPLC method for determining concentrations of metallothionein-I (MT-I) and metallothionein-II (MT-II) in rat liver has been developed. MT-I and MT-II, separated on an anion-exchange column with a gradient of Tris-HCl mobile phase, eluted at 7.5 and 10.4 minutes, respectively. Quantitation by AAS was based on the determination of Cd content in MT peaks from Cd-saturated and subsequently heat-denatured rat liver cytosols. To establish standard curves, protein concentrations of purified MTs were determined by the Kjeldahl method and saturated with Cd (final concentration of 50 ppm). Recovery of MTs exceeded 95% and a detection limit of 5 μg MT/g liver was established. Only MT-II was detected in untreated rats, whereas following treatment with Cd or Zn, both forms of MT were detected. Hepatic concentrations of total MT determined by HPLC-AAS and the Cd-hemoglobin radioassay compared favorably. However, HPLC-AAS offers the advantage of determining the concentrations of MT-I and MT-II in tissues and thus should be useful for studying the induction and regulation of both proteins. (Supported by USPHS grants ES-01142 and ES-07079 and a Proctor & Gamble fellowship).
INDUCTION OF HEPATIC METALLOTHIONEIN (MT) BY ALCOHOLS. W.M. Bracken and C.D. Klaassen, Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS

The purpose of the present study was to determine the ability of various short chain alcohols to induce MT in the liver and to determine whether the induction results from a direct action of alcohol on liver or an indirect action mediated by zinc, glucocorticoid or catecholamines. Mice were administered alcohol by gavage and hepatic MT was quantitated by the Cd-hemoglobin radiolysis assay. Ethanol, methanol, isopropanol and propanol increased MT content to 6 times that of control liver. In vitro, ethanol did not increase MT concentrations in rat hepatocyte cultures, indicating that the in vivo induction is not a direct effect of ethanol on the liver. Adrenergic receptor blockade did not reduce the MT content of ethanol-treated mice, indicating that catecholamines are probably not involved in the MT induction. Corticosterone and zinc concentrations in plasma were increased in mice 1 hr after ethanol treatment. Corticosterone, given in vivo, was a less effective inducer of MT than ethanol treatment. In conclusion, (1) hepatic MT was increased by several alcohols, (2) the induction was not due to direct action of alcohol on the liver, and (3) while the mechanism of alcohol induction of MT is unclear, it may be due to an alteration in zinc and glucocorticoid homeostasis. (Supported by USPHS grants ES-01142 and ES-07079).

Role of Calmodulin in The Mechanism of Cadmium Induced Cell Injury. B.A. Ferrino and J.N. Chou, Dept. of Microbiology, Boston University School of Medicine, Boston, MA. Sponsor: A.E. Rogers

Cadmium, a known health hazard, has various toxic effects on cell growth and metabolism, although the mechanisms of its cellular toxicity are still unclear. Using immunofluorescent techniques, we have shown that micromaneral CdCl₂ causes disassembly of the cytoplasmic microtubule complex (CMC) in 3T3 cells. To investigate the mechanism of Cd induced MT disassembly, 3T3 cells are extracted with a nonionic detergent in a MT stabilizing buffer to remove cellular soluble components, but leaving behind an intact CMC which can be preserved for hours. This provides a system for studying the CMC induced MT disassembly without interference from such soluble components. Incubation of the CMC with micromolar CdCl₂ causes a time and dose dependent disassembly of MT. CdCl₂ acts similarly, in accordance with the known Ca₃ activity of MT. The ubiquitous regulator protein, calmodulin(CaM), modulates many Ca-mediated effects including MT disassembly. When trifluoperazine, a specific CaM inhibitor, is added during incubation, MT disassembly caused by Ca or Cd is inhibited, indicating that Cd-induced MT disassembly may be due to activation of CaM by Cd. Our data support Cheung's hypothesis(Fed. Proc. 43:2995, 1984) that Cd toxicity may result, in part, from inappropriate activation of CaM. Supported by a Grad Stud Res. Award(BAP) and NIH grant ES 03574.

Dramatic Cytoskeletal Perturbation Induced by Epigenetic Carcinogenic Metals. I.N. Chou, Department of Microbiology, Boston University School of Medicine, Boston, MA. Sponsor: A.E. Rogers

Carcinogenic metals consistently fail in genotoxicity tests and are classified operationally as epigenetic agents. To understand the mechanism of cell injury by metal insult, we have studied changes in cytoskeletal organization, particularly, microtubules (MT) and microfilaments (MF) of mouse 3T3 cells exposed to As(III), Cd(II), Co(II), Cr(VI) or Ni(II). Fluorescence microscopic visualization of MT and MF of the same cell was performed by double staining of the fixed, detergent-extracted cytoskeletons of control and metal-treated cells. Severe MT disassembly and MF derangement occur in a time and dose dependent manner upon exposure of 3T3 cells to As and Cd in the micromolar range. In contrast, both Ni and Co induce not only dramatic reorganization (but not disassembly) of MT but also unusual redistribution of MF such that MT and MF are localized to mutually exclusive areas of the cells. Treatment with Cr, the only metal showing weak responses in genotoxicity tests, results in a marked thinning out of MT and loss of MF. The very different patterns of cytoskeletal perturbation may be important in the epigenetic mechanism(s) of cellular toxicity by metal insult. Supported by NIH grant ES 03574.
Several methods have been developed for estimation of metallothionein (MT). In the present study, Cd-hem assay (TAP 63: 270, 1982) is compared for sensitivity and specificity with a newly developed Ag-binding assay in the determination of MT from various tissues. Ag-hem method was more sensitive than Cd-hem (0.5ug vs. 10ug) when known amounts of purified rat liver Zn-MT-2 was used. Ag-hem method is similar to Cd-hem method except for the use of 0.5M glycine buffer and the addition of 20ppm AgNO3 labelled with 110mAg. In Ag-MT, a stoichiometry of one sulfur to one Ag was obtained in comparison with one Cd to 3 sulfur in Cd-MT. Estimation of MT in control rat tissues by the two methods showed good agreement in all tissues except in testes where Ag-hem gave lower values than Cd-hem. In adult rats injected with copper salts (5mg/Kg for 5 days), higher values of MT were measured in both liver and kidney by the Ag-hem method. This is probably due to a complete displacement of Cu by Ag and not by Cd under our experimental conditions. A stepwise removal of Cd, Zn and Cu from MT was observed when Ag was added in increasing concentration in Ag-hem assay. The results show that Ag-hem assay may be superior to Cd-hem in measuring tissue MT, containing high copper. (Supported by MRC, Canada).

The pyrimidine analog, azacytidine, increases expression of the metallothionein (MT) gene and induces tolerance to cadmium. Since incorporation of azacytidine into DNA seems to be required the deoxynucleoside should also be effective. This study assessed the effect of azacytidine (AZA-Cd) on cadmium-induced toxicity and MT expression. Cultured rat liver cells (TRL 1215) in log phase of growth were exposed to AZA-CdR (0.4, 0.8, 4 and 8 uM); after 48 h 10 uM cadmium was added. MT was measured 24 h later. AZA-Cd caused modest, dose-related increases in MT (2.3-fold maximum) while cadmium resulted in a 9.2-fold increase. Combined AZA-CdR pretreatment and cadmium caused 19- to 23-fold increases in MT at all doses of AZA-CdR. The DNA synthesis inhibitor hydroxyurea prevented the enhancing effect of AZA-CdR on cadmium-induction of MT synthesis. AZA-CdR pretreatment also reduced the time for increased cadmium-induced MT synthesis (0-2 h) compared to control (4-8 h). Suspensions of AZA-CdR-treated cells were less sensitive to cadmium (126 uM), cytotoxicity as reflected by reduced glutamic-oxaloacetic transaminase loss. AZA-CdR treatment did not alter cadmium uptake. Results suggest that AZA-CdR induces tolerance to cadmium cytotoxicity because of a greater rapidity and increased capacity of MT synthesis.

In male mice the influence of sublethal iv doses of cadmium chloride (3 mg/kg), mercuric chloride (2 mg/kg) or vanadate (NaVO3, 6 mg/kg) on glutathione (GSH) content and GSH S-transferase activities (substrates: 1-chloro-2,4-dinitrobenzene; 1,2-epoxy-3-(p-nitrophenoxy)-propane) were estimated in liver, kidney, lung and brain. Liver GSH-content was not altered within 24 h by any of the metal ions, whereas kidney GSH was slightly elevated upon application of HgCl2. Treatment with either metal resulted in a tendency to lower GSH concentrations in lung and brain tissues. GSH S-transferase activities (CDNB) were enhanced in all tissues up to 24 h after treatment with any of the three metals in the order CdCl2 < NaVO3 < HgCl2. GSH S-transferase activity towards the epoxide-substrate which was only measurable in the liver, was suppressed during the first two hours after administration of HgCl2 or NaVO3. In conclusion, the organ-specific toxicity of the three metals investigated seems not to be related to an impairment of the GSH-conjugating enzyme system. Moreover, these in vivo findings are contradictory to previous in vitro results (J. Toxicol. Env. Hlth. 7, 139, 1981) showing that metals deplete glutathione, induce lipid peroxidation and cellular damage.

The effectiveness of zinc (Zn) in reducing existing cadmium (Cd) body burden and/or in reversing the Cd induced alterations in selected tissue morphology and biochemistry was studied in calves. Bull calves were given 5 alternate days s/c injections of 0.05 mM Cd/Kg body weight as CdCl2. Seven days following last Cd dose, calves were given either a basal (no Zn) or a high Zn (1000 ppm) diet for a 45 day period. The results indicated that multiple doses of Cd at 0.01 mM/kg in growing animals can: a) produce morphological testicular and renal damage that is partially reversible by Cd withdrawal, b) reversibly reduce serum alkaline phosphatase levels indicative of reduced osteoblastic activity, and c) inhibit the activity of epidemicinal 5α-reductase and 3α,5α hydroxysteroid dehydrogenase enzymes. Dietary Zn supplementation following prior Cd loading resulted in: i) reduced Cd concentration in tissues compared to those in calves on a basal diet, and b) almost complete reversal of the inhibitory effects of Cd on the epididymal enzyme activities which remained low following Cd withdrawal with no Zn supplementation. Evidence of beneficial effects of Zn on Cd induced changes in tissue morphology was inconclusive. College of Veterinary Medicine CDR Grant #2-01949.
432 SUPERFICIAL BINDING AND INFUX OF CADMIUM AND CALCIUM USING CULTURED HEPATOCYTES. E.M.B. Scrensen, Department of Pharmacology and Toxicology, The University of Texas, Austin, TX

Primary cultures of parenchymal hepatocytes from neonatal rats were used to assess the interaction between calcium and cadmium at the plasma membrane. Competitive interaction between the two divalent cations at this membrane could lead to increased cadmium influx during intervals when extracellular levels of calcium are low. These experiments were conducted to determine if differences existed in either calcium influx into- or superficial binding to-cultured hepatocytes. Influx of 109Cd into hepatocytes was reduced (p<0.01 or p<0.001) when calcium was added to culture media. Experiments conducted to determine whether the presence of extracellular calcium reduced the levels of 109Cd adhering to membranes of cultured parenchymal hepatocytes showed that significantly higher concentrations of cadmium were bound in the absence of calcium following 2-hour, but not 30 minute, exposures. Reciprocal experiments were conducted using 45Ca and stable cadmium under the same conditions employed for the isotopic cadmium influx studies. Although the presence of calcium did not effect the influx of 45Ca, the presence of stable cadmium significantly increased the superficial binding of 45Ca (p<0.001). The presence of cadmium apparently altered the plasma membrane, exposing additional binding sites which were subsequently occupied by 45Ca. Such alterations in plasma membranes would not be expected to occur if calcium were omitted from the incubation medium. These results indicated that the presence of calcium in the culture media of hepatocytes reduced both the superficial binding and influx of cadmium.


Female C57 mice were fed diets containing 0.25, 5, or 50 ppm cadmium (Cd) from 70 to 320 d of age. 70-80 mice per diet group were bred for 6 consecutive 42-day breeding cycles and pregnancy and lactation (PL mice); 60-75 mice per group were nonpregnant (NP) controls. PL mice that were consecutive breeders (and their NP controls) were sacrificed at the end of PL cycles 1, 2, 4, and 6. Kidney concentrations of Cd (Cd-K), Zn (Zn-K), and Cu (Cu-K) and urine concentrations of protein, glucose, amino acids, creatinine, and Cd were measured. In all groups, Cd-K increased steadily with time. Each dietary Cd level, Cd-K was 2-5x higher in PL than in NP mice. After 6 PL rounds at 50 ppm Cd (252 days), Cd-K was high (155 μg Cd/g) and urinary Cd was low (0.1 μg Cd/ml); urinary protein, glucose, and amino acids showed no consistent increases in mice at 50 vs. 0.25 ppm Cd. Zn-K and Cu-K increased only slightly (1.5x and 1.3x, respectively) with large increases in Cd-K (650x). In summary, PL mice after multiple rounds of pregnancy and lactation at 50 ppm Cd had high Cd-K levels with no clear indications of renal tubular dysfunctions. Work supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38.

434 EFFECT OF CADMIUM AND SELENIUM INTERACTION ON Ω-AMINOLEVULINIC ACID SYNTHETASE IN MALE RATS. M. B. Iziard and J. L. Early, College of Pharmacy and Pharmaceutical Sciences, Florida A&M Univ. Tallahassee, FL 32307. Sponsor: R. Craig Schmell.

Previous reports from this laboratory have shown that selenium antagonizes cadmium-induced increases in Ω-aminolevulinic acid dehydratase activity. The purpose of the present investigation was to determine if the effect of selenium on Ω-aminolevulinic acid synthetase (ALAS) is the mechanism whereby selenium protects against cadmium-induced toxicity. Male 150-175 g Sprague-Dawley rats received sodium acetate (1.23 mM/kg, control), sodium selenite (1.6 mg Se/kg), cadmium acetate (0.84 mg Cd/kg), or both selenium and cadmium via the intraperitoneal route. Animals were sacrificed 72 hr following treatment. Hepatic microsomal ALAS activity was quantitated spectrophotometrically. Selenium decreased ALAS activity to 96% of control values. Cadmium increased ALAS activity to 112% of controls. Treatment with both cadmium and selenium resulted in an increase in ALAS activity to 161% of control. These results indicate that selenium alone slightly decreases, while cadmium slightly increases ALAS activity. Thus, the concurrent administration of both selenium and cadmium markedly enhance the activity of ALAS. (Supported by NIH grant RR 00111).

435 EFFECTS OF ZINC, N-ACETYLCYSTEINE AND SODIUM SULFATE ON TISSUE CADMIUM AND ZINC LEVELS IN MICE PRETREATED WITH CADMIUM, M.A. Martino, F.K. Mohanad and C.S. Reddy, Department of Veterinary Biomedical Sciences, Univ. of Missouri, Columbia, MO. Sponsor: M.F. Raisbeck.

Many studies have shown the protective role of zinc (Zn) pretreatment against cadmium (Cd) induced toxicities in rodents. The effects of Zn, N-acetylcysteine, and sodium sulfate on liver and kidney Cd and Zn concentration in Cd pretreated mice were studied. Male Charles River CD 1 mice were given 5 i.p. injections of Cd every other day followed by a 14 day oral administration of either saline, Zn, sodium sulfate or N-acetylcysteine. Cadmium treatment increased the liver and kidney Cd concentration; yet the body weights were not greatly affected. Gross pathological lesions seen in the Cd injected mice consisted mainly of swollen liver and peritoneal adhesions. Zinc significantly reduced Cd concentration in the kidney, spleen, and pancreas as well as the total tissue Cd burden. Sodium sulfate also decreased Cd levels in the kidney. N-acetylcysteine did not affect Cd levels in the kidney or liver. Further studies are needed to examine the effects of Zn and sodium sulfate on Cd contents in different organs and to understand the mechanism of Cd mobilization from the kidney and other tissues in mice.
This study was carried out to elucidate the mechanisms of sex difference in renal toxicity of inorganic mercury (Hg) in rats. The renal uptake of Hg and its toxicity was studied in SD rats given 1.0, 0.5, or 0.2 mg HgCl₂/kg intravenously and sacrificed 24 hrs later. No significant differences in the accumulation of Hg in whole kidney or in renal cortex were observed between the male and female rats. Metallothionein levels in the renal cortex were also very similar. Renal toxicity, as evaluated by urinary excretion of γ-glutamyl transpeptidase and protein, was evident in males at all doses and in females only at the highest dose. This suggests that the sex difference in renal toxicity observed at the two lower doses is not due to the difference in renal Hg accumulation. The male rats also showed a significant decrease in renal cortex glutathione level and a significant increase in lipid peroxidation. Castration of male rats made them less sensitive to Hg; treatment with testosterone, however, reversed this effect. It is concluded that testosterone potentiates renal toxicity of mercury and that glutathione depletion and lipid peroxidation may be the probable mechanisms of Hg toxicity. (Supported by USPHS Grant No. ES 03187.)

CROMOSAL EFFECTS OF NITRILOTRIACETIC ACID (NTA) AND HEAVY METALS. A. Montalda, L. Zentilin, E. Capuano, and A. G. Levi. Dept. of Biology and Inst. of Environmental Health, Padua, Italy. Sponsor: M. Lotti

We studied the interaction of NTA, a substitute for polyphosphates in household detergents, with heavy metals in the induction of genotoxic effects in cultured mammalian cells. Chromosomal aberrations (c.a.) and micronuclei (m.n.) were detected in human lymphocytes, and sister chromatid exchanges (s.c.e.) were scored in a hamster cell line (CHO). NTA did not enhance the induction of s.c.e. by soluble metals (HgCl₂, NiCl₂, MnCl₂, CdCl₂, CuCl₂), while it significantly increased the frequencies of s.c.e. induced by insoluble metals (HgCl₂NiCl₂, PbCl₂, CdCl₂, CuCl₂, PbSO₄). A statistically significant increase of c.a. (both gaps, mono- and iso-chromatid breaks and fragments) and m.n. was also induced by the insoluble metals (particularly HgCl₂ and CuCl₂) in the presence of NTA. As NTA is able to mobilize heavy metals from insoluble precipitates in water sediments and sludges, the present results raise some concern about the possible genotoxic risk deriving from an extensive use of NTA in detergents. (Supported by C.N.R., P.F. "Medicina Preventiva e Riabilitativa").


Female BALB/c and HRS/J mice (5 weeks old; averaging 18 g) received (i.p.) 2.5 mg Hg [as methylmercury (MeHg) + Me²⁰³Hg] per kg b.w. at a specific activity of 200 µCi. Five animals of each strain were sacrificed at 0, 25, 0, 5, 1, 2, 4, 16, and 32 days postadministration. MeHg uptake and elimination rates were determined by gas chromatography and gas isotope dilution mass spectral analysis. The order of (apparent) maximum tissue/organ Hg uptake for BALB/c mice was: kidney > liver > spleen > muscle > fat > skin > brain > cerebellum > lens > hair. For HRS/J mice, it was kidney > liver > spleen > blood > muscle > fat > skin > brain > cerebellum > lens. Blood had the highest Hg elimination rate (shortest T 1/2); lens and BALB/c hair, the slowest. Cerebrum and cerebellum also exhibit slow rates of release. Except for lens, the T 1/2 for the tissue/organs and carcass were slower for the BALB/c mouse. Except for kidney and cerebrum, maximum tissue/organ Hg concentrations were greater in the BALB/c mouse up to 2 days postadministration. Urinary Hg excretion rate was constant for both strains. The amount of Hg eliminated by this route was 10% for the BALB/c mouse and 6% for the HRS/J. The rate of fecal Hg excretion was much higher for the HRS/J mouse up to 14 days postadministration. In 32 days, it excreted 86% of the dose while the BALB/c mouse excreted 64%.


HgCl₂ treatment markedly increases renal microsomal EH activity in Sprague-Dawley (SD) rats, but only marginally alters renal EH in F-344 (F) rats. Possible mechanisms underlying this strain difference were examined. Male F and SD rats were killed 1 hr to 27 days after a single ip injection of HgCl₂ or 200 µg HgCl₂ (1 mg/kg). Maximal increases in renal EH were observed in F rats (400% of control) at 3 days, and in F rats (200%) at 4 days. Nephrotoxicity (fructose-1,6diphosphatase activity), and renal and hepatic [²⁰³Hg] concentrations were similar in both strains. Hepatic metallothionein (MT) concentrations were increased to 300% and 400% of control in F and SD rats, respectively, at 1 day. Renal MT increases were similar in both strains (300% of control). In pretreatment did not alter EH increases elicited by HgCl₂ in either strain, suggesting that the slight MT differences do not contribute to the EH strain difference. Up to 48 hr after HgCl₂ treatment, renal glutathione (GSH) was increased slightly more in SD than F rats, but control values were consistently higher in F rats. Hepatic GSH was unaffected by HgCl₂ in either strain. These data suggest that differences in basal renal GSH may play a role in the strain difference observed in EH elevation by HgCl₂. Alternatively, regulation of renal EH may differ between the strains.
Methylmercury (MeHg) exposure during the prenatal and/or postnatal periods has been associated with the abnormal migration of cortical neurons. A cell recognition assay based on the ability of dissociated cells to reaggregate was used to test whether MeHg exposure could alter cell recognition thereby influencing the fate of migrating neurons. Cells freshly isolated from 3 days postnatal mouse cerebella were exposed to 0, 0.5, 1.0 and 4.0 μM MeHgCl. Reaggregation of the dissociated cells was monitored through 190 hours in vitro (hiv) by measuring reaggregate diameters from low power photomicrographs. A dose-dependent inhibition of reaggregation with an ID_{50} of 1.5 μM at 24 hiv was observed. Reaggregation was completely inhibited with 4 μM MeHg. Following initial inhibition, exposed groups showed a dose-dependent acceleration in reaggregation. At 190 hiv, average diameter from the 1 μM group was double that of controls. In vivo exposure to 4 mg/kg MeHg per os 24 hours prior to cell isolation did not affect aggregation seen at 24 hiv. However, as in the in vitro studies, diameters exceeded control diameters at later time points. Histologically, reaggregates following in vitro and in vivo exposure could not be distinguished from controls. (Supported by NIEHS grants ES07026 and ES01248).

Both application of MeHg increases spontaneous release of ACh at the neuromuscular junction, an effect observed electrophysiologically as increased miniature endplate potential (MEPP) frequency. The objective of the present study was to test whether the mitochondrial inhibitors dinitrophenol (DNP, 100 μM), dicoumarol (DC, 100 μM), or rotenone (1 μM) could block the MeHg-induced increase in MEPP frequency. MEPPs were recorded continuously from the rat hemidiaphragm using conventional methods during pretreatment with a mitochondrial inhibitor and subsequently with the inhibitor plus MeHg. MeHg (100 μM) given following DNP or DC pretreatment increased MEPP frequency sharply after approximately 20 min; peak MEPP frequencies were 55 MEPPs/sec (Hz). MEPP frequency subsided to pre-MeHg levels 10 min later. Application of MeHg for up to 80 min following RR pretreatment failed to increase MEPP frequency. RR did not deplete releasable transmitter stores or decrease postsynaptic sensitivity to ACh, since subsequent treatment with La^{3+} (2 mM) increased MEPP frequency to 12.5 Hz within 10 min. Thus, RR blocked the stimulatory effects of MeHg on MEPP frequency while uncouplers of oxidative phosphorylation did not. The results are consistent with the proposal that MeHg acts on mitochondria to release Ca^{2+} which stimulates spontaneous release of ACh. (Supported by NIH grant ES03293).

The biliary excretion of MM is thought to be related to the biliary excretion of sulfhydryls (SH) in rats. Species differences in biliary excretion of glutathione (GSH) and related SH are unknown; therefore, the relationship between biliary excretion of endogenous SH and MM in five species was studied. Reduced and oxidized SH excretion in bile was 369, 192, 102, 48 and 19 nmol/min/kg for mice, rats, hamsters, guinea pigs and rabbits, respectively. GSH was the main component in bile of mice, hamsters and rats whereas guinea pig and rabbit bile mainly contained cysteinylglycine (Cys-Gly). The larger percentage of Cys-Gly in guinea pigs and rabbits corresponded to a greater activity of hepatic γ-glutamyltranspeptidase (GGT) than in the other species. Biliary excretion rate (nmol/min/kg) of MM was 0.8 in mice, rats and hamsters compared to significantly lower rates in guinea pigs and rabbits (0.15 and 0.03, respectively). It is concluded that (1) the composition of biliary SH is related to hepatic GGT activity and biliary excretion rate of SH; and (2) species differences in biliary SH excretion do not entirely account for species variation in MM excretion, indicating other factors may also be involved. (Supported by USPHS grants ES-03192, ES-01142 and ES-07079).

GENETIC FACTORS AFFECTING METHYL mercury METABOLISM AND EXCRETION. R.A. Doherty. University of Rochester Medical Center, Rochester, NY. R. Wood

35 inbred mouse strains have been screened for genetic variation in biotransformation (metabolism) and/or excretion of methylmercury. Our strategy was to examine mice from as many different origins as possible to cast a broad genetic (evolutionary) net to sample the largest possible array of genes which might influence metabolism of mercury compounds. Following a single, non-toxic dose of radiolabeled methylmercury chloride, extents of initial absorption and half-times of elimination were determined by least squares linear regression analyses of retained whole body radioactivity during an 8-day period. Feces and urine were separately collected and analyzed for mercury content. Blood, brain, liver, kidney, testes and remaining carcasses were weighed and counted on day 8 to determine mercury distribution. Large interstrain differences in half-times of excretion of methylmercury (13.1 days to 3.2 days) were observed. Large differences in male vs. female rates of mercury excretion were seen in some mouse strains but not in others. Our studies should aid in elucidating specific cellular and molecular processes involved in the biotransformation of mercury compounds and other metals. It is expected that formulation of genetic models will enable better delineation of possible hyper- and -susceptible human subpopulations, which must be taken into account for rational hazard evaluation, including safe limits of exposure for all life cycle stages. (NIHES ES01247 & 1248)

ORAL ADMINISTRATION OF ORGANOTIN COMPOUNDS: TISSUE SPECIFIC ACTION OF α-BUTYLLIN DERIVATIVES ON CYTOCHROME P-450. D.W. Rosenberg and A. Kapoor. The Rockefeller University Hospital, New York, NY

Since the GI tract is a major route by which human populations are exposed to environmental chemicals, we have examined the effects of oral administration of organotin compounds in the small intestine, an organ which exhibits highly active drug and chemical metabolisms. Organotins were given by gavage to male Sprague-Dawley rats in single doses up to 150 mg/kg body wt. The di- and trialkyltin produced dose- and time-dependent decreases in the content and function of intestinal P-450, together with an elevation (3-fold) in heme oxygenase activity. The effects of di-n-butylltin extended to the liver and kidneys within 12 hours after oral exposure, while bis(tri-n-butylltin)oxide did not affect the liver until much later (6 days). Graphite furnace atomic absorption analysis revealed a high degree of structure-dependence on the concentrations of tin recovered from viscera, with the greatest concentrations produced by the di- and trialkyltins. These studies define important toxicological effects of organotins in the gut, and raise the possibility that concurrent oral ingestion of environmental pollutants can affect P-450-dependent metabolism of other chemicals in the intestine.


Acetaminophen (A), is detoxified by conjugation with sulfate and glucuronic acid (G) and also as mercapturate (M) via its toxic intermediate mediated through microsomal mixed function oxidase. In order to study the effect of subchronic exposure of styrene (ST) on the metabolism and liver toxicity of A, 6 groups of male Sprague-Dawley rats received either saline, corn oil 0.5 mL/kg, or ST 500 mg/kg/day, 5 days a week, for 3 weeks. 250 or 750 mg/kg of A were given at the end of pretreatment with ST or corn oil. Rats were sacrificed 24 h after the last exposure and urine and tissues were collected during this period. SGPT and SDH activities were assayed for liver damage. Urinary metabolites of A and ST were determined by HPLC. Pretreatment with ST decreased the toxic response of A at 750 mg/kg as shown by the SGPT and SDH levels. At the same time an increase in G formation, and a decrease in M were noticed; ST metabolites were also decreased in their recoveries, including thiethers. These results indicate a potential protective effect of ST on A hepatotoxicity in rats. (Supported by CAFIR, Université de Montréal).

Experiments were conducted to determine if Celiprolol (Celi), a carboxyselective beta-receptor antagonist with antihypertensive & antianginal activity, altered CCl4-induced hepatotoxicity in young Sprague-Dawley rats. In the 24 hours post CCl4, 1 cc/kg, sc., most rats showed lower body weights (BW) & food consumption (FC). Histopathologically, liver cells had massive vascular degeneration & multiple foci of necrosis. Serum glucose levels were markedly decreased. By 72-96 hrs the liver parenchyma had returned within normal limits. Celi. 50 mg/kg, p.o., produced minimal effects on BW & FC & moderate degrees of disorganized, intact hepatic cords of cells, which had returned to normal by 72-96 hrs. Rats administered Celi at 24 hours post CCl4 experienced less severe & less extensive forms of hepatocellular vascular degeneration & necrosis than after CCl4 alone. After 8 days of Celi (10 mg/kg/day, p.o.), CCl4 generally produced smaller changes in BW & FC & greatly decreased hepatotoxicity than after CCl4 alone. Control rats administered 0.9% NaCl, 1 cc/kg, s.c., &/or distilled water, 5 cc/kg, p.o., didn't exhibit these changes. The mechanism of Celi's action to attenuate CCl4's toxicity may be related to its beta antagonist activity.

449 POTENTIATION OF NONREGROGENIC AND CHOLESTATIC LIVER INJURY BY METABOLITES OF METHYI ISOBUytL KETONE. M. Vezena and G.L. Plaa. Depament de pharmacologie, Université de Montréal, Montréal.

Ketonic and ketogenic chemicals can potentiante haloalkane-induced hepatonecrogenicity. We demonstrated that methyl isobutyl ketone (4-methyl-2-pentanone; MIBK) and 4-methyl-2-pentanol (4MPOL; a metabolite) possess this property. We evaluated if another metabolite, 4-OH-4-methyl-2-pentanone (4OH-MIBK) was also a potentiator. We derived a dose-response curve of potentiation CHCl3-hepatotoxicity by 4-OH-MIBK using biochemical parameters (ALT, OCT). The compound dissolved in corn oil was administered po to Sprague-Dawley rats 24 h before an ip CHCl3 challenge. The non-effective dose (NED) and the minimal effective dose (MED) for potentiation were about 3.75 and 5.60 mmol/kg, respectively. MIBK and 4MPOL (1.88 mmol/kg/day; 3 days) can also potentiate the cholestatic properties of a manganese-bilirubin combination. MIBK, 4MPOL, and 4-OH-MIBK (7.5 mmol/kg/day; 3 days) caused the appearance of cholestasis in rats receiving only manganese. Thus, MIBK and its two metabolites enhanced both necrogenic and cholestatic forms of liver injury. (Supported by NRC).

450 VITAMIN A POTENTIATES THE HEPATOTOXICITY OF CARBON TETRACHLORIDE AND ALLYL ALCOHOL. A.E. ElSisi, D. Earnest, I.G. Sipes, Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

Hepatotoxicity produced by high doses of vitamin A is well established. However, the ability of vitamin A to modulate the actions of other hepatotoxins is less well known. Therefore, vitamin A (retinol) was given by gavage to male SD rats (180-200g) in daily doses of 100,000, 150,000, 290,000 or 250,000 IU/Kg for 3 weeks. Then they were challenged by CCl4 (0.15 ml/Kg, i.p.). In duration of treatment studies, retinol (250,000 IU/Kg/day) was given for 1 day, 1, 2, 3 or 5 weeks prior to CCl4. Similarly, allyl alcohol (40 uI/Kg, i.p.) was administered to rats treated with retinol (250,000 IU/Kg/day) for 1 or 3 weeks. Hepatotoxicity was assessed 24 hrs after the challenge by increased in SGPT activity and histological evaluation. In the 3 week dose-response study, doses greater than 100,000 IU resulted in significant (8 to 22-fold) increases in SGPT activity and increased hepatic cell necrosis. In the duration of treatment studies, equivalent potentiation of CCl4 liver injury occurred at all times except 1 day. Dramatic potentiation of allyl alcohol-induced hepatotoxicity occurred at both 1 and 3 weeks of retinol treatment (30 and 50 times, respectively). In conclusion, short-term administration of high doses of retinol can potentiate the hepatotoxicity of CCl4 and allyl alcohol. The underlying mechanism of this potentiation remains to be elucidated. (Supported by SPO1 CA-27502.)


Allyl alcohol (AA), a periportal hepatotoxicant, is activated by alcohol dehydrogenase (ADH) to acrolein, the ultimate reactive metabolite. The interaction of AA and methoxyflurane (MFO), a commonly used animal anesthetic with limited hepatotoxic activity, has been evaluated. Male F-344 rats were treated with MFO for 9 min after loss of the righting reflex. MFO anesthesia occurred 192 or 48 hr before, or 4 hr after the administration of AA (32 mg/kg, ip). Animals were killed 24 hr after AA administration. Hepatotoxicity was assessed by measuring serum alanine aminotransferase (ALT) activity. MFO effects on liver cytosolic ADH activity and rate of acrolein formation were determined 48 hr after MFO treatment. MFO treatment, 192 or 45 hr before, or 4 hr after the administration of AA, increased AA-induced serum ALT activity by 80, 300 & 1350%, respectively. ADH activity and AA activation to acrolein were increased 15%. 48 hr after MFO treatment. MFO enhancement of allyl alcohol hepatotoxicity can be explained by increased ADH activity which increases the toxic activation of allyl alcohol to acrolein.
The potential effects of copper on hepatic toxicity and metabolism of diethylnitrosamine (DEN) were studied in female Sprague-Dawley rats. Concurrent administration of copper sulfate (1.44 mg Cu/kg, ip) protected against DEN-induced alterations in subcellular enzyme distribution, decreased cytochrome P450 content and against pathologic damage induced by DEN (125 mg/kg, ip).

Concurrent administration of copper with [14C]DEN resulted in increases in total [14C]DEN-equivalents of 58% in the plasma, 38% in the packed red cells, 28% in the urine and marked decreases in all subcellular liver fractions. Maximal inhibition of in vitro metabolism of DEN by 59 fractions was measured by metabolite production and by recovery of unreacted DEN, was observed following introduction of 8 × 10−5 M copper. The mechanism by which copper decreased metabolism of DEN appeared to involve uncompetitive inhibition.

Evidence for the involvement of mitochondria and lysosomes in chlordecone potentiation of CHCl₃ hepatotoxicity in the rat. L.A. Hewitt, S. Masson and G.L. Plaa, Dép. de pharmacologie, Université de Montréal, Montréal, Québec, Canada.

Chlordecone (CD), but not mirex (M), a nonketo- nic analogue potentiates CHCl₃ hepatotoxicity. The phenomenon cannot be explained on the basis of differential induction of biotransformation. This study investigates the possible involvement of mitochondria or lysosomes in this interaction. Rats received corn oil, or 50 mg/Kg CD or M and were killed 18 hr later. Mitochondrial respiration, permeability, Ca²⁺ pump and fragility were not modified. However, similarly treated rats subsequently given 0.5 ml/Kg ¹⁴CHCl₃ and killed 3 hr later, showed a significant increase in irreversibly bound ¹⁴C in the mitochondrial fraction of the CD group. This observation is not due to increased biotransformation as the ¹⁴C bound in the CD group is greater than that in a corn oil group given 1.0 ml/Kg ¹⁴CHCl₃, in which the biotransformation rate is similar. Lysosomes were significantly more fragile in the CD treated group. CD-treated rats, administered 0.5 ml/Kg CHCl₃ released lysosomal enzymes to a greater extent than corn oil-pretreated rats administered 1.0 ml/Kg CHCl₃. Results suggest CD-modified evolution of CHCl₃ hepatotoxicity may result in part from changes at the organelle level. (Supported by NSERC and IRRSTQ).


A number of compounds have been shown to produce liver enlargement and hepatic peroxisome proliferation in rodents. We have compared the dose response relationships for hepatic peroxisome proliferation by 4-2-ethylhexyl phthalate (DEHP) and 4-2-ethylhexyl adipate (DEHA). Individually housed 6 week old male and female Fischer F-344 rats were fed diets containing 0 (control), 0.01-2.5% DEHP or 0.1-2.5% DEHA for 3 wk. Both compounds produced dose-related increases in liver size and dose-related decreases in perportal neutral lipid in liver sections stained with Oil Red O. Ultrastructural examination revealed dose-related increases in peroxisome numbers and, particularly at high doses lacked the normal crystalline core. Biochemical studies demonstrated dose-related increases in peroxisomal palmitoyl-CoA oxidation and microsomal lauric acid (ω-1) and particularly ω-hydroxylation. Generally DEHP was more potent than DEHA and male animals were more responsive than females. The experimental procedures as described in these studies form a suitable protocol for evaluating the relative potencies of compounds to produce hepatic peroxisome proliferation in rodents. (Supported by the CMA Phthalate Esters Panel)

Certain peroxisome proliferators are known to be hepatocarcinogenic in rodents and it has been suggested that tumour formation is linked to increased peroxisomal H$_2$O$_2$ production. We have investigated whether or not peroxisome proliferation results in increased lipid peroxidation. Male Sprague-Dawley rats were fed diets containing either 0.5% or 2% DEHP for 2 years. Both compounds produced liver enlargement and induced peroxisomal and microsomal fatty acid oxidising enzyme activities. Increased lipid peroxidation was observed by measurement of ethane exhalation in vivo and conjugated dienes and fluorescent products, but not thiobarbituric acid reactive material, in liver homogenates. Microsomal NADPH-dependent lipid peroxidation was also stimulated. Histological examination revealed extensive lipofuscin deposition in liver sections from treated but not control rats. These results demonstrate that peroxisome proliferation can result in lipid peroxidation and that lipid peroxidation is best assessed by several different methods. (Supported by U.K. Ministry of Agriculture, Fisheries and Food).

Peroxisome proliferators (PP), such as nafenopin (NAF) and methylcyclofenamate (MCP), represent a novel class of non-mutagenic hepatocarcinogens, and their administration to rodents results in hyper trophy (peroxisome and smooth endoplasmic reticulum (SER) proliferation) and hyperplasia (increased DNA synthesis and mitosis). Previous publications have reported peroxisome and SER proliferation in vitro, here we report PP-elicted DNA synthesis and mitosis in rat hepatocyte cultures. Cells were isolated and cultured for up to 5 days (Mitchell et al. Arch Toxicol 55 239 (1984)). NAF and MCP were added to cells 24hr after isolation and then at 24hr intervals. Cells undergoing DNA synthesis (S-phase) were determined autoradiographically after a 1hr pulse of $^3$H-thymidine; or following a bromodeoxyuridine pulse and flow cytometric analysis. The addition of NAF or MCP to the cultures resulted in dose- and time-dependent increases in S-phase activity (cells labelled) from less than 0.2% to 12%. Increases in the mitotic index of the cultures were also seen. These data were similar to data obtained in vivo and demonstrated both proliferative and hypertrophic effects of epigenetic carcinogens can be studied in vitro.


A wide variety of chemicals are known to produce liver enlargement and hepatic peroxisome proliferation in rodents and the increase in organelle numbers and induction of associated enzyme activities has been extensively studied in primary hepatocyte cultures. We have investigated the structure activity requirements for enzyme induction by 3 series of compounds, namely hypolipidemic drugs, phthalate monoesters and chloroenoxyacetic acid herbicides. Rat hepatocytes were isolated by collagenase perfusion, cultured with the compounds for 3 days, and the activities of palmitoyl-CoA oxidation, carnitine acetyltransferase and lauric acid hydroxylation determined. Compound potencies were compared with electronic structural parameters obtained by molecular orbital calculations employing a MINDO/3 computer program. The results demonstrate relationships between chemical structure and potency to induce enzyme activities and indicate the potential usefulness of primary hepatocyte cultures for screening compounds for peroxisome proliferation. (Supported by U.K. Ministry of Agriculture, Fisheries and Food).

Species Sensitivity to the Induction of Peroxisome Proliferation by Trichloroethylene and Its Metabolites. A.B. DeAngelis1, S. Herren-Freund1, M.A. Pereira1, N.E. Schults2 and J.E. Klaunig2. U.S. Environmental Protection Agency, Cincinnati, OH and Medical College of Ohio, Toledo, OH.

Trichloroethylene (TCE), a drinking water contaminant, has been reported to increase the incidence of liver cancer in mice, but not rats. Evidence suggests that the species sensitivity to TCE resides in its ability to induce peroxisome proliferation (PP) in mice, but not in rats. Chloroenoxyacetic acid (CEAA) is a major metabolite in both species; dichloroenoxyacetic acid (DCAA) and monochloroenoxyacetic acid (MCAA) are minor ones. In this study PP was scored by measuring the induction of carnitine-acetyl transferase, palmitoyl CoA oxidase, and the PPA-80 protein in rat and mouse liver after a 14 day exposure to daily dosing of TCE (1 mmole/kg, p.o.) in corn oil or to TCAA, DCAA or MCAA in the drinking water. TCE induced PP only in the mouse. TCAA (1, 3 and 5 g/l) induced PP in a dose dependent manner in mice, but was inactive in rats. DCAA (1, 3 and 5 g/l) was active in mice and rats. MCAA was not active in either species. Cytotoxicity studies using primary cultures of rat and mouse hepatocytes reflected a sensitivity to the TCE metabolites seen for PP induction in vivo. These data support the idea that mouse liver sensitivity to induction of PP by TCAA in part underlies the carcinogenicity of TCE in that species. This abstract does not necessarily reflect EPA policy.

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Previous work in this laboratory indicated that compound LY171883 (1-2-hydroxy-3-propyl-4-4′-(1H-tetrazol-5-yl)butoxy-phenyl)ethaneone) induced hepatic peroxisomal enzymes and peroxisome proliferation in rats. The studies reported here examined structural requirements for induction of peroxisomal β-oxidation (8-ox) in rats and in cultured rat hepatocytes. Increasing the length of the alkyl chain between the phenyl and tetrazole from four methylenes to six increased induction of 8-ox while decreasing to one methylene abolished the effect. Replacement of the hydrogen on the tetrazole with a methyl also prevented induction. Insertion of a phenoxymethyl between the phenyl and tetrazole yielded a compound which was inactive as an inducer. The addition of a methylene in the 4-position did not lead to induction of 8-ox but two methylenes were mildly active and three caused profound induction. A single methylene in the 3-position also resulted in profound induction of 8-ox. The data indicated that the length of the alkyl chain adjoining the acidic tetrazole is a factor which modulates induction of 8-ox. When the alkyl tetrazole is linked to a phenyl ring, its position on the ring is also a factor. The data generated in cultured hepatocytes corresponded well with the in vivo results.

IN VIVO AND IN VITRO INDUCTION OF RAT HEPATIC PEROXISOMAL ENZYMES BY VALPROIC ACID. Y. Singh, G. Liu, V. Shrirattti and G. Krishna, NHLBI, Bethesda, MD. Spon.: J.R. Gillette

Antiepileptic drug, Valproic acid (VA) (2-propyl pentanoic acid) when injected into male Sprague Dawley rats weighing 150-250g at a dose of 100-200 mg/kg ip daily for 30 days caused a 2-3 fold increase of carnitine acetyltransferase (CAT) in mitochondria. This indicated that VA may be peroxisomal proliferator as we have examined the VA induced peroxisomal proliferation in cultured rat liver cells. Liver cells were incubated with VA dissolved in the medium (pH 7.4) for 24, 48 and 72 h. Cells were solubilized in emulgen buffer and assayed for CAT, carnitine palmitoyltransferase (CPT) and cytochrome P-450 levels. VA markedly increased CAT in a dose and time dependent fashion. A maximal increase of 6-fold in activity of CAT was induced by 3 mM VA at 72 h. CPT was increased only 2-fold and cytochrome P-450 was not increased. When VA treated cells were stained for peroxisomes and examined by electron microscopy, there was no apparent increase in the number of peroxisomes. An increase in CAT alone may not be a good predictor of peroxisomal proliferation induced by some groups of drugs.


Hepatic peroxisome proliferation (HPP) has been detected by monitoring changes in peroxisomal fatty acid oxidizing activity or the increase in the amount of the 80-1000 Dalton HPP-associated polypeptide (PF480). PF480 has been reported to be a multifunctional protein having enoyl CoA hydratase (ECH) and hydroxacyl CoA dehydrogenase (HCDH) activities. Peroxisomal changes were examined by measuring activities of enzymes involved in peroxisomal β-oxidation, catalase, and levels of PF480 in livers of F344 rats treated with DEHP (2g/kg) for 1, 2, 3, 4, 7, or 14 days. Fattyacyl CoA oxidase (FCO) activity was increased 2.5-fold after one day, and 8-fold after 14 days. There was no apparent change in ECH activity after one day; however, there was a 2.2-fold increase after 2 days and 6 fold increase after 14 days. There was no significant increase in HCDH after 4 days of treatment; the activity of this enzyme at 7 and 14 days was only about 60% greater than that of controls. Changes in catalase activity were similar to those of HCDH. The increase in PF480 was apparent after 2 days of dosing and continuous for 14 days. The different rates of change indicate that there are at least two events in HPP and that the induction of HCDH and PF480 are not simultaneous. FCO induction is an early indicator of peroxisomal changes.

METABOLISM AND BIOCHEMICAL EFFECTS OF 2-ETHYLHEXYLADIPATE (DEHA) IN RATS. J.C. Lhuiguenot and C.R. Elcombe, University of Dijon, ENSBANA, 21100 Dijon, France, and Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, U.K. Spon.: E.A. Lock.

The important peroxisome proliferators (PP) derived from DEHP are ω-1 oxidised metabolites of mono-(2-ethylhexyl)phthalate. These studies have examined, 1) whether ω-1 oxidised monooester metabolites are produced from DEHA, and 2) which are the important proximate PP derived from DEHA. Male rats were administered [1,6-14C]-DEHA (250-2000μg/kg) and 24 hr. No intact oxidised metabolites of mono-(2-ethylhexyl) adipate (MEHA) were found. The relative proportion of 2-ethylhexanoic acid (EHA) and its glucuronide increased with increasing doses of DEHA. In vivo peroxisome proliferation studies showed EHA to be considerably more potent than DEHA or ethylhexanol. In vitro experiments utilising rat hepatocyte cultures demonstrated no peroxisome proliferation due to DEHA (little biotransformation was seen), little due to MEHA, but a marked effect of EHA. These studies suggest that EHA is an important proximate PP derived from DEHA and that increasing dose levels of DEHA lead to proportionally increased quantities of EHA and hence more peroxisome proliferation.
Inhibition of hepatic ketogenesis may be an early event which leads to proliferation of peroxisomes by ethylxanol. Therefore, the purpose of this study was to evaluate whether other peroxisomal proliferators structurally similar to ethylxanol inhibit hepatic ketogenesis. Control rates of ketogenesis (β-hydroxybutyrate + acetoacetate production) by perfused livers from fasted rats were about 40 μmol/g/h, values which were inhibited about 60% by ethylxanol (200 μM). Equimolar concentrations of the structurally similar compounds, 2-ethylbutanol and 3-octanol, inhibited rates of ketone body production by 39 and 18%, respectively. In contrast, perfluorooctanoic acid inhibited ketogenesis by 80%. The potency of these compounds to cause peroxisomal proliferation has been reported as follows: perfluorooctanoic acid > 2-ethylhexyl phthalate > 2-ethylbutyl phthalate > 3-octyl phthalate. Phthalic acid did not inhibit hepatic ketogenesis nor did it cause peroxisomal proliferation. These results suggest that inhibition of hepatic ketogenesis may be a common early event by which plasticizers cause proliferation of peroxisomes possibly by causing the accumulation of hepatic lipid.

Electrical Comparisons of Liver Regeneration, Hyperplasia and Neoplasia in the Rat.
J.A. Styles and C.R. Elcombe.
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Sponsor: E.A. Lock

The development of different ploidy and nuclear classes in the rat liver proceeds by cell cycles that alternate suppress mitosis and cytokinesis. The phenomena of regeneration, hyperplasia and neoplasia were induced by 2/3 partial hepatectomy, administration of 25mg/kg methyl clofenapate or 5mg/kg 6-p-dimethylaminophenylazobenzothiazole respectively. Ploidy, nuclearity and DNA synthesis were analysed by a combination of flow cytometry and cell sorting, using propidium iodide staining for total DNA content and BUdR incorporation/BUdR monoclonal antibody for DNA synthesis. In liver regeneration there is an overall increase in ploidy due to an increased rate of maturation of the liver. In hyperplasia there is a marked reduction in the incidence of binucleated tetraploid cells and a concomitant increase in mononucleated tetraploid cells. In neoplasia there is a decrease in the incidence of binucleated tetraploid cells and an increase in mononucleated diploid cells. Thus in both hyperplasia and neoplasm the binucleated tetraploid cell is sensitive to the chemicals but the cytological responses of the liver are different.

Detecting Toxicity Using Primary Hepatocyte Cultures. J. E. Simmons and J. Lewtas.

Sponsor: William F. Durham

We are evaluating the usefulness of a primary hepatocyte culture assay to screen complex mixtures for hepatotoxic effects. Viable hepatocytes were isolated from 47-55 day old male Sprague-Dawley CD rats (235-260 grams) using an in situ collagenase perfusion. We modified previously described perfusion and culture media (In Vitro, 17: 1004, 1981). These modifications included buffering the collagenase perfusion medium and the hepatocyte culture medium with HEPES buffer; adding CaCl2 to the collagenase perfusion medium; and adding L-glutamine and a higher concentration of glucose to the culture medium. Testing of a series of aliphatic chlorinated hydrocarbons has been initiated using the release of intracellular enzymes as toxicity indicators. Treatment with 0.0625, 0.125, 0.25, 0.5, 10, and 20 mM carbon tetrachloride for 20 hours resulted in concentration-dependent increases in the release of GOT and GPT into the culture medium. In addition, a concentration-dependent increase in the number of cells detached from the surface of the tissue culture flask was observed.

In Vitro Hepatotoxicity Study of Chlorpromazine and Erythromycin Analogs
K.C. Norbury1, P. Carhage1, E.M.B. Sorensen2, G. Acosta3, and G.J. Davis1
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An interlaboratory comparison was conducted in two laboratories to assess the potential in vitro toxicity of chlorpromazine (CPZ), erythromycin estolate (EE), and erythromycin stearate (ES). Parenchymal hepatocytes were isolated, plated, and incubated according to the Acosta et al. procedures (J Tiss. Cult. Meth. 6:35, 1980). Primary cultures were exposed to various concentrations of the individual drugs and monitored to determine the release of both lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). Cultured cells released both soluble cytoplasmic enzymes in a dose-dependent fashion. Significant increases occurred at concentrations of 40-160 μM for CPZ and 80-160 μM for EE. Compared to untreated controls, enzyme release was significantly different at the same concentrations in both laboratories. Exposure of cells to ES did not result in significant release of either enzyme even at 160 μM, the highest concentration tested. The results from these in vitro studies with known in vivo hepatotoxins compare favorably between laboratories (r2 > 0.98) and also demonstrate the reproducibility of primary cultures of parenchymal hepatocytes to evaluate the relative cytotoxicity of various compounds.
468 THE EFFECT OF CELLULAR GLUTATHIONE (GSH) LEVELS ON CYTOTOXICITY OF T-2 MYCOTOXIN (T-2) IN RAT HEPATOCYTES. W.L. Thompson and R.F. Fricke, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD.

Treatment of cultured hepatocytes with GSH prodrugs (oxo- or methyl-thiazolidine carbonylate) or GSH suppressors (buthionine sulfoximine or acetalaminophen) results in the following levels of intracellular GSH (micromoles/10^6 cells): prodrugs, 90 and 120; suppressors, < 2.0 and 16, respectively; as compared to control, 32. Since the prodrugs have shown protection in vivo against T-2, a potent protein synthesis inhibitor, these drugs were tested in vitro by exposure of drug-treated hepatocytes to various doses of T-2. There was no evidence of extracellular leakage of LDH and CPK enzymes in the drugs treated cells, with or without T-2 exposure, indicating that no membrane leakage occurred. Drug treatment had no effect on the ability of T-2 to inhibit protein synthesis as measured by shifts in the SOX protein synthesis inhibition assay. Using thin-layer radiochromatography, no significant difference was seen in the metabolism of [3H]-T-2 by control and GSH-stimulated cells, while little additional metabolism to some of the more polar intermediates was seen in the GSH-depleted cells. These studies suggest that although GSH may aid in the metabolism of T-2, it does not affect cytotoxicity at the cell level.

469 KINETIC EVALUATION OF CARRIER-MEDIATED TRANSPORT OF OUABAIN AND TAUCROHOLIC ACID IN ISOLATED RAT HEPATOCYTES. EVIDENCE FOR INDEPENDENT TRANSPORT SYSTEMS. D.L. Eaton and J.A. Richards, Dept. Environmental Health, University of Washington, Seattle, WA.

Ouabain and taurocholic acid (TcH) share in common the steroid nucleus, and both are concentrated in the liver by hepatic sinusoidal carrier-mediated transport processes. To determine if the ouabain transport system is related to transport of TcH, kinetic studies were conducted to examine the nature of the competition-inhibition of ouabain and TcH. TcH was found to inhibit ouabain uptake in a competitive manner, with a Ki 10 times less than the Km for ouabain. However, ouabain failed to inhibit total TcH uptake in a competitive manner when added to the system at the same time as the ouabain substrate. Pre-incubation of cells with ouabain resulted in non-competitive inhibition of Na-dependent TcH uptake, but had no effect on Na-independent TcH uptake. Ouabain had a weak competitive inhibitory effect on Na-independent transport of TcH, but the Ki was approximately 10 times greater than the Km for ouabain. These results demonstrate that the ouabain transport system is distinct from both the Na-dependent and Na-independent transport systems for TcH. TcH apparently binds competitively to the ouabain transport system, but is not transported across the cell membrane by this system. (Supported by USPHS Grant ES-03719).

470 INFLUENCE OF CHLORINATED HYDROCARBONS ON CULTURED HEPATOCYTE FUNCTION. R.G. Lamb, J.B. Coleman, L.W. Condie* and J.F. Borzelleca, Depts. of Pharmacology/Toxicology and Internal Medicine, Medical College of VA, Richmond, VA 23298 and U.S.E.P.A., Cincinnati, OH 45268.

Cultured hepatocytes were incubated (2 to 72 hr) with various concentrations (0.1 to 1.0 mM) of carbon tetrachloride (CCl4), trichloroethylene (TCE), perchloroethylene (PCE) and chloroform (CHCl3) to assess the hepatotoxicity of these agents in vitro. Cultured hepatocytes maintained with supplemented Waymouth 752/1 medium were able to generate (48 h) 14C-CCl4 metabolites that were covalently bound to cellular protein, phospholipid and glycerides. CCl4 rapidly (1 hr) alters the formation and degradation of liver cell phospholipids and increases (24-72 hr) GOT release; however, TCE, PCE, and CHCl3 have little effect on these cell functions. When CCl4 metabolism is reduced (non-supplemented medium), the CCl4-dependent changes in liver cell GOT release and phospholipid metabolism are decreased. These results suggest that cultured hepatocytes may be an appropriate model in vitro for identifying hepatotoxic agents and elucidating the mechanisms by which these chemicals produce liver cell injury. (Supported by NIH AM 31115 and EPA Cooperative Agreement 812558.)

471 EFFECT OF VOLATILE ANESTHETICS ON PROTEIN SYNTHESIS AND SECRETION IN RAT HEPATOCYTE SUSPENSIONS. A.B. DiRenzo, A.J. Gandolfi, K. Brendel, G. Krak, Arizona Health Sciences Center, University of Arizona, Tucson AZ.

The effect of volatile anesthetics on protein synthesis and secretion by isolated rat hepatocytes in suspension was investigated. Halothane and enflurane (0.15-60 mM) inhibited protein synthesis in a dose-dependent manner (25-49%; 4 hr incubation; 21 % Co). Diethyl ether had little effect on protein synthesis while isoflurane caused a mild inhibition. This effect was more pronounced in hepatocytes from phenobarbital treated male rats when compared to control hepatocytes (1.4 fold, isoflurane; 1.5 fold, enflurane; 1.8 fold, halothane). Protein synthesis in hepatocytes from phenobarbital treated female rats was inhibited similar to that seen with control male rat hepatocytes. Isoflurane, enflurane, and halothane caused a dose-dependent inhibition of protein secretion (45, 64, 64%, respectively; at 0.6 mM). Protein secretion is inhibited more than synthesis with the fluorinated anesthetics. The biotransformation of the fluorinated anesthetics proceeded in a dose and time dependent manner and parallelized the inhibition of protein synthesis and secretion. From these studies it appears that inhibition of protein synthesis/secretion might be an early and sensitive indicator of cellular injury by volatile anesthetics. (NIH AM 16715)
OXMETIDINE is a potent and specific inhibitor of the histamine-H₂ receptor. Oxmetidine is cytotoxic to isolated rat hepatocytes by inhibiting mitochondrial oxidative phosphorylation. The purpose of this investigation was to test a variety of H₂ antagonists that are structural analogues of Oxmetidine in an attempt to identify a critical structural component of the Oxmetidine molecule causing cytotoxicity. Six histamine H₂-receptor antagonists were tested. The minimum drug concentrations that caused 100% cell death (leakage of lactate dehydrogenase and loss of intracellular potassium; EC₅₀(90) ranged from 0.87 to 22.5 μM. All of the drugs tested produced a rapid decrease in hepatocyte O₂ consumption and ATP content at toxic concentrations. The toxicity of these drugs did not correlate well with their potency as histamine H₂-receptor antagonists but did correlate well with their respective octanol/water partition coefficients. These data suggest that lipid solubility is a key factor in the cytotoxicity of this class of drugs to isolated rat hepatocytes.

HAPATOTOXIC ELEVATE CYTOSOLIC Ca²⁺ STIES WITH QUNL, R.M. Long and L. Moore, Dep. of Pharmacology, Uniformed Services University, Bethesda, MD 20814. Sponsor: A.P. Alves

Carbon tetrachloride (CCl₄) and 1,1-dichloroethylene (DCE) inhibit nequestration of Ca²⁺ by rat hepatic endoplasmic reticulum. As a result, cytosolic Ca²⁺ levels may become elevated in rat liver cells. Previously, we have demonstrated in vivo and in vitro that CCl₄ causes a prolonged increase in the activity of glycogen phosphorylase a, a Ca⁺⁺-responsive cytosolic enzyme. We have now examined cytosolic Ca²⁺ concentrations in isolated rat hepatocytes with the fluorescent Ca⁺⁺ indicator dye, furaß. Basal Ca²⁺ levels in these cells averaged 247 ± 26 nM. Ca²⁺ concentrations were increased to 829 ± 129 nM after 2 mM CCl₄, 592 ± 60 nM after 4 mM DCE, and 997 ± 136 nM after 10 μM phenylephrine (PE, adrenergic agent that mobilizes Ca²⁺). These changes were apparent within 20 seconds. Thus, CCl₄ and PE rapidly mobilized Ca²⁺ to approximately the same extent, while DCE was somewhat less effective. Because CCl₄ and DCE are hepatotoxins and PE is not, we speculate that there are differences in the durations of elevated Ca²⁺ levels produced by these agents. Prolonged elevation of cytosolic Ca²⁺ may cause excessive activation of Ca⁺⁺-responsive enzymes (e.g., phospholipases, proteases, kinases) capable of mediating toxic cell injury. (This work was supported by grant ES03437 from the U.S. Public Health Service.


Since media commonly used for hepatocyte culture differ in their content of sulfur amino acids, we have examined the influence of medium composition on glutathione (GSH) levels in cultured hepatocytes. Over a 2 hr period, hepatocytes maintained in RPMI 1640 or Williams E media showed a progressive decline in GSH content to around 5 mM/mg protein. In contrast, GSH levels were maintained at around in vivo levels (25-30 mM/mg protein) in cells cultured in L15 medium or RPMI 1640 in which the methionine concentration was increased from 0.1 to 0.5 mM. There was little difference in hepatocyte cytochrome P=450 content in any of these media. Hepatocytes cultured in RPMI 1640 containing 0.5 mM methionine were much less sensitive to the toxicity of allyl alcohol and hydrogen peroxide than cells cultured in the standard medium, as judged by LDH release, stimulation of lipid peroxidation and inhibition of protein synthesis. Thus, the outcome of toxicity studies in cultured hepatocytes with chemicals whose detoxification involves GSH may be profoundly influenced by the choice of culture medium. (Supported by U.K. Ministry of Agriculture, Fisheries and Food)

AN IN VIVO/IN VITRO MODEL FOR ASSESSING HEPATOXIN-INDUCED CELL INJURY, J.R. MacDonald and E.A. Smuckler, Department of Pathology, UCSF, San Francisco, CA, 94143

Primary monolayer cultures of hepatocytes from male, Sprague-Dawley rats were established following in vivo administration of carbon tetrachloride (CCl₄) or galactosamine (GAL). Fasted rats received CCl₄ (2.5 ml/kg, po, 50% in mineral oil) or GAL (1 g/kg, po) 30 min prior to portal vein cannulation for cell isolation. GAL (400 mg/kg, im) was given to fasted rats 1 hr before cell isolation. Initial cell viability, yield, and attachment efficiencies were the same for cells from toxin treated rats and their respective controls. After 1 hr attachment monolayers were washed, fresh media added and cell viability found to be 85% in all cultures. By 24 hr after in vivo CCl₄ or GAL treatments, viable cell density was decreased 50% compared to 1 hr post-attachment densities. Controls had 10% decline in cell density over 48 h in culture. Cells from CCl₄, but not GAL, treated rats continued to deteriorate between 24 and 48 h in culture. Media LDH activities reflected the decline in cell density in cultures from toxin treated rats. This model combines initiation of cell injury in vivo with the potential advantages of subsequent analysis of cellular injury in culture. In addition, the progression of cell injury can be assessed over a time course similar to the development of hepatotoxin-induced cell death in vivo. (Supported by UCSF grant M5C-68 and NIH grants ES05359 and AM19843)
CHLOROBENZENE, CARBON TETRACHLORIDE
AND TRICHLORETHYLENE INHIBIT METABOLIC
FUNCTIONS IN ISOLATED RAT HEPATOCYTES
BEFORE DETECTABLE MEMBRANE DAMAGE.
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Isolated hepatocytes in suspension are a useful model
for the assessment of acute hepatotoxicity. 
However, tests for damage vary significantly and
tend to focus on plasma membrane integrity. In this
work the effects of chlorobenzene, CCl₄ and
trichlorethylene on protein and glycogen synthesis
were also investigated. Primary suspensions of
hepatocytes isolated from male Fischer 344 rats were
incubated in culture medium with nominal concentrations
of the compounds ranging from 0.1 to
10 mM. Samples were obtained at 30 min, 1, 2 and 3
hr. Cellular membrane damage was evaluated by
LDH leakage, while metabolic competence was
evaluated by glycogen and protein synthesis
capabilities. CCl₄ inhibited protein synthesis by
nearly 40% at 0.5 mM and glycogen synthesis by 20%
at 2.0 mM, both after 1 hour. Chlorobenzene at 2
mM inhibited both processes by at least 20% after 1
hour. TCE decreased protein and glycogen synthesis
by up to 20% at 0.5 mM after 30 minutes. LDH
release was not significantly affected by any of these
treatments. In every case, the biochemical markers indicate that toxic events occur at earlier times and
at lower concentrations than indicated by LDH
release. (Supported by NTP-NOl-ES-3-5031.)

HEPATOXICITY OF THREE DICHLOROBENZENE
ISOMERS IN HUMAN LIVER ORGAN CULTURE.
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O-Dichlorobenzene (o-DCB) and m-chlorobenzene
(m-DCB) and p-chlorobenzene (p-DCB) were tested for
their hepatotoxic effects on precision cut human
liver explants maintained for 6 hr in dynamic organ
culture. All results are from tissue obtained from 46
human donors. Dose and time-dependent cell injury was
based on intracellular K⁺ content, LDH leakage, and
protein synthesis. Incubation of explants with 1 mM
of any DCB did not result in apparent toxicity, but o- and
m-DCB produced minor decreases in protein synthesis
at 6 hr. When explants were incubated with 2 mM
all isomers resulted in substantial toxicity. To determine
if cytochrome P-450 is involved in this DCB toxicity, a
P-450 inhibitor, SKF 525-A or metyrapone, was incubated in the presence of 2.0 mM DCB. The toxicity of
o-DCB at 4 hr, was blocked with metyrapone
(0.5 mM) but not SKF 525-A. m-DCB toxicity was
blocked with SKF 525-A (0.25 mM) but not metyrapone.
The toxicity of p-DCB was not blocked by either inhibitor. These results are similar to those obtained in
noninduced rat liver explants in that all isomers are
toxic at 2 mM, but not at 0.1 mM. In addition these
inhibitors show similar selectivity between o- and
m-DCB in explants from rats as in those from humans.
In conclusion, it appears that human liver cultures will
increase our ability to extrapolate animal data to
humans. (Supported by NIEHS NOl-ES-55112.)

BROMOBENZENE AND ALLYL ALCOHOL HEPATOTOXICITY IN
CULTURED RAT LIVER SLICES: II. INHIBITION OF
TOXICITY BY SELECTIVE ENZYME INHIBITORS.
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Bromobenzene and allyl alcohol hepatotoxicity were
studied in vitro using organ-cultures of
depancreas liver slices from newborn rats of the
dawley strain. These slices were found to retain their
biotransformation ability for at least 6 hr
based on maintenance of cytochrome P-450 content
and 0-deethylase activity. Either compound caused
dose (.01-1.0 mM) and time (0-6 hr) dependent
cell injury as indicated by the loss of slice K⁺,
inhibition of protein synthesis, and leakage of
lactate dehydrogenase (LDH). By 2 hr, a significant
(p<.05) inhibition of protein synthesis was
noted in allyl alcohol (0.5 mM) treated slices.
At 4 and 6 hr, significant loss of slice K⁺, LDH
inhibition of protein synthesis were evident in
slices exposed to allyl alcohol (.25 mM) or
bromobenzene (.5 mM). The toxicity of other
compound could be modulated by selective enzyme
inhibitors. The toxicity of either allyl alcohol
or bromobenzene, at 4 hr, was blocked when slices
were preincubated for 30 min with pyrazole (1.0
mM) or SKF 525-A (.1 mM), respectively. This
system provides a new tool for the study of hepatoxocity under conditions where hepatocellular
functional integrity, biotransformation, and
architecture are maintained. (NIEHS-ES-070-91)

THE DICHLOBENZENES IN
ISOLATED RAT HEPATOCYTES
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The three dichlorobenzenes are widespread
contaminants of water supplies. In vitro experiments
with rats have indicated that the ortho-isomer of
dichlorobenzene is hepatotoxic, at lower doses than the
meta- or para-isomers, as assessed by enzyme
leakage and histology. The object of this work was to
determine whether this differential toxicity could be
observed in isolated hepatocytes. Hepatocytes were
isolated from uninduced mature male F-344 rats and
incubated in culture medium (0.5 million cells/ml)
and sampled at 1, 2 and 3 hours. The parameters measured
were LDH leakage, as an indicator of cell membrane
integrity, and protein synthesis and secretion, as
indicators of cellular biochemical integrity. The
nominal concentrations of the dichlorobenzenes studied
were 10, 25, 50, 100, and 500 µM for each isomer. LDH
leakage was affected at the highest concentration of
each isomer studied, but there was no difference
between the isomers in the percentage of enzyme
released (40%). Protein synthesis and protein secretion
are inhibited in a similar dose dependent manner by all
three isomers of dichlorobenzene (at concentrations
above 50 µM). However, neither the inhibitory
effects on protein synthesis and secretion or cytosolic
enzyme leakage suggest any one form of
dichlorobenzene to be markedly more toxic than any
other using this in vitro system. (Supported by
NTP-NOl-ES-3-5031.)

The hepatotoxicity of many aromatic hydrocarbons has been attributed to bioactivation to reactive intermediates with subsequent covalent binding to tissue macromolecules. In vivo toxicity studies in male F-344 rats demonstrate a marked structure-toxicity relationship for the 3 isomers of dichlorobenzene (DCB) following ip exposure. Plasma GPT activity 24-hr post exposure (time to maximal elevation) is elevated at approximately 4000 units/ml following a dose of 1.8 mmol/kg o-DCB, while at 4.5 mmol/kg, m-DCB and p-DCB produce little or no elevation (306 and 24 units/ml, respectively). Ortho- and m-DCB deplete hepatic glutathione to the same level (3.8 and 3.7 umole/g tissue, respectively) 90 min following a dose of 1.8 mmol/kg, while p-DCB treated animals are not different from controls (3.7 and 5.8 umole/g, respectively). Furthermore, preliminary in vitro data show that the degree of covalent binding to rat liver microsomal protein is comparable between o- and m-DCB, despite a lack of correlation with in vivo hepatotoxicity. These data indicate that end organ hepatotoxicity for the DCBs cannot be explained solely by P-450 bioactivation. In vivo pharmacokinetic studies are underway to determine if the observed structure-toxicity differences result from differences in distribution, metabolism, or elimination. (Supported by NTP-NOI-ES-5-5231 and NIH T32 ES 07391.)

HEPATOTOXICITY OF DICHLOROBENZENE ISOMERS IN SPRAIGE DAWLEY RAT LIVER ORGAN EXPLANTS. R. Fisher, P.F. Smith, A.J. Gandolfi, I.G. Sipes and K. Brendel. Center of Toxicology, University of Arizona, Tucson, AZ.

o-Dichlorobenzene (o-DCB), m-dichlorobenzene (m-DCB) and p-dichlorobenzene (p-DCB) were tested for their hepatotoxic effects on precision cut rat liver explants obtained from male Sprague Dawley rats. The explants were maintained in dynamic orgm culture for up to 6 hr. Toxicity was evaluated by intracellular K+ content, LDH leakage, and protein synthesis. Incubation for 2.4 or 6 hr with 1 mM of any DCB did not result in toxicity in noninduced rat liver explants. In the presence of 2 mM each DCB resulted in toxicity, but the time response was different. When liver tissue obtained from phenobarbital-induced rats was incubated with 1.0 mM of either o-DCB or m-DCB toxicity was seen, but p-DCB exhibited no toxicity. To further explore the role of biotransformation in DCB-induced toxicity, the isomers were incubated in the presence of the cytochrome P-450 inhibitors, SKF 525-A or metyrapone. The toxicity of o-DCB, after 3 hr, was blocked with metyrapone (0.5 mM) but not SKF 525-A. m-DCB toxicity was blocked with SKF 525-A (0.25 mM) but not metyrapone. These results indicate the possible involvement of different cytochrome P-450 isozymes in the metabolism and toxicity of o- and m-DCB. (Supported by NIH-NOI-ES-5-5231 and T32 ES 07391.)

ULTRASTRUCTURAL EFFECTS OF CARBON TETERACHLORIDE (CCl4) AND DIGITONIN ON ISOLATED RAT HEPATOCYTES. J.M. Waltman, F.-P. Fang, P. Sims, J. Freeland, D-C Wang and M.A. Evans, Department of Pharmacology, University of Illinois College of Medicine, Chicago, Illinois, 60612.

Previous investigators have suggested that the early histological changes observed in isolated rat hepatocytes following CCl4 administration is related to damage and subsequent leakage of calcium across the plasma membrane. Digitonin is used as a mild specific detergent for solubilization of membranes with minimal protein damage. Dose response studies were performed to compare the early ultrastructural and biochemical effects of CCl4 (0.1-2.0 uM) on isolated rat hepatocytes with those of digitonin (5.0-50 uM protein). Treatment of hepatocytes with CCl4 (1.0 uM) for 30 min resulted in a breakdown of internal structures of the nucleus and mitochondria, cellular protrusions and a rearrangement of the nucleoli. Digitonin, at doses of 20 and 50 uM protein, produced an alteration of the cellular membrane with loss of the outer layer of the bilayer membrane and without cellular protrusions. Increased cellular concentrations of calcium was observed following both CCl4 and digitonin exposure. No alteration of the internal milieu was noted after any dose exposure to digitonin. It is suggested that the cellular protrusions observed in the hepatocyte following CCl4 treatment is not related to plasma membrane damage.

TRALOMETHRIN HAS INTRINSIC ACTIVITY ON NERVE MEMBRANE SODIUM CHANNELS. L.D. Brown and T. Narahashi. Department of Pharmacology, Northwestern University Medical School, Chicago, IL

Considerable controversy exists over the question of whether tralomethrin has intrinsic activity or merely acts as a propyrehond of deltamethrin. To resolve this question, voltage clamp experiments were performed with internally perfused squid giant axons. Under this condition, pyrethroids could be applied directly to the internal membrane surface with no chance of enzymatic degradation due to a high concentration of fluoride in the internal perfusate. Furthermore, the kinetics of sodium channel gating could be measured with the highest degree of accuracy. Both tralomethrin and deltamethrin increased and prolonged the sodium tail current associated with a step repolarization. The apparent dissociation constants to increase the tail current amplitude were estimated to be 0.2 and 0.3 uM for tralomethrin and deltamethrin, respectively. Despite such similarities in action, the rate of decay of slow tail current was vastly different between them. The mean time constants were estimated to be 4,200 msec for tralomethrin and 860 msec for deltamethrin representing a 5-fold difference. Such a large difference is incompatible with the notion that tralomethrin is a prodrug only after having been converted into deltamethrin. Supported by UCLA Corporation and NIH grant NS14143.

Whereas the actions of pyrethroids have been well characterized in peripheral nerves, little is known of their neurotoxic effects in the mammalian central nervous system. We have examined the central actions of pyrethroids using slices of the guinea pig olfactory cortex. Field potentials were recorded from the surface of the olfactory tubercle in response to a single or paired (50 msec interval) electrical pulse (0.05 msec duration) applied to the lateral olfactory tract. The stimulation induced a slow negative wave which mainly reflects the excitatory postsynaptic potentials. An application of delta-methrin or fenvalerate (100 nM) markedly increased the amplitude of the slow negative wave. When paired stimuli were delivered, the amplitude of the slow negative wave evoked by the second pulse was much smaller than that evoked by the first pulse. This was in sharp contrast with the observation in the control solution where the second slow negative wave was consistently larger than the first. Such a reversal of the responses to paired stimuli in the pyrethroids was offset by a reduction of external Ca^2+ concentration. These results indicate that pyrethroids facilitate neurotransmitter release in the mammalian central synapses. Supported by NIH grant NS14743.

REFRACTORINESS OF THE NEUROMUSCULAR TOXICITY OF DITHIOBIURET IN RATS. K.D. Williams and R.E. Peterson, School of Pharmacy, University of Wisconsin, Madison, WI 53706

A 3 day latency was thought to govern the onset of dithiothreitol (DTB)-induced flaccid muscle tone (FMT) in rats given daily doses exceeding 2 mg/kg. Correspondingly, FMT impaired treadmill performance developed after 3 days of DTB treatment (5 mg/kg, ip). Also, the tension during individual tetanic gastrocnemius muscle contractions elicited by nerve stimulation was depressed in magnitude and faded excessively. Suprisingly, rats receiving larger doses of DTB (12 mg/kg/day x 3 days) were refractory to the neuromuscular toxicity. FMT, impaired treadmill performance, and tetanic contractile abnormalities were absent even though other signs of toxicity (weight loss, diuresis, dehydration) were severe. Treatment for 2-4 additional days caused the rats to become moribund (treadmill performance then declined) and die without prior development of FMT. The severe toxicity suggested that diminished bioavailability of DTB was not the cause of the refractoriness. Inhibition of acetylcholinesterase (ACHE) was considered a likely mechanism since this could counter an underlying deficit in ACh release (the presumed cause of FMT with low doses of DTB). However, ACHe activity in brain and skeletal muscle homogenates of DTB-treated rats (12 mg/kg/day x 3 days) was similar to control. Thus, the cause of the refractory condition remains unresolved. (Supported by NIH Grant ES01906)


The peripheral effects of organophosphates (OP) in the hen are well known, but detailed electrophysiologic studies have not previously been reported. White Leghorn hens were dosed po with tri-o-cresyl phosphate (TOCP, 30 and 750 mg/kg), 2,2-dichlorovinyl di-n-butylphosphate (DBCP, 4 mg/kg) or corn oil vehicle. 24 hrs after dosing lymphocytic neurotic esterase was inhibited by 54, 87 and 88% for low TOCP, high TOCP and DBCP, respectively. 21 days after dosing hens were anesthetized and electrophysiologic activity was measured. All OP treatments resulted in an increased refractoriness of the sciatic and slightly decreased refractoriness of the tibial nerve. Strength-duration curves indicated an increased activation threshold. The TOCP treatments (but not DBCP) produced significant decreases in nerve compound action potential (AP) conduction velocity and amplitude and an increased AP duration. DBCP and high TOCP produced clinical signs such as unsteady gait and ataxia. Histologic evaluation revealed marked pathology in the high TOCP and DBCP groups but none in the low TOCP group. These data demonstrate that electrophysiological measurements may be sensitive indicators of OPIDN and that these effects occur in hens with no clinical or histologic deficits.

FUNCTIONAL AND MORPHOLOGICAL CHARACTERIZATION OF DITHIOBIURET NEUROTOXICITY IN RATS. K.D. Williams B.G. Boysen, and R.E. Peterson School of Pharmacy, University of Wisconsin and Hazleton Laboratories of America, Inc., Madison, WI.

Rats treated with dithiothreitol (DTB, 1 mg/kg/day, ip) were evaluated for the appearance of flaccid muscle tone (FMT, detected by impaired treadmill performance) to determine its association with muscle contractile deficits (fading, low-tension nerve-elicited tetanic gastrocnemius muscle contractions) and other signs of toxicity. Treadmill performance was impaired by Day 4, and contractile deficits were noted on Day 5. On Day 6, FMT, depressed tetanic contractions, weight loss, dehydration, and hypothermia were severe. Nevertheless, acid/base balance, arterial blood concentrations of O2 and CO2, serum levels of various electrolytes, urea nitrogen, and glucose were similar to controls. Thus, disturbed electrolyte concentrations, hypoxia, or hypoglycemia do not cause FMT. Light microscopic evaluation of liver, kidney, lung, thyroid, skeletal muscle, sciatic nerve, brain, and other organs was unremarkable. Based on the early detection of contractile deficits without coincident appearance of toxicity in non-neuromuscular organs, a direct neuromuscular action of DTB seems likely. (Supported by NIH Grant ES01906)

Power spectral analysis was utilized to delineate dose and temporal variations observed in cortical EEG in the rat following acute i.v. doses of phystogyrine. Related changes in behavioral activity were also measured.

General behavioral arousal and increased latency to sleep occurred following all doses. Tremor, salivation, urination and defecation occurred at higher doses, and duration of tremor increased with increased dose.

EEG changes included (1) significant increases in theta wave activity (4 to 8 Hz) that returned to control values after 15 or 20 minutes following the lower doses; (2) significant increases in theta wave activity and delta activity (0 to 4 Hz) that persisted beyond 20 minutes following the highest dose; (3) intermittent spiking in the EEG following the highest dose; and (4) significant slowing of theta wave frequency (6.6 Hz to 5.6 Hz) following the higher doses.

Power ratios for the absolute power across time (0 to 5 minutes:15 to 20 minutes) were calculated for the various treatments within each frequency band and subjected to linear regression analysis. In every case, there was a strong linear association between dose and power ratios.

MODULATION OF TRIMETHYL-INDUCED NEUROGENESIS BY EXCITATORY PATHWAYS. Y. Theoret, D.A. Lindemann and M.R. Krigman, University of North Carolina, Chapel Hill, N.C.

We have shown (Soc. Neurosci. Abstr., Vol.11, Part 1, p. 493, 1985) that electrolytic lesion of the entorhinal cortex (EC) produces selective neuronal necrosis of the CA1 pyramidal neurons. The evidence suggests that the excessive firing at the terminals of the EC neurons may cause directly (EC→CA1) and/or indirectly (EC→granule cells→CA3→CA1) excitotoxic postsynaptic damage to CA1 pyramidal neurons. In the single dose model of trimethyltin (TMT) that we have developed, neuronal necrosis involved primarily the CA4 and CA1 pyramidal neurons of the hippocampal formation and the EC neurons. Therefore, it is conceivable that the excitatory pathways which interconnected these two structures may play a role in the pathogenesis of TMT. We propose that TMT may by its direct neurotoxic effect favor toxic synaptic cooperativity between CA1 pyramidal neurons and EC neurons. Adult male Long-Evans rats (300-400g) were sham operated or had knife cuts placed in the EC (unilaterally or bilaterally). On day 7, rats were orally administered either saline or TMT (7mg/kg) and were sacrificed on day 14. The results will be presented and the interaction of TMT with excitatory pathways will be discussed. (P01 ES01104, T32 ES07126, T32 ES07017 and IRSSH Fellowship to YT)


Trimethyltin (TMT) is a neurotoxic alkylmercury which damages several regions of the CNS, particularly structures of the limbic system. Studies of TMT toxicity in primates have generally used single exposures. We examined the pathology resulting from multiple exposures to TMT over a period of up to 15 weeks. Adult Macaca fascicularis were administered doses ranging from 0.5 to 4.0 mg TMT/kg/wk orally and tested for behavioral changes. Animals were then sacrificed under anesthesia by intracardiac glutaraldehyde perfusion, and samples taken for light and electron microscopy. Light microscopy revealed scattered neuronal loss in the CA-3 and CA-1 regions of the hippocampal Ammon's horn, accompanied by a slight astrogliosis. Occasionally, degenerating neurons were noted in the amygdala, Tascia dentata, cerebral cortex and retina. Ultrastructurally, atypical lysosomes and concentrically laminated organelles were noted within pyramidal neurons of hippocampus and cerebral cortex, while hippocampal granule cells were minimally affected. The absence of extensive neuronal loss at total dosages exceeding those causing death when given acutely suggests that TMT does not cause cumulative neurological damage in M. fascicularis. (Supported by NIH Grant ES0-3461.)


Trimethyllead (TMB) and trimethyltin (TMT) produce selective neurotoxicity in the limbic system but the mechanisms are still to be defined. We investigated the effect of these organometal species on the survival of hippocampal neurons which are metabolically compromised by high levels of corticosterone. Male Long-Evans rats weighing 250-300 g were treated with a daily s.c. injection of corticosterone (10 μg/ml/animal) in corn oil for 14 days. On day 7, the treatment, rats were given a p.o. dose of either TMB (20 or 35mg/kg) or TMT (5 or 8 mg/kg p.o.), or a non-neurotoxic intrahippocampal dose of kainic acid (0.0175 μg in 1 μl of saline and ascorbic acid). As previously reported by Sapolsky (J. Neuroscience 5(5):1226, 1985) persistent elevated corticosterone levels amplify the neurotoxic potential of kainic acid. However, the neurotoxicity of these organometal species was not changed by corticosterone treatment. It is possible that organometals may affect the vulnerability of hippocampal neurons to kainic acid. The results of intrahippocampal injection of kainic acid in TMT and TMB treated animals will be presented. (P01 ES01104, T32 ES07126, T32 ES07017 and IRSSH Fellowship to YT.)

Trichloroethylene (TCE) is an unsaturated chlorinated hydrocarbon used as an industrial degreaser. Identified primarily as a central nervous system toxin in adult systems, TCE's effect on developing systems is unclear. The concern of this study was to determine whether the developing hippocampus is a target for TCE, particularly the pyramidal cell layer of the region CA 1. Female rats were exposed to concentrations of either 625 ppm TCE or 312 ppm TCE through drinking water for two weeks prior to breeding, and until the pups were weaned at 21 days of age. The pups were sacrificed and the hippocampi were paraffin embedded for morphometric quantification. The volume of the pyramidal cell layer in the CA 1 region from rats exposed to 625 ppm TCE was significantly reduced. No significant reduction was observed in cell number, cell density or CA 1 volume of 312 ppm TCE exposed rats, or in cell number or cell density in 625 ppm TCE exposed rats. Changes in this region could be occurring in the axon collaterals of the cell bodies. (This does not necessarily represent official EPA policy; Supported by EPA Coop. Agreement CR809618).

EFFECT OF SUPPLEMENTAL CORTICOSTERONE AND SOCIAL STRESS ON ORGANOPHOSPHORUS-INDUCED DELAYED NEUROPATHY (OPIDN) IN CHICKENS. M. Ehrich and W. B. Gross, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

A previous study (Toxicol Appl Pharmacol 70:249) demonstrated that roosters moved from low social stress (LSS) to high social stress were more susceptible to clinical signs of OPIDN induced by triorthotolyl phosphate (TOTP). This was also noted after disopropyl phosphorofluoridate (DFP) 1 mg/kg sc (clinical scores 1-2 on Day 18 if LSS, >3 if moved; 0-4 = nonaffected to paralyzed). To determine if increased levels of corticosterone caused by social stress could contribute to exacerbation of OPIDN in 10-mo-old White Leghorn roosters moved from LSS (3 mo in individual cages), we fed 200 ppm corticosterone beginning 24 hr before and continuing 10 da after TOTP, 160 mg/kg po. This regimen increased heterophil/lymphocyte (H/L) ratios in LSS birds by 3.9x, which compared to 3.4x when LSS chickens were removed from their individual cages and placed in groups of 5 birds, with individuals in the group changing daily. 10 days after TOTP, moved roosters had higher clinical scores (3 > 0.8, x > 50, N > 4) than did LSS chickens (1 > 0.5). Roosters provided corticosterone had clinical scores of 3 > 0.5. This indicates that dietary corticosterone in a concentration capable of mimicking the effects of social stress on H/L will have the same detrimental effect on OPIDN. (Supported in part by NIH grant ES03384)


The putative neurotoxicity of the organophosphite TPP was evaluated in rats. Animals (Long-Evans, male, 60d) were exposed to two 1.0 ml/kg (1.184 mg/kg) injections (ac) of TPP spaced 1 week apart and sampled (for biochemical and neurological examination. Fourteen days after initial exposure rats displayed dysfunctional changes including tail rigidity, circling, and hind-limb paralysis. Neuropathic damage, consisting of myelin ellipsoids and giant axonal swellings filled with smooth endoplasmic reticulum, was noted in the lower brainstem and ventral and lateral columns of all spinal cord levels. Wallerian-like degeneration was observed in the spinal roots, the sciatic nerve and tibial branches. Biochemical assessment of whole brain acetylcholinesterase and neurotoxic esterase activity was determined 1, 4, 24, 48, and 72 hr after the second TPP treatment. Both enzyme activities were depressed maximally at 48 hr post-exposure by 30% and 35%, respectively. Serum cholinesterase, sampled 48 hr after the second TPP exposure was depressed by 33%. Data from this preliminary study indicate that subacute exposure to the organophosphite TPP produces neurotoxic effects which differ significantly from those previously described in rats with OPIDN.

USE OF VITAMIN E AND SELENIUM AS POSSIBLE TREATMENTS FOR ORGANOPHOSPHATE INDUCED DELAYED NEUROPATHY (OPIDN) IN CHICKENS. C. Brown, L. Force, B.S. Jortner and M. Ehrich, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Adult White Leghorn chickens were dosed with trip- orthotolyl phosphate (TOTP), 360 mg/kg po, 1 hr and 24 hr prior to 10 daily sc injection of vitamin E (70 mg/kg) and Na selenite (.32 mg/kg). The vitamin E-selenium treatment had no effect on blood esterase activities, but elevated activity of glutathione peroxidase. Onset of clinical signs was evident by 10 days post-TOTP in all groups of birds. Although less in chickens treated within 1 hr of TOTP (i.e., score = 1.0±0.3 at 18 da, x±SE, N=5) than in birds with treatment delayed 24 hr (2.4±0.7), neither group was significantly different from chickens given only TOTP (1.6±0.3). Because events occurring between OP administration and onset of clinical signs are not all defined, it is possible that these early treatments were too late to prevent TOTP from initiating OPIDN. Clinical signs did, how- ever, correlate with quantitative histopathological examination of teased myelinated peripheral nerve fibers. For 3 of 4 chickens with clinical scores of 3 or 4, the ratio of fibers undergoing wallerian degeneration to normal fibers (out of over 50 total) was reduced. (Supported in part by NIH grant ES03384)

Studies indicate that in utero exposure to methylmercury (MM) may interfere with neural differentiation and migration within the CNS. While the toxic mechanism of MM is unknown, microtubules (MTs) are highly sensitive to MM. We examined the effects of MM on the cytoskeleton of embryonal carcinoma (EC) cells derived from pleuripotent stem cells of malignant murine teratocarcinomas. EC cells were induced to differentiate into neurons and glia by retinoic acid. Cultures were treated with 10^{-5} to 10^{-7} M MM, and cell cytoskeletons were visualized by double indirect immunofluorescent staining with anti-neurofilament, anti-glial filament, or anti-tubulin antibodies. MM effects on MTs were dose-dependent. MM at 10^{-5} M caused depolymerization of MTs and loss of neurofilaments. At 3.3 x 10^{-6}, MTs in both neurons and astroglia were fragmented, while neuro- and glial filaments were intact. MTs were generally intact but reduced in number at 10^{-6} M. At 10^{-7} M, no changes in neuronal or glial cytoskeleton were observed. Data from immunofluorescent antibody staining indicate that MTs are more sensitive to MM than neurofilaments or glial filaments. Contrary to existing in vivo data, the neuronal cytoskeleton does not appear selectively vulnerable to MM-induced injury. (Supported by NSERC.)

PHENYTOIN SUPPRESSES IMMUNE FUNCTIONS AND CAUSES DAMAGE TO DNA IN VITRO AND IN VIVO. N.C. Margareten, J.R. Hincks, R.P. Warren, and R.A. Coulombe, Jr., Center for Environmental Toxicology, Utah State University, Logan, UT. Sponsor: R.P. Sharma

Phenytoin, a widely used antiepileptic drug, has been associated with immune system abnormalities and lymphoma in man. Previously, we showed that phenytoin depressed basal natural killer (NK) cell activity of human peripheral blood mononuclear cells in vitro in a dose-dependent manner. We now report that phenytoin depresses interferon-augmented NK cell activity and antibody-dependent cell-mediated cytotoxicity (ADCC) at concentrations of drug considered therapeutic, 10 and 20 µg/ml, to neurotoxic in range, 40 µg/ml. DNA damage in human lymphocytes exposed to phenytoin for 18 or 72 hr in vitro was measured by gravity-flow alkaline elution. The 72 hr exposure produced a significant increase in DNA single-stranded breaks (SSB) at all three concentrations. Phenytoin given to male NFS mice for 18 days suppressed NK cell activity of splenic cells at doses of 20 and 40 mg/kg, indicating a long-term effect on this function. No effect on female mice NK cell activity was found. However, splenic cells from the female mice showed an increase in DNA SSB at the 20 and 40 mg/kg doses. These results indicate that phenytoin possesses a possible tumorigenic potential. Supported in part by USPHS grants ES03591 and ES07097.

ALTERATIONS IN THE PATTERN OF SEROTONIN PROJECTIONS FOLLOWING NEONATAL EXPOSURE TO 5,7-DIHYDROXYTRYPTAMINE. K. Jensen, NTD, USEPA, Research Triangle Park, NC. Sponsor: R.S. Dyer

Previous studies have shown that neonatal exposure to the serotoninergic neurotransmitter, 5,7 dihydroxytryptamine (DHT), results in a decrease in serotonin in the spinal cord and an increase in serotonin in the brainstem. We have examined the distribution of serotonin immunoreactivity in animals treated neonatally with DHT to evaluate the underlying changes in serotonin projections. Long Evans rats were pretreated with 25 mg/kg desipramine and 30 minutes later systemically administered 100 mg/kg of DHT on postnatal day (PND) 0 and PND 1. The animals were sacrificed between PND 20 and 30 and brains and spinal cords processed for immunohistochemistry. The pattern of serotonin immunoreactivity in animals treated with DHT when compared with controls indicates an increase in local serotonin projections within the brainstem and a decrease in extent of the serotonin projections to the spinal cord. The differential response to neurotoxic injury of these two regions may reflect differences in the extent of their development in the neonate.


The purpose of the study was to evaluate the toxicity of theophylline, a compound present in tea and used in a variety of clinical applications. Fourteen-day toxicity studies were conducted in 36 CF1 mice and 344 rats of both sexes using dosed feed (0, 500, 1000, 2000, 4000 and 8000 ppm) or gavage (12.5-2x/day, 25, 50, 50-2x/day, 100, 200, 200-2x/day and 400 mg/kg). Exposure to the feed at concentrations up to 8000 ppm induced no significant toxicity, but palatability problems occurred at the level precluded administration of higher concentrations. In the gavage study, 400 mg/kg was lethal, but mice and rats differed in that 200 mg/kg, 2x/day was lethal in rats but not in mice. No weight-gain depression was evident in mice; weight-gain was depressed in male rats at 100 mg/kg (or 50 mg/kg, 2x/day) and 200 mg/kg. Clinical signs in mice were squinting and distorted tastes in males, and in rats, rapid respiration (all doses), squinting and hunching. Gross necropsies, organ weights, clinical pathology, and pathology identified no target organs in mice, while histopathologic observations in rats suggested a number of possible target organs, including heart, stomach, lungs, thymus, bone marrow, spleen and uterus. The data are consistent with acute toxicity once a critical dose is reached.
SUBACUTE TOXICITY OF A PHENYLENE(OXY)-BIS 2,2-
DIMETHYLPENTANOIC ACID, A LIPID REGULATING AGENT.
E.J. McGuire, J.A. Anderson, C.C. McGehee, and
Parke-Davis Pharm. Res., and Env. Ind. Health,
University of Michigan, Ann Arbor, MI. Sponsor:
F.A. de la Iglesia

Subacute toxicity of a lipid regulating agent (CI-924), chemically described as 5,5'-(1,1-
biphenyl)-2,2-diylbis(oxy)bis[2,2-dimethyl-
pentanoic acid], was investigated in rat, dog and
monkey. CI-924 was well-tolerated in rats for 13
weeks at doses of 25, 150 and 300 mg/kg. Body
weight gain suppression was dose-related in both
sexes. Slight increases in liver enzymes in males
given 300 mg/kg, and slightly lowered hematocrit
and hemoglobin levels in females given 300 mg/kg
were the principal clinical laboratory findings. Reversible
dose-related pathologic findings
occurred in the liver, were more prominent in
males, and consisted of hepatocellular hyper-
trophy, increased cytoplasmic eosinophilia, and
fat content. CI-924 was well-tolerated by dogs
for 13 weeks at oral doses of 50, 300 and 600
mg/kg. There were no remarkable clinical
clinical laboratory, or pathologic findings. In
monkeys at an oral dose of 50 mg/kg, CI-924 was
well-tolerated for 13 weeks. Higher doses of 250
and 500 mg/kg resulted in gastrointestinal signs
and subsequent mortality or early termination of
one female in each group. Except for gastro-
enteritis in these two animals, there were no
clearly drug-related pathologic findings.

INTERSTITIAL PNEUMONIA INDUCED BY TRIETHYLENE-
TETRAMINE DIHYDROCHLORIDE (TRIEN) IN B6C3F1 MICE
FED AIM-76A DIET. U.L. Greenman and R.L.
Morrissey*, National Center for Toxicological
Research, Jefferson, AR and Pathology Associates

Trien is a chelating agent proposed for treatment
of patients with Wilson's disease. To evaluate the
safety of Trien, male (M) and female (F) mice
(B6C3F1) were given 0, 120, 600 or 3000 ppm Trien
in their drinking water and fed either AIM-76A or
NIH-31 diets for 90 days. A control group fed
Cu-deficient AIM-76A diet was also included.
Trien was without notable effects at 120 or 600
ppm. At 3000 ppm, it depressed body weight gain
in males on AIM-76A (19%) and on NIH-31 (9%).
There was no effect on weight gain of females.
Both sexes maintained on the Cu-deficient diet
gained more than controls fed the unaltered
AIM-76A diet. Kidney weights were depressed and
the incidence of renal tubule vacuolization was
decreased in males fed AIM-76A but only at the
high dose. Chronic fibrosing interstitial
pneumonia developed in both M (14/20) and F
(16/20) mice receiving 3000 ppm Trien and AIM-76A
diet. The lung lesion did not occur in AIM-76A
controls, lower dose groups, Cu-deficient controls
or in NIH-31 diet groups. This lesion and the
body and kidney effects appear related to an
interaction between the high Trien dose and the
AIM-76A diet.

CHRONIC TOXICITY AND CARCINOGENICITY EVALUATION
OF VIDARABINE IN WISTAR RATS. S.I. Grecon,
D.S.B. Watson, C.J. DiFonzo, C.S. Smith,
Tox. Dept., Warner-Lambert/Parke-Davis Pharm.
Res., Morrisville, NC and Ann Arbor, MI.
Sponsor: F.A. de la Iglesia

Vidarabine (Vira-A®) is a nucleoside derivative
with antiviral activity by virtue of its inhibi-
tion of DNA polymerases. The carcinogenic
potential of Vidarabine was evaluated in a 2-
year study in Wistar rats. Weekly IM injections
of Vidarabine in phosphate buffer were adminis-
tered to groups of 60 rats per sex for 92 weeks
at doses levels of 0, 3, 7 and 15 mg/kg. The
animals were observed for an additional 12 weeks
without treatment prior to sacrifice. An addi-
tional control group was untreated. Survival
rates were similar for treated and both control
groups, and Vidarabine did not elicit signs of
overt toxicity during the study. Frequently
encountered tumors in treated and control
gemales were pituitary adenoma, mammary fibro-
adenoma and carcinoma. In males, pituitary
adenoma, adrenal adenoma and pancreatic islet
cell tumors were diagnosed most frequently. No
significant differences were found in tumor
incidence or latency between Vidarabine-treated
and control groups, and Vidarabine was not
carcinogenic in Wistar rats.

MORPHOMETRIC ANALYSIS OF CULTURED HEPATOCYTES EXPOSED
TO BENOXAPROFEN. F.M.B. Sorensen. Department of Pharmacology
and Toxicology, The University of Texas, Austin, TX.

Benoxaprofen (BP) is a nonsteroidal anti-inflammatory
treatment which was approved for the treatment of arthritis in the United
States, but was withdrawn from the world market by the manufacturer after British reports of deaths and serious
hepatotoxic reactions in patients treated with the drug. This study
was undertaken to quantitate morphological changes in liver cells
exposed to BP 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 12 hours to 0, 100, 500, or 1000
μM concentrations of BP. 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 
BP 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 and 1000 μM BP had
significantly reduced percentages of Type I cells compared to
cultures exposed to lower concentrations of BP (p<0.01). In
contrast, the percentages of other progressively more damaged cell

types (i.e. types II, III, IV) increased as the concentration of BP
was increased. These morphometric results paralleled those obtained from functional assessments. Therefore, the use of
morphometric techniques provided a good assessment of BP-induced cytotoxicity and demonstrated the value of quantitative
estimates in the evaluation of cellular injury.
POLY (N-ACRYLOYL-β-ALANINE-N'-METHYL HYDROXYACID) (PA-11) INDUCED RENAL LESIONS IN RATS.
J.C. Siglin1, W.D. Johnson1, A. Winston2, P.J. Beccal1, 1 Food and Drug Research Laboratories, Inc., Waverly, NY. 2 Dept. of Chemistry, West Virginia University, Morgantown, WV. PA-11 is an iron chelating agent developed for potential therapeutic use in patients suffering with Cooley's Anemia. To test its potential toxicity, PA-11 was administered by daily subcutaneous injection to 6 rats/sex for 14 days at levels of 20, 70 or 100 mg/kg BW. A control group, 6 rats/sex, was administered USP water for injection under the same conditions. Three rats/sex/group were sacrificed after completion of dosing. Remaining animals were maintained untreated for an additional 2 weeks to evaluate the reversibility of treatment-related effects. Gross necropsies and histopathological examinations were performed. All animals survived for the duration of the study. Body weight gain, food consumption, food conversion and organ weights were comparable among the groups. PA-11 induced cortical tubular epithelial degeneration was observed in the kidneys of animals at the 20 and 70 mg/kg levels. The severity of this lesion increased with increasing PA-11 dose. Presence of the renal lesion in recovery animals indicated that this effect was not reversible after a recovery period of 2 weeks. No toxicological or pathological effects were evident in animals administered PA-11 at 2 mg/kg BW. (Supported by NIH Contract No. NOI-AM-2255).


Caramel Color (IV), a type of caramel color made by the sulfite ammonia process, was evaluated for chronic toxicity in rats, and carcinogenicity in rats and mice. Caramel Color (IV) was mixed with deionized water and given to the drinking water to groups of 25 animals/sex at levels of 0, 2.5, 5, 7.5, and 10 g/kg body weight (BW) for 12 months in the chronic toxicity study, and to groups of 50 animals/sex at levels of 0, 2.5, 5, and 10 g/kg BW for 24 months in the carcinogenicity studies. There were no treatment-related clinical observations in mice. Treated rats had soft feces and lower BWs, and all treated animals showed lower food and water consumption compared to those of controls. There were no treatment-related differences in survival or the incidence of tumors between treated and control groups. There were no toxicologically important pathologic findings. Based on these studies, Caramel Color (IV) was not toxic or carcinogenic in F344 rats or B6C3F1 mice.


Subchronic toxicity studies were performed using food grade enzyme product from Bacillus stearothermophilus (natural enzyme) or from a recombinant B. subtilis containing the stearothermophilus alpha-amylase gene (cloned enzyme). Beagle dogs and F344 rats were fed diets containing 0, 36, or 72 alpha-amylase units per gram of feed. Dogs were treated for 13 weeks; parental rats were treated before breeding through weaning of pups (F1); and F1 rats were treated for an additional 13 weeks. Treated animals had sporadic significant differences in body weights and food consumptions when compared to those of the control. There were no treatment-related clinical observations, reproductive effects, or ophthalmic, hematologic, microscopic, or microscopic findings. No changes considered to be of toxicologic importance were found in serum chemistry data from treated rats and dogs.


A National Research Council Committee evaluated data pertaining to the carcinogenicity of cyclamate including metabolic studies, short-term tests, animal bioassays and epidemiological studies. The committee concluded that the evidence indicates that cyclamate is not carcinogenic by itself, but that certain in vitro studies and in vivo studies suggest that it may have cancer-promoting or co-carcinogenic activity. Epidemiological evidence shows no overall increase in risk among users of cyclamate-saccharin mixtures, but cannot rule out increased risk in long-term or heavy users. Repetition of two studies was recommended: an experiment in the rat that suggested possible tumor promotion, and another in mice that suggested cocarcinogenic activity. Irrespective of the outcome of the additional studies, a wider analysis is required to learn the significance of these types of studies to human health. Moreover, the committee recommended that epidemiological monitoring be continued in those countries where cyclamate is being or has been used, and that cancer sites other than the bladder should be studied. Furthermore, it recommended additional assays for mammalian cell DNA damage and gene mutation for cyclamate, DNA damage tests for cyclohexylamine, and more definitive cytogenetic studies.
A lifespan study dosing mice with gentian violet (hexamethyl-p-rosamine and pentamethyl-p-rosamine) was reported in which gentian violet was shown to be a carcinogen in mice producing neoplasm of the liver and of the lymphoreticular system in several organs, i.e., uterus, bladder, ovaries, and vagina. This study reports a subsequent study in Fischer 344 rats at the same doses (100, 300 and 600 ppm in the diet) under identical conditions as the mice. A total of 570 males and 570 females were sacrificed at 12, 18 and 24 months. Body weights and food consumption were decreased slightly in the highest dose. A dose response for mortality existed in all dose groups and was greater in females. Mortality in the controls of both sexes was approximately 3% at 24 months, but was about 65% in the females and 48% in the males in the high dose. Body weight changes, food consumption and mortality data in rats were similar to that noted in the mice study. However, the pathology examination revealed much lower neoplastic involvement. Hepatocellular adenomas were noted in females only at 24 months at 10% in the high dose. Also, follicular cell adenocarcinomas of the thyroid gland showed a slight dose-response trend. Leukemia showed a time-to-response trend. Males exhibited no neoplastic histopathology. Other responses consisted of liver lesions in both sexes. Gentian violet exhibited a low level but positive dose response in rats which was considerably less than that demonstrated by mice.

Whole blood, liver, kidney, heart, neck and diaphragm muscles, fat, reticulum, and rumen contents from California beef cattle receiving selenium (Se) rumen pellets were analyzed for Se. Each 30 g pellet was composed of 10% elemental Se and 90% powdered iron and is commercially available in the United Kingdom. The tissues were recovered 137-272 days after treatment, highest values being in kidney, liver, and muscle of treated animals receiving 2 pellets each. Controls were lower. Conservative and worst-case estimates of human exposure indicate a possible additional intake of 13-37 ug Se/day from consumption of beef from cattle receiving 2 pellets, which, when added to the highest estimate of 169 ug daily intake, is comparable to values considered adequate and safe. Additional data from cattle receiving 0-8 pellets each suggested similar levels of Se in the 2-pellet animals regardless of control tissue levels, and indicated a plateau effect at 4 or more pellets.

A whole blood survey of the Central Valley of California indicated many deficient animals and no toxic levels. This is pertinent in view of the current toxicological concerns about reported elevated levels of Se in parts of the valley. Se deficiency causes significant production losses in California livestock annually.

A radioimmunoassay test for etorphine was used to screen for the presence of etorphine in post-race horse urines. Most horse urines contained materials which reacted positively in this radioimmunoassay. These materials are apparently endogenous to horses and were called endogenous etorphine equivalents. Their level was about 0.1 ng/ml, the population distribution was log normal, and individual horses showed levels of up to 0.8 ng/ml. Dosing of horses with etorphine at rates from 100 ug horses to 1 ug/horse yielded levels of etorphine equivalents in urine of 1.0 to 25 ng/ml. These levels of endogenous etorphine equivalents (EETE) were easily distinguished from background EEE. Radioimmunoassay for etorphine is therefore sufficiently sensitive to allow control of illegal use of etorphine in racing horses.

Supported by a grant entitled "Immunoassay Tests for High Potency Narcotic Analgesics" from the Kentucky State Racing Commission, Kentucky Horse Racing Commission and the Kentucky Equine Drug Research Council.

Uterotrophic response in sexually immature female rats has been used to rank the relative estrogenic potencies of 6 resorcylic acid lactones (RALs), and to compare their activities with 17ß-estradiol. On oral administration, the estrogenic potency relative to 17ß-estradiol is as follows: α-zearalenol, 20 times less; zeranol, 100 times less; zearalenone, zearalanone, taleranol, all 500 times less; β-zearalenol, 3500 times less. On subcutaneous administration, zeranol is 350 times less estrogenic than 17ß-estradiol. Significant conclusions from these findings are: 1) Major phase 1 metabolites of zeranol (viz. zearalanone and taleranol) are substantially less hormonally active than zeranol itself. This implies that the estrogenic activity of the residues of zeranol in meat is markedly reduced in comparison with the activity of unmetabolized zeranol. 2) The RAL myco-estrogen α-zearalenol (major metabolite of zearalenone) exhibits 5 times more hormonal activity than zeranol. This implies that the residues of RAL myco-estrogens occurring naturally in human food are likely to be collectively more hormonally active than the residues of zeranol and its metabolites present in meat from implanted cattle.
512 EFFECTS OF ACUTE AND SUBCHRONIC OZONE EXPOSURE ON FUNCTIONAL PROPERTIES OF RABBIT ALVEOLAR MACROPHAGES. K.E. Driscoll, T.A. Vollmuth, R.B. Schleninger, Dept. of Environmental Medicine, New York University Med. Ctrn. New York, NY

The alveolar macrophage (AM) plays an important role in the defense against inhaled particulates. This study examined the numbers and functional properties (substrate attachment, mobility, phagocytosis) of AM isolated from rabbits given either a single 2 hr exposure to 0.0 (control), 0.1 or 1.2 ppm O₃; or repeated exposures to 0.0 or 0.1 ppm O₃, 2 hr/d X 13d. In the acute regime, groups of 5 rabbits were sacrificed immediately, 1d or 7d post exposure; in the subchronic regime, sacrifices were 3d, 7d or 14d after the initial 03 exposure. Acute exposure to 0.1 ppm produced an increase in AM numbers and a depression in AM function (phagocytosis). Exposure to 1.2 ppm resulted in a transient increase in PMN's with no change in AM numbers. Alterations in AM function at the 1.2 ppm level were more pronounced and persistent than for the 0.1 ppm group. Repeated exposure to 0.1 ppm O₃ resulted in increased numbers of AM and PMN's and alterations in AM function (phagocytosis). These results demonstrate that acute and subchronic exposure to 0.1 ppm O₃, a level below the current National Ambient Air Quality Standard, produces significant changes in the numbers and functional properties of AM.

514 OZONE (O₃) INHIBITS CYCLOOXYGENASE (C) ACTIVITY IN CULTURED PULMONARY ENDOTHELIAL CELLS (PC). M.C. Macken, T.E. Eling, G.C. White II, and M. Friedman. Center for Environ. Medicine and Curr. in Toxicology, Univ. of North Carolina, Chapel Hill, NC and MERS, Research Triangle Park, NC. Sponsor: T.S. Riva

O₃, a major photochemical toxicant, inhibits EC prostacyclin (PGI₂) synthesis (Toxicologist, 5:830a, 1985). This inhibition of EC PGI₂ synthesis could be due to inhibition of C and/or PGI₂ synthetase activities. To examine the site of PGI₂ inhibition induced by O₃, confluent EC grown in roller bottles were exposed to 0.0 or 1.0 ppm O₃ for 2 hr in serum-free media (this level of O₃ exposure results in a 90% reduction in PGI₂ synthesis). After O₃ exposure, EC were washed; incubated with 4 µM PGI₂ for 2 min; the media removed, centrifuged (500 x g, 10 min), and the amount of 6-keto-PGF₁α, (the stable metabolite of PGI₂) in the supernatant measured by radioimmunoassay. After PGI₂ incubation, EC previously exposed to O₃ produced 82 ± 10% (n=7) of the level of PGI₂ produced by air-exposed EC (p<.05) indicating normal PGI₂ synthetase activity. These data suggest that O₃ alters EC arachidonic metabolism by inhibiting C. (Supported by EPA Grant CR807392)

513 EFFECT OF OZONE EXPOSURE ON DNA SYNTHESIS IN PULMONARY ALVEOLAR MACROPHAGES. E.S. Wright, Biomedical Science Dept., General Motors Research Labs, Warren, MI. Sponsor: E. W. Lee

An apparent increase in the alveolar macrophage population can be induced by a number of toxic insults to the lung. This increase could arise from an influx of monocytes cells from the vascular or interstitial compartments, or from proliferation of alveolar macrophages in situ. While the proliferative response of alveolar type II cells has been well documented, this reaction in free alveolar macrophages has not been described. In these studies, rats were exposed continuously to air or 0.12, 0.25, or 0.50 ppm ozone for 1, 2, 3, 7, or 14 days. DNA synthesis measured in lung homogenates increased in a dose-related manner with a maximal 10-fold increase occurring after two days of continuous exposure to 0.50 ppm. Labeling index in free alveolar macrophages was measured in fixed tissue sections and increased dramatically after two days of exposure to 0.25 and 0.50 ppm of ozone, but returned to control levels by the end of one week of exposure. This transient, dose-related response in alveolar macrophages was qualitatively and quantitatively similar to that seen in type II cells. These results suggest that like type II cells, free alveolar macrophages are capable of entering the cell cycle and synthesizing new DNA in situ in response to low-level, short-term ozone exposure.

515 POSSIBLE PROTECTIVE ROLE OF GLUTATHIONE IN OZONE-INDUCED PULMONARY FIBROSIS. J.D. Sun, S.I. Mclaughlin, J.A. Pickrell, F.F. Rahn, and R.F. Henderson, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

The oxidant gas ozone (O₃) is a common air pollutant known to cause pulmonary fibrosis (PF). The reductive capacity of glutathione (GSH) to protect against O₃-induced PF was tested. Male B6C3F₁ mice were exposed to 0, 0.2, and 0.5 ppm O₃ for 5 hr/day, 5 days/week, for 2 weeks. During exposures, half the mice at each O₃ level were given drinking water containing 30 mm bithionol sulfoximine (BSO) to lower in vivo GSH levels. BSO lowered GSH in lungs, liver, kidney, and blood by 40%, 54%, 68%, and 17%, respectively, in air-exposed mice. O₃ exposure increased lung GSH levels up to 3-fold in both BSO-treated and non-treated rats. However, mice treated with BSO and exposed to O₃ showed lung GSH levels ~50% lower than water-treated, O₃-treated mice. Blood glutathione synthetase activity (GSH biosynthetic enzyme inhibited by BSO) was decreased 49% by BSO and increased by 2- to 2.5-fold by O₃ exposures. Data show O₃ exposures increase lung GSH by increasing its biosynthetic rate. Such a response suggests GSH may serve to protect lungs against oxidant gas injury. Histopathological evaluations for differences in lung lesions are in progress. (Research supported by U.S. DOE Contract No. DE-AC04-76EV0113.)
516 AN APPROACH FOR PROVIDING COMPREHENSIVE ANALYSES OF O₃ AND NO₂ HEALTH EFFECTS DATA. E.D. Smokol, R.L. Wolpert, D.J. McKeen, and R.E. Moneal. Depts. of Pharmacology and Medicine, Comprehensive Cancer Center, Duke Medical Center, Durham, NC and OAQPS, USEPA, Research Triangle Park, NC.

A major problem confronting the USEPA in setting National Ambient Air Quality Standards is the utility of animal toxicity data. The analytical approach presented here is an effort to further a more quantitative utilization of studies reporting results from animal experimentation. The methodology is comprised of three basic phases: assessing the quantitative utility of data reported in animal health effects studies; predicting regional lung dose with a dosimetric biologic model (e.g., the Miller O₂ Model reported in Miller et al., Toxicol. Appl. Pharmacol. 79: 11-27, 1985), and combining these two data sets to determine relationships. Lung doses predicted by the Miller Model are correlated with quantitative representations of O₂ effects from the literature for endpoint classifications such as edema, pulmonary function, infectivity, and biochemistry. Linear dose-response relationships are suggested. O₂ animal toxicity data are also compared with corresponding NO₂ data. Application of this methodology aids in identifying data gaps and areas requiring additional research. (Supported by EPA Contracts 68-02-3869 and A46751 and by NIH Grants CA14236 and R01669.)

518 NITROGEN DIOXIDE PEROXIDATION OF LIPOSOMES AND VITAMIN E PROTECTION. C.R. Shoaf, J.R. Sandy, and R.E. Moneal. Departments of Pharmacology and Medicine, Comprehensive Cancer Center, Duke University Medical Center, Durham, NC.

To predict human health effects from ambient NO₂, a mathematical model of toxic reactions in the lung is being developed. Liposomes, a model membrane system, were peroxidized by 0 to 10 ppm NO₂ in air. Dose-inhibition measured at 235 nm for dillinoenoloyl lecithin (di-18:3 L) or at 233 nm for dillinoenoloyl lecithin (di-18:2 L) showed that the initial rate of diene conjugation was second order for di-18:2 L and di-18:3 L at all NO₂ concentrations. The rate of oxidation for di-18:3 L peaked and then decreased after 30 min of reaction with 2, 5, and 10 ppm NO₂. Only malonaldehyde (MA) was formed on peroxidation of di-18:3 L, but di-18:2 L oxidation produced a TBA-reactive product which was not MA. When vitamin E was incorporated into di-18:3 L liposomes at 10 mole %, MA formation and unsaturation conjugation were prevented, even at 10 ppm NO₂. Vitamin E was preferentially oxidized to tocopherone. NO₂-initiated peroxidation of membrane lipids can be quantitated by this model system and more physiological oxidation rates measured. (Supported by EPA Cooperative Agreement CR809715 and NIH Grants RR01693 and CA14236.)


The relative importance of antioxidant substances in determining the sensitivity of lung tissue to inhaled oxidants was investigated. Lung non-protein sulphydryl (NPSH) content in rats was lowered using BSO prior to exposure to the edemagenic gases. Lung AA levels were lowered in guinea pigs by feeding rabbit chow for 2 weeks. Injection of guinea pigs with up to 1.0 g/kg BSO did not affect lung NPSH levels, although liver levels were decreased. Injections of BSO (0.125 mg/kg) reduced rat lung NPSH levels by 28% and increased susceptibility of the animals to 1.0 ppm O₃ and 0.2 ppm COCl₂ (4 hr), but did not appear to alter the effects of NO₂ exposure (10.0 ppm, 4 hr). BSO also slightly lowered lung α-tocopherol and liver AA levels. AA deficiency in guinea pigs led to enhanced susceptibility to NO₂ (1.6-5.0 ppm, 3 hr) but did not affect sensitivity to O₃ (0.25-0.75 ppm, 4 hr) or COCl₂ (0.25-0.5 ppm, 4 hr). These results suggest that AA is important in protecting the lung from NO₂, while NPSH may be more critical in protecting against O₃ and COCl₂. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

519 DIABETES DECREASES SUSCEPTIBILITY TO OXYGEN TOXICITY IN MICE BUT NOT RATS. W.M. Haschek, J.E. Rutherford, R.S. Shimkus and K.H. Elistine, Department of Veterinary Pathobiology, University of Illinois, Urbana, IL 61801.

The effects of hyperoxia were studied in 10-11 wk male diabetic (db/db) mice and rats. C57Bl/KaJ genetically diabetic mice (db/db) with lean non-diabetic littermates (db/+), and chemically-induced diabetic mice (160mg/kg streptozotocin (st) ip 2 wk previously: +/- st) with controls (citrate buffer: +/-) were exposed to 100% O₂. By 57 h post-exposure 16/18 control (db/-/+ and +/-) but no diabetic mice were dead. By 68 h 17/18 control and 0/8 (db/db) and 3/11 (+/- st) diabetic mice were dead. Similar results were obtained with Balb/c mice but not with Sprague Dawley rats. No difference in survival time was noted between st (45mg/kg) treated and vehicle control rats. The hypothesis that species differences are due to alteration of epithelial but not endothelial cell function in db was examined by putrescine (PT) and 5-hydroxytryptamine (5HT) uptake in lung slices from st treated mice (180mg/kg) and rats (50mg/kg) following 100% O₂ for 80 and 60 h respectively. PT uptake (epithelial) was decreased slightly in diabetic animals exposed to O₂ or air, and increased markedly in both diabetic and control rats and mice. Little effect was noted on 5HT uptake (endothelial). Thus diabetic mice, but not rats, are less susceptible to hyperoxia than non-diabetic. Further studies are required to elucidate mechanisms.
PULMONARY O3, TOXICITY AND ENDOGENOUS PROSTAGLANDINS


Prostaglandins (PG's) are vasodilatative and can contribute to the vascular congestion and edema characteristic of pulmonary O3 toxicity. Male rats were exposed to 100% O3 for 24 or 48 hrs, killed, and an isolated perfused lung (IPL) system prepared which directly superfused an isolated rat stomach strip (RSS). RSS contraction, perfuse protein, malondialdehyde (MDA) and lung dry wt/wet wt (D/W) were determined. Indomethacin (10μg/ml) when added directly to the IPL perfuse blocked this RSS contraction. Furthermore, when rats were treated with (I) (1.8 mg/kg/day for 7 days) before exposure to 100% O3 for 24 or 48 hrs, the RSS contraction also was blocked. In both studies with (I), perfuse protein and MDA increased. Pyrimidene pretreatment (25 mg/kg 4 x a day) reported to block PG2 and PGF2α formation, increased survival of rats in O3 by 30%. However, in perfusion studies employing pyrimidene, RSS contraction, perfuse protein, and MDA increased and D/W decreased. Chloropirimidine (7.14 mg/kg 4 x a day) had no effect upon rat survival in O3, but protected against lung damage as determined by D/W. These studies suggest that PG's seem to play a role in pulmonary O3 toxicity. The mechanism, although uncertain, may include other elements of the arachidonic acid cascade. (Supported by a contract from the U.S. Air Force. #33615-83-P-0601).


Animal studies may be used more accurately for human risk assessment if quantitative extrapolations are possible. The objective of this project is to provide tissue sensitivity data for the quantitative extrapolation of inhalable particulate toxicity data from animals to humans. This will be accomplished by comparing dose-response curves obtained in animal cells in vivo and in vitro to those obtained in human cells in vitro. These studies are unique in that both rat and human nasal turbinate epithelial (NTE) cells are obtained from fresh, normal tissue. As an initial study O3 toxicity is being evaluated in human and Fisher 344 rat NTE cells exposed 2 hr in vitro, and in Fisher 344 rats exposed 2 hr by nose-only. Methods were developed for isolating and culturing NTE cells from rat and human tissues, and for measuring nucleotides by HPLC. C18 and nucleotide levels are expressed in terms of TCA content. Human NTE cells show a dose-dependent decrease in cell growth after treatment for 2 hr with 0, 0.01, 0.1, 1.0, and 10 μg C18/gm ATP, ADP, AMP. C18 levels increased, while ADP and AMP levels increased with increasing C18 concentrations. HPLC analysis revealed an unknown compound in cells exposed to 1 and 10 μg C18/gm but not in lower doses or control cells. The results of these studies along with dosimetric data, should permit quantitative extrapolation of inhalable particulate toxicity data from animals to humans. This abstract does not necessarily reflect EPA policy.

523 PULMONARY FUNCTION CHANGES IN GUINEA PIGS EXPOSED TO HIGH PEAK LEVELS OF FREQUENTLY GENERATED ULTRA-FINE ZnO. H.F. Lam, D. Ainsworth, S. Peoples and M.O. Andrus, Department of Applied Biological Sciences, M.I.T., Cambridge, MA. 02139

During the course of our study on animals exposed for 3 hr/day for 5 consecutive days to freshly formed ultrafine ZnO (CMC 0.05 μm, ζ 2.0) at the currently recommended TLV of 5 μg/m3, there occurred short 1 hr peaks. Since high peak levels commonly occurred for short periods of the work day especially among welders working in enclosed spaces, we decided to examine the effects of such peaks on the lung function of male Hartley guinea pigs. In the first exposure a peak of 25 μg/m3 ZnO occurred during the first hour of the first exposure day. In the second exposure a peak of 34 μg/m3 ZnO occurred during the first hour of the second exposure day. Exposure was then discontinued but pulmonary function measurements were performed as scheduled. In both exposures, the peak levels caused reductions in lung volumes greater in magnitude (by day 3) than in the last day of a 5 day exposure where no peak occurred. Peak exposures similarly caused reductions (40 to 60%) in diffusing capacity. Flow resistance was increased and compliance was decreased. This study suggests that short peak exposure is very effective in inducing changes in lung function. We believe that in the establishment of safe exposure levels consideration must be given to the possible effect of peak levels that occur for short periods. (Supported: NIH ES-02429).
Ultratine particles (count median diameter less than 0.1 μm) with surface layers enriched with zinc oxide (ZnO) and the oxides of other volatile trace metals enter the atmosphere during fossil fuel combustion. The effects of inhalation of ultratine ZnO were studied in male Hartley guinea pigs exposed in a nose-only apparatus to freshly generated ZnO (count median diameter 0.05 μm) at 0, 2, 6, or 13 mg/m³ and examined after 1, 2 or 3 daily 3-hour exposures. ZnO exposure caused concentration-dependent increases in protein, total number of cells and activities of beta-glucuronidase, acid phosphatase, alkaline phosphatase, lactate dehydrogenase and angiotensin converting enzyme (ACE) in pulmonary lavage fluid. Three exposures to 13 mg/m³ ZnO increased lavage protein and activity of most enzymes to 10–15 times control values. Centrilobular lung injury was present only in guinea pigs exposed to 13 mg/m³ ZnO. Lesions were mild in guinea pigs exposed to 6 mg/m³ but were severe in guinea pigs exposed to 13 mg/m³. Microscopic lesions were a less sensitive indicator of lung damage than abnormalities in lavage fluid. (Supported by grant FO1 ES02429 from NIEHS.)

Nickel sulfate is a soluble nickel compound with the potential for inhalation exposure of man in the electroplating industry. The purpose of this study was to evaluate the inhalation toxicity of NiSO₄. F344/N rats and B6C3F₁ mice of both sexes (N = 10-20) were exposed to aerosols of nickel sulfate hexahydrate (NiSO₄·6H₂O) 6 hr/day for 12 days. Target exposure concentrations were 60, 30, 15, 7.5, 3.5, and 0 mg/m³. All mice exposed to 60, 30, 15, or 7.5 mg/m³ died following 5 days of exposure, while all mice exposed to 3.5 or 0 mg/m³ survived the exposure. Mortality was less extensive in rats with 7, 2, and 1 rat dying in the 60, 30, and 15 mg/m³ groups, respectively. Clinical signs of toxicity in both species included rapid breathing, lethargy, and weight loss with the severity dependent on exposure level. Histopathological lesions in rats and mice were most severe in the lung and bronchiolar lymph nodes. Results indicate that NiSO₄ has a relatively high toxicity following inhalation. (Research conducted under IAA Y01-ES-3010B between U.S. DOE Contract No. DE-AC04-76EV01013 and NIEHS/NTP.)
To estimate the human health risk from the inhalation of nickel aerosols, a complete toxicokinetic model of Ni in the rat is being developed. The removal of Ni from the lung to the blood has been shown to be saturable. The removal of i.v. Ni from the body was followed using $^{65}$NiCl$_2$. Ni is rapidly cleared from the blood to the kidneys. 2% of the injected dose is present in the kidney after 1 hr. Less than 1% of the injected dose was found in each of the following tissues: brain, liver, spleen, lung, adrenals, pancreas, thymus, intestine, testes, heart, muscle and fat. All tissues were cleared of Ni by 24 hrs. Terminal elimination in the urine was rapid with Ni appearing in the urine 16 min after injection. These data have been incorporated into a multicompartmental model with the non-linear clearance of Ni from the lung to predict lung burdens following Ni inhalation. (Supported by NIH Grant ES07031, RR01693 and CA14236.)

In this study, we aimed at studying the lung morphology, with special emphasis on the distal airways, following chronic exposure of guinea pigs to cotton dust (CD). The lungs from 2 groups of animals (control N=17; exposed N=17) were obtained from the Unv. of Pittsburgh. They had been exposed to CD 6 h/d, 5 d/w for 52 weeks, at a concentration of 21 mg/m$^3$, as described by Ellakani et al., Proc. Ninth Cotton Dust Res. Conf., 1985. Lung tissues were fixed in formaldehyde and embedded in glycol methacrylate (GMA). Various stereological parameters were determined on histological sections, through a multi-stage sampling approach. After one year of exposure, the lung volume was increased. The volume densities ($V_v$) of the airway and vascular compartments were increased, while the parenchymal zone was decreased. Bronchioles from orders 5th to 9th of the guinea pig respiratory tract were characterized by a raised wall/lumen ratio; in particular, the $V_v$ value of the bronchial epithelium was markedly increased. The stereological results for the airways correlate well with the physiological measurements done on these animals: three subtypes of responders could be identified within the exposed group. Supported by MRC of Canada and UoM internal funds, CAIFR.

The morphological aspect of the alveolar parenchyma after chronic cotton dust (CD) inhalation was studied in male guinea pigs, through stereological measurements performed on histological material. The lungs from 2 groups of animals (control N=17; exposed N=17) were obtained from the Unv. of Pittsburgh. They had been exposed to CD 6 h/d, 5 d/w for 52 weeks, at a concentration of 21 mg/m$^3$, as described by Ellakani et al., Proc. Ninth Cotton Dust Res. Conf., 1985. Lung tissues were fixed in formaldehyde and embedded in glycol methacrylate (GMA). Various stereological parameters were determined on histological sections, using a multi-stage sampling approach. The volume density ($V_v$) of the parenchymal zone was decreased after CD exposure. The $V_v$ and mean thickness of alveolar septa were markedly increased: this caused the surface density ($S_v$) of the alveolar epithelium to decrease. Also, the percentage of alveolar septum functional for gas exchange was significantly lowered in this group. Thus, on a statistical basis, two distinct patterns of response of the lung alveolar zone were identified in the exposed animals, suggesting differential susceptibility to chronic cotton dust inhalation. Supported by MRC Canada and UoM internal funds, CAIFR.

Effluents of coal-fired utility boilers and smelters contain both sulfur dioxide and submicrometer metal oxides. The interaction between these products form irritant aerosols with potential health effects. Stable inorganic S(IV) species have volumes and efficiencies from power plants, smelters and in ambient air. The role of inhaled S(IV) aerosol in irritating the respiratory system is not well defined. There was dispute over the species responsible for the sensory irritant effects of S(IV). Effects of a transition metal S(IV) aerosol on the muco-ciliary clearance system were inconclusive. Other studies suggested S(IV) produces biochemical changes but not direct irritant effects. Earlier studies had shown the Cu as an effective potentiator of irritant response of the lung to SO$_2$ inhalation. Interaction of SO$_2$ and Cu could lead to the formation of S(IV) species. We mixed SO$_2$ and copper oxide fume in a furnace under controlled temperatures and humidities. Sulfur species were determined by ESCA and suggested the existence of S(IV) species. Guinea pigs were exposed to these atmospheres and altered pulmonary functions produced were compared with those obtained during inhalation of sodium sulfate aerosol at comparable particle size. (Supported by NIEHS PO1-ES02429).

SRC Solid is a major product of Solvent Refined Coal I (SRC-I) syngas technology. A 2-year inhalation study has been conducted to determine the long-term carcinogenic potential of SRC Solid. Groups of 80 Charles River CD rats of each sex were exposed to an aerosol (90% <10μm) of SRC Solid at concentrations of 0, 5, 10, and 50 mg/m³ for 6 hr/day, 5 days/week for 12 months. At 6 months there were no exposure-related effects on appearance, behavior, mortality, body weight, food consumption, or urine analysis. The leukocyte count was increased in the highest exposure females. Liver and lung weights were elevated in the highest dose animals. There was gross discoloration with black foci in the lungs, corresponding to pigmented macrophages in alveolar lumens, adenomatous hyperplasia, and squamous metaplasia in alveolar lining cells. Macrophages containing brown pigment were seen in the thoracic lymph nodes. At 12 months there were no exposure-related effects on appearance, behavior, mortality, and the eyes. In the highest dose group, there was a decrease in mean body weights of males and females, in the latter the leukocyte count was increased. Lung weights were elevated in both males and females of the highest dose group. The increase seen in the females was about 3 times that for males. Males and/or abscesses were observed in the lungs of the highest dose females. Both males and females of the highest dose group exhibited squamous metaplasia, pulmonary adenomatosis, and pigment containing macrophages in the lungs. The most significant lesion to date has been found in 10/15 females from the highest dose group. It has been characterized as a benign keratinizing squamous epithelium, which are cyst-like structures lined by squamous epithelium with centrally located keratin debris. Similar lesions were not seen in males. (Supported by subcontract 01-13116 from DOE through APLC.)


Many synthetic materials, including polyacrylonitrile, produce hydrogen cyanide upon pyrolysis. Several cyanide antagonists are available. α-Ketoglutarate has recently been shown to be an effective cyanide antagonist. It was of interest to test the efficacy of the cyanide antagonists in the prophylaxis against the inhalation products produced by the gaseous pyrolytic products of polyacrylonitrile. Male ICR mice (24-26 g) were pre-treated with either sodium nitrite (80 mg/kg, i.p., 30 min prior to challenge), sodium thiourea (1 g/kg, i.p., 15 min prior to challenge), cobalt edetate (20 mg/kg, i.p., 10 min prior to challenge), 100 mg/kg H2O2 (for 15 min prior to challenge), or α-ketoglutarate (2 g/kg, i.p., 10 min prior to challenge). They were subsequently introduced into a dynamic inhalation chamber and exposed to cooled and filtered smoke produced from the pyrolysis of 1.7 g of polyacrylonitrile at 640°C. All control mice died within 3 min after combustion as thiourea, thiourea, oxygen, and cobalt EDTA pre-treated animals. Sixty percent of the α-ketoglutarate pretreated animals and 40% of the sodium nitrite pretreated animals survived challenge. (Supported by grant 96090001-8, Center for Fire Toxicology, National Bureau of Standards.)

534 PATHOLOGICAL RESPONSES OF RATS TO ACUTE INHALATION EXPOSURE TO PYROLYSIS PRODUCTS. G.V. Alexeef, D. Thorning*, M.L. Howard*, L.D. Hudson* and Y.C. Lee. Weyerhaeuser Company; Longview, WA; *University of Washington, VA Medical Center, *Harborview Medical Center; Seattle, WA.

The pulmonary response to smoke is an important determinant for potential treatment of fire victims. Studies were undertaken to compare the pathological damage due to smoke produced by Douglas fir (DF), polyisocyanurate foam (PIF) or polyvinyl chloride (PVC). Smoke was generated using a radiant furnace at an energy level of 2.5 W/cm². Rats were exposed for 30 minutes to smoke concentrations that approximated the materials' L50's. At 24-hours post-exposure, lung samples were taken for light microscopy analysis. Injury from DF smoke was concentrated in the proximal portion of the trachea. Exposure to PIF smoke produced an injury in the distal portion of the trachea and in the proximal portion of the large airways. PVC smoke produced an injury involving all airways, and several animals exhibited signs of pulmonary edema, All the smoke produced an acute airway injury consisting of degeneration, necrosis and sloughing of columnar epithelial cells with polymorphonuclear infiltration. However, the injuries differed in location and the PVC smoke produced a more extensive and more severe injury. (Supported in part by NIH grant no. GM 24990-07.)


Five groups of 48 M and 48 F 1:1(D:SD)BR rats were exposed 6 hr/day, 5 days/week to a non-ionic acryl polymer dust at 0, 14, 56, 134, and 275 mg/m³ (total dust) (Groups 1 to 5) with MMO's from 4.4-5.4 um and SSD's from 2.6-2.9. Grp 5 was exposed for 4 wk only, and subgroups were killed 0, 3, and 6 mo post-exposure (PE). Rats in the other groups were killed after 4 or 13 wk exposure, and after 13 wk exposure and 3 or 6 mo PE. At 4 wk, increased lung was observed in Groups 4 and 5. Grp 5 had increased lung was at 3 and 6 mo PE. Increased lung was seen in Groups 3 and 4 after 13 wk and at 3 and 6 mo PE. Nn hematological effects were detected at 4 wk; at 13 wk, there was an increase in the SECS in the F and MMOs in M in Grp 4. Histopathologic changes occurred only in the lungs and associated lymph nodes. Bronchiol-centric interstitial pneumonia was first detected at 4 wk in all rats in Groups 3 to 5, but in only 2 rats in Grp 2. In Grp 2, the incidence increased at 13 wk, and at 3 and 6 mo PE. Bronchiolization of the alveoli was seen at 13 wk in Groups 3 and 4. Lymphoid hyperplasia and nodular histiocytosis in the bronchial and mediastinal lymph nodes were first seen at 4 wk with incidences increasing with concentration and exposure duration. Frozen sections of the lungs and lymph nodes contained bifringent particles associated with the above lesions. There were no indications of significant fibrosis or parenchymal cell necrosis. The responses were those expected from the inhalation of an insoluble respirable dust at concentrations in excess of the normal clearance capacity of the lungs.
536 RESPONSES OF ALVEOLAR MACrophages TO INHALATION OF α-QUartz DUST. R.S. Anderson, L.L. Gutshall, Jr., and S.A. Thomson. Chemical Research and Development Center, Aberdeen Proving Ground, MD.

Contact with inhaled particulates can markedly alter the subsequent activities of pulmonary alveolar macrophages (PAM). The effects of α-quartz on PAM obtained by bronchoalveolar lavage were determined after a single 4-hr exposure to 100 mg/m³ aerosolized Min-U-Sil. Cells were harvested at 1, 3, 7, 14 days and 3 months from the lungs of exposed and control Fischer 344 rats. Trypan blue exclusion tests indicated normal cell viability at all sample times; however, high levels of polymorphonuclear leukocytes suggested a prolonged quartz-induced inflammatory response. Morphological PAM changes included multinucleated forms and apparent nuclear fragmentation. There were signs of macrophage activation in all PAM samples from the exposed rats; these included elevated phagocytic indices and increased intracellular myocidal activity. Other parameters, such as chemotaxis and oxygen uptake by resting or phagocytically-stimulated PAM, were not significantly changed by the experimental treatment. A single exposure to α-quartz produced both morphological and functional abnormalities in rat PAM that were evident 24 hrs post-exposure and persisted for at least 3 months.


A murine model of pneumoconioses evaluated the inflammatory and fibrotic potential of glass insulation fibers (GF; MW-100) compared to silica and latex beads. Male C3H/HeJ mice (20g) were intratracheally instilled with particulates (.5 mg) or saline. After one week the lungs were removed, lavaged and the fluid was analyzed for total/differential cells and protein. The total pulmonary hydroxyproline (THP) was also determined. The inflammatory cells (3.2-3.8 x 10⁵/ml) in the lavage of the saline and latex injected animals were 93-99% macrophages. Instillation of GF doubled the inflammatory cells while silica treatment caused a 4-fold elevation (15-21% PMN and 79-85% macrophage in both cases). Despite the cellular inflammation evoked by GF, the lavage protein content from these animals did not differ from saline and latex treated mice whereas silica treatment caused a large increase. Treatment with GF produced THP similar to that seen with silica (.23 μg/g, respectively) and twice that of other treatments. These studies suggest that GF may be potentially inflammatory and fibrotic, but perhaps minimally cytotoxic as evidenced by normal lung lavage protein levels. (HL 31754)


Measurement of angiotensin converting enzyme activity (ACEA) has been investigated as an index for measuring pulmonary damage. In this study, ACEA data was obtained during the development of silica-induced pulmonary fibrosis. Twelve groups of male Sprague-Dawley rats received either a single intratracheal instillation of 20mg SiO₂ suspended in saline, or saline as a control. Six exposed and six control rats were sacrificed at 1, 8, 15, or 29 days post-instillation for ACEA evaluation. Due to time constraints, the remaining rats (4 exposed/4 control per time point) were sacrificed at either 2, 9, 16, or 30 days for biochemical and histopathology assessment. The ACEA in the lavage fluid from the exposed animals was increased at all time points, while ACEA in the serum was not. Cell counts, protein, and albumen measurements were also performed on the lavage fluid, and all 3 variables were elevated at all time points compared to the controls. Biochemical data showed increased levels of hydroxyproline, elastin, DNA, and protein after 4 wks. The histopathology results supported the biochemical and compositional evidence of a developing fibrotic lesion. The results of the ACEA measurements, as related to the structural findings, will be evaluated as a possible method for monitoring pulmonary injury.


Intratracheal injection of silica into the lungs of rats caused marked hyperplasia and hypertrophy of alveolar Type II cells as indicated by light and electron microscopy. In order to quantitate the proliferative response we developed a histochemical method based on alkaline phosphatase staining of Type II cells in glycol methacrylate embedded lung. With this technique Type II cells were easily identified and electron microscopic histochemistry confirmed that in the alveolar epithelium only the Type II cell contained alkaline phosphatase activity. The histochemical procedure was used to quantitate over time the proliferative response induced by a single intratracheal dose of 10 mg silica. Type II cells were significantly increased relative to the corresponding control at all time points examined. By 28 days following silica Type II cells had increased to 252±(16) x 10⁵ cells per lung compared to a control value of 141±(32) x 10⁵ cells. The method presented is a simple procedure permitting the examination of Type II cell population kinetics. (*Supported by ES 07126)
EFFECTS OF ORAL PIPERFENIDONE ON CHRYSTOSITE ASBESTOS-INDUCED PULMONARY FIBROSIS IN HAMSTERS. S.W. Grimm, D.A. Wiersma, J.W. Clayton, S.E. Wilson. Dept. of Pharmacology and Toxicology, Col. of Pharmacy, University of Arizona, Tucson, AZ.

The present study was conducted to evaluate the efficacy of piperfenidone, 1-phenyl-3-methyl-2-pyridone, in the amelioration of pulmonary fibrosis induced by chrysotile asbestos. Male and female Golden Syrian hamsters were dosed intratracheally 0.5 mg chrysotile asbestos twice weekly for two weeks. At 60 days after instillation, groups 3, 4, and 5 received daily oral doses of 25, 250, or 500 mg piperfenidone/kg/day, respectively, for 40 days; group 6 received 1 mg cortisone acetate/day. Group 1 were controls while group 2 received asbestos only. 3 to 5 hamsters from each group were terminated for histopathology, biochemistry, and analyses of lung lavage fluid enzymes between 30 and 100 days post-instillation. Prolyl hydroxylase activity was increased at 30 and 60 days and hydroxyproline content was increased at 90 days. Lactate dehydrogenase and alkaline phosphatase activities in lavage fluid of group 2 animals were increased at 30 days. Decreases in inflammation and the number of collagen and reticulin fibers for male piperfenidone treated groups (4 and 5) were observed at several time points. In males, cortisone decreased inflammation and number of collagen and reticulin fibers at 70 and 80 days. In groups 4 and 5, lesions were less severe, inflammation was decreased, and the number of collagen fibers was decreased. These suggest an improvement in the disease state as compared to the untreated asbestos group.

THE INFLUENCE OF DISULFIRAM OR ETHANOL ON 1,2-DICHLOROETHANE CARCINOGENICITY. J.W. Chalakas, K.L. Cheever, R.M. Kovach, and E.K. Weisberger. MKR, Kansas City, MO; NIOSH, Cincinnati, OH; PAI, Ijamsville, MD; and NCI, Bethesda, MD.

A 24-month inhalation study of 1,2-dichloroethane (EDC), with concurrent disulfiram (DS) or ethanol administration, was conducted in Sprague-Dawley rats. Animals received either 50 ppm EDC, 0.05% DS in the diet, 5% ethanol in the drinking water, 50 ppm EDC plus 0.05% DS (EDC/DS), 50 ppm EDC and 5% ethanol, or control air. The rate of body weight gain for DS treated rats was significantly reduced during the entire study. However, survival times for treated rats were unaffected when compared with the controls. The combined treatment of EDC/DS resulted in a high incidence of intrapulmonary bile duct cholangiomas in both male (6%) and female (34%) rats. This combined treatment also resulted in an increased incidence of neoplastic nodules (hepatocellular adenomas) in male rats (12% for EDC/DS treated group vs 0% for controls), interstitial cell tumors in the testes (22% for EDC/DS group vs 4% for controls), and mammary adenocarcinomas in female rats (25% for EDC/DS group vs 8% for controls). The synergistic interaction of DS with EDC results in increased carcinogenicity, an effect previously observed for 1,2-dibromoethane.
Groups of 50 rats and mice were exposed to PERC (0, 209, or 400 ppm for rats; 0, 100, or 200 ppm for mice) 6 hr/day, 5 days/week, for 103 weeks. Exposure to 400 ppm reduced survival of male but not female rats. Survival of dosed male and high dose female mice was reduced. Both levels of PERC were associated with increased incidences of mononuclear cell leukemia (MCL) in male rats. In female rats, the low dose increased the incidence of MCL and both doses decreased the time to diagnosis of this disease. Exposure to PERC also produced renal tubular cell karyomegaly in both sexes of rats and renal tubular cell hyperplasia and neoplasms in males. In both sexes of mice, PERC produced dose-related increases in the incidences of hepatocellular neoplasms and renal tubular cell karyomegaly. One low dose male mouse had a renal tubular cell adenocarcinoma. There were no neoplastic changes in the respiratory tracts of either species but there was squamous metaplasia in the nasal cavities of dosed male rats. The early deaths among high dose male rats were considered to be due to MCL; early deaths among mice were considered to be due to hepatocellular carcinoma. The results of these studies provide evidence of carcinogenicity of PERC in F344 rats and B6C3F1 mice.

Inhalation of 12.5, 25 and 50 μg Cadmium in CdCl2·H2O for about 150 h/week induced lung carcinomas in rats (Takanaka et al., 1983). Therefore the carcinogenic effect of other cadmium compounds and the susceptibility of other species should be investigated. Male and female Syrian hamsters and female mice (NMR2) were exposed to 4 different cadmium compounds (mass medium aerodynamic diameter 0.2-0.6 μm), usually 19 h/day, 5 days/week. The following exposure groups were used (48 hamsters and 48 mice per group):

- 30 and 90 μg Cd/m³ in CdCl2, CdSO4
- 270 μg Cd/m³ in CdO, CdS
- 1000 μg Cd/m³ in CdS

The exposure to 90 μg Cd/m³ in CdCl2, CdO and CdSO4 and 1000 μg Cd/m³ in CdS was finished after 10 months at the latest because of low body weight or higher mortality. Histopathological findings and the Cd content of lung, liver, and kidneys of animals that died within 26 months of the start of the exposure, and the number of lung tumors found in mice are presented.

Male C57Bl mice were exposed daily to smoke (SM) from the University of Kentucky Reference cigarette (2R1) under standardized conditions. Room (RC) and sham (SH) controls were maintained for comparison. At different exposure points, radiolabeled B16 melanoma or YAC-1 cells were injected intravenously and the pulmonary clearance of these cells determined four hours later. The retention of B16 cells increased slightly with the duration of treatment but the differences among the groups were not significant. YAC cell clearance was, however, significantly inhibited both in the SM and SH groups at the 14 th 19 wk exposure times. Pulmonary tumor cell metastasis, determined by injecting B16 melanoma cells intravenously after different exposure times showed a slight but consistent increase in the number of lung tumors at 5-8, 10-13 & 20-23 wk exposure times in the SM group. At 20-23 wk exposure, the numbers of lung tumors per mouse were significantly higher in both the SM and SH groups than in the RC group. These results suggest an inhibitory effect of stress alone and stress in combination with cigarette smoke on the pulmonary effector cells responsible for the control of tumor cell metastasis. (Supported by KTRB 5A533).
Calcium sodium metaphosphate fibers (phosphate fibers) potentially can replace asbestos in some applications (e.g., brake composite material). Using two relevant lung cell culture systems, rat lung epithelial cells (LEC) and rat alveolar macrophages (RAM), two properties of the phosphate fibers related to biodegradation and cytotoxicity, were studied. Using 32P-labeled phosphate fibers, we found that both LEC and RAM significantly enhanced the dissolution of the fibers as indicated by the appearance of radioactivity in the 0.45 um filtrate of the medium and the solubilized cells. Using LDH release as an endpoint of cytotoxicity, we found that the cytotoxicity of the phosphate fibers were significantly lower than that of crocidolite and chrysotile asbestos, and were similar to that of two noncarcinogenic glass fiber samples. Similar ranking of cytotoxicity was observed in Chinese hamster ovary cells using inhibition of colony formation as an endpoint. Our in vitro conditions that these phosphate fibers were biodegradable and had a low order of cytotoxicity are encouraging that the phosphate fibers should be further studied in vivo as a possible safe replacement for asbestos.

S-Sulfonates are formed by the addition of sulfite (SO3), hydrated sulfur dioxide, across disulfide bonds, forming glutathione-S-sulfonate (GSSGSH) from glutathione disulfide (GSSG). GSSGSH is a potent inhibitor of the glutathione S-transferases, and a substrate for glutathione reductase. A549 cells, a human lung tumor line, were treated with 1,3-bis(2-chloroethy1)-1-nitrosourea (BCNU), then exposed to SO3 for 1 hour at 37°C in medium without cysteine. The cytosol was analyzed for glutathione (GSH), GSSG, and GSSGSH by HPLC. SO3 exposure resulted in a dose-dependent increase in GSSGSH; 0.86, 3.22, and 5.98 nmol/10^6 cells from cells exposed to 0.5, 5, and 20mM SO3 respectively. SO3 exposure at 0.5 and 20mM increased GSH and GSSG decreased as SO3 concentration increased. No GSSGSH was detected in control cells. The ratio of GSH/GSSC increased, and the ratio of GSH/GSSGSH decreased in a dose-dependent manner. No transport of GSSGSH out of A549 cells was detected. Results show that GSSGSH is formed in human lung cells by SO3 exposure. GSSGSH may accumulate in lung cells, impairing the ability of the lung to detoxify reactive intermediates of xenobiotic compounds. (Supported by NIH Grants ES02916, ES07031, CA14236 and RR01693).

The functions of the bronchiolar Clara cells are not known although current opinions favor a secretory role for the cell. We have shown that Clara cells (85% pure) isolated from the lungs of rabbits and incubated in the presence of 35S-methionine, synthesize and release a major low molecular weight (LMW) (6 Kd - determined by using SDS-PAGE under reducing conditions) protein into the culture media. Approximately 40% of the radioactive activity associated with proteins released from the cells over the course of a 4 hr incubation was found with the 6 Kd protein. Release of the 6 Kd protein was stimulated by phorbol myristate acetate and inhibited by the tubule inhibitor vinblastine. Using antisera prepared against Clara cells, we have also detected a LMW protein in bronchoalveolar lavage effluents from the lungs of rabbits that is immunochemically similar to that released by Clara cells under in vitro conditions. This extracellular protein had a MW of about 6 Kd and coelectrophoresed with the protein from the Clara cells. These studies support a secretory role for the Clara cell, identifies the 6 Kd protein as a major secretory product of the Clara cell, and indicate that the the protein may be a normal constituent of the pulmonary extracellular lining.

Dibasic esters (DBE) is a solvent (typical 67% dimethyl glutarate, 17% dimethyl succinate, 16% dimethyl adipate) used in the coating industry. Thirty Sprague-Dawley rats of each sex were exposed for 6 hours a day, 4 days a week for 15 weeks to 0, 160, or 400 mg/m3 of DEP vapor or to 1000 mg/m3 of DBE aerosol-vapor mixture. Ten rats/sex/group were necropsied after 15 weeks. The remaining 20 rats/sex/group were exposed in the reproduction study through breeding, gestation and lactation periods. Ten pups/sex/group were necropsied at age 21 days. In the teratology study 24 pregnant rats per group were exposed to DBE on gestation days 7-16 and killed on day 21. Effects seen after 15 weeks were slightly depressed body weights and depressed male liver weights at 1000 mg/m3 and dose-related depressed liver weights in all exposed females. There were no histological findings other than a dose-related mild degeneration of the olfactory epithelium in all DBE exposed groups. There were no reproductive effects except for depressed pup weights in the 1000 mg/m3 group, and no effects in the teratology study except for an increased incidence of delayed renal papillary development which was seen only in the presence of depressed maternal body weight at 1000 mg/m3.
In a pilot two-week inhalation toxicity study, rats were exposed to levels of 0, 33, 60, 119, and 241 ppm of CTFE, six hours/day, five days/week for two weeks. In a second phase of this study, groups of five pregnant rats were exposed to the same levels of CTFE from Day 6 through Day 19 of gestation. Effects were limited to depression in rates of body weight gains, elevated kidney/body weight ratios, and toxic nephrosis in groups exposed to 241 ppm, and depressed body weight gains in males and pregnant females exposed to 119 ppm. There were no reproductive or developmental effects.

In the subchronic study, male and female Fischer 344 rats were exposed six hours a day, five days a week, for 13 weeks at 30, 60, and 120 ppm. No animals died due to being exposed to CTFE. Indications of effects on the kidneys included increased organ weights, alterations in clinical chemistry parameters and clinical observations, and alterations in microscopic structure. Dose response was evident in both sexes, with 30 ppm being a clear (but limited) effect level in the males. A group of animals maintained for two weeks after the completion of exposure showed marked remission from the observed effects.

Ribavirin is a broad spectrum antiviral agent useful for treatment of infants. To study the effects in developing mamalian lungs, 4 groups of jill ferrets and their litters were given whole body inhalation exposures for 6 hours a day for 10 or 30 consecutive days to Ribavirin aerosols. The effects including changes in lung observations in suckling kits were evaluated after exposures, at weaning and at puberty. First the high dose, then the mid dose jills developed lactation failure. Probably due to the consequent nutritional deficiency, half of the mid dose (355 ug/L) and three quarters of the high dose (620 ug/L) kits died. There were no test compound related deaths in the vehicle control or low dose (162 ug/L) groups. Dose-related reductions in body weight occurred, with partial recovery in survivors. There were no gross or histopathologic lesions in the lungs or tracheas of suckling ferrets attributable to the test article. Ribavirin exposure had no effect on the lavageable cell pool, and while some dose-related effects were seen in the other special studies, specifically in the lung DNA to protein ratios, some pulmonary function parameters and alveolar size, most were reversible. The low level, which corresponds to an exposure dose 4 to 7 times that used clinically, appeared to be a no-effect level.

An IgM monoclonal antibody (Ab) has been purified and labeled with FITC which binds to ≥1% of resident alveolar macrophages (AM) lavaged from the rat’s lung. Twenty-four hours after the intra-nasal deposition of 4 x 10^6 2-μm diameter latex particles, between 25-35% of the lavaged AM bind the Abs. However, the detected antigen (Ag) is also expressed by 25-35% of resident AM incubated in unconditioned medium for 24 h in the presence or absence of particles; the percentage of cells expressing Ag does not further increase when the AM are incubated for up to 72 h. The Abs also bind to ≥1% of blood monocytes, and those that are Ag are predominantly peroxidase negative (PO-). In a recent study (Lehman et al., J. Immunol. and Environ. Health, in press, 1985), we found that the intrapulmonary deposition of 4 x 10^6 microparticles in the rat results in a doubling in the size of the AM population 24 h later with only 13% of the AM being PO-. The results of the present study are consistent with the possibility that this increase in the AM population may be due to the preferential recruitment of Ag blood monocytes into the alveolar compartment. Alternatively, the Ag AM found 24 h after particle deposition may also represent resident AM that in some manner are stimulated by the presence of the lung particles to express the Ag and/or the Ag cells are migrating interstitial macrophages, which are also PO-. In the rat (Lehman et al., Exp. Lung Res., in press, 1985). [This work was conducted under the auspices of the D.O.E.]

Four groups of ten female Hartley guinea pigs each were exposed once a week for 10 weeks by the intratracheal (IT) route to 0.1 ml volumes of saline solutions containing 250 μg of a detergent along with either 3, 1, 0.3, or 0.1 μg of protein of the proteolytic enzyme, Subtilisin A, Evaluation of respiratory responses immediately following each IT exposure revealed a positive dose-response relationship commencing after the fourth IT dose and continuing through the remainder of the experimental period. Weekly serological measurements of allergic antibodies and of precipitating antibodies revealed a similar dose-response relationship. Comparison of the results from this IT study with those of an inhalation study with combinations of the same detergent and enzyme (abstract by McNeill, D.A., et al., SIG Meeting, 1986) revealed that the kinetics of the antibody response and the association between the appearance of allergic antibodies and the onset of respiratory symptoms in both studies were quite similar.

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A systematic study was undertaken to develop a rapid, reproducible method to measure cyanide levels in small samples of blood (250 μl). The most commonly used techniques require larger blood samples (2 ml), 4 hr equilibration times, and produce variable results if the samples are stored before processing. In our procedure, sodium nitrite is added to the blood to stabilize the cyanide levels. The sodium nitrite is believed to act by increasing the blood methemoglobin which binds with cyanide and produces the stable complex cyano-methemoglobin. The blood samples, in sealed vials, are acidified and heated to release HCN into the head-space. After equilibrium (30 min), gas samples are taken with an automated head-space sampler and analyzed with a gas chromatograph equipped with a nitrogen-specific detector. The effects of different types and volumes of acids as well as the equilibrium temperatures were tested in an effort to optimize cyanide recovery. This procedure proved to be simple, fast, reproducible and stable over time.

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A system was developed for continuously monitoring the temperature of guinea pigs in whole body exposure chambers. Principal components included the transmitter (Mini-Mitter, Inc.), a 150 kHz AM tuner and amplifier, and an IBM XT. An algorithm was developed which sampled at 5000 per second for 250 usec. The transmitter was coated with paraffin, sterilized, and surgically stitched to the peritoneal wall. In guinea pigs monitored for 24 hour periods, temperatures were found to vary 0.5°C. No recurrent patterns were observed. Determinations were within 0.2°C of temperatures measured rectally. In 5 guinea pigs monitored from six weeks to five months, the temperatures varied from about 38.4 to 39.4°C. No appreciable age-associated changes in core temperature were noted. Experiments were conducted in which guinea pigs were treated with agents which induced transient hyper- or hypothermia. For the former, temperature increases of 1.5°C were detected. In the latter case, decreases of as much as 3.4°C in 3 hours were observed. Use of this system will enable determination to be made of pyrexia for the study of conditions such as hypersensitivity pneumonitis and Mill Fever. Supported by NIAMS ES01532.

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Quantitative measurements of the constituents in lavage fluids are of diagnostic value for lung disorders, including acute lung injury. We have developed a new method by which 11 fractions from lavage fluids of rats are isolated using high performance liquid chromatography (HPLC): fractions 1-5 are eluted from a Bondapak C18 column with a water-acetonitrile gradient containing 0.2% trifluoroacetic acid; fractions 7-11 are subsequently eluted with methanol. Nine of the constituents are proteins, three of which are common to the lavage fluids and blood compartment, i.e., albumin and immunoglobulins. All the other constituents appear to be unique to the lung. The ability to use HPLC to identify and quantify changes in lavage fluid constituents was evaluated using two experimental conditions. In one study, fluids obtained from rats 7 days after thoracic X-irradiation at doses of 7.5 and 15 Gy were analyzed. The X-irradiation produced dose-dependent decreases in fractions 4, 5, and 6 (which include the albumin and immunoglobulins) and dose-independent increases in fractions 10 and 11. In another study, lavage fluid harvested from rats 24 h after the intrapulmonary deposition of 7 μg cadmium (as CdCl2) were analyzed. The acute inflammatory response to this toxic agent was induced by a 30-fold increase in fraction 3, fraction 4 (albumin), and in fraction 5 (immunoglobulins). In comparison, changes in fractions 1, 2, 6, and 9-11 were markedly less pronounced. These results demonstrate the utility of HPLC for studies of environmental insults to the lung. [This work was performed under the auspices of DOE.]
Inhalation of cotton dust has been associated with development of byssinosis. In order to understand the progression of the disease, an animal model was developed in which guinea pigs were exposed to atmospheres of cotton dust for twelve months and respiratory function was monitored daily pre and post-exposure. Four stages of the disease were identified. Upon first exposure, a severe effect is observed characterized by an increase in respiratory frequency (f), decrease in tidal volume (VT) and airflow interruption. After 2-12 weeks of exposure, a second stage is identified characterized by a response on "Mondays" with small daily reactions. Following 12-24 weeks of exposure, respiratory reactions occurred with regularity on Mondays and on other weekdays. A fourth stage was identified following 24-52 weeks of exposure. Animals had daily responses and reduced VT and increased f prior to daily exposure. The four stages of disease noted in the guinea pigs closely parallel those described in cotton dust workers. Supported by USDA Cooperative Agreement No. 58-7830-2-426 and ES02747.

Single-pass perfusion of mouse livers in situ with the phosphorothioate pesticide parathion (PS) resulted in formation of the cholinesterase inhibitor paraoxon (PO), p-nitrophenol, p-nitrophenyl sulfate, and p-nitrophenyl glucuronide. Daily pretreatment of mice with phenobarbital (PB) (ip, 80mg/kg) induced hepatic cytochrome P-450 content, as well as activation of PS to PO in vitro. However, PB pretreatment did not alter the rate of production of PO from PS in mouse livers perfused in situ. Moreover, pretreatment of mice with PB or saline resulted in a mortality rate of 20% following a challenge dose of PS (ip, 5mg/kg). These results indicate that PB pretreatment clearly induces the formation of cytochrome P-450 catalyzing conversion of PS to PO. Yet increased production of PO from PS does not occur in the intact liver following PB pretreatment, indicating that additional factors such as induction of alternate metabolic pathways for PS must be considered. (Supported by Grant ES 03435 from NIEHS, and a grant from the Edward G. Schleider Foundation).

Inducibility of the hepatic mixed function oxidase (MFO) responsible for 7-ethoxyresorufin (7-ER) metabolism has been shown to be significantly different between strains of laboratory mice. Metabolism of 7-ER has been used as a measure of cytochrome P-448 activity in rats and mice. Mice of both sexes from B6C3F1, (B6) or Swiss-Webster (SW) strains were pretreated with IP injections of normal saline (NS), or phenobarbital (P; 80 mg/kg) for 3 days; or corn oil (CO) or Aroclor 1254 (A; 100 mg/kg) for 1 day. Liver microsomes were prepared 24 hours after the last injection. Metabolism of 7-ER was evaluated using a fluorometric technique [Burke and Mayer (1974) Drug Metab. and Dispos. 2(6):583-88]. The difference between sexes for a given strain was insignificant; therefore, the data for both sexes of each strain were pooled. The data expressed as mean ± S.E. of resorufin formed (nanomoles/mg protein/hour) were as follows: B6: NS - 15.10 ± 0.37, CO - 16.71 ± 0.64, P - 35.66 ± 1.35*, A - 108.66 ± 7.34*, SW: NS - 13.52 ± 0.56, CO - 10.49 ± 0.53, P - 20.97 ± 0.99*, A - 34.73 ± 4.11*. While induction by A was an expected finding, it was not for P. These data demonstrate that there is a marked strain difference in MFO metabolism of 7-ER and that P-450 may play a role in 7-ER metabolism in mice.

The kidney of the male Syrian golden hamster is a target for estrogen-induced carcinogenesis. The mechanism is believed to involve hormonal factors and covalent binding of reactive metabolite(s) to critical macromolecules. Irreversible binding of [3H]DES to hamster renal cortical protein was observed when 50 nM [3H]DES was incubated with renal cortical slices. No radioactivity beyond background was associated with DNA purified from the same slices. Samples of protein adducts examined by SDS-PAGE revealed radioactive peaks corresponding to molecular weights of 53 kDa and 37 kDa. Phenobarbital or 3-methylcholanthene did not increase the irreversible binding of [3H]DES to protein, while metyrapone and SKF 525-A decreased irreversibly bound [3H]DES by 70% and 55% respectively. Decreased production of oxidative metabolites of [3H]DES was observed with SKF 525-A, Arachidonic acid, a substrate, and indomethacin, an inhibitor of prostaglandin synthetase did not alter the levels of irreversibly bound [3H] DES. These data suggest that NADPH-dependent monoxygenases metabolize DES to reactive intermediates that covalently bind to protein. Covalent binding to specific proteins may be an important step in the mechanism of estrogen-induced carcinogenesis in the male hamster.
The role of the hepatic mixed-function oxidase system in the bromine (TB) metabolism was investigated by measuring in vitro and in vivo metabolism and kinetic parameters in control and induced male Sprague-Dawley rats. Treatment with phenobarbital (PB) and 3-methylcholanthrene (3MC) resulted in two- and seven-fold increases, respectively, in the formation of 3,7 dimethyluric acid (3,7DMU - the major TB in vitro metabolite) by isolated liver microsomes. PB induction also selectively increased production of dimethylallantoin (DMA), whereas 3MC had little effect. Inclusion of cytosol or reduced glutathione catalyzed a dose-dependent conversion of 3,7DMU to 6-amino-5-[N-methylformylaminol-l- methyluracil (6AMMU). In vivo, 6AMMU is the major metabolite in the rat.

Induction with 3MC decreased mean TBR by 1/2 from 1.6 to 0.6 hr, and increased mean plasma C1 from 3.8 to 14.6 ml/min/kg. Selective increases in urinary excretion of 6AMMU indicated the role of a 3MC inducible P-450 isozyme in this metabolic pathway. PB treatment had no effect on overall TBR kinetics or metabolism, but significantly increased conversion to DMA (a minor metabolite). Further characterization studies with other inducing agents and cytosol are in progress.

**567** TREATMENT WITH POLY I.C. ENHANCES LIPID PEROXIDATION, ACTIVITY OF XANTHINE OXIDASE AND DECREASES HEPATIC P-450 SYSTEM IN MICE AND RATS. A. Koizumi, L. Hasegawa and T. Imaura. Division of Toxicology and Physiology, University of California, Riverside, CA.

Treatment of mice and rats with polyribosinosinic acid-polyribocytidylic acid (poly I.C., 5 mg/kg i.p.), a potent interferon inducer, decreased hepatic cytochrome P-450 systems without causing any effect on P-450-independent xenobiotic metabolizing enzymes. Treatment with poly I.C. decreased the content of P-450 by 28% in mice (P<0.05) and 30% in rats (P<0.05) while it did not decrease activity of cytochrome c reductase. The effects on P-450-dependent systems was accompanied by an increase in xanthine oxidase (XO) activity and the enhancement of lipid peroxidation. The activity of XO was increased by 87% in mice (P<0.01) and 30% in rats (P<0.01). Lipid peroxidation was enhanced by 82% in mice (P<0.01) and 95% in rats (P<0.05), respectively. These results suggest the possibility that a part of the depression of P-450 systems might be caused by an enhancement of lipid peroxidation associated with an increased activity of XO.

This study quantitated the enzyme inducing activity of the fungicide NPSM. Male Fischer 344 rats were treated with an acute dose of 0.4 or 0.8 mmol/kg NPSM, ip. Controls received an equal vol/kg of sesame oil. In a second series of expts., rats were injected with 0.2 or 0.4 mmol/kg NPSM daily for 3 days. Twenty-four hr after the last NPSM treatment, in vivo aminopyrine demethylase, aniline hydroxylase, and hexobarbital oxidation activity were quantitated in hepatic 9,000g supernatant. A single 0.4 mmol/kg dose of NPSM produced a 13% (ns) and 29% (p<0.05) increase in aminopyrine demethylase and aniline hydroxylase, resp. while an acute 0.8 mmol/kg NPSM dose resulted in a 20% (ns) and 18% (ns) increase, resp. Aminopyrine demethylase, aniline hydroxylase, and hexobarbital oxidation were increased 9.3%, 57% (p<0.05), and 25% (ns), resp. by 3 day pretreatment with 0.2 mmol/kg NPSM and 34% (p<0.05), 126% (p<0.05), and 21% (ns), resp. by 3 day pretreatment with 0.4 mmol/kg NPSM compared to controls. Enzyme induction occurs following a 3 day treatment with NPSM. (Supported by NIH grant AM 21210.)


Rodent and human microsomal cytochrome P-450 systems, both hepatic and extrahepatic, hydroxylate fatty acids at the ω or ω-1 carbon. Extensive evidence indicates that fatty acid ω-hydroxylase activity can be specifically induced by hypolipidemic agents such as clofibrate, fenofibrate and Wyeth 14,663. Recently, we demonstrated LTB4 ω and ω-1 hydroxylase activity in hepatic microsomes. Therefore, these studies were designed to compare the effects of the inducers phenobarbital (PB), 3-methylcholanthrene (3MC) and Wyeth 14,663 (WY) on rat liver and kidney LTB4 and LA hydroxylases. Hepatic LA ω-hydroxylase was not induced by PB or 3MC but was induced 16 fold after WY pretreatment. Similarly, LA ω-1 hydroxylase was not induced by PB or 3MC but was increased 2 fold by WY pretreatment. In contrast, hepatic LTB4 ω and ω-1 hydroxylases were not induced by PB, 3MC or WY. renal LA ω and ω-1 hydroxylases were not affected by PB or 3MC but were doubled by WY pretreatment. renal LTB4 ω-hydroxylase was not induced by any pretreatment regimen while LTB4 ω-hydroxylase was induced two fold after WY pretreatment. These results demonstrate that hepatic LTB4 ω and ω-1 hydroxylases are not induced by hypolipidemic agents and suggest that different forms of cytochrome P-450 are involved in the metabolism of LA and LTB4.

EFFECT OF T-2 TOXIN ON ACTIVITIES OF TRANS-STILBENE OXIDE-INDUCED HEPATIC MICROSOMAL ENZYMES. R.P. Fricke, Pathophysiology Division, US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

The effects of T-2 toxin, a 12,13-epoxy trichothecene, on trans-stilbene oxide (TSO)-induced hepatic microsomal enzyme activities were studied. Mice were pretreated with vehicle only (control) or TSO (50g/kg body weight/day, 3d). Following pretreatment, mice were further exposed to either T-2 toxin (55g/kg body weight, s.c.) or toxin vehicle only. Microsomes were prepared 6 hr later, and assayed for cytochrome P-450 (P450), epoxide (stereose oxyde) hydroxylase (EH), 7-ethoxy-coumarin-0-deethylase (EOD), and NADPH cytochrome reductase (Cr). The activities of control, T-2 toxin only, TSO only, and TSO + T-2 toxin were, respectively: P450 (nmol/mg protein), 0.623 ± 0.11, 0.413 ± 0.02, 1.45 ± 0.05, and 1.12 ± 0.13; EH (nmol/mg protein/min), 2.48 ± 0.29, 1.61 ± 0.43, 7.53 ± 0.52, and 7.72 ± 0.51; Cr (μmol/mg protein/min), 91 ± 5, 80 ± 17, 227 ± 11, and 210 ± 8; and EOD (μmol/mg protein/min), 124 ± 14, 87 ± 15, 313 ± 12, and 320 ± 9. In summary, TSO significantly (p < 0.001) increased the activities of all the microsomal enzymes assayed. T-2 toxin did not have a significant effect on activities of either EH, Cr, or EOD but caused a significant (p < 0.05) decrease in P450 levels for both control and TSO-induced microsomes.


Female Sprague-Dawley rats were administered by gavage, 500 mg/kg of DCPD for 4 days. The specific content of cytochrome P450 was unchanged (0.92 ± 0.06 nmole/mg hepatic microsomal protein in control and treated animals, respectively). SDS gel electrophoresis of these microsomal proteins demonstrated an increase in bands that co-migrated with epoxide hydroxylase (MW 49,000) and cytochromes P450c (MW 56,000) and P450b or d (MW 52,000). The aryl hydrocarbon hydroxylase activity was increased ca. 2.6 times in microsomes from DCPD treated animals as compared to microsomes from control rats. This compound (DCPD) is an in vivo and in vitro metabolite of the fungicide/herbicide 2,6-dichloro-4-nitroaniline which has been shown to produce similar biochemical alterations in microsomes from female rats.

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Diethylphenylphosphine is rapidly metabolized to diethylphenylphosphine oxide by the FAD-containing monoxygenase with a K<sub>m</sub> value lower than 2.5 μM. Reconstituted cytochrome P-450 dependent monoxygenase systems also metabolized diethylphenylphosphine to the phosphate oxide. NADPH is required and the amount of oxide formed is time and cytochrome P-450 dependent.

Purified phenobarbital (PB)-induced cytochrome P-450 produces more oxide per mole enzyme than the purified constitutive cytochrome P-450 isozymes tested. The phosphate oxide is also formed in lesser amounts in incubation mixtures containing only NADPH-cytochrome P-450 reductase and NADPH. Diethylphenylphosphine binds to oxidized PB-induced cytochrome P-450 and to the constitutive cytochrome P-450 isozymes with K<sub>m</sub> values of 16 μM and 11-18 μM, respectively.

Diethylphenylphosphine is also a competitive inhibitor of p-nitroanisole O-demethylation catalyzed by a reconstituted PB-induced cytochrome P-450 dependent monoxygenase system, with a K<sub>i</sub> value of 5 μM. The phosphate oxide produces no observable optical difference spectrum with oxidized PB-induced cytochrome P-450 and causes no inhibition of p-nitroanisole O-demethylation.

Both the cytochrome P-450 dependent monoxygenase system and the FAD-containing monoxygenase catalyze the sulfonation of thioether-containing organophosphorus insecticides. Using purified FAD-containing monoxygenase and cytochrome P-450 isozymes isolated from mouse liver, the stereospecificity of the oxidation of phorate to (+)- and (-)-phorate sulfoxide and the further oxidation of (+)- and (-)-phorate sulfoxides to the sulfone and sulfoxide oxon was examined.

The FAD-containing monoxygenase catalyzed the formation of (-)-phorate sulfoxide, while two cytochrome P-450 isozymes (cytochrome P-450-B2, a constitutive form, and cytochrome P-450-P8, the main form induced by phenobarbital) produced (+)-phorate sulfoxide. The other three constitutive cytochrome isozymes examined yielded racemic mixtures. No additional oxidation of phorate sulfoxide by the FAD-containing monoxygenase was detected using either (+)- or (-)-phorate sulfoxide or (+)- or (-)-phorate sulfoxide as substrate. By contrast, all cytochrome P-450 isozymes tested formed additional oxidation products; preliminary results indicate that (+)-phorate sulfoxide is the preferred substrate for cytochrome P-450 isozymes.

The in vitro exposure of microsomes obtained from male BALB/c mouse livers to t-4HN, t-4HH and t-2H, resulted in the loss of cytochrome P450. Microsomes (3.17 mg protein/ml ± 0.12), in the presence of 2.0, 1.0 and 0.5 mM T-4HN plus a NADPH generating system, resulted in a 52.9, 26.4 and 14.7% decrease in cytochrome P450 in relation to controls without aldehyde. Reducing the microsomal protein concentration (0.97 mg/ml ± 0.03) resulted in a 52.9, 49.1, 28.9, 16.2 and 19.5% reduction in cytochrome P450 for the respective concentrations of t-4HN, 1.0, 0.5, 0.1, 0.05 and 0.025 mM.

Exposure of microsomes (1.19 mg/ml ± 0.08) to t-4HN produced a 61.4, 53.3, 41.8, 26.8 and 15.9% reduction in cytochrome P450 for the respective doses 1.0, 0.5, 0.25, 0.1 and 0.05 mM. Trans-2-hexenal also reduced the levels of microsomal cytochrome P450. Hexanal (2 mM) was unable to reduce the concentration of cytochrome P450. Microsomes incubated with t-4HN and t-4HH, under N₂ minus an NADPH generating system, showed considerable conversion of P450 to P420. The mechanism by which these sulphydryl reagents act may be initially through the conversion of P450 to P420; the latter being unstable under aerobic conditions disappears when incubated in the presence of oxygen. [Supported by NIH, ES03343]
AGE-RELATED CHANGES IN THE ACTIVITIES OF EPoxide HYDROLASES IN DIFFERENT TISSUES OF MICE. S. Kaur and S. S. Gill. Division of Toxicology and Physiology, University of California, Riverside, CA 92521.

Age-related changes in epoxide hydrolase (EH) activity in the liver, kidney, lung, and intestine were studied in male C57BL/6 mice of 1 through 30 months of age. EH activity in the cytosolic and mitochondrial fraction was monitored by trans-stilbene oxide hydrolysis. Hepatic cytosolic EH activity increased until 15 months after which there was a decline of 59% between 30 months. Hepatic EH activity in the mitochondrial fraction increased until 4 months and decreased thereafter. Using cis-stilbene oxide as substrate, liver microsomal EH activity increased until 6 months followed by a decline of 32% by 30 months. Renal cytosolic EH showed maximum activity at 6 months after which the activity decreased significantly with age. However, renal EH activity in the mitochondrial fraction, in general, did not change with age. EH activity in the cytosolic, mitochondrial and microsomal fractions of kidney was 2.5-, 2-, and 10-fold less, respectively, than that found in similar subcellular fractions of liver. Cytosolic EH activity in lung and intestine and lung microsomal EH showed variations with age.


Oxidation by microsomal monoxygenase is the rate-limiting step in the excretion of many organochlorine insecticides. The high degree of variation in monoxygenase activity between different classes of vertebrates may be important in determining the toxicity of these compounds in the environment. Multiple forms of cytochrome P-450 were separated from the hepatic microsomes of untreated male rats, rainbow trout and two avian species - feral pigeon and ronbill, using an identical procedure of anion exchange chromatography on DEAE-cellulose. In some cases, further purification of P-450 was carried out on hydroxyapatite and CM-sephadex. Aldrin epoxidase activity and hydroxylation of the dieldrin analogue MCE were examined in intact microsomes and in reconstituted systems containing microsomal lipid, cytochrome P-450 reductase purified from uninduced rats and the purified cytochromes P-450. Significant differences were seen in the distribution of P-450 forms and, in spite of the close structural similarity between the two substrates, in the distribution of catalytic activity between the cytochrome P-450 isozymes of each species.

HEPATIC DRUG METABOLISM IN QUAIL, DUCKS, GEESE, CHICKENS, TURKEYS AND RATS. K. R. Dalvi and V. A. Nunn. School of Veterinary Medicine, Tuskegee University, Tuskegee, AL.

Although general pattern of biotransformation of drugs is common to all species, many interspecies differences in drug metabolism have been shown. The present study was carried out to compare the in vitro drug-metabolizing ability of quail, ducks, geese, chickens, turkeys and rats. No significant differences in relative liver weights were noted for the avian species. Concentration of liver microsomal protein in quail and ducks was much higher than that of other avian species. The rat was highest in these parameters. Hepatic cytochrome P-450 content was highest in rats followed by turkeys, geese, chickens, ducks and quail. Rate of benzenesulfonamide N-demethylation was higher in geese, turkeys and chickens than that for quail, ducks and rats. Geese, turkeys and chickens were almost similar in the rate of aniline hydroxylation. However, the rate was markedly low for quail and ducks. These results provide a basis for prediction and comparison of phase 1 biotransformations in various avian species. (Supported by FDA Grant No. FD-U-004064-01).

RELATIVE CONTRIBUTION OF THE FAD-CONTAINING MONOXYGENASE AND THE CYTOCHROME P-450 MONOXYGENASE SYSTEM IN THE MICROSONAL OXIDATION OF PHORATE. S. Kimler and E. Hodgson. North Carolina State University, Toxicology Program, Box 7633, Raleigh, NC.

The FAD-containing monoxygenase and the cytochrome P-450-dependent monoxygenase system are the major enzymes catalyzing the metabolic oxidation of organophosphate pesticides. A number of organophosphates are oxidized by both systems, to varying degrees. In the current study, the relative involvement of these two enzymes in the oxidation of phorate to phorate sulfoxide was investigated in mouse liver microsomes by selectively inactivating either the FAD-containing monoxygenase or the cytochrome P-450-dependent monoxygenase system, prior to incubation with 14C-ethyl phorate and NADPH. Microsomes were heated at 37°C for 90 minutes to inactivate the FAD-containing monoxygenase; or the cytochrome P-450-dependent monoxygenase system. Prior to incubation with 14C-ethyl phorate and NADPH, microsomes were heated at 37°C for 90 minutes to inactivate the FAD-containing monoxygenase; or the cytochrome P-450-dependent monoxygenase system. Prior to incubation with 14C-ethyl phorate and NADPH, microsomes were heated at 37°C for 90 minutes to inactivate the FAD-containing monoxygenase; or the cytochrome P-450-dependent monoxygenase system. Prior to incubation with 14C-ethyl phorate and NADPH, microsomes were heated at 37°C for 90 minutes to inactivate the FAD-containing monoxygenase.
580 THE ROLE OF DESACETYLATION IN THE DETOXIFICATION OF CEPHALOTHIN. F. D. Williams, G. H. Rottendorf and D. A. Laska, Department of Experimental Toxicology, Bristol-Myers Company, Syracuse, NY. Sponsor: R. Madigos.

The toxicities of three cephalosporin antibiotics (cephalothin, CEPH, ceftazolin, CZL, cephaloridine, CLOR) were compared in a rabbit kidney cell line (LLC-RK) in vitro. Rabbit kidney cells were exposed for 48 hours to various concentrations of the antibiotics in monolayer cultures. CEPH was markedly toxic to kidney cells resulting in 25% cell viability at a concentration of 0.5 mg/ml. The same concentration of CZL and CLOR produced 68 and 25% cell viability, respectively. The addition of rabbit kidney microsomal S9 to the incubation media decreased the toxicity of CEPH (90% viability) and provided the proper in vivo nephrotoxicity ranking of the cephalosporins (CLOR > CZL > CEPH). The S9 fraction was found to desacetylate CEPH. An esterase inhibitor, aminocarb, blocked the in vitro desacetylation and detoxification of CEPH. Since CEPH is extensively metabolized in vivo to desacetylcephalothin, these studies suggest that the desacetylation process may represent an unrecognized mechanism of detoxification of CEPH.

582 PARTIAL PURIFICATION AND CHARACTERIZATION OF A PEROXIDASE ACTIVITY FROM NEONATAL RAT SKIN.

B.H. Strohm, A.P. Kulkarni, Toxicology Program, The University of Michigan, Ann Arbor, MI 48109

Peroxidase was isolated from neonatal (3-6 day) rat skin. The membrane bound peroxidase activity was extracted using 0.5 M CaCl2 and monitored spectrophotometrically at 470 nm using 2-mercaptoethanol (guaiacol) and H2O2 as substrates. Subcellular distribution studies indicate the activity to be highest in mitochondria and nuclei, lowest in microsomes and absent in cytosol. The peroxidase activity was partially purified by affinity chromatography on Con A-Sepharose 4B and by gel filtration using Sephadex G-100 and Bio-gel P-150. Purification factors from these two steps were about 25 and 3, respectively. Peroxidase extraction in the presence of 2mM NEM increased activity about 2 fold. The combination of 2mM NEM and 10% (v/v) glycerol were found to be optimal for preservation of activity. Peroxidase activity was found to increase linearly with increase in protein concentration, time, and guaiacol concentration. Benzyl peroxide could support the reaction with guaiacol as substrate. Activity was inhibited 75% by 0.1mM KCN or 0.05 mM NaN3. Data indicate pyrogallol, hydrogen peroxide, p-cresol, catechol, benzidine and 3,3'-dimethoxybenzidine can act as substrates for rat cutaneous peroxidase.

581 PARTIAL PURIFICATION OF HUMAN TERM PLACENTAL PEROXIDASE BY AFFINITY CHROMATOGRAPHY. J.L. Nelson and A.P. Kulkarni, Toxicology Program, The University of Michigan, Ann Arbor, MI 48109

Peroxidases are known to metabolize a variety of xenobiotics, including the carcinogens benz(a)-pyrene and DES to reactive intermediates capable of forming adducts with proteins and nucleic acids. The extent to which this enzyme system may directly or indirectly contribute to fetotoxicity has been largely unexplored. In the present study, human term placental peroxidase was purified from Concanavalin A Sepharose 4B affinity chromatography from CaCl2 extracts of the particulate fraction. Peroxidase activity was measured colorimetrically using a model substrate, guaiacol (o-methoxyphenol), in the presence of H2O2. Peroxidase activity was eluted with a methyl-D-mannopyranoside in fractions devoid of hemoglobin contamination. Active fractions were combined and assayed for peroxidase activity and protein content. Affinity chromatography provided an efficient method of purification, typically resulting in a 30-fold increase in specific activity with a 92% recovery. The results show that placental peroxidase appears to be a glycoprotein like other peroxidases. Currently, further purification of placental and fetal peroxidase is being undertaken by HPLC techniques, and evaluation of the ability of peroxidase to metabolize fetotoxic chemicals in vitro are in progress.


Reactive metabolites generated by BP monoxygenase (AHH) interact with nucleophiles in macromolecules such as DNA and protein, and cause mutation and carcinogenesis. We studied the effect of Panax ginseng C. A. Meyer, which induce epoxide hydratase activity without concomitant induction of AHH activity, on the binding of BP metabolites to DNA in vitro. Sprague-Dawley rate(200 g) were treated with ginseng extract for 3 days at a daily oral dose of 250 mg/kg b.w. Incubation mixture consisted of 20.6 µM 3H-BP(14.5 nCi/mole), 1 µg DNA, 0.7 mM NADPH, NADPH generating system, microsomal protein (0.75-1 mg) and 0.1 M phosphate buffer, pH 7.4 containing 2.5 mM MgCl2, 0.1 mM EDTA in a final volume of 1 ml. DNA-BP adducts were chromatographed on a Sephade DH-20 column employing a linear gradient of 45-75% MeOH/H2O. Of the 5 peaks tentatively assigned to 7,8-diaryl-9,10-oxide(A), 7,8-oxide(B), 4,5-oxide(C), and further metabolites of 9-OH BP(C & D), peak A, C, D, and E were reduced to 30, 15, 20, and 65% of controls, respectively, and there was no significant change in peak B. These findings strongly suggest that ginseng component has the potential to inhibit, at least in part, the mutagenic and carcinogenic processes effected by the binding of BP-metabolites to the nucleophiles of critical macromolecules in mammalian tissues.
Induction of mammary tumors in female Sprague-Dawley rats by DMBA is dependent on hormonal status. Hormonal status is known to influence metabolic activation of many xenobiotics in the liver. In this study the perfused rat liver was used to examine endocrine influence on DMBA metabolism. Livers from intact (IN) and ovariectomized (OV) rats were perfused for 120 min. Five μCi (20 μmol) 125I-DMBA were infused from 0-60 min. Samples of perfuse and bile were collected periodically. Analysis of perfusate extracts by HPLC showed that the rate of DMBA metabolism increased linearly from 5-60 min. The maximum rate of appearance of metabolites in the perfuse and the bile was higher in the IN than the OV rat (perfusate, 33.2 vs. 28.8 nmol/g liver/hr; bile, 142 vs. 125 nmol/g liver/hr). The maximum rates of production of certain polar metabolites were higher in the OV than the IN rat. Two of the peaks detected co-eluted with the 7,12-bis-hydroxybenzyl and 3,4-dihydrodiol metabolites of DMBA. These results indicate that the endocrine status of the female Sprague-Dawley rat influences the hepatic metabolism of DMBA. (Supported by the American Cancer Society, Ohio Division, and NSF Graduate Fellowship RCD-8450052).


Inhalation exposure of rats to acrylate esters results in a selective degeneration of olfactory (OE) but not respiratory (RE) epithelium. It has been postulated that this effect results from esterase mediated metabolism in OE to the corresponding toxic acid. The purpose of this study was to compare the carboxylesterase activity in RE and OE of rats and identify individual cell types of high esterase activity. p-Nitrophenylbutyrate esterase activity was measured in homogenates of OE and RE. For OE, Km = 16.3 μM and Vmax = 49.4 μmoles/min/mg. For RE, Km = 12.9 μM and Vmax = 5.5 μmoles/min/mg. Thus, Vmax/Km was 6.6 times higher in OE than RE indicating more efficient metabolism at saturating concentrations in OE. Histochemical localization of α-naphthyl butyrate esterase activity demonstrated very strong reaction product in ciliated respiratory epithelial cells. In OE, esterase activity was intense in the olfactory sustentacular cells, acini of Bowman's glands, and seromucous glands. These results are consistent with the hypothesis that intraepithelial production of acidic metabolites of inhaled esters occurs to a greater extent in OE than RE of rats and may be responsible for the pathologic changes observed in the olfactory epithelium.

**Effect of Selenium on Acetaminophen (AAP) - Induced Hepatotoxicity in Male Hamsters.** A. Blakker and R.E. Schnell, Dept. Pharmacody. Toxicology, Univ. Neb. Med. Ctr., Omaha, NE 68105.

Male, Golden Syrian Hamsters pretreated with sodium selenite (0.25, 0.5 or 1.0 mg Se/kg, sc) 24 hours prior to receiving a hepatotoxic dose of AAP (400 mg/kg, ip) exhibited significantly attenuated toxicity in comparison to animals receiving only AAP. Serum enzyme levels of SDH and ALT (24 hr after AAP dosing) in the Se + AAP treatment groups ranged from 11 to 39% of the AAP-treated group and were statistically similar to control values. Hepatic glutathione levels of all animals dosed with AAP were significantly increased above control. Cytochrome P-450 levels and activities of microsomal or cytosolic enzymes in the liver, (i.e., ethylmorphine N-demethylase, aniline hydroxylase, glucuronyl transferase, GSSG-reductase, glutathione peroxidase, and GSH- S-transferase) were not significantly different from control. 24 hr after Se (1.0 mg/kg, sc) administration. Further studies are required to determine if Se is alleviating AAP-induced hepatotoxicity via an alteration in Phase II AAP metabolism. Supported by PMA Starter Grant (AB) and a Burroughs-Wellcome Toxicology Scholar Award (RCS).

**Effect of Hormonal Status on the Hepatic Metabolism of 7,12-Dimethylbenz(a)anthracene (DMBA) in the Sprague-Dawley Rat.** S.T. Vater, D.M. Baldwin and D. Warshawsky, Deps. of Env. HLth. and Physiol., Univ. of Cincinnati College of Medicine, Cincinnati, OH. Sponsor: R.C. Shertzer.
588  OLIPRAZ (OTP) - INDUCED CHANGES IN ACETAMINOPHEN (AAP) DISPOSITION IN MALE HAMSTERS. M.H. Davies, A. Prous, S.J. Neth and R.C. Schnell, Dept. Pharmacodyn-Toxicology, Univ. NE Med. Ctr. Omaha, NE 68105.

OTP (500 mg/kg), a natural component of cruciferae, or vehicle (25% glycerol-1% CMC) were administered (po) at 96 and 48 hr prior to experiments. Plasma pharmacokinetics of AAP, glucuronide (AAP-GLUC) and sulfate (AAP-SULF) conjugates and urinary concentrations of these compounds were measured after an iv bolus of AAP (100 mg/kg). Plasma AUC data fit a one compartment model using PCNONLIN from estimates generated by CSTRIP. The overall first order elimination constant for AAP was increased by OTP treatment, while apparent volumes of distribution, total clearance and AUC were statistically similar for both groups. AAP-GLUC AUC increased, while AAP-SULF AUC decreased and urinary concentrations of AAP, AAP-GLUC and -SULF were markedly increased in OTP-dosed animals. AAP UDP-glucuronyl transferase activity was elevated nearly two-fold by OTP treatment. Oral AAP bioavailability was decreased 53% in OTP-treated hamsters. These results provide evidence that OTP-induced protection from AAP toxicity may, in part, be due to altered AAP disposition. (Supported by Burroughs-Wellcome Toxicology Scholar Award (RCS) and Sandoz Research Institute Fellowship (MHD)).

589  HEPATIC UDP-GLUCURONIC ACID REGULATION DURING ACETAMINOPHEN BIOTRANSFORMATION IN RATS. J.J. Hjelle and N.G. Ship. School of Veterinary Medicine and the Environmental Toxicology Center, University of Wisconsin, Madison, WI.

The purpose of this study was to determine whether acetaminophen (AA)-induced depletion of hepatic UDP-glucuronic acid concentration occurs because of decreased availability of the precursor UDP-glucose or limited UDP-glucose dehydrogenase activity. Hepatic UDP-glucuronic acid concentration was markedly decreased 30, 60 and 120 min after administration of AA to adult male Sprague-Dawley rats. UDP-glucose levels remained unchanged after 30 min but did drop at later time points (approx. 60% of control values after 60 and 120 min) whereas glycogen levels were affected at the 30 min interval only (78% of control values.) Studies with rat liver UDP-glucose dehydrogenase revealed that NADH is a potent competitive inhibitor of NAD (NADH = 0.7 μM). Enzyme response experiments demonstrated that AA increased liver lactate/pyruvate ratios 30 min after administration of 150, 300 and 600 mg AA/kg (14%-173% of control values) and that increases in lactate/pyruvate ratios were coincident with declines in UDP-glucuronic acid. Therefore, depletion of UDP-glucuronic acid is probably related to inhibition of UDP-glucose dehydrogenase secondary to an accumulation of NADH in the cytoplasm. (Supported by a PMAF starter grant.)

590  INHIBITION OF ACETAMINOPHEN GLUCURONIDATION BY DIAZEPAM: SPECIES DIFFERENCES IN VIVO. A.A. Lavo, Dep't of Toxicology and Pathology. Hoffmann-La Roche Inc. Nutley, New Jersey 07110. Sponsor: R.M. McLarn.

In previous studies we demonstrated that diazepam is an inhibitor of acetaminophen glucuronotransferase (GT) in hepatic microsomes but that the inhibition differed markedly depending on the species (Lavo et al. The Toxicologist 5:78). In hepatic microsomes from male rats the 50% inhibition of acetaminophen GT was 25 μM diazepam, while hepatic microsomes from rat, monkey, and human had IC50's of 65, 55, and 10 μM. This in vivo study compared the effects of diazepam on acetaminophen disposition in a sensitive species (dog) and an insensitive species (rat). Acetaminophen, its glucuronide and sulfate were determined by HPLC in the serum of rats (10μg/kg) and dogs (60μg/kg) treated with 100+50 or 100+25 μg/kg acetaminophen + diazepam and dogs (60μg/kg) treated with 100+0 or 100+25 μg/kg acetaminophen + diazepam. Both coadministration of diazepam did not alter the disposition of acetaminophen as determined by area under the concentration vs time curves (AUC). In dogs treated with 25 μg/kg of diazepam, the mean peak acetaminophen glucuronide concentration was markedly reduced and the AUC for acetaminophen was increased by 1.8 fold. There were no significant alterations of acetaminophen sulfate concentrations or AUC. Thus, in the dog but not the rat, treatment with acetaminophen and diazepam decreased the glucuronidation of acetaminophen and resulted in increases in acetaminophen available for metabolic activation. This alteration of acetaminophen disposition might be the mechanism for the species specific diazepam-inhibited potentiation of acetaminophen toxicity in the dog (McLarn et al. The Toxicologist 5:78). Since the inhibitory effects of diazepam are observed in the dog at doses many fold higher than human therapeutic doses and since in vitro the IC50's for the acetaminophen GT from the dog were 20 fold less than that of human, the inhibition of GT by O2 should not have clinical significance.


RA potentiates acetaminophen (APAP) hepatotoxicity while CM protects against APAP toxicity in F344 rats. The main route of biotransformation of APAP is conjugation by UDPGT and ST enzymes. We characterized the inhibitory effects of RA and CM on isozymes of UDPGT using APAP, p-nitrophenol (PNP), and p-hydroxybenzylphenyl (PBPB) as substrates and the effects on ST with APAP and PNP as substrates. Rat liver cytosol was incubated with 0.04-5.5 μM [14C]APAP or 0.07-7.5 μM [14C]PNP, 15 μM (APAP) or 10 μM (PNP) PAPS, in 0.1 M Tris-HCl, pH 8.5 (APAP) or 0.1 M sodium phosphate, pH 6.0 (PNP). No inhibitory effects on ST activity were observed with RA or CM at concentrations up to 5 μM. Incubations of rat hepatic microsomes with 0.05-0.5 μM PBPB or 0.25-2 μM PNP, 10 μM UDPGA, 0.5% Brij 58, and 1 mM MgCl2 in 0.1 M Tris-HCl (pH 7.5) revealed no significant inhibition of UDPGT by CM or RA at concentrations up to 0.5 μM. Incubations with 2.5-15 μM [14C]APAP were performed in the presence of 0.1-2 mM RA or CM in Tris-HCl buffer with 0.5% Brij 58, 1 mM MgCl2, and UDPGA (15 μM). The KI's with RA and CM of 18 μM and 140 μM, respectively, for APAP-UDPGT. These results show that UDPGT is inhibited in vitro by RA to a greater extent than by CM which may explain the potentiation of APAP-induced toxicity by RA.
The feasibility of using miniature swine in drug metabolism and toxicological research was examined. The pharmacokinetics of commonly used compounds, APAP (40 mg/kg), VAN (20 mg/kg) and AN (15 mg/kg), were examined to assess rates of conjugation, renal clearance and oxidation, respectively. Drugs were administered i.v. via a vascular access port.

The pharmacokinetic parameters for APAP were:
- Clearance (CL): 9.0±1.3 ml/min/kg, volume of distribution at steady state (Vdss): 0.76±0.53 L/kg, mean residence time (MRT): 864±14 min and half-life (T1/2): 67±4.1 min.
- For VAN:
  - CL: 10.6±2.9 ml/min/kg
  - Vdss: 5.8±1.1 ml/min/kg
  - MRT: 68±7.3 min
  - T1/2: 63±23 min.

The free fraction of VAN in serum was 67±5.0%.

Using the vascular access port to facilitate blood sampling, these studies were easily performed and provided baseline data for comparison to other species. These studies suggest that the miniature swine may be a useful animal for investigative toxicological research.

The rate of UDP-glucoronate (UDPGA) synthesis may be a capacity-limiting factor in hepatic glucuronidation. An HPLC method was developed to quantitate in vivo synthesis rates of UDPGA and UDP-glucose (UDGP), its immediate precursor. Rats were injected with C-14 orotate (50 µCi in 4.2 µmol/kg, ip). At various times, rats were killed by cervical dislocation and liver samples freeze-clamped. A perchloric acid extract was prepared and injected onto either an Adsorbosphere HS C18 5 µm reverse phase column (mobile phase: 100 mM K acetate, 0.01% n-octylamine, pH 5.6, 53°C, 2 ml/min) to quantitate UDPGA and UDPG at 254 nm or a Partisil SAX 10 µm ion-exchange column (mobile phase: 550 mM KCl, 100 mM ammonium phosphate, pH 5.2, 20°C, 2 ml/min) to quantitate UTP, the precursor to UDPG, at 262 nm. Effluent fractions containing these compounds were collected and their radioactivity determined. Synthesis rates were calculated using the rate of change in the compound's and its precursor's specific activities and the concentration of the compound. The rate of synthesis of UDPGA was 99 ± 1 nmol/g/min and that of UDPG was 107 ± 5 nmol/g/min

The compound lindane has been used as a model substrate to study the ontogeny of metabolism in the developing rat. The distribution and metabolic fate of the model substrate at 2, 5, 10, and 20 mg/kg, respectively, were investigated following subcutaneous administration of 20 mg/kg of lindane containing 0.5 µCi[U14C]-lindane in peanut oil. Groups of ten pups (5 male and 5 female) were sacrificed at 4 hr intervals during the 24 hr period following dosing. Adrenals, blood, brain, heart, lung, liver, and kidneys were analyzed for radioactivity. Urine samples were analyzed for radioactivity and metabolic fate of lindane. There was a significant age-dependent increase in the metabolism of lindane in the rat. High levels of radioactivity in the lung and increased reductive dechlorination suggest that the lung may play a greater role in metabolism of lindane by young rats.phase I reactions increased significantly while anaerobic dechlorination of lindane to 4-chloro-3,5-dichloro-trans-stilbene increased significantly with age. Phase II sulfate and glutathione conjugations decreased significantly and glucuronide conjugation increased significantly with age. Metabolism and excretion of the model substrate appears to parallel development of the hepatic enzymes involved in phase I and phase II reactions.
The influences of age and hepatic GGT on biliary excretion of GSH and related thiols were studied in rats. Total thiol excretion in bile of 2-week-old rats was low, with GSH being the major component. Cysteinylglycine (CG) and cysteine (CYS) markedly increased in bile by 4 weeks of age, each exceeding that of GSH. The increase in CG and CYS in bile corresponded to an increase in hepatic GGT activity. GSH in bile of 7-10 week-old rats was 9-times higher than at 4 weeks, while CYS and CG excretion and hepatic GGT remained unchanged. In 4-week-old rats, acivicin inhibited hepatic GGT activity, elevated GSH (up to 10-fold) and decreased CG and CYS in bile (-70%) without altering biliary excretion of total thiols (GSH + CG + CYS). The effect of acivicin on biliary thiol excretion was considerably less in 2-3 and 7-10 week-old rats. In conclusion, (1) biliary excretion of total thiols increases with age, (2) thiol composition in bile depends on hepatic GGT activity and the rate of hepatic thiol transport of GSH, and (3) hepatic GGT does not promote reabsorption of thiols from bile, as its inhibition does not enhance total thiol excretion. (Supported by USPHS grants ES-03192 and ES-07079).

A rapid and sensitive HPLC method for quantitation of picomole levels of GSH, glutathione disulfide, cysteine, cystine, cysteinylglycine, cystinylglycine disulfide and cysteine glutathione mixed disulfide in biological samples is described. The compounds were separated on a reverse-phase column by ion-pair chromatography. Aqueous buffer containing 0.1 M monochloroacetic acid and 3.3 mM 1-heptanesulfonic acid (pH 2.60) was mixed with methanol and N,N-dimethylformamide (96.5:3:0.5 v/v). After separation, the oxidized SHs were reduced by a potential (-1.0 V) from a battery, with subsequent detection of all thiols by electrochemical oxidation (+0.15 V) with a dual gold-mercury electrode. SH concentrations were determined in tissue extracts (liver and kidney) and fluids (bile and plasma) from control rats and rats treated with acivicin, an inhibitor of γ-glutamyltranspeptidase. A marked increase in biliary GSH concentration was observed in treated animals with a corresponding decrease in cysteine and cysteinylglycine concentrations. The results suggest that this method is useful for measuring the degradation of GSH in tissues and fluids. (Supported by USPHS grants ES-03192, ES-01142, and ES-07079 and a Stauffer Fellowship).

The stereoselectivity of purified human glutathione S-transferases (GST), μ, and α-ε varies markedly with arene and alkene oxide substrates. L. A. Dostal, C. Gutenberg, B. Mannervik, and J. R. Bend. NIES, P.O. Box 12233, Research Triangle Park, NC 27709 and University of Stockholm, S-106 91, Stockholm, Sweden.

The stereoselectivities of three biochemically distinct human GST purified from placenta (+), liver (μ, and α-ε) were determined with (+)-β-nortropane 4,5-oxide (BPO), pyrene 4,5-oxide (PO) and (R,S)-styrene 7,8-oxide (SO) as substrates. The μ enzyme was highly selective (95%) for reaction of glutathione (GSH) with S-configured oxirane carbons of BPO and PO, while the α transfersase was highly selective (95%) for R-configured carbon atoms of BPO and PO. The basic liver transfersase (α-ε) showed low stereoselectivity with these substrates; GST reaction at R-configured oxirane atoms was preferred but only by about 2-fold. With BPO as substrate μ and α-ε were strongly enantiosselective for (+)-4R,5S-BPO (about 6-fold), whereas it showed little enantioselectivity. With SO as substrate all three GST were enantiosselective for (S)-SO although the enantioselectivity was weaker (1.3- to 1.8-fold). μ preferentially catalysed the reaction of GSH with the benzylic oxirane carbon of SO, whereas α-ε GST preferentially catalysed reaction with the terminal epoxide carbon. These data demonstrate that stereoselectivity with epoxides serves as one useful parameter for the functional characterization of GST isozymes.
600 INHIBITION OF HUMAN GLUTATHIONE S-TRANSFERASES BY 2,4-DICHLOROPHENOXYACETATE (2,4-D) AND 2,4,5-TRICHLOROPHENOXYACETATE (2,4,5-T). S.V. Singh and Y.C. Awasthi, University of Texas Medical Branch, Galveston, TX 77550. Sponsor J.P. Saunders.

Epidemiological surveys point to an increased risk of soft tissue sarcoma and malignant lymphomas among the workers occupationally exposed to phenoxy acid herbicides such as 2,4-D and 2,4,5-T. Since glutathione S-transferases (GST) are a family of enzymes involved in the detoxication of xenobiotics by catalyzing their conjugation to GSH and also by non-catalytic binding, we have studied the effect of 2,4-D and 2,4,5-T on GST purified from human liver and erythrocytes. The results of present studies indicate that 2,4-D and 2,4,5-T inhibit all known GST isoenzymes of liver and erythrocytes. The maximal inhibition is 100% for both the compounds vary significantly for different GST isoforms. Except for the less anionic isoenzyme, 2,4-D inhibited all the isoenzymes of human liver GST non-competitively with respect to 1-chloro-2,4-dinitrobenzene (CDNB). 2,4,5-T inhibited all the isoenzymes of liver competitively with respect to CDNB. GST peroxidase II activity expressed by the cationic form of liver GST is also inhibited by both these compounds and it may be speculated that substantial exposure of humans to these herbicides may affect the mechanisms for the detoxication of xenobiotics and endogenous lipid hydroperoxides. (USPHS GM 32304).

601 COMPARISON OF ADENOSINE 3'-PHOSPHATE 5'-PHOSPHOSULFATE (PAPS) CONCENTRATIONS IN TISSUES FROM DIFFERENT LABORATORY ANIMALS. E.A. Barzenicka, G.A. Hazleton, and L.D. Kilasen. Dept. of Pharmacol., Toxicol. & Therap., Univ. Kansas Medical Center, Kansas City, KS

PAPS plays a critical role as the co-substrate for the sulfation of xenobiotics. However, the concentration of PAPS in tissues is not known. In the present study, PAPS levels were determined in several tissues of rats, mice, hamsters, rabbits and dogs. PAPS was quantitated with a radiometric method that measures the formation of 14C-1-naphthyl sulfate from 14C-1-naphthol and PAPS (limiting substrate) via a sulfotransferase catalyzed reaction. The concentration of PAPS in rat livers (about 77 nmol/g) was 5 to 10 times higher than in any other extrapleptic tissue examined (about 15 for kidney, 9 for intestine and lung, and 7 nmol/g for brain). PAPS concentration in livers of mice, hamsters, rabbits and dogs was about one-fourth to one-half that in rats (29, 33, 33 and 17 nmol/g, respectively). The concentration of PAPS in extrapleptic tissues of these animals was generally similar to those found in the extrapleptic tissues of rats. In summary, both species and tissue differences in the co-substrate concentration for sulfation (PAPS) was described. The availability of PAPS may be of importance in determining species and organ differences in the sulfation of xenobiotics. (Supported by USPHS grant ES-03192).

602 STUDIES ON THE CONJUGATIVE CAPABILITIES OF α,β-UNSATURATED CHEMICALS. I.S. Silver and W.C. Dauterman. North Carolina State University, Toxicology Program, Box 7631, Raleigh, NC.

Diethylmaleate (DEM) and diethylfumarate (DEF) are members of a group of α,β-unsaturated chemicals used as synthetic intermediates in manufacturing agricultural, industrial, and pharmaceutical chemical products. DEM has been shown to deplete hepatic glutathione levels (GSH) and has been widely used for this purpose in toxicological studies.

The present study investigated the role of DEM, DEF, and α,β-unsaturated chemicals as electrophilic substrates for GSH. In rats treated orally with 800 mg/kg, hepatic GSH was depressed 55.7% and 78.3% of normal levels 2 hrs after treatment with DEM and DEF, respectively; 4 hrs after treatment hepatic GSH was depressed 82.6% and 94.0%, respectively.

Conjugation rates of α,β-unsaturated compounds to GSH were measured in vitro. Non-enzymatic studies revealed that DEF is more reactive than DEM. However, in the presence of rat liver enzyme the opposite situation was observed. The rate of conjugation for DEF was 17.43 µmol/min/mg protein, while for DEF the rate was 3.43 µmol/min/mg protein.

603 NEPHROTOXICITY OF S-(2-CHLOROETHYL)-GSH (CEG), AND S-(2-BROMOHYDROQUINONE)-GSH (BHQG): EVIDENCE FOR MULTIPLE GSH CONJUGATE TRANSPORT SYSTEMS IN THE KIDNEY. R.A. Kramer, K. Greene, National Cancer Institute, Bethesda, MD., and G.L. Fourman and D.J. Reed, Oregon State University, Corvallis, OR.

The renal selectivity of toxic GSH conjugates, is presumably due to initial processing by γ-GT(γ-glutamyltranspeptidase) leading ultimately to mercapturic acid formation. We describe here initial studies, in F344 rats, on the role of renal γ-GT and probenecid-sensitive anion transport systems in mediating the toxicity of two such renal toxic GSH conjugates, CEG and BHQG. Within 24 hr, CEG(100 mg/kg) and BHQG(200 mg/kg) produced necrosis to the pars recta of the proximal tubule and a corresponding increase in the urinary excretion of glucose and LDH activity. Urinary glucose and LDH levels were more markedly elevated in BHQG treated rats, whereas, BUN levels were greater after CEG treatment. Administration of AT-125 (Aclavinin, 2.5-40 mg/kg) produced a dose-dependent decrease in renal γ-GT activity(40-97% inhibition) and corresponding increase in urinary GSH (0.5-37 umol/16 hr). AT-125 pretreatment did not protect against CEG nephrotoxicity but was protective in BHQG nephrotoxicity. In contrast, pretreatment with probenecid(143 mg/kg) protected against CEG nephrotoxicity without affecting BHQG.

These findings indicate that GSH conjugates may enter renal tubular cells by separate transport systems.
Previous studies have shown that o-, m- or p-dinitrobenzene (DNB) is conjugated rapidly with glutathione in isolated Fischer-344 rat hepatocytes. In order to assess the toxicological significance of this reaction, male Fischer-344 rats were given o-, m- or p- DNB (10 mg/kg) by gavage. Blood was withdrawn from the heart via an indwelling jugular vein cannula for determination of the percentage of hemoglobin in the methemoglobin (MB) form. Twenty min after p-DNB administration a peak Mhb value of 72.1 ± 5.58 (mean ± SE, n=5) was recorded. m-DNB administration resulted in a peak Mhb value of 26.5 ± 1.8% at 180 min. In contrast, o-DNB caused only a slight (peak of 2.8 ± 0.2%) Mhb production at 20 min. Even when the o-DNB dose was increased to 100 mg/kg little Mhb resulted (peak of 4.4 ± 1.4% at 10 min). However, when this dose of o-DNB followed 30 min after administration of the glutathione depleting agents phenone (300 mg/kg) and buthionine sulfoximine (8 mmole/kg) a peak Mhb value of 16.8 ± 4.6% was recorded at 75 min. These results suggest that rapid conjugation of o-DNB with glutathione protects Fischer-344 rats from o-DNB-induced Mhb.

The influence of dietary administration of DEHP, a hepatocarcinogen and peroxisome proliferator, on epoxide hydroxylase activities in various mouse strains was evaluated. Epoxide hydroxylase activity in the cytosolic and mitochondrial fraction was monitored following the hydrolysis of trans-stilbene oxide whereas microsomal epoxide hydroxylase activity was determined by following the hydrolysis of trans-stilbene oxide (CSO). Administration of DEHP (2% in diet for 10 consecutive days) induced cytosolic epoxide hydroxylase (CEH) and microsomal epoxide hydroxylase (mEH) activities in livers of all the mice strains examined. The percent induction of CEH and mEH activities following DEHP was independent of the basal level of these enzymes in the various strains. DEHP treatment caused either no change or a decrease in EH activity in the mitochondrial fraction. Dietary administration of DEHP caused no alteration in the rate of CSO conjugation catalyzed by glutathione S-transferase in most of the strains examined except CBA, NZB and SW where an increase in the conjugate formation was noted. The mechanism of induction of CEH in various mouse strains by DEHP is not known.

Conjugation with glutathione (GSH) is a major pathway for the inactivation of AFB, and may be the principal determinant of species differences in sensitivity to the hepatotoxic and carcinogenic effects of AFB. The effects of GSH depletion with BSO (1 g/kg), DEM (0.7 ml/kg) or BSO+DEM on hepatobiliary disposition of 3H- AFB (0.25 mg/kg, 25 μCl/kg, ip) was determined in male rats. Bile was collected for 2 hrs, the liver was removed, homogenized and used for measurement of enzyme activities, GSH concentrations and covalent binding to DNA. A 60% reduction in GSH levels with BSO had no effect on the biliary excretion of AFB-GSH or covalent binding of AFB to DNA. An 80% reduction of GSH levels with DEM reduced biliary excretion of AFB-GSH, but had no significant effect on covalent AFB-DNA binding. Further reduction of GSH levels with BSO+DEM greatly decreased the excretion of AFB-GSH, increased the excretion of AFB-glucuronide, and increased binding to DNA by 1.7-fold. These data suggest that GSH must be depleted below a critical level before it becomes rate-limiting in the inactivation of AFB, thus resulting in increased hepatotoxicity and carcinogenicity. (Supported by NIH Grants ES-03719 and T32 ES-07032).

MM is thought to be excreted into rat bile as a glutathione (GSH) complex. Therefore, inhibition of GGT, which hydrolyzes GSH to cysteinylglycine (CG) and ultimately yields cysteine (CYS), may increase the biliary excretion of GSH and MM. In 4-week-old rats, acivicin (100-1000 umol/kg, iv) inhibited GGT activity in liver and kidney up to 98%. It enhanced biliary excretion of GSH up to 8-fold but decreased that of CYS and CG by 60-70%. Thus, total thiol (GSH + CG + CYS) excretion in bile did not change. After acivicin administration, the urinary excretion of GSH, CYS, CG and total thiols was increased 2700-, 9-, 10- and 130-fold, respectively. Inhibition of GGT did not affect hepatic and renal levels of GSH. Pre-treatment with acivicin (100 umol/kg, iv) failed to influence the biliary excretion of MM. In contrast, excretion of MM into urine increased 13-fold. Find out that inhibition of hepatic GGT failed to enhance biliary excretion of MM because it did not increase hepatobiliary transport of total thiols. The enhanced urinary excretion of MM is due to the vastly increased excretion of thiols after inhibition of renal GGT. (Supported by USPHS grants ES-03192, ES-01142 and ES-07079).
607A FURTHER STUDIES ON PHARMACOKINETIC MODEL FOR THE STEADY-STATE CONCENTRATION OF REACTIVE METABOLITES. R. Chen and J.R. Gillette. NHLBI, Bethesda, MD

The amount of intracellular glutathione is an important factor in determining the concentration of toxic short-lived reactive metabolites formed within the cells. Changes in the amount of glutathione govern the rate of formation of metabolite and the rate of formation of glutathione conjugate. A simple pharmacokinetic model is presented as a first approximation of metabolite formation, glutathione conjugation, and covalent binding. The model assumes: a) first order kinetics govern the formation of reactive metabolite and covalent binding, b) rate of elimination of the reactive metabolite is rapid so that the concentration of reactive metabolite reaches a virtual steady-state almost instantaneously, and c) glutathione conjugate formation is governed by second order kinetics. Simulation of this model is by integration of equations for the steady-state concentration of the metabolite, the concentration of glutathione, the rate of formation of glutathione conjugate, and the rate of covalent binding employing MATLAB (Modeling Laboratory). The metabolism of acetaminophen 4-HAA and 3-hydroxyacetanilide (3-HAA) will be used to test the validity of this model.

609 α-TOCOPHEROL AND GLUTATHIONE-DEPENDENT INHIBITION OF NADPH-INDUCED PEROXIDATION OF ISOLATED RAT LIVER NUCLEI. M.A. Tirmenstein and D.J. Reed. Oregon State University, Corvallis, OR

The present study was conducted to assess the ability of the antioxidants glutathione (GSH) and α-tocopherol (αT) to protect the cell nucleus against oxidative stress. 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 determined by measuring thiobarbituric acid-reactive products (TBA-RP). Nuclear preparations contained 0.07 mol % αT and were oxidized at rates of 0.20 nmoles TBA-RP/min per mg protein. Nuclei preincubated with αT and containing αT levels above 0.04 mol % peroxidized at rates of 0.01 nmoles TBA-RP/min per mg protein. GSH produced a lag period prior to the onset of lipid peroxidation. The duration of this GSH-induced lag period was dependent on the concentration of GSH present. This GSH-induced lag period did not occur if nuclei were immersed in boiling water for 2 min. In boiled nuclei, GSH promoted not prevented lipid peroxidation even though αT levels remained at 0.02 mol %. The data suggest that both GSH and αT can protect nuclei against lipid peroxidation, but that GSH requires the presence of a nuclear protein to be effective. (Supported by USPHS Grant ES01978.)

608 PROXIDANT AND ANTIOXIDANT EFFECTS OF GLUTATHIONE AND ASCORBIC ACID ON Fe³⁺-/H₂O₂-INDUCED PEROXIDATION OF LIPOSOMES CONTAINING α-TOCOPHEROL. D.C. Liebler, D.S. Kling, and D.J. Reed. Oregon State University, Corvallis, OR

The α-tocopherol (αT) status of cell membranes may govern the balance between pro- and antioxidant effects of ascorbic acid (AA) and glutathione (GSH) during Fe-induced lipid peroxidation. Soy lecithin liposomes (SLL) were peroxidized by Fe⁺³-/H₂O₂, in 50 mM Tris HCl/50 mM NaCl at pH 7.0. αT above a threshold level (0.2 mol %) inhibited lipid peroxidation (LORP), measured as thiobarbituric acid-reactive products. Fe⁺³-/H₂O₂ did not deplete αT in dipalmitoyllecithin liposomes, indicating a requirement of lipid hydroperoxides for αT oxidation. AA (0.1-1.0 mM) stimulated Fe⁺³-/H₂O₂-induced LORP of αT-free SLL but 5 mM AA was inhibitory. αT (0.3 mol %) abolished the stimulation of Fe⁺³-/H₂O₂-induced LORP by 0.1 mM AA. AA also prevented depletion of αT and enhanced its antioxidant efficiency. GSH-stimulated LORP in αT-free SLL and did not prevent αT oxidation or LORP in SLL containing 0.3 mol % αT. Moreover, GSH diminished LORP inhibition by the AA/αT combination. The data indicate that AA augments the antioxidant action of αT, presumably by regenerating αT from its oxidation products. Peroxidation products of GSH may obscure any similar interaction of GSH with αT. (Supported by USPHS Grants ES05309 and ES01978.)

610 AGE-DEPENDENT CHANGES IN ANTIOXIDANT ENZYME ACTIVITIES IN FOUR TISSUES OF NORMAL AND MUSCULAR DYSTROPHIC CHICKENS. Michael J. Murphy, and James P. Kohrer. Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX 78712

Inherited muscular dystrophy in chickens has been used as a model of Duchenne muscular dystrophy. The activities of five antioxidant enzymes were measured in normal and muscular dystrophic chickens at 1, 7, 14, and 28 days after hatching. The pectoralis major muscle, which is damaged in chickens with this disease, showed increased levels of catalase (CAT) one day after hatching, compared to controls. Increased levels of Cu²⁺- and Mn²⁺-superoxide dismutase (SOD), gluthione peroxidase (GPX) and glutathione-S-transferase (GST) were evident 7 days after hatching. The soleus muscle, liver and lung from dystrophic chickens were considered to be undamaged by this disease, but increased activities of some antioxidant enzymes were found in each of these tissues. Soleus muscle contained significantly increased levels of Mn-SOD and GPX in 1 and 7 days old birds, and increased GST in 1, 14 and 28 day old birds. Liver from dystrophic chickens showed increased CAT activity at 1 and 7 days after hatching, and increased Cu²⁺-SOD activity over all of the time points examined. GPX was significantly elevated in lung from 1, 7 and 14 day old dystrophic chickens. These results suggest that there is some type of oxidative stress in tissues of chickens with inherited muscular dystrophy which leads to enhanced activity of antioxidant enzymes. This supports the possibility that excess oxygen free radicals or inadequate antioxidant defenses in pectoralis muscle play some role in the pathogenesis of muscular dystrophy. (This work was supported by BRSG #RR05849. JPK is the recipient of RCDA HL01435.)
611 PARAQUAT TOXICITY IN OBLIGATELY THERMOPHILIC BACTERIA. G. S. Allgood and J. J. Perry. Toxicology Program and Department of Microbiology, NC State University, Raleigh, NC.

Examination of oxygen toxicity in obligately thermophilic bacteria revealed a limited response to oxygen stress. Paraquat (PQ$^{++}$) generates toxic oxygen radicals and the effect of PQ$^{++}$ was examined in five strains of obligately thermophilic bacteria. The level of the oxygen defense enzymes superoxide dismutase, catalase, and peroxidase were determined after growth in the presence and absence of paraquat. In general, the superoxide dismutase level in the thermophiles did not change on PQ$^{++}$ addition and peroxidase levels had a varied response. PQ$^{++}$ addition did result in minimally a three-fold increase in catalase levels. PQ$^{++}$ increased cyanide-resistant univalent respiration in these thermophiles. PQ$^{++}$ was reduced by an NADH: or NADPH:paraquat diaphorase, and the reduced PQ$^{++}$ was reoxidized, producing the toxic oxygen byproducts superoxide anion and hydrogen peroxide.

612 RADICAL MEDIATED RELEASE OF IRON FROM FERRITIN. C. E. Thomas and S. D. Aust, Department of Biochemistry and Center for Environmental Toxicology, Michigan State University, East Lansing, MI

The generation of organic and oxygen free radicals in biological systems is thought to result in cellular damage via lipid peroxidation (LP). The initiation and propagation of LP is known to require iron. Accordingly, we have demonstrated LP when ferritin was incubated with liposomas, paraquat, and NADPH-cytochrome P450 reductase. Superoxide (O$_2^-$) generated by redox cycling released a small amount of iron from ferritin which then promoted LP. When incubated aerobically superoxide dismutase was only able to inhibit paraquat-dependent release of iron from ferritin 50-60% while xanthine oxidase-dependent iron release was inhibited nearly 100%. This suggests that both O$_2^-$ and the organic radical can catalyze the release of iron from ferritin and in agreement, when incubated anaerobically, cytochrome P450 reductase and paraquat rapidly released all of the iron from ferritin. Subsequent studies showed that diquat, benzyl viologen, adriamycin and COCl$_2$ all catalyzed radical-dependent release of iron from ferritin. These studies indicate that release of iron from ferritin may be a common feature contributing to free radical mediated toxicities. (NIH GM33443).

613 IN VITRO COVALENT BINDING OF $^{14}$CCl$_4$ TO NEUTRAL LIPIDS. B. S. Kappalai and C. A. S. Ansari, Division of Chemical Pathology, The University of Texas Medical Branch, Galveston, TX 77550. Sponsor: Ahmed E. Ahmed

The hepatotoxic effect of carbon tetrachloride (CCl$_4$) may be attributed to its covalent binding to cell constituents which are important for normal cell function. In the present work we studied the binding of CCl$_4$ with liver microsomal lipids obtained from phenobarbital-treated rats. Following anaerobic incubation of microsomes with $^{14}$CCl$_4$ in the presence of NADPH generating system, lipids were extracted with chloroform:methanol (2:1, v/v). The total recovery of radioactivity in the lipid fraction was 14.4%. The lipids were separated using silica Sep-Pak cartridge into neutral and phospholipids containing 0.6% and 6.6% radioactivity, respectively. Neutral lipids were further separated into two radioactive fractions by thin layer chromatography, followed by high performance liquid chromatography. The observed binding could be either due to the reaction of CCl$_4$ with carbon-carbon double bond or to the allylic carbon of the lipid. The formal characterization of these compounds using mass spectrometry is presently being conducted. It is possible that binding of CCl$_4$ to lipids may alter the membrane composition and function and thus contributing to the observed hepatotoxicity. (Supported by NIH Grant No. AM 27135).

614 IN VITRO COVALENT BINDING OF 1,2-DIBROMOETHANE WITH PLASMA PROTEINS. Jose C. Can and C. A. S. Ansari, Div, of Biochemistry, The University of Texas Medical Branch, Galveston, Texas 77550 (Spon: Mary Treinen Moslen).

1,2-Dibromoethane (DBE) is a known carcinogen and environmental contaminant. In order to study the feasibility of employing plasma proteins as markers of chemical exposure, $^{14}$C-DBE (25 uCi, 2.5 x 10$^6$ dpm/mg) was incubated with human plasma (10 ml) at 37°C from 2 min to 8 h. At various time intervals samples were taken, extensively dialyzed against distilled water, lyophilized and the protein-bound radioactivity determined. The chemical was found to rapidly bind to plasma proteins; maximal binding occurred in 2 h with a specific radioactivity of 250 dpm/mg. When the lyophilized material was subjected to molecular sieve chromatography on a Sephadex G-100 column and monitored at 280 nm, two protein peaks emerged. The first peak contained very little radioactivity while the second had greater than 90% of the total radioactivity applied. The second peak was pooled, dialyzed lyophilized and subjected to HPLC on an anion exchange column. Three major protein peaks were separated when monitored at 230 nm which emerged in the following order: α$_1$-antitrypsin, albumin and α$_2$-acid glycoprotein as identified by radial immunodiffusion. Two major radioactive peaks were observed, one associated with the albumin (65%) and the other with the α$_2$-acid glycoprotein (22%), thus showing preferential binding. (Supported by NIH Grant No. AM 27135).
615 REACTIVE METABOLITES OF O-DIBROMOBENZENE (DBB), IN VITRO TRAPPING WITH N-ACETYLCYSTEINE (NAC). N. Narasimhan, J.A. Buben, and R.P. Hanzlik, Dept. of Medicinal Chemistry, Univ. of Kansas, Lawrence, KS.

DBB, like bromobenzene, undergoes metabolic activation and protein covalent binding in vitro and in vivo, and causes dose-dependent hepatic injury in vivo. The covalent binding has been proposed to involve reactive electrophilic intermediates such as arene oxides, but our work on bromobenzene covalent binding suggested the involvement of more highly oxidized intermediates. In an effort to identify the reactive metabolites of DBB responsible for its covalent binding, we have investigated its metabolism in vitro with rat liver microsomes. Several oxygenated metabolites of (3H)-DBB are formed, including 2,3- and 3,4-dibromohexanal, and a dihydrodial (probably 4,5-dibromo). NAC blocks covalent binding of DBB without inhibiting its metabolism, leading to the formation of a new acidic metabolite which is dual labelled when (3H)-DBB and (14C)-NAC are used. The structure of this adduct is under investigation. Supported by NIH-GM-21784.

617 EFFECT OF L-2-OXOTHIAZOLIDINE-4-CARBOXYLIC ACID (OTCA) ON THE ASSESSMENT OF INTERNAL EXPOSURE TO REACTIVE INTERMEDIATES AFTER REPEATED BROMOBENZENE (BB) TREATMENTS. R. Goyal and J. Brodeur. Dép. méd. trav. hyg. mll., Faculté de médecine, Université de Montréal, Québec, Canada.

This study was conducted to explore the relationship between dose, toxicity and metabolism of BB, and the use of urinary metabolite excretion as an index of internal exposure to reactive BB-3,4-oxide. BB (0.5 or 0.75 mmol/kg twice a day i.p. for 18 days) was given to mice and urine was collected at intervals of 0-6 hr and 6-24 hr, at the end of days 1, 4, 11 and 18. After 18 days, both dose levels produced a marked decrease in all BB metabolites during 0-6 hr: mercapturic acid (MA), o-, m- and p-bromophenol (BP); a decrease in MA and p-BP was also present in 0-24 hr samples. This was accompanied by increases in plasma aminotransferases (ALT, AST). Treatment with OTCA (1 or 2 mmol/kg, i.p., from day 4) known to increase hepatic glutation synthetase, prevented hepatotoxicity and reduction in metabolite excretion due to repeated BB administration. A significant negative correlation between MA and ALT was found. These results suggest the use of OTCA for a more accurate estimation of internal chemical exposure to reactive BB-3,4-oxide; in addition, they provide evidence that the oxide can be better quantitated as the sum of urinary MA and p-BP. (Supported by NSC Canada, and IRST, Québec).

616 MECHANISM OF BROMOBENZENE COVALENT BINDING. J.A. Buben, N. Narasimhan, and R.P. Hanzlik, Dept. of Medicinal Chemistry, Univ. of Kansas, Lawrence, KS.

The identity of the reactive metabolite(s) of bromobenzene which covalently binds to liver protein is uncertain; both epoxide and quinone intermediates have been proposed. Using 3H/14C dual-labelled bromobenzene, the oxidation state of the covalently bound material was determined based on its tritium/carbon-14 (T/C) ratio (Toxicologist 5:109 (1985)). With both in vivo and in vitro exposures, the T/C ratio of bromobenzene which became covalently bound to liver protein was 0.3, versus 1.0 for the starting or reisolated substrate. Metabolites formed from the microsomal incubation of dual-labelled bromobenzene were isolated. The major metabolites, p-bromophenol, o-bromophenol, and bromobenzene dihydrodiol, which arise through an epoxide, were found to have T/C ratios of 20.9. Several other minor metabolites, including bromobenzozquinone, had T/C ratios of <4. N-acetyl cysteine (NAC), added to the incubation mixture to trap the reactive intermediate, led to a new metabolite with a T/C ratio of 0.3 which is being characterized. The results implicate the involvement of an intermediate more highly oxidized than an epoxide in the bromobenzene covalent binding process. Supported by NIH-GM-21784.


It has recently been proposed that muconaldehyde (MUC), a six carbon C=O-unsatuated aldehyde, may be a hepatotoxic metabolite of benzene (BZN). The present studies indicate that incubation of 14C-BENZ in liver microsomes (obtained from female CD-1 mice pretreated with 440 mg/kg of BBZ) in the presence of NADPH results in the formation of a ring-opened product. This product was identified by trapping with 2-chloroboronic acid (TBA), which resulted in the formation of an adduct with a 490 nm absorption maximum and a 510 nm fluorescence emission maximum. These maxima are identical to those observed after reacting authentic trans,trans-MUC with TBA. Separation of MUC, both with and without trapping by TBA, from other BZN metabolites in the incubation mixture was accomplished by high performance liquid chromatography (HPLC). The radioactivity profile of fractions collected during HPLC analysis contained two peaks which coeluted with MUC and the MUC/TBA adduct. Additional peaks were identified as phenol, catechol, hydroquinone and muconic acid, based on known standards. The results support the finding that BZN is metabolized in vivo to a ring-opened product with spectral and chromatographic characteristics consistent with trans, trans-muconaldehyde. Supported by NIH grant ES02558.
THE ROLE OF N-HYDROXYPHENETIDINE IN PHENACETIN-INDUCED HEMOLYTIC ANEMIA. Charles B. Jensen, Steven A. McLees, Veronica F. Price and David J. Jollow, Department of Pharmacology, Medical University of South Carolina, Charleston, SC

A variety of arylamine-derived drugs, such as phenacetin, are known to provoke hemolytic and methemoglobinemic responses in laboratory animals and man via active/reactive metabolite mechanisms. Phenolic and N-hydroxy metabolites of arylamines have been shown to possess methemoglobin-generating capacity, but the identity of the hemolytic metabolite(s) is less well known. Recent studies in this laboratory indicate that the hydroxylamine metabolites of aniline and dopa are directly responsible for the hemolytic activities of these arylamines. We now propose that the hemolytic and methemoglobinemic effects of phenacetin are similarly caused by its metabolite, N-hydroxyphenetidine (PNOH). Single doses of phenacetin, p-phenetidine and PNOH decreased the survival half-time of $^{51}$Cr-labelled erythrocytes (T$_{50}$Cr) in rats. PNOH had greater potency in producing this hemotoxic effect; further, in vitro exposure of erythrocytes to PNOH decreased the T$_{50}$Cr of these treated cells in the rat. Significant methemoglobin production was also observed with in vitro exposure of erythrocytes to PNOH. These results support the proposal that PNOH, a metabolite of phenacetin, acts directly to induce hemolytic anemia with acute doses of phenacetin in the rat. (HL 30038)

THE ROLE OF ALCOHOL-INDUCED CYTOCHROME P-450 IN CARBON DISULFIDE (CS$_2$) METABOLISM TO A REACTIVE METABOLITE. E.C.G. Snyderwine, R. Kroll and R.J. Rubin. Johns Hopkins University, Baltio., Maryland 21205

Ethanol (E) has been previously shown to induce an isozyme of cytochrome P-450 responsible for the enhanced hepatic microsomal metabolism of a select group of substrates such as aniline and nitroanisole. In order to investigate the role of alcohol-induced cytochrome P-450 in CS$_2$ metabolism, we have studied the effects of E, methanol (M), isopropanol (IP) or isobutanol (IB) pre-treatments (dose < 1/5 LD50) on in vitro microsomal aniline hydroxylation (AH), nitroanisole O-demethylation (ND), aminopyrine N-demethylation (AD) and CS$_2$ desulfuration (CD) in rats. The sensitivity of CD to induction by the alcohols paralleled that seen for AH and ND (i.e., IP > M > E > IB) and was distinctly different from that seen for AD which was not significantly induced by any treatment. Furthermore, CS$_2$ given in vivo (1 mg/kg ip - 3 hours) significantly inhibited only the alcohol-induced AH and ND but had no effect on AD. These results suggest that the alcohol-induced isozyme of cytochrome P-450 that metabolizes nitroanisole and aniline also metabolizes CS$_2$. The CS$_2$-induced inhibition of AH and ND also supports this conclusion and further suggests that the metabolism of CS$_2$ leads to a localized destruction of a specific cytochrome P-450.


The increased resistance to acetaminophen (ACET) hepatotoxicity induced by STZ-diabetes is largely due to increased clearance of the drug by glucuronidation (JPET 220,504,1982), which in turn results from enhanced capacity to produce UDPGA during metabolic demand (BP, in press). Mechanism studies revealed that the enhanced rate of UDPGA synthesis was not due to increased basal levels of UDP-glucose dehydrogenase, NAD$^+$ or UDPG. Upon challenge with a hepatotoxic dose of ACET (800 mg/kg,ip), UDPG levels were depleted more rapidly in diabetic rats, suggesting greater flux of glucose through the glucuronosyl-xylulose pathway. ACET induced significant increases in blood glucose levels in both normal and fasted rats, but a marked fall in blood glucose in diabetic rats. Both fasted and diabetic rats lack hepatic glycogen, and glucose metabolism is dependent on enhanced glucogenesis (ONG). Thus, in fasted rats given high doses of ACET, a significant fraction of ONG-derived glucose is lost from the liver. In contrast, ACET-treated diabetic rats appear to retain more hepatic ONG-derived glucose. These data suggest that during ACET metabolism, the diabetic liver has a higher ratio of activities of phosphoglucomutase to glucose-6-phosphatase and hence is more efficient in directing ONG-derived glucose toward UDPGA formation. (GS 30546)
HELENALIN: MECHANISM OF TOXIC ACTION: J. Merrill, H.L. Kim and S.H. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77842.

Toxic plant sesquiterpene lactones are believed to be the agents responsible for the loss of grazing livestock. The objective of this study was to determine the effects of modulating cellular glutathione (GSH) levels on the toxicity of the sesquiterpene lactone, helenalin, in male mice. A toxic dose of helenalin (isolated from Helium microcarpum DC) was administered to male mice and resulted in a 58% reduction in hepatic GSH within 12 hours of exposure. The administration of L-2-oxothiazolidine-4-carboxylate (OTC), an intracellular cysteine delivery system, resulted in increased hepatic GSH values. The coadministration of this compound 6 hr prior to a toxic helenalin challenge (25 mg/kg) resulted in significant protection from the lethality of this toxin. In the group treated with helenalin 5/6 animals died within 6 days. In contrast only 1/6 animals receiving both helenalin and OTC died within 6 days. This in vivo study demonstrates the protective role of GSH against helenalin toxicity and suggests that alkylation of cellular thiol may be an important determinant in helenalin-induced toxicity (Supported by the Texas Agricultural Experiment Station).

ORGAN SENSITIVITY TO NAPHTHALENE-INDUCED PEROXIDATIVE INJURY: ROLE OF GLUTATHIONE PEROXIDASE. M.Germansky and I.S. Jamall
Toxicology Program, St. John's University, NY

Weanling, male Blue-Spruce rats were fed a standard Purina Chow and treated, per os every other day for 10 weeks, with graded doses of naphthalene (NAP) dissolled in corn oil (100 mg NAP/Kg body weight to 750 mg/kg body wt.). Controls received an equivalent volume of corn oil. Half of the treated rats were given 0.5 ppm selenium (Se) as sodium selenite in their drinking water. The activity of the selenoenzyme, glutathione peroxidase (GSH-Px) was significantly reduced in the liver of NAP-treated rats. The decrease in selenoenzyme activity was accompanied by a significant increase in lipid peroxidation in this organ. Rats receiving Se concurrently had GSH-Px activities similar to controls. The Se-independent GSH-Px activity was significantly increased in the liver of all NAP-treated rats. The eye, heart and lung of treated rats showed no significant enzyme reductions and no increase in lipid peroxidation.

IN VIVO INHIBITION OF CYTOCHROME P-450 MECHANISM OF TOXIC ACTION: J. Merrill, H.L. Kim and S.H. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77842.

Suicide inhibitors of cytochromes P-450 have been extensively utilized to investigate the mechanism of heme biosynthesis, degradation and regulation. ABT225 efficiently destroys cytochromes P-450 in studies with highly purified enzymes, rat liver microsomes and whole animals. The effect of ABT225 on the in vivo metabolism of other substrates has not been examined. We have investigated the effect of ABT225 on phenacetin elimination and on metabolism to acetaminophen in male Sprague-Dawley rats. Phenacetin (40 mg/kg in propylene glycol, IV) was rapidly eliminated with a half-life of 36 min (AUC = 2,350 ng/ml/min) and the conversion of phenacetin to acetaminophen (AUC = 760 ng/ml/min) was observed. In contrast, after pretreatment with ABT225 (50 mg/kg in saline, IV, one hour pretreatment), the half-life of phenacetin was 230 min (AUC = 34,600 ng/ml/min) and acetaminophen was not detected in plasma. The elimination of intravenously administered acetaminophen (40 mg/kg in saline) was not altered by ABT225 pretreatment. These results suggest that ABT225 is a selective inhibitor of oxidative metabolism in vivo and does not affect conjugation reactions. ABT225 may be a useful experimental tool when selective inhibition of cytochrome P-450 is desired.
Induction of rat hepatic cytochrome P-450 and monoxygenase activities was studied with a series of 4-alkyl MDPs, 4-t-butyl MDP, and n-butylidoxaloxime. The 4-n-alkyl MDP series caused induction of cytochrome P-450 and aryldiazenine hydroxylase (AhE) but had little or no effect on amineoxidase N-demethylase (AFDM) or aniline hydroxylase; induction of AhE increased with increasing length of the alkyl chain and was optimal with the C-6 derivative. In contrast, 4-t-butyl MDP and n-butylidoxaloxime induced AFDM. Gel electrophoresis of microsomes from induced animals indicated that the pattern of cytochrome P-450 in isozymes induced by 4-n-alkyl MDPs resembled that from animals treated with 8-naphthoflavone. The electrophoretic patterns in hepatic microsomes from rats treated with 4-t-butyl MDP and n-butylidoxaloxime were similar to those from phenobarbital-treated animals. Different isozyme compositions in the variously treated animals were confirmed by HPLC analysis of cytochrome P-450. The results suggest that the induction patterns observed might be related to the planar (4-n-alkyl MDP) and nonplanar (4-t-butyl MDP and n-butylidoxaloxime) structures of the test compounds.

Tris(2,3-dibromopropyl)phosphate (Tris-3BP) a widely used flame retardant in the 1970's is a potent promutagen and nephrotoxin. The nephrotoxicity is believed to be caused by the metabolite Bis(2,3-dibromopropyl)phosphate (Bis-3BP). In an attempt to establish a structure-toxicity relationship, a series of specifically methylated derivatives of Tris-3BP and Bis-3BP were synthesized and tested. The mutagenic potentials of the methylated Tris-3BP analogs were assessed using Salmonella Teller Strain 1A 100, phenobarbital-induced microsomes and substrate amounts ranging from 0.15 microM/plate. The results demonstrated that methylation decreased mutagenicity. In particular, the 3,3-dimethyl and the 2-methyl analogs of Tris-3BP were non-mutagenic. Furthermore, all methylated analogs of Bis-3BP were significantly less nephrotoxic at equimolar doses of Bis-3BP (0.3 mM/KG) that caused extensive damage in rats. Results of these structure-activity relationships are consistent with mechanisms that we have previously proposed for the formation of a reactive mutagenic metabolite for Tris-3BP and should help us further define the pathogenesis of toxicity caused by this halogenated compound.

Sulfur dioxide (SO2) potentiates the carcinogeticity of poly cyclic aromatic hydrocarbons. To investigate the mechanism of SO2 synergism, the effect of sulfite, a hydrated form of SO2, on the covalent binding of benzo(a)pyrene (BP) metabolites to DNA in vitro was measured. 14C-BP (17 μM) was incubated with rat liver or lung postisocytodrial supernatant (5%, 3 μg/ml), an NADPH generating system, calf thymus DNA (1 mg) and sodium sulfite (0-40 mM) for 30 min at 37°C. In the presence of liver S9, the level of covalent reaction increased linearly from 0.12 nmol to 0.27 nmol BP metabolites per mg DNA with increasing sulfite concentration. Similar increases were observed using lung S9 except that the levels of reaction were two orders of magnitude lower than those mediated by the liver S9. Incubation of rat liver S9 with 0.40 mM sulfite resulted in a dose response formation of glutathione S-sulfonate (GSSG), a known inhibitor of glutathione S-transferases mediating the conjugation of glutathione (GSH) and BP epoxides. These results suggest that sulfite increases the covalent binding of reactive BP metabolite to DNA by inhibiting, via formation of GSSG, the conjugation of GSH and BP epoxides. (Supported by NIH Grants ES02918, RR01093 and CA14236.)
FORMATION AND FATE OF S-[2-(-N\textsuperscript{7}-GUANYL)ETHYL]-GLUTATHIONE IN DNA FROM ETHYLENE DIHALIDES. N. Koga, P. Inskeep, W.J. Meredith, T.M. Harris, and F.P. Guengerich. Departments of Biochemistry and Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37232.

Genetic damage by 1,2-dihaloethanes requires bio-transformation by glutathione (GSH) conjugation. Reaction of 1,2-dibromoethane (DBE) in vitro with rat liver cytosol, GSH, and DNA, isolation of the DNA, neutral thermal hydrolysis, and chromatography gave S-[2-(-N\textsuperscript{7}-guanyl)ethyl]GSH as the only major DNA adduct. The structure was determined using two-dimensional correlated (COSY) NMR and fast-atom bombardment (FAB) mass spectrometry. The same adduct was the only one detected in rat liver or kidney after EDB administration (37 mg/kg ip). The level of liver and kidney DNA adduct formation was not altered by the y-glutamyl transpeptidase inhibitor AT-125 in in vitro or in vivo experiments. Administration of 1,2-dichloroethane to rats (150 mg/kg ip) resulted in considerably less DNA adduct formation than observed with DBE. The major hepatic DNA adduct was S-[2-(-N\textsuperscript{7}-guanylethyl)GSH, in the kidney, this was only one of several adducts formed. Freshly isolated human hepatocytes are capable of forming DNA adducts from DBE. Monolayer cultures of human hepatocytes showed DBE-dependent unscheduled DNA synthesis that could be blocked by depletion of GSH with diethylmaleate. The half-life of the DNA adduct ranged from 70-100 hours in rat liver, kidney, lung, and stomach.


Neutrophil-derived oxidants have been implicated in both damage to biomolecules and the metabolic activation of xenobiotics. Since the bone marrow is a relatively neutrophil-rich tissue which is subject to xenobiotic toxicity, we have characterized the oxidant generating capability of neutrophils isolated from femurs of male C57BL/6J mice. Addition of the phorbol ester TPA to neutrophil preparations (70-15% ring neutrophils and metamyelocytes) elicited superoxide anion generation, as indicated by SOD-inhibitable acetylated cytochrome c reduction, and oxidant-dependent chemiluminescence (CL) from luminol or lucigenin. The interaction of benz[a]pyrene-7,8-dihydradiol (BP-diol), a proximate carcinogenic metabolite of BP, with TPA-stimulated bone marrow neutrophils resulted in azide-inhibitable CL (75%) indicative of its myeloperoxidase-dependent oxidation to a 9,10-dioxetane intermediate. Recently, our laboratory has shown that in addition to CL, TPA-stimulated human polymorphonuclear leucocytes can activate BP-diol to an intermediate which covalently binds to DNA and elicits mutagenicity in Salmonella typhimurium TA100 (PNAS 82:5194, 1985). These observations combined with our current results suggest a possible role for neutrophil-derived oxidants in the mechanisms of chemically-induced bone marrow toxicity, such as that which occurs with oral BP administration. Supported by NIH ES07141; ES03760, and the American Cancer Society #IN-11X.


We have recently demonstrated that BHA and other phenolic compounds can enhance both the in vitro peroxidative biotransformation of BHT to BHT-quinone methide and the covalent binding of BHT to microsomal protein (Pharmacologist 27:263; 1985). BHT-quinone methide has been proposed to be the BHT metabolite responsible for causing lung damage in mice. In order to assess whether the peroxidative interaction of phenolic compounds with BHT might have any in vivo significance, we measured the effect of various doses of BHA on BHT-induced lung toxicity (increased lung/body wt. ratio) in male CD-1 mice (4-5 weeks old). BHA alone had no effect on the lung/body wt. ratio up to a dose of 500 mg/kg (s.c.). When injected 30 minutes prior to subthreshold doses of BHT (0-250 mg/kg, i.p.), BHA (50 or 250 mg/kg) was observed to significantly enhance (p<0.05) the lung/body wt. ratio in a dose-dependent manner. This enhancing effect was also observed when BHA was given i.p. or up to hours (s.c.) prior to BHT. Using mouse and human lung microsomes, we observed that BHA could enhance the H2O2-dependent covalent binding of BHT to protein. These results suggest that peroxidase enzymes may play a role in BHT-induced mouse lung toxicity and that concomitant exposure to BHT and BHA may lower the margin of safety for BHT toxicity. Supported by NIH OH01833-02 and NIH ES07141.
Scallops (*Placopecten magellanicus*) were exposed in seawater to 10 µg Cu/L (Low Cu), 20 µg Cu/L (High Cu) or equimolar 10 µg Cu/L plus 17 µg Cd/L (Low Cu + Cd). The animals were sacrificed at 2, 4, 6 and 8 weeks. Initial Cd concentrations increased markedly over the 8-week exposure period for the Low Cu + Cd group becoming statistically significant by 8 weeks. Ultrastructure of kidney epithelial cells from scallops in the Low Cu + Cd group was indistinguishable from controls except for loss of concretions while kidney cells from the High Cu group showed both marked loss of membrane integrity and concretions. Sphadex chromatography of the cytosol fractions from the control and Low Cu + Cd groups showed extensive binding of Cd, Zn, and Cu to the 45,000 dalton protein and large Zn binding peak in the low molecular weight fractions. Cu-only treatment groups showed increased Cu and decreased Zn in these components. These data suggest that Cu exposure alters normal molecular mechanisms for renal intracellular Zn homeostasis and that increased Cu binding to the 45,000 dalton peak in Cd-treated scallops appears to provide sufficient metal-binding sites to reduce Cu cellular toxicity.

Recently, the induction of tolerance to cadmium was demonstrated in adult fathead minnows (*Pimephales promelas*). Based on acute toxicity tests, those animals which had received 35-day prior exposure to 30 and 50 µg Cd/L were 63 to 68% more tolerant of cadmium than were previously unexposed animals. The purpose of the present investigation was to determine if tolerance could be induced in early life-cycle stages of fish. Freshly fertilized eggs of the fathead minnow and rainbow trout (*Oncorhynchus mykiss*) were exposed to 0, 5 and 50 µg Cd/L for 4 and 24 days, respectively. These periods represent the embryonic development time for each species. Survival frequencies during acclimation ranged from 57 to 90%, except at the 50 µg Cd/L concentration at which no fathead minnows survived. Based on acute toxicity tests, minnows which had been exposed to 5 µg Cd/L and trout which had been exposed to 50 µg Cd/L showed significantly increased cadmium tolerance over controls. Although tolerance was induced in these newly hatched larvae, the embryo-larval stages of fish were more sensitive to the effects of cadmium acclimation than were the adults.

Information on the accumulation of cadmium in cytosolic proteins of Great Salt Lake brine shrimp (*Artemia salina*) was obtained from animals collected directly from the lake and also from animals hatched and maintained in three sublethal concentrations of cadmium (0.5, 2.0, 5.0 ppm) in salt water aquaria. Brine shrimp growth under these conditions was monitored by measuring body lengths during a 7-day exposure period. Heat stable, cadmium-binding ligands were isolated and identified by Sephadex G-75 chromatography and atomic absorption spectrophotometry. Cadmium was found to be equally distributed between high and low molecular weight proteins in animals collected from the lake and the 0.5 ppm cadmium group. There was also a slight growth stimulation noted in the 0.5 ppm group. Higher cadmium incorporation was noted in low molecular weight fractions with increasing cadmium concentration in the exposure media. Low molecular weight fractions were also found to have high U.V. absorption characteristics at 250 nm and low absorption at 280 nm. Molecular weight of the cadmium-binding ligands was found to be 11,000 as estimated by the gel filtration method. Denovo synthesis of this protein was increased as a function of cadmium concentration in the media.

Freshwater sunfish (*Lepomis*) were collected from two reservoirs containing excess levels of selenium in the water, sediments, flora, and fauna—Martin Lake in east Texas and Belews Lake in North Carolina. Of a number of other toxicants and physico-chemical parameters which were measured, only selenium was accumulated to a degree sufficient to warrant concern. Organs from green sunfish (*L. cyanellus*) and redear sunfish (*L. microlophus*) were preserved and examined for histopathological changes at the optical level. The liver (i.e. hepatopancreas), kidney (i.e. mesonephros), and several other organs were examined histopathologically. Individuals which had accumulated about 20 ppm (wet weight) in the liver exhibited central necrosis. Whereas, sunfish accumulating about 11 ppm in liver showed focal necrosis, fatty infiltration, proliferation of Kupfer cells, focal intercapillary proliferative glomerulonephritis, periglomerular fibrosis, tubular casts, and desquamation of tubular epithelium were found. Secondary lamellae of the gill filaments were swollen and vacuolated. Numerous swollen, necrotic, and ruptured gill follicles were observed in female green sunfish. Pericarditis and myocarditis, possibly secondary to glomerulonephritis-induced uremia, were also observed. These changes were not observed in reference sunfish. The occurrence of extensive pathological lesions in fish with elevated tissue selenium levels confirms earlier conclusions regarding the adverse effects of selenium on fish in Martin Lake and Belews Lake.
SLOW BINARY ELIMINATION OF METHYLMERCURY IN THE MARINE ELASMOBRANCHS, RAJA ERINACEA AND SQUALUS ACANTHIAS. N. Ballatori and J.L. Royer. Liver Center, Yale Univ. School of Medicine, New Haven, CT, and Mount Desert Island Biological Laboratory, Salsbury Cove, ME. Sponsor: T.W. Clarkassen.

Methylmercury (MM), a major toxic contaminant of the marine biosphere, exhibits a long biological half-time in fish. The reasons for the avid accumulation of MM by marine species are not known. We studied the ability of two marine elasmobranchs (R. Erinacea, little skate, and S. Acanthias, spiny dogfish shark) to excrete MM into bile, a major excretory route in mammals. 203Hg-Labeled CHTAgCl was administered via the caudal vein, and bile collected through exteriorized cæcale in the free swimming fish. Skates and sharks excreted a small fraction of the 203Hg into bile over a 3-day period: in the skate, the 3-day cumulative excretion (as a % of dose) was 0.44±0.10 (n=4, ±SD), 0.71±0.23 (n=6), and 1.00±0.34 (n=4) for doses of 1, 5, and 20 μmol/kg, respectively, while the shark excreted only 0.15±0.15 % (n=6) at a dose of 5 μmol/kg. As in mammals, the availability of MM was a determinant of the biliary excretion of MM in these species. The slow hepatic excretory process for MM in the skate and shark was attributed to an inordinately slow rate of bile formation: from 1-4 ml/kg/day. An inefficient hepatic excretory process in fish may account for the long biological half-times for MM in marine species.

CYTOCHROME P-450 ISOZYMES IN THE ENGLISH SOLE (Parophrys vetulus). U. Varanasi, T.K. Collier, D.E. Williams, and D.R. Buhrer. NOAA, NMFS, TWAFC, Seattle, WA 98117; Medical College of Wisconsin, Milwaukee, WI 53226; Oregon State University, Corvallis, OR 97331.

The benthic fish, English sole, is shown to develop hepatic neoplasia in chemically contaminated areas. To investigate the ability of these fish to metabolize chemical carcinogens, we have initiated studies to characterize their hepatic cytochrome P-450 system. Using immunological techniques, we have shown, in liver microsomes of sole from two areas of Puget Sound, the presence of two cytochromes P-450 which crossreact with rabbit antibodies to the purified rainbow trout liver cytochromes P-450, LM4a and LM4b. The level of isozyme that crossreacted with the antibody to trout LM4a, a P-450 type isozyme, was strongly correlated with hepatic AH activity. The isozyme that crossreacted with the antibody to trout LM4a, the constitutive form in trout, did not correlate as well with hepatic AH. Moreover, the LM4a-LG strongly inhibited AH activity in sole liver microsomes, whereas LM4a-LG did not have any effect on AH activity. These results are consonant with earlier findings showing the tendency of English sole to metabolize carcinogenic aromatic hydrocarbons into their bay-region dioxin epoxides. Supported by NIH Grants ES-00040, ES-00210 (JRA); NICHS Aquatic Biomedical Ctr Grant ES-01965 (DWE).


Rainbow trout, yellow perch, carp, bluegill, large mouth bass, and bullhead were treated with graded doses of TCDD (1, 5, 25, 125, 100, and 25 μg/kg, ip) or vehicle. The lethal potency of TCDD was greater in yellow perch, carp, and bullhead (LD50 at 80 days, 1.5 μg/kg) than in the other 3 species (5-25 kg/kg). All species exhibited a dose dependent decline in body weight gain and perch, carp, and bullhead were more sensitive to this effect than the other 3 species. Cutaneous lesions were observed in all species by Day 50 posttreatment. Metabolism of TCDD was also examined 7 days following exposure to purified [14C]-TCDD (60 μg/kg, ip.). Gallbladder bile was collected, lyophilized, extracted with methanol, and analyzed by reverse phase HPLC. Bile from all species was found to contain at least 3 metabolites of TCDD, with 5-25% of the [14C] eluting as parent compound. Biliary metabolite profiles were generally similar for most species, with the exception of yellow perch where the major TCDD metabolite eluted at 10 min compared to 26-28 min in the other 5 species. These results demonstrate that the lethal potency of TCDD in freshwater fish species is generally similar to the most sensitive rodent species, and like mammals, fish species have the capacity to biotransform TCDD. (Supported by UW Sea Grant and NIH grants ES01332 & ES02693).

COMPARATIVE INDUCTION OF CYTOCHROME P-450 ISOZYMES BY 2,4,5,1'-TETRACLOROBIPHENYL (PCB) 3,4,5,1'-PCB, PHENOBARBITAL (PB) AND BENZAPRIDE (BAP). IN RABBIT AND RAINBOW TROUT. K.M. Kleinow, M.L. Haasch, D.E. Williams and J.J. Lech. Medical College of Wisconsin, Milwaukee, WI.

Previous studies have indicated that rainbow trout (RBT) are refractory to induction of hepatic monooxygenase activity (MO) by the PB class inducing agents when administered ip. The effects of the MO inducing agents 2,4,5-PCB; 3,4,5-PCB; BNF and PB upon translational incorporation of 35S methionine into hepatic microsomal protein were comparatively investigated for RBT and the rat. The correlation of exposure route (ip) and bioavailability to hepatic tissues (2,4,5-PCB) as well as P-450 isozyme specificity to hepatic MO induction rates were also investigated for RBT. The induction of MO activities and the de novo synthesis of hepatic protein was evident in both species for 3,4,5-PCB and BNF while the response for 2,4,5-PCB and PB, immunological quantification of trout P-450 isoforms indicate significant increases in the LM4b form with 3,4,5-PCB and BNF administration, whereas the only significant difference demonstrated for the LM3 form was a decline following phenobarbital administration. The results from these experiments suggest that the refractoriness of RBT to PB class agents is neither route nor distribution related and can be demonstrated at the translational level. (Supported by ES 01680 and ES 01985).

Sexually-immature yearling rainbow trout were fed diets containing BNF (500 mg/kg of diet) for 2 weeks and then starved for 4 days prior to sacrifice. BP hydroxylase (BPH) activity in both males and females was significantly increased whereas EH activity was significantly reduced by BNF pretreatment at incubation temperatures of 0°C and 25°C. While the temperature optimum for BPH activity was 25°C, EH activity of control and treated fish was found to be 1.8 to 2-fold higher at 37°C than at 25°C. When the incubation temperature was lowered from 25°C to 0°C, a much greater reduction occurred in EH activity than in BPH activity of BNF-treated fish microsomes. Covalent binding of BPH metabolites to microsomal protein from BNF-induced trout at 0°C was as high as that observed at 25°C. Greater proportions of phenols and lower proportions of BP-7,8-diol and BP-9,10-diol were formed at 0°C than at 25°C. Presumably, at 0°C, less reactive intermediates were deactivated by EH. The unique response of trout EH to BNF pretreatment and to changes in incubation temperature may have important implications in the characterization of in vitro and in vivo metabolism of BP in fish. (Supported by NIH grants ES00040 and ES00240)

644 THE EFFECT OF 2-NAPHTHOFLAVONE AND PIPERONYL BUTOXIDE ON HEPATIC MONOOXYGENASE ACTIVITY AND THE TOXICITY OF ROTENONE TO RAINBOW TROUT, SALMO GAIRDNERI. D.A. Erickson, M.S. Goodrich, and J.J. Lech. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI

The difference between species in the rate of biotransformation of rotenone to less toxic compounds has been suggested to be responsible for its selective toxicity. Therefore, it was of interest to determine if altering hepatic monooxygenase (MO) activity with 2-naphthoflavone (BNF) or piperonyl butoxide (PBO) would affect the toxicity of rotenone to rainbow trout. Treatment with PBO produced an initial inhibition of MO activity followed by an induction of MO activity during a 7-day constant-renewal exposure. The induction of MO activity by PBO was dose-dependent and could be delayed by using low concentrations of PBO, e.g., 10 ppb of PBO by bath produced an inhibition of MO activity for about 4 days. Rotenone toxicity to rainbow trout during a 7-day constant-renewal exposure was greatest in the PBO-treated group, intermediate in the control group, and least in the BNF-treated group. The level of MO activity was correlated with the degree of toxicity of rotenone to rainbow trout. The data suggest that the rate of biotransformation of rotenone by MO activity may be an important determinant of the degree of toxicity of rotenone.

645 THE EFFECT OF TRICAINNE ANESTHESIA ON SELECT P-450 DEPENDENT MONOOXYGENASE ACTIVITIES IN RAINBOW TROUT (Salmo gairdneri). K.M. Kleinow, M.L. Haasch and J.J. Lech. Medical College of Wisconsin, Milwaukee, WI

The effect of tricaine anesthesia (neutralized) upon select hepatic monooxygenase activities and cytochrome(s) P-450 of rainbow trout was investigated. In contrast to findings in studies with other fish species, tricaine produced no observable effect upon cytochrome(s) P-450 levels. Similarly, ethoxycoumarin-O-deethylase (ECOD), benzphetamine-N-demethylation (BEND) and antipyrine-N-demethylation (APD) activities in rainbow trout liver were not affected. Ethoxyresoru- ffin-O-deethylase (EROD) activities appeared to be marginally decreased suggesting perhaps a limited inhibitory effect upon P-450 like cyto- chromes. The induction of the de novo synthesis of beta naphthoflavone (BNF)-inducible P-450 form(s) or associated monooxygenase activities were unaffected by a previous tricaine exposure in regards to the magnitude or time course of the induction. In vivo spectral studies indicate that neutralized and unneutralized tricaine at physiological levels produce a low amplitude P-450 type II inhibitory spectrum. The amplitude of the inhibitory spectrum increases with tricaine concentration.

(Supported by ES 01080 and ES 01985)

646 CONTROL OF TOXIC CHEMICALS IN NUCLEAR SUBMARINE ATMOSPHERES, A HISTORY. R.C. Wands and B.W. Darhart. Ralph C. Wands & Assoc., Inc., Burke, VA and U.S. Naval Research Laboratory, Washington, DC

The introduction of nuclear power into submarines eliminated the need for daily surfacing to replenish the oxygen supply. However, prolonged submergence with oxygen from electrolysis of sea water was accompanied by high levels of airborne toxic materials generated by the equipment and the crew. This paper will present the work of the U.S. Naval Research Laboratory, with the assistance of the National Academy of Sciences, to provide clean air during prolonged submergence cruises. Today's nuclear-powered submarines can operate for 90 days of continuous submergence with no significant effects on the crew.
647 A NOVEL BIOLOGICAL WATER PURIFICATION RECYCLING SYSTEM FOR USE IN FISH TOXICOLOGY STUDIES. Anthony J. Verlanger, Ron M. Lewis and Marvin C. Wilson, Department of Pharmacology, School of Pharmacy, Univ. of Mississippi, University, MS 38677. Sponsor: W.M. Davis.

The toxic build-up of ammonia and nitrite in laboratory aquaculture is a common cause of mortality in acclimation/holding tanks. The following system utilizes nitrification bacteria to convert ammonia and nitrite to innocuous nitrate without the use of any conventional type filter. This system is used to maintain and acclimate blue channel catfish to the laboratory environment prior to pesticide LC₅₀ studies. This system could be used for other types of fish as well. The system is composed of a 500 liter glass holding tank (HT) and a filter tank (FT, 28 x 29 x 14 in.). The FT contains 3 ft³ of plastic matrix (Munters Corp., Fort Myers, FL) which provides the surface area for bacteria growth. A suspension of nitrification bacteria (Aqua-Bacter-Ald, Water Quality Control, Inc., Northfield, Ill.) was added at 10 ppm, initially, and thereafter daily at 3 ppm. The flow thru rate of the system is 5 gpm and the fish load 6 gm/ml. Initial values for ammonia (0.65 ppm) and nitrite (1.65 mg/l) dropped to a 25 day mean of 0.19 ppm and 0.32 mg/l, respectively. The pH remained constant at 7.4. The mortality rate (26 days) was 0% with a load of 360 fish. The system provides a method for preventing the toxic build-up of ammonia, H⁺ and nitrite in a closed laboratory aquatic environment. (Supported by the Department of Pharmacology).


A toxicological investigation was conducted to examine the possible human health effects of the elevated levels of selenium (Se) found in parts of the San Joaquin Valley. Selenium occurs naturally in the soil on the west side of the valley. Elevated levels found in the Kesterson National Wildlife Refuge, which received agricultural drainage water, have been linked to low hatchability of fish and aquatic birds in the area. Findings in fish, waterfowl, and aquatic birds suggested unsafe levels of Se. No health hazards were indicated by the undetectable or low levels of selenium in air and in drinking water. Limited data on animals and animal products did not suggest a source of high exposure by ingestion. Data on crops were inadequate for health assessment. Biological monitoring of workers did not show abnormal levels of Se in blood or urine. A health survey was conducted among local residents matched by age and sex with a control population. It included a community health survey consisting of a questionnaire, hematology, blood chemistry, and analysis of Se in blood, urine, and hair. Organochlorines and bromide were also analyzed. Results did not show any trend of adverse health effects in the study population. Further studies are planned.

650 COMPARISON OF SUBACUTE TOXICITIES OF DICHLORO-ACETATE AND TRICHLOROACETATE IN FEMALE RATS. M.E. Davis, Dept. of Pharmacology and Toxicology, VA Univ. Med. Ctr., Morgantown, WV 26506

Dichloroacetate (DCA) and trichloroacetate (TCA) are chlorination by-products found in drinking water at concentrations comparable to trihalomethanes. Previous studies on DCA have shown that DCA activates pyruvate dehydrogenase and its metabolite oxalate inhibits pyruvate carboxylase and kinase. The studies reported here were designed to determine if these effects occur following subacute exposure to DCA or TCA. Female Sprague-Dawley rats were maintained on reagent grade water containing either DCA (30, 125, 500 or 1875 mg/l) or TCA (38, 158, 652, or 2577 mg/l) for 14 days. The highest dose of either caused reductions of food and water consumption and body weight. DCA treatment caused a biphasic response, with the highest dose showing the expected reductions of lactate and pyruvate concentrations in tissue, and elevated renal phosphate-dependent glutaminase activity. Excretion of ammonia was slightly, not significantly increased, in the high dose group. These rats compensated for the acid load by increased formation and excretion of ammonia. TCA did not have similar effects on lactate metabolism or ammonia synthesis. These results suggest that TCA does not have effects on intermediary metabolism similar to those of DCA; differences in metabolism likely contribute to this. (This research was supported by the US EPA.)
LEAD POISONING IN SWANS IN THE LOWER COEUR

Tundra swans Cygnus columbianus migrating through the Coeur d'Alene River Valley are exposed to soils and sediments containing extremely high amounts of lead (1-8,000 ppm). In some years several hundred swans have died. Since 1982, when approximately 200 swans (of an estimated 1200 birds) died, intensive efforts have been made to establish cause of death Clinical signs of affected birds included depression and weakness, some being unable to fly. Necropsy revealed emaciation, dark liver, congested kidneys and small spleens. In some cases the proventriculus was packed with horsetail fern Equisetum sp. No lead shot were found. High amounts (6-170 ppm) of lead were present in livers and kidneys, and proventricular contents contained up to 705 ppm Pb. Lead is bioavailable to animals in the valley. Sediments and perhaps horsetail fern may be toxicologically significant sources of lead to swans and other waterfowl.

COMPARISON OF BLOOD TOULUENE LEVELS AFTER ORAL AND INHALATION ROUTES OF EXPOSURE. M.J. Sullivan and R.B. Conolly, Kresge Hearing Research Institute, 2Toxicology Program, School of Public Health, University of Michigan, Ann Arbor, MI 48109.

Groups of 30 rats were gavaged with 0.10, 0.25, 0.50 or 1.00 ml toluene/kg or exposed for up to 6 hr to 200 or 1000 ppm toluene. Blood was sampled by cardiac puncture from rats in each dose group 0.5, 1.0, 2.0, 4.0, 6.0 and 24.0 hr after gavage or the start of inhalation exposure and blood toluene levels quantitated with a gas-chromatographic method. A 4 parameter model (Benignus et al., Environ. Res. 33:441) was fit to the orally dosed blood toluene data. The area under the curve generated by this model for 6 hr after dosing was compared to the area under blood toluene curve for 6 hr inhalation exposure. The function:

\[ \ln(\text{oral dose}) = 1.1108 + \ln(\text{inhalation concentration}) - 8.0116 \]

was found to describe the relationship between 6 hr inhalation and oral exposures. These data show that gavage dosing can be used to achieve blood levels and peak concentrations of toluene over 6 hr similar to those generated by 6 hr inhalation exposure. (Supported by the American Petroleum Institute)

ABSORPTION OF STYRENE VAPOURS ADMINISTERED BY INHALATION IN HUMANS UNDER EXPERIMENTAL CONDITIONS. H. Wieczorek, J.K. Pietkowski, Radiation Biology and Biophysics, University of Rochester NY Sponsor: G. Oderbroter.

Because of the suspected genotoxic properties of styrene there is a tendency nowadays towards lowering its hygienic standard. In spite of the biological monitoring, there have been no satisfactory methods for evaluating exposure to styrene at low concentrations in the air (<100 mg/m³).

Volunteers were exposed by inhalation to styrene within a concentration range of 20 to 200 mg/m³. The average retention of styrene vapours in the respiratory tract was 71%. Within 24 hrs, 30% of the absorbed styrene was metabolized to mandelic acid and 17% to phenylglyoxylic acid, respectively. The determination of mandelic acid in urine collected immediately after the exposure was used as a measure of exposure. The excretion rate of this metabolite correlated best with the absorbed dose. The relative standard deviations of the concentrations in urine related to actual dose level varied, depending on the analysed concentration range, from 0.21 to 0.33.

Quantitative interpretation of the test is possible for styrene concentrations in the air exceeding 20 mg/m³. An inhaled concentration of 100 mg/m³ (TLV in Poland) corresponded to a mandelic acid excretion rate of 15 mg per hour.

NONLINEAR PHARMACOKINETICS OF INHALED PROPYLENE GLYCOL MONOMETHYL ETHER (PGME) IN FISCHER 344 RATS. D.A. Murgott and R.J. Nolan. HAES, Dow Chemical USA, Midland, MI. Sponsor: A.M. Schumann.

The kinetics of PGME and its demethylated metabolite, propylene glycol (PG), were studied in rats receiving single doses (300, 750, 1500, & 3000 ppm) or ten daily (300 & 3000 ppm) 6-hr inhalation exposures. Blood PGME increased throughout the exposure period suggesting absorption was limited by respiration. PGME kinetics were nonlinear following all exposures; end exposure blood PGME levels (108, 344, 618, & 2113 µg/ml) were not proportional to the single exposure concentrations, and the clearance determined from end-exposure blood levels decreased from 3.1 ml/min-kg at 300 ppm to 0.5 ml/min-kg at 3000 ppm. Blood levels of PG were similar after the 1500 & 3000 ppm exposures, indicating saturation of PGME demethylation. PGME was excreted faster by Fischer 344 rats after a 3000 ppm exposure, despite lower PG blood levels. PGME did not accumulate upon daily exposure and blood PGME levels were actually 50% lower after the 10th versus the 1st 3000 ppm exposure. Repeated exposures to 3000, but not 300 ppm PGME, also increased liver weights by 24% and hepatic P450 by 27%. These data suggest that the liver weight increases observed in rats exposed repeatedly to 3000 ppm PGME were due to the induction of microsomal enzymes. (Co-sponsored by ARCO Chemical Co.)
The effect of dose on absorption and excretion of $^{14}C$ benzene administered by gavage or by inhalation was studied in 13-week-old male F344 rats and B6C3F1 mice. Gastrointestinal absorption of benzene was >97% at doses of 0.5 and 150 mg/kg for F344 and Sprague-Dawley rats and B6C3F1 mice. At oral doses below 15 mg $^{14}C$-benzene/kg body weight, >90% of $^{14}C$ excreted was in urine as polar metabolites. Above 15 mg/kg in both rats and mice, an increasing amount of administered benzene was exhaled unmetabolized, suggesting saturation of benzene metabolism. Total metabolites (as determined by $^{14}C$ in urine, feces and carcass) formed by B6C3F1 mice and F344 rats was not linearly related to dose above 15 mg/kg. In inhalation exposures, the percent of benzene retained after 6-hour exposure decreased from 33 to 6% to 159% in rats, and from 50 to 15 to 102% in mice as the exposure concentration was increased from 10 to 900 ppm. Total metabolite formation was exponentially related to exposure concentration with one-half the maximal metabolite formation occurring at 100 ppm for mice and 280 ppm for rats. (Research supported by NIEHS through Interagency Agreement under U.S. DOE Contract No. DE-AC04-76ER03013.)


2,5HD has been proposed as a possible index of exposure conditions leading to the potentiation of halokane-induced hepatotoxicity by MnBK (2-hexanone). Since MEK (2-butanone) can modify the kinetic profile of MnBK metabolism after repetitive exposures, the effect of MEK on the urinary excretion of 2,5HD was investigated in rats after acute exposure to MnBK. Unconjugated 2,5HD was measured by GC 6, 12, 18 and 24 h after a single oral dose of a MEK-MnBK mixture (15, 10, 5 mmol MEK/kg: 5.7, 1.9 mmol MnBK/kg). The same mixtures were also orally administered 18 h prior to a challenge of COCl$_2$ (0.1 ml/kg, ip) or corn oil (4 ml/kg, ip). Plasma ALT activities and bilirubin levels were assessed 48 h after the challenge as indices of hepatic dysfunction. The pattern of free 2,5HD excretion was altered when the MEK dosage was increased, leading to a 3- to 5-fold rise (at 24 h) compared to controls, regardless of the MnBK dosage administered. However, the potentiation of the COCl$_2$-induced liver injury resulting from the mixtures was additive (sum of the potentiations induced by the two ketones separately). We therefore conclude that 2,5HD as a predictive index of MnBK exposure should be used with caution when MnBK is present as a mixture. (Supported by IRSST Québec and NRCC).


DGBA is widely used as a coalescing aid in latex paints. The in vitro hydrolysis in rat blood and the in vivo metabolism and disposition of DGBA was studied in male Sprague-Dawley rats. For the in vitro study, 3 ml DGBA was incubated with heparinized rat blood obtained by cardiac puncture. Analysis by gc showed that the DGBA was hydrolyzed to diethylene glycol monobutyl ether (DGBE) with a half-life of <3 min. For the metabolism studies, rats were dosed orally with 20 or 2000 mg/kg [U-14C-diethylene DGBA. 14C was rapidly excreted in urine (ca. 80% of the dose in 24 hrs for both doses). A mean total of 2.0% and 3.1% of the dose was recovered in the feces, 5.0% and 4.9% in the breath (predominantly as CO$_2$) and 4.12 and 4.2% in the carcass and tissues 72 hrs after dosing with 200 and 2000 mg/kg, respectively. The major urinary metabolite was 2-(2-hydroxyethoxy)acetic acid (ca. 50% of the urinary $^{14}C$ for both doses). Diethylene glycol and an unknown metabolite tentatively identified as a diol together accounted for 2% to 3% of the urinary $^{14}C$ for both doses. No unchanged DGBA or DGBE was detected in the urine of rats from either dose group. No evidence was found for the production of the molecular weight alkoxyacetic acids, which is consistent with the low acute and subchronic toxicity of DGBA and DGBE.


Toxicological evaluation of chemical substances has typically relied upon dose-response parameter estimates (e.g. LD50 or EC50). These estimates are subject to considerable variation. Understanding the determinants of this variation will enable the design of better experimental protocols. Data from a previous work (Rees et al., Neurobehavioral Toxicology and Teratology, in press) was reanalyzed to evaluate the influence of the dose-metameter on the calculation of both the LC50 and EC50 (effective concentration at which 50% of the animals are affected) for both logistic and probit models. Different groups of mice were examined for effects on lethality and motor performance on an inverted screen. Following inhalation exposure to either isocyan, n-butyl, or isobutyl nitrite. The motor performance measure was scored as either affected or not affected. EC50 and LC50 estimates and confidence intervals were found to vary depending upon the dose-metameter chosen. Probit and logistic models produced similar estimates. A statistical procedure is described which permits choosing the most appropriate dose-metameter for a given data-set.

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Diethylene glycol monobutyl ether (DEGBE), a primary component of aqueous-film-forming-foams used by the Navy in shipboard firefighting systems, was assayed for acute and subchronic toxicity in male and female Fisher 344 rats. The estimated LD50 oral was 6.53g/kg and 5.08g/kg, respectively. Rats (16/group) were treated 5 days/wk for 13 wks by gavage with: DEGBE, in amounts equal to 1.5, or 25% of the LD50; ethylene glycol monobutyl ether (EGBE), administered at 25% of the reported LD50 (positive control); or H2O (negative control). All animals received equivalent dose volumes. Male rats receiving DEGBE (high dose) showed a significant and persistent reduction in body weight after 1 wk exposure; food consumption for these animals was lower for wks 1 and 2 of exposure. Female body weights were unchanged from controls. After 13 wks exposure, mortality for rats receiving the high dose DEGBE was 80%, with lower and EGBE treated animals unchanged from controls. All rats exposed to DEGBE had increased liver and spleen weights and exhibited lowered, RBC, lymphocytes, and mean corpuscular hemoglobin concentration (MCHC) and elevated mean corpuscular volume. A dose-related decrease in MCHC was determined for all animals exposed to DEGBE. No dose-related gross lesions were noted in any rats.


A number of branched chain ketones, including methyl isobutyl ketone and methyl isooctyl ketone, have been shown to produce hyaline droplet nephrosis when vapors were inhaled by male rats. The present study was conducted to determine if a similar response is produced by DBK. Four groups of 10 rats of each sex were exposed to DBK vapors at concentrations of 0, 98, 300 or 905 ppm for nine 6-hour exposures over an 11-day period. An additional 10 rats of each sex exposed to 0 or 905 ppm were allowed 2-weeks to recover after the exposure regimen. Light microscopy revealed a greater severity of hyaline droplet nephrosis in the 905 ppm and, to a lesser extent, in the 300 ppm males. The lesion in the 905 ppm group was characterized by electron microscopy as an increase in the size and number of electron dense lysosomes in the cytoplasm of the proximal tubular epithelium. Large irregular phagolysosomes, often containing internal crystals, occurred more frequently in this group than in controls. Kidney lesions were not observed in female rats or in males exposed to 98 ppm. The effect either regressed or was not present in the 905 ppm males held for a 2-week recovery. The significance of this lesion to human health is not known since it has not been reported in other species exposed to branched chain ketones.

TOXICITY OF POLYETHYLENE GLYCOL 400. L.C.K. Wong, B.S. Bras and R. Chau. Department of Drug Safety Evaluation, Research and Development Division, Revlon Health Care, Tuckahoe, NY

Polyethylene glycol 400 (PEG 400) has been used extensively in pharmaceutical formulations. In acute studies in our laboratories, after oral administration of 20 ml/kg of PEG 400 to rats (10/sex/group), diarrhea with anogenital staining was observed. Subcutaneous (SC) administration of PEG 400 to rats and mice (10/sex/group) at 25 ml/kg produced ataxia and tremors. Bloody urine and anogenital staining was observed following intravenous (IV) administration of 2.5 ml/kg. Histopathologic examination of the injection sites and surrounding tissues in rats and mice after SC or IV administration revealed severe irritative changes or lesions due to PEG 400. In a 21 day dermal study in 4 rabbits/sex/group, concentrations of 4, 10, and 20% PEG 400 in petrolatum applied to the skin of rabbits were associated with various degrees of erythema and edema after 14 days of application at 1 g/kg. Similarly, in a 21 day ocular study in which the same concentrations were instilled BID 0.1 g into the eyes of 4 rabbits/sex/group, PEG 400 produced irritation in the cornea leading to a moderate inflammatory reaction with lymphocytes and other mononuclear cells under conjunctival epithelium. In summary, data from these studies suggest that PEG 400 has some biological activity.


A number of branched chain ketones, including methyl isobutyl ketone and methyl isooctyl ketone, have been shown to produce hyaline droplet nephrosis when vapors were inhaled by male rats. The present study was conducted to determine if a similar response is produced by DBK. Four groups of 10 rats of each sex were exposed to DBK vapors at concentrations of 0, 98, 300 or 905 ppm for nine 6-hour exposures over an 11-day period. An additional 10 rats of each sex exposed to 0 or 905 ppm were allowed 2-weeks to recover after the exposure regimen. Light microscopy revealed a greater severity of hyaline droplet nephrosis in the 905 ppm and, to a lesser extent, in the 300 ppm males. The lesion in the 905 ppm group was characterized by electron microscopy as an increase in the size and number of electron dense lysosomes in the cytoplasm of the proximal tubular epithelium. Large irregular phagolysosomes, often containing internal crystals, occurred more frequently in this group than in controls. Kidney lesions were not observed in female rats or in males exposed to 98 ppm. The effect either regressed or was not present in the 905 ppm males held for a 2-week recovery. The significance of this lesion to human health is not known since it has not been reported in other species exposed to branched chain ketones.

ACUTE TOXICITY OF METHYLENE CHLORIDE; TUMORIGENIC IMPLICATIONS FOR B6C3F1 MICE. D.L. Eisenbrandt and R.H. Reitz. Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, MI 48674.

Effects of 4000 ppm methylene chloride were evaluated in male, B6C3F1 mice. The inhalation experiment consisted of a 6-hour, single exposure study as well as a repeated exposure study through 3 weeks. Liver weights were increased after 3 days, 2 weeks, and 3 weeks of exposure; increased mitotic activity was observed in hepatecyes after 2 weeks of exposure. Significant pulmonary lesions were present 1 day after a single exposure to methylene chloride. The bronchi and bronchioles had necrotic epithelial cells and non-ciliated (Clara) cells were swollen and vacuolated; a slight increase in mitoses also was noted. Repeated, daily exposure to methylene chloride resulted in necrosis of occasional epithelial cells in the bronchi and bronchioles and reactive hyperplasia of adjacent lymphoid tissue in a few animals. Pulmonary injury in the mouse, however, may not be representative of man and other animals because the mouse differs significantly in the ultrastructure and number of Clara cells. The cytotoxic effects of methylene chloride in lung and liver may be a basis for chronic alterations which could increase the rate of spontaneous tumors in B6C3F1 mice exposed to high levels of methylene chloride for two years.
TIME COURSE OF p-XYLENE'S EFFECT ON RAT LUNG CYTOCHROME P450, NADPH CYTOCHROME C REDUCTASE ACTIVITY AND BaP METABOLISM. A.E. Roberts, D.R. Brown, R. A. Schatz and D. Silverman. Northeastern University, Toxicology Program, Boston, MA 02115.

p-Xylene has been shown to reduce lung cytochrome P450 content 24h after inhalation (1000 ppm, 4h). Work in our laboratory has shown that the metabolic detoxication of the lung carcinogen, benzo(a)-pyrene (BaP), was inhibited at this same time point. Specifically, three hydroxy (3-OH) BaP formation was inhibited 41% 24h after p-xylene (1 ml/kg in soybean oil, i.p.). The formation of 3-OH BaP in rat lung microsomes, measured fluorometrically as aryl hydrocarbon hydroxylase activity (AHH), was also inhibited 15 min (40%), 1h (42%), and 4h (39%) after p-xylene. Cytochrome P450 content was measured at these early time points in an attempt to correlate the observed changes in BaP metabolism with destruction of P450. Rat lung microsomal P450, measured as the CO binding spectra, was 0.061 ± 0.009 nmol/mg in vehicle treated controls. P450 levels fell below limits of detection (0.02 nmol/mg protein) in 4 of 7, 7 of 7, and 6 of 7 rats sacrificed 15 min, 1h and 4h after p-xylene respectively. NADPH cytochrome c reductase activity was found to be unchanged in lung microsomes from rats sacrificed 15 min and 1h after p-xylene. In summary, the destruction of lung P450 and resultant inhibition of BaP detoxication occurs as early as 15 min after p-xylene and persists for at least 4h.

MACROPHAGE UPTAKE OF A LIPOPROTEIN-SEQUISTERED TOXICANT. M.E. Kaminski, D.S. Wells, J.F. Roberts, W.C. Dauterman, and F.E. Guthrie. Toxicology Program, North Carolina State University, Raleigh, NC

An experimental system was chosen to investigate the bioactivity of a lipoprotein (LP)-sequistered toxicant at the cellular level based on recent studies demonstrating receptor-mediated uptake of LP by macrophages. Rat peritoneal exudate cell (PEC) suspensions were exposed to DDT and LP-sequistered DDT, followed by measurement of DDT uptake, metabolism and cellular toxicity. In vitro uptake assays demonstrated that PECs treated for 10, 20, and 30 min with 2.5 μM LP-sequistered DDT had approximately a two-fold increase in the amount of DDT associated with PECs than PECs treated with 2.5 μM free DDT. PECs were assayed for DDT metabolites to serve as a measure of the cellular internalization of the toxicant after treatment. In vitro for 18 hrs with 1.5 μM DDT and LP-sequistered DDT. Evidence of DDT metabolism was only observed with PECs which had been treated with LP-sequistered DDT. Assays measuring macrophage phagocytic activity indicated that macrophages treated for 4.5 hrs in vitro with 2.5 μM LP-sequistered DDT showed significant inhibition in their ability to phagocytose yeast particles. These results suggest that serum LP may facilitate the cellular uptake of LP-sequistered toxicants leading to altered cellular function. (Supported by NEHS ES-00644)

IMMUNOSUPPRESSION INDUCED BY CHEMICALS REQUIRING METABOLIC ACTIVATION IN MIXED CULTURES OF RAT HEPATOCYTES AND MURINE SPLEOCYTES. K.H. Yang*, B.S. Kim*, A.E. Munson and M.P. Holasekle. Korea Advanced Institute of Science and Technology, Seoul, Korea*, and Medical College of Virginia/VCU, Richmond, VA

Primary cultures of adult rat hepatocytes (Fisher 344) were used as an in vitro metabolic activation system in immunotoxicology assays. Rat hepatocytes were isolated by a collagenase perfusion technique and cultured for 20-24 hr to allow the formation of a monolayer on collagen-coated polystyrene dishes. Spleen cells isolated from C5BL/6 x C3H/1F1 mice were co-cultured with hepatocytes along with the chemicals. Cyclophosphamide (CP) and Atlatoxin B1 (AFB1) were effectively activated in the co-culture system and produced a dose-related suppression of the in vitro antibody responses to LPS, DNA-Ficol, and SRBC in 3 hr. Neither CP (1μM) nor AFB1 (10-4M) cultured with spleen cells alone produced any effects. Both CP and AFB1 also produced a dose-related suppression of the proliferative responses to LPS, Con A and PHA. In contrast, up to 100μM of n-nitrosodimethylamine (NDMA) did not suppress any of these assays after a 3 hr incubation in the co-culture system. These results indicate that a co-culture system can be used to characterize the activity of immunosuppressive chemicals requiring metabolic activation. (Supported in part by NIH ES50594 and ES0356).
Benzo-(a)-pyrene (BaP) inhibits antibody responses to T-dependent antigens. We examined the cellular mechanisms of BaP immunomodulation. Female B6C3F1 mice were exposed to 40 mg/kg BaP in polyvinylpyrrolidone (PVP) or PVP alone for 14 days. Reconstitution of antibody forming cultures using combinations of adherent and nonadherent cells from BaP-or vehicle-treated animals demonstrated that both populations were sensitive to BaP modulation. Low numbers of BaP-exposed macrophages were found to enhance in vitro antibody responses. Admixture of purified T or B cells from BaP or vehicle-exposed mice with normal macrophages demonstrated that both T and B cell populations were sensitive to BaP immunomodulation. These results indicate that in vivo exposure to BaP causes modulation through macrophage activation and through effects on both T and B lymphocyte populations. Supported by PHS grants ES-03366 and CA-09210.

Benzo(a)pyrene (BaP) inhibits the responses of macrophages, T and B lymphocytes. Because BaP also inhibited the production of IL-2 it was of interest to determine if the capacity to respond to IL-2 was impaired. Three different experimental systems that induce the presence of IL-2 receptors but not IL-2 synthesis were utilized. BaP was found to inhibit IL-2 induced proliferation, as measured by thymidine uptake, in lymphocytes exposed either in vivo or in vitro. To determine the affected cell type(s) purified macrophages and T cells from vehicle and/or BaP exposed animals were admixed. There was a significant decrease in the IL-2 responsiveness of normal T cells when cultured with macrophages from BaP treated animals as compared to normal macrophages. There was no difference in the responsiveness of BaP or vehicle-exposed T cells when cultured with vehicle-treated macrophages. These results suggest that one site of BaP-induced inhibition of humoral immunity is the macrophage. Supported by PHS grants ES-03366 and CA-09210.

Work in our laboratory has demonstrated that Benzo-(a)-pyrene (BaP) inhibits humoral antibody responses, macrophage phagocytosis, and antigen presentation. Here we report the effects of BaP on the production of interleukins 1, 2 and 3. Subcutaneous exposure of B6C3F1 mice to 40 mg/kg of BaP in polyvinylpyrrolidone vehicle for 14 days resulted in a 70-80% reduction in IL-2 but not IL-1 production when isolated spleen cells were stimulated with Con A. In vivo BaP treatment did not affect IL-1 production by macrophages purified from peritoneal exudate cells (PEC) and stimulated with LPS in vitro. A dramatic increase in IL-1 production was observed when PEC macrophages were incubated with BaP and LPS in vitro. IL-1 release was accompanied by macrophage lysis. This finding correlates with the loss of macrophages in the spleens of BaP treated mice. Alterations of interleukin regulation may represent one of the main mechanisms of BaP immunotoxicity. Supported by PHS grants ES-03366 and CA-09210.

Murine lymphocytes exposed in vivo to dimethylnitrosamine (DMN) show decreased CHI responsiveness. Therefore the production of interleukins following DMN treatment was examined. DMN had no effect on the production of IL-2 or IL-3. There was no effect on the ability of DMN exposed cells to respond to IL-2. DMN exposure resulted in the production of IL-1 from unstimulated macrophages (Mph) comparable to that observed in LPS stimulated vehicle control Mph. LPS stimulation of DMN-exposed Mph did not result in an increase in the production of IL-1. These results suggest: (1) the DMN-induced alterations of cellular immunity are not mediated through alterations in IL-2 production or responsiveness; (2) DMN treatment stimulated spontaneous production of IL-1 in the absence of LPS stimulation; (3) DMN does not affect the total amount of IL-1 released after LPS stimulation as compared to Mph from vehicle treated animals; and (4) DMN-induced alteration of Mph maturation is not due to changes in the production of IL-3. Supported by PHS grants ES-03466 and CA-09210.
EFFECTS OF 0.0,8-TRIMETHYL PHOSPHORODITHIOIC ANION ON IMMUNE FUNCTION. K.E. Rodgers, N. Leung, C.F. Ware and T. Imamura. Divisions of Biomedical Sciences and Toxicology and Physiology, University of California, Riverside, CA 92521.

The effect of acute administration of 20-60 mg/kg O,0,8-trimethyl phosphorodithioic (OSS-TMP) to C57Bl/6 female mice was examined. The doses included body weight, cholinesterase (ChE) levels, splenic and thymic size. Acute administration of 60 or 80 mg/kg OSS-TMP led to a decrease in ChE levels and thymic size. At a dose of 30 mg/kg OSS-TMP, the animals exhibited body weight loss. The effect of OSS-TMP on the generation of in vivo primary (1ª) and in vitro secondary (2ª) cellular and humoral immune responses was also determined. At nontoxic doses of the compound, the in vivo generation of a 1ª cytotoxic T lymphocyte (CTL) response to alloantigen was significantly elevated but was unaffected following restimulation in vitro. The generation of an in vivo 1ª and in vitro 2ª humoral responses to sheep red blood cells (SRBC) was elevated following a single dose of 40 mg/kg OSS-TMP. Administration of toxic doses of OSS-TMP did not alter the ability of splenocytes to generate a 1ª or 2ª CTL response; however, it suppressed the generation of humoral immune responses. These results differ from those observed in a similar system following acute administration of a structural analog, O,0,8-trimethyl phosphorothioate which has potent immunosuppressive activity. Supported by NIH ES03105.

EFFECTS OF EXPOSURE TO 2,3,7,8-TCDD (TCDD) ON PROTEIN PHOSPHORYLATION IN B LYPHOCYTES. G.C. Clark, D. Gormloe, and M.I. Luster. National Institute of Environmental Health Sciences, Research Triangle Park, NC

In vitro exposure of lymphocytes to TCDD results in a dose dependent suppression of differentiation to antibody forming cells in response to the polyclonal B lymphocyte mitogen lipopolysaccharide (LPS). To investigate potential mechanisms by which TCDD inhibits humoral immunity, we examined one of the early events following B lymphocyte stimulation, the phosphorylation of endogenous proteins. Purified B lymphocytes exposed to TCDD in vitro were labeled with 32P orthophosphate, stimulated with LPS, and the phosphorylation of endogenous cytoplasmic proteins determined by SDS polyacrylamide gel electrophoresis. Stimulation of splenic lymphocytes with LPS resulted in the phosphorylation of a cytoplasmic protein with an apparent molecular weight of 160,000. Exposure to TCDD inhibited the phosphorylation of this 160,000 molecular weight protein. These results suggest that TCDD alters protein phosphorylation which is controlled by protein kinases. Since protein kinase activity is associated with receptors that control growth and differentiation, one potential mechanism for TCDD suppression may be through the alteration of protein phosphorylation and/or protein kinase activity of receptors that control B lymphocyte growth.

IN VITRO AND IN VIVO EFFECTS OF STYRENE OXIDE ON NATURAL KILLER CELL ACTIVITY IN C57BL/6 SPLENOCYTES. M.H. Grayson and S.S. Gill. Division of Toxicology and Physiology, University of California, Riverside, CA 92521.

Styrene oxide is used extensively in the manufacture of reinforced plastics. It has relatively low acute toxicity, but chronic occupational exposure occurs at fairly high levels. Therefore, we have examined the effects of styrene oxide exposure (both in vitro and in vivo) on cell-mediated immunity. Chromium-Release assays were used to examine cytotoxic T-lymphocyte (CTL), natural killer (NK), natural cytotoxic (NC) response of female C57BL/6 splenocytes. Styrene, styrene glycol, allylbenzene, ethylbenzene and toluene were also studied for comparative purposes. In vitro treatment of styrene oxide, styrene, or allylbenzene depressed NK activity but had no effect on NC activity. Allylbenzene also inhibited CTL response. Styrene glycol, ethylbenzene, and toluene had no effect on any of the cell-mediated functions. Oral administration five times weekly for 2 weeks with doses ranging from 250-750 mg/kg caused no signs of toxicity (weight loss, spleen and thymus weights and cellularity). NC and CTL levels were not affected by this treatment, but NK response was increased by 50% or more. Interleukin 2 production by cultured lymphocytes was also increased.


The immunotoxicity of 1.25, 2.5 and 5 mg/day of VCH, a widely used intermediate in epoxy resin manufacture was assessed in B6C3F1 female mice. Effects of VCH on body and relative organ weights, on host resistance and immune function were examined beginning on Day 16. Although VCH exposure had no effect on body weight, there were significant increases in kidney/body weight ratios. The number of antibody forming cells (APCs) to sheep erythrocytes (SE) were quantitated in animals immunized during VCH exposure. VCH suppressed AFC to SE 4 days (Day 15) after immunization. However on Day 16, the values were comparable to vehicle controls. Resident peritoneal macrophage phagocytosis of 3Hr-labeled chicken erythrocytes was significantly impaired while cytostasis of tumor cells and ectoencezyme levels were unaltered. Host resistance was significantly decreased following challenge with Herpes simplex type 2 virus (HSV-2) and was essentially unchanged with Listeria monocytogenes, influenza virus, HSV-1 and PYF6 tumors. This immunologic profile demonstrated that VCH is immunotoxic and that dermal application can exert systemic effects distant from the site of application. (Research supported by NHSE Contract N01-ES-1-5000.)
675 ABSENCE OF INFLUENCE OF AN GENOTYPE ON IMMUNOSUPPRESSION BY 7,12-DIMETHYLBENZ AUDIO-ANTHRACENE (DMBA) IN MICE. L.M. Thurmond, L.D. Lauer, F.V. House, and J.H. Dean, CILIT, Research Triangle Park, NC 27709
Recent reports suggest that the immunotoxicity of certain polycyclic aromatic hydrocarbons is associated with the Ah locus in mice. To test whether DMBA-mediated immunosuppression is influenced by the Ah locus, several endpoints of immune function were measured in B6C3F1 (Ah+), DBA/2 (Ah-), and in Ah- (B6) and Ah+ (B6D1) congenic C57BL/6 mice dosed (i.p.) with 2 mg DMBA (300 μmol/kg) in corn oil. Antibody plaque-forming cells (PFC) measured 4 days after iv SRBC immunization were suppressed 96% in B6C3F1 and 97% in DBA/2 mice. The mixed lymphocyte responsiveness (MLR) of B6C3F1 and DBA/2 splenocytes exposed in vitro to 4 log of DMBA was suppressed 44% and 30%, respectively. Antibody PFC after in vitro immunization to SRBC were equally suppressed in both B6D1 and B6D2 mice. The MLR was suppressed 76% in B6D1 and 85% in B6D2; cytokotic lymphocyte generation was suppressed 65% in B6D1 and 75% in B6D2. The differences between immunotoxic responses in splenocytes from B6D1 and B6D2 were not significant. Splenocytes with induced cytochrome P-450 monooxygenase activity showed DMBA-mediated immunosuppression comparable to controls. These data suggest that DMBA has a direct immunotoxic action on splenic target cells which is independent of Ah genotype.

676 COMPARISON OF MULTIPARAMETER FLOW CYTOMETRY AND FUNCTIONAL TESTS FOR THE ASSESSMENT OF 7,12-DIMETHYLBENZ(A)ANTHRACENE (DMBA) IMMUNOTOXICITY IN B6C3F1 MICE: P. W. Burghiel, S. L. Barton, J. F. Fincher, T. W. Min, W. W. Hadley, and J. H. Dean. 1. The University of New Mexico College of Pharmacy, Albuquerque, NM 87131, and 2. The Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.
DMBA is a potent carcinogen and immunosuppressant in rodents. Previous studies (Dean, et al) have demonstrated a prolonged inhibition of both humoral and cell-mediated immunity in response to DMBA exposure. The purpose of the present study was to use monoclonal antibodies and multiparameter flow cytometry to determine the differential effect of DMBA on subpopulations of murine lymphoid cells, and to compare these findings with results from functional tests. Young adult female B6C3F1 mice were treated by daily s.c. injection for ten days with DMBA in corn oil at doses of 0, 0.5, 5, and 10 μg/g/day. Studies were conducted 4 and 8 weeks after the last dose. The results demonstrated a significant dose-dependent decrease in the total number and percentage of spleen cells expressing B cell markers (mu heavy chain, kappa light chain, and 14B) and T cell markers (Thy-1, Lyt-1, and Lyt-2). Mac-1 expression was increased on spleen cells by DMBA. DMBA produced suppression of T and B cell mitogen responses and inhibited the in vitro primary humoral immune response to SRBC, TNP-Ficoll, and TNP-LPS.

677 PROTECTION FROM ORGANOPHOSPHATE IMMUNOSUPPRESSION. K.E. Rodgers, D.L. Haviland, J. Immuno and C.F. Ware. Division of Biomedical Sciences and Toxicology and Physiology, University of California, Riverside, CA 92521.
Acute sublethal exposure to O,O-trimethyl phosphorothioate (OOS-TMP) is a potent immunosuppressive agent which has been shown to block the in vivo generation of cytotoxic T lymphocytes (CTL) and antibody secreting cells (Ab). The effects of an antagonist of the delayed toxicity of OOS-TMP, O,O,O-trimethyl phosphoro-thionate (COO-TMP) and repeated exposure (4x with 25% dose each time) to OOS-TMP prior to challenge with OOS-TMP on OOS-TMP induced immune suppression was studied. Co-administration of OOS-TMP with OOS-TMP and of pretreatment with OOS-TMP effectively antagonized OOS-TMP induced alterations in the generation of CTL and IL-2 production. Proliferative responses to the mitogen concanavalin A and lipopolysaccharide were elevated by all treatments. The ability of splenocytes to generate an Ab response was elevated following acute administration of OOS-TMP or pretreatment with 2.5 mg/kg OOS-TMP followed by 10 mg/kg OOS-TMP, but co-administration of OOS-TMP with OOS-TMP did not alter the Ab response. The failure of OOS-TMP to antagonize the immunosuppressive effect of acute OOS-TMP administration on the antibody response is consistent with the increased sensitivity of this immune parameter to OOS-TMP induced toxicity. Supported by PHS ES03075.

678 INVOLVEMENT OF THE COMPLEMENT COMPONENT C8 IN 0,0,5-TRIMETHYL PHOSPHOROTHIOATE INDUCED LUNG INJURY. D.D. Ellefson, H.K. Thomas, and J. Immuno. Division of Toxicology and Physiology, University of California, Riverside, CA 92521.
0,0,5-Trimethyl phosphorothioate (OOS-TMP), an impurity of commercial malathion, is a potent inducer of pulmonary injury in rats. We have investigated the role of the complement component C8 in OOS-TMP induced alveolar inflammation. C5 deficient strains of mice (D2/MSN) displayed reduced alveolar inflammatory response over C5 sufficient mice (D2/O5N) at a treatment dose of 30 mg/kg OOS-TMP. Immunofluorescent studies utilizing anti-C5 antibody revealed marked increases in fluorescence on days 3 and 5 after treatment. Fluorescence was apparent as both generalized and focal about the alveolar membrane in C5 sufficient and not deficient mice, most likely outlining the pattern of complement deposition in the lung after treatment. Fluorescent foot could be attributed to activation of the complement cascade with injury. The capacity of OOS-TMP to stimulate macrophages was also studied. Treatment of macrophages in vitro with OOS-TMP in the presence of glutathione resulted in the increased cytokine production from macrophages against P815 tumor target cells. These data suggest that C5 plays a role in OOS-TMP induced pulmonary injury in rats and mice.
The effect of O,S,S-trimethyl phosphorothioate (OSS-TMP) on immune responses such as antigen presentation, antibody production and cytotoxic T-lymphocyte (CTL) function was examined in vitro. The roles of non-enzymatic and enzymatic glutathione (GSH) conjugation of OSS-TMP in these responses were studied. Antibody responses to T-dependent and T-independent antigens were evaluated after (i) direct culture with spleen or B-cells; (ii) cocultivation of B-cells with T-cells with and without preincubation of OSS-TMP with GSH fortified cytosol. Antigen presentation by macrophages was also assessed after such treatment as compared to untreated controls. OSS-TMP preincubated with GSH had an inhibitory effect on the cytotoxic T-lymphocyte and the direct hemolytic plaque forming cell responses. This was found to be mediated by a direct inhibitory effect on macrophages, T- and B-cells of the immune system and not through the generation of regulatory suppressor T-cells. Thus, the mode of suppressive action of OSS-TMP in vitro is due to inhibition of lymphocytic proliferation. This is only possible in the presence of glutathione which was determined to be a prerequisite for the induction of OSS-TMP suppressive effect.

To determine the immunotoxic effect of O,S,S-trimethyl phosphorothioate (OSS-Me) on the immune response in mice an in vitro model was utilized. The involvement of glutathione (GSH) in enhancing the immunotoxic effect of OSS-Me was also investigated. Antibody responses to a T-dependent and T-independent antigen were studied either in direct culture with spleen or B-cells or after cocultivation of B-cells with T-cells treated with preincubated OSS-Me with GSH enriched liver cytosol. Macrophage function was also tested after such treatment as compared to untreated controls. Data suggest that OSS-Me when preincubated with GSH becomes inhibitory to cytotoxic T-lymphocyte, macrophage and plaque forming cell functions. Thus, it was found to be toxic to cells of the immune system and does not inhibit immune function through activation of regulatory suppressor T-cells. This immunotoxic effect was observed after preincubation of OSS-Me with glutathione.

The immunotoxicological effects of DOTC have been studied extensively in the rat but little is known of its effect in other species. We have examined the effect of DOTC on some immune parameters in the mouse, and in particular monitored alterations in immune responsiveness towards both self and heterologous cell membrane components. The investigation was based on the observation that the weekly injection of rat erythrocytes into normal mice initiates both an anti-rat erythrocyte antibody response as well as antibodies with specificity for cross-reacting determinants expressed on self erythrocytes. The weekly oral administration of graded doses of DOTC was shown to suppress significantly the anti-self erythrocyte response at the highest dose level (P<0.001) as well as depressing the anti-rat response. Although suppression was only seen at a dose of DOTC that also caused thymic atrophy, thymectomy alone had no effect on the antibody responses. The delayed type hypersensitivity response to oxazolone, a T-lymphocyte mediated immune response, was not influenced by DOTC treatment. These data demonstrate that DOTC suppresses specific aspects of murine immune competence. (Supported by the UK Ministry of Agriculture, Fisheries and Food)

Benzene myelotoxicity occurs after in vivo metabolism to compounds such as HQ. The goal of this study was to determine if exposure to HQ for short periods altered the ability of B-cell precursors to mature to surface IgM expression in vitro and proliferate in agar. marrow cells from B6C3F1 mice were first depleted of mature (sIgM+) B-cells, exposed to 10^{-M} HQ for one hour and plated in liquid culture. Pre-B (sIgM+) cells were then allowed to mature in culture for 2 days. Phenotypic analysis by immunofluorescence detected a 36% reduction in the frequency of newly generated B-cells (sIgM+) compared with control cultures. To determine the functional integrity of these newly generated B-cells, cells from day 2 cultures were plated in soft agar and stimulated with mitogen (LPS). Only mature (sIgM+) B-cells will proliferate and form colonies in agar (CFU-B). CFU-B from HQ treated suspensions were reduced by 35% compared with control cultures. These results demonstrate that HQ can alter marrow lymphopoiesis in vitro at a concentration which can theoretically be attained in vivo after exposure to benzene. Supported by NIH grant GM01542.
1-Nitropyrene (NP) is a potent bacterial mutagen and carcinogen. Rats were exposed by inhalation to 6.6 mg/m³ NP for 4 weeks (2 hr/day, 5 days/week). In addition, groups of rats were exposed to this compound in combination with GaO₃ particles and SO₂. The rats were assessed for both in vivo and in vitro effects. The in vivo effects were determined by measuring immune responses and splenic cellularity, while the in vitro effects were assessed by analyzing the activity of certain enzymes. The results showed that NP exposure led to a decrease in thymus and spleen weight, and an increase in the number of lymphoid organs. The macrophage cell line SF20A was also used to assess the effects of NP on the immune system. The data suggest that NP exposure can have significant effects on the immune system, and further research is needed to fully understand the mechanisms involved.

IN VITRO IMMUNOSUPPRESSION BY GAMMA-CHLORDANE. K.W. Johnson, N.E. Kaninski, and A.E. Munson. Department of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA

Gamma-Chlordane (CLD) from 0.1 to 100 μM produced a marked suppression of cell-mediated and humoral immune responses of mouse splenocytes upon in vitro exposure. The MRL was suppressed by 40, 81, and 95% at 1, 10, and 100 μM, respectively. The Ab response to SRBC was not altered by 10 μM CLD since the peak suppression occurred on the peak day of response. Day of addition studies with EtOH vehicle and 20 μM CLD to the 5 day Ab response resulted in most marked suppression on days 1 and 2. Since previous studies with CLD failed to produce in vivo immunosuppression, the role of protein binding was evaluated by demonstrating a reversal of CLD-induced suppression of 3H-TdR uptake in mouse bone marrow cells by the addition of mouse or human AB serum. The possibility that CLD was metabolized to a less immunosuppressive form in vivo was demonstrated by the inability of 25 and 50 μM CLD to suppress the Ab response when pre-incubated with a PB-induced S9 preparation. In summary, the failure of CLD to produce in vivo immunosuppression in contrast to marked in vitro activity may be related to extensive association of CLD with serum components and metabolism of the parent compound to less immunosuppressive form.

THE EFFECT OF 2,3,7,8-TCDD ON PROTEIN KINASE C ACTIVITY IN EL4 TUMOR CELLS. G. C. Neumann, J.J. Sando and M.P. Hollands. University of Virginia, Charlottesville, VA and *Medical College of Virginia, Richmond, VA.

In vivo administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) produces an increase in the level of protein kinase C (PKC) in the plasma membrane (Bombeck et al., BBRC 127:296, 1985). We have studied the effects of TCDD on cultured EL4 thymoma cells, which contain a large amount of PKC and respond to phorbol esters with rapid translocation of PKC to the membrane, followed by growth inhibition, adherance to substrate and production of interleukin 2 (IL2). TCDD (0-1000 nM) did not displace binding of 3H-PDB to cytosolic PKC in the presence of optimal (96 μg/ml) or suboptimal (32 μg/ml) phosphatidylserine (PS). TCDD had no effect on PKC activity measured in vitro (phosphorylation of histone H1) in the presence of optimal PS (40 μg/ml), optimal dioln (30, 1 μg/ml), suboptimal (20 μg/ml) PS (0.8 μg/ml) D0, or in the absence of PS and D0. Thus, TCDD does not bind to or directly activate PKC in EL4 cells. TCDD (0-1000 nM) did not inhibit EL4 cell growth, as measured by 3H-thymidine incorporation, or affect growth inhibition by suboptimal (5 nM) PMA, and failed to stimulate production of IL2 or affect PMA-stimulated IL2 production. Thus, TCDD does not appear to stimulate PKC in EL4 cells.
The results of previous studies have demonstrated that the humoral immune response of mice was not affected with subchronic exposure to NA, whereas benzo(a)pyrene (BAP) exposure results in a decreased humoral response. In this study, the effects of NA metabolites on the *in vitro* T-dependent antibody forming cell (AFC) response to sheep red blood cells were investigated. Direct addition of NA did not affect the AFC response. However, the response was significantly decreased with the addition of BAP, 1-naphthol and 1,4-naphthaquinone to splenocyte cultures. With the addition of liver S9 from phenobarbital (PB)-pretreated mice to splenocyte cultures, NA (50, 100 and 200 uM) exposure resulted in a decreased AFC response, while liver S9 from bete-naphthoflavone (BNF)-pretreated mice mediated a decreased response only at the high dose of NA (200 uM). Liver S9 preparations from PB- and BNF-pretreated mice were able to augment the suppressive effects of BAP to a similar extent. These data suggest that, within splenocyte populations, NA metabolites are not generated and the major isozymes of cytochrome P-450 are similar to the BNF-induced isozyme. (Supported by PHS ES05317.)

**INCREASED SUSCEPTIBILITY TO STREPTOCOCCUS PNEUMONIAE (STREP) BY EXPOSURE TO DIMETHYLITROAMINE (DMN).** M.P. Holsapple, K.L. White, Jr., S.C. Bradley, and K.W. Johnson, Medical College of Virginia/VCU, Richmond, VA

*Strep* is a pathogen which is defended against by granulocytic phagocytes, by the activity of complement and by the production of specific antibody. In female adult B6C3F1 mice subchronically exposed to DMN, there was a dose-related increase in *Strep*-induced lethality. With an inoculum of 5.2 x 10⁶ CFU/mouse, 1/8 vehicle mice died, while 8/8 mice treated with 5 mg/kg DMN died. A comparable DMN regimen produced either no effect or a slight increase in the number of PMNs and in the functional activity of complement (CH50). These results indicated a defect in the antibody component of the host defense capability to *Strep* and suggested that the B-lymphocyte was a primary target for DMN. This defect was verified as a dose-related suppression of the antibody response to the T-dependent antigen, SRBC, with a significant suppression of 81% reached at the highest dose. The antibody response to a T-independent antigen, DNP-Ficoll, was also significantly inhibited, but only at the highest dose of DMN and only by 50%. These latter results are somewhat paradoxical since the *Strep* antigen elicits an antibody response which is also T-independent. (Supported in part by NIH ES-1-5001.)

**A STRUCTURE ACTIVITY RELATIONSHIP OF DIOXIN SUPPRESSION OF COMPLEMENT ACTIVITY AND SEGREGATION WITH THE Ah LOCUS.** H.H. Lysy, J.A. McCey, and K.L. White, Jr., Deps. of Biostatistics, and Pharmacology & Toxicology, Medical College of VA/VCU Richmond, VA. Sponsor: M.P. Holsapple.

Previous studies from our laboratory have demonstrated that in female B6C3F1 mice, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin were capable of suppressing serum complement activity. Structure activity studies demonstrated that 14 days of exposure to 2,7-dichlorodibenzo-p-dioxin or octachlorodibenzo-p-dioxin at doses up to 10 µg/kg do not affect serum complement activity. Others have shown that these congener do not induce Ahh. Similar exposure to 3-methylcholanthrene (10 mg/kg), 3,4,7,8-PCDD (1 mg/kg), and α and β naphthaquinone (10 mg/kg) also failed to decrease CH50 activity or C3 levels. Acute exposure to TCDD, which significantly decreased C3 levels in B6C3F1 (Ah+ mice, failed to do so in the DBA/2 (Ah-) mice. Genetic regulation of TCDD effects on complement is further supported by guinea pig studies where doses of TCDD up to 1 µg/kg did not affect complement activity. Since TCDD (100 ng) added directly to mouse serum did not affect total hemolytic activity, effects of TCDD added *in vitro* to complement producing cells are being studied. Complement activity may represent another of the pleotropic responses modulated by dioxin exposure. (Supported by NIH ES50054).
NF can produce pulmonary and hepatic reactions in humans; the latter is thought to be associated with a certain HLA haplotype. To reproduce such reactions in experimental animals, we used 14 strains (A/J, A/SnJ, B.10M/Sn, BALB/cByJ, BUB/Bry, C3H/Ouj, C3H/HeJ/Sn, C3H/JR/Sn, C57BL/6J, LP/J, PL/J, RIIS/J, SM/J and SMR/J) of inbred mice bearing different haplotypes of H-2 complex. Ten 3- to 4-months-old female mice from each strain were given NF in the diet for 6 months at a concentration of 0.025%, which yielded an approximate daily dose of 40 mg/kg body wt. Another group of 10 mice from each strain served as controls. Periodic urine bilirubin and antinuclear antibody determinations showed no significant changes. No treatment-related deaths occurred during the study. At the termination of the study, mice were killed and lung, liver, kidney, heart and spleen were examined histologically. The incidence and the character of histopathologic changes were comparable in each group of control mice with the exception of NF-treated BALB/c mice which showed a high incidence of hepatocellular degeneration, vacuolated hepatocytes and periportal perivascular lymphocytic collection. The mechanism of this NF-induced hepatic injury remains to be studied.

DOSE RESPONSE STUDIES OF 2,3,4-TRIMETHYLPTANE (TMP)-INDUCED NEPHROTOXICITY AND RETENTION IN THE MALE RAT. B. Short, V. Burnett, M. Cox, J. Bus, and J. Swenberg. Chemical Industry Institute of Toxicology, RTP, NC 27709

Previous studies conducted in our laboratory have detected a non dose-related accumulation of large crystalloid protein droplets representing phagolysosomes in the P2 segment of the male rat kidney 72 hours after gavage with 50-2000 mg/kg TMP. Additionally, the kidney was the primary site for [14C] retention after a similar single dose regimen with 50-2000 mg/kg [14C]TMP (range 187-1626 nmol eq/gm kidney). In the present experiment a single oral dose of 0.9 (corn oil control), 1, 5, 10, 20, or 50 mg/kg [14C]TMP was administered to male F344 rats. Marking of kidney slides based on severity and extent of crystalloid protein droplet accumulation 72 hours after giving the dose to each group showed that the treated groups had a greater incidence of both acute and chronic changes. This study indicates that chronic studies can only partially delineate the dose response relationship for nephrotoxicity induced by TMP. Multiple exposures and the addition of end points such as cell proliferation will be required.


TCDD can act directly on murine thymic epithelial (MuTE) cells to alter MuTE-dependent maturation of thymocyte precursors (Cook et al., 1985). These actions are mediated by a specific receptor protein for TCDD (designated the Ah receptor). To determine the potential for TCDD to alter thymocyte maturation in humans, ten strains of HuTE cells have been established. The Ah receptor is present in all human strains examined (10-45 fmol/mg protein). HuTE cells support thymocyte maturation as judged by the expression of co-cultured thymocytes to the mitogens concanavalin A (Con A) (9-14-fold over control) and phytohemagglutinin (PHA) (3-7-fold over control). Treatment of HuTE cells with TCDD produces a concentration-dependent inhibition of thymocyte maturation (at 100 nM TCDD: Con A = 50-76% of control; PHA = 45-77% of control). TCDD suppresses HuTE-dependent thymocyte maturation mediated by both cell-cell contact and soluble factors produced by the epithelial cells. It is proposed that these actions are regulated by the human Ah receptor. These data suggest that TCDD acts directly on HuTE cells to alter normal patterns of thymocyte maturation in vivo and thus would appear to have the potential to produce immune dysfunction in humans.

EVIDENCE OF [14C]-2,2,4-TRIMETHYLPTANE BINDING IN URINE AND SERUM OF F-344 RATS. M.J. Kluos, M.G. Cov, E.M. Norton, and J.S. Bus. Merck Sharp & Dohme Research Labs, West Point, PA 2 and Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Renal toxicity produced in male rats exposed to unleaded gasoline has been attributed to saturated, branched, and trifunctional aliphatic compounds, such as 2,2,4-trimethylpentane (TMP). Approximately 50% of [14C]-2,2,4-TRIMETHYLPTANE has been shown ultimately to appear in the urine of rats 72 hours after a single oral dose. This study was designed to investigate the nature of this urinary radioactivity and also to examine the effect of nephrectomy on the serum levels of [14C]-2,2,4-TRIMETHYLPTANE. Bilaterally nephrectomized and control male and female rats were treated with 0.3 g/kg p.o. (5 μCi) [14C]-2,2,4-TRIMETHYLPTANE; 5 hr serum and 24 hr urine samples were collected. Approximately 12% and 4%, respectively, of urine radioactivity in control male and female rats was covalently bound (non-dialyzable) to a protein with molecular weight between 3500 and 6000 daltons. Analysis of serum radioactivity showed 13% and 30%, respectively, of the radiolabel bound to a component greater than 3500 daltons in male and female rats. Nephrectomy caused a two-fold increase in covalently bound serum radioactivity in male rats, but did not alter that of female rats. These data suggest that TMP interacts with low molecular weight serum components which are filtered by the kidney and excreted into the urine.

In an effort to determine causal factors involved in hydrocarbon-induced nephropathy in male rats, the effect of a single oral dose (1.0 ml/kg) of 2,3,4-trimethylpentane (TMP) on renal lesion development was examined using both castrated and normal male rats (6 per group/sacrifice time). At 3, 6, 12, 24, 48 and 96 hours after dosing, rats were killed and kidneys and livers collected for both light and electron microscopic examination. Urine and serum samples were collected for clinical analyses at 12, 24, 48 and 96 hours. Serum testosterone was undetectable and urinary protein was significantly lowered following castration. Histopathologically, a significant increase in hyaline droplet formation was observed, using electron microscopy, in both the castrated and non-castrated animals as early as 3 hours postexposure. Severity of the lesion was somewhat less in castrated rats. Similar findings were observed in the elevation of enzymes (Alk. Phos, GGT, LDH and NAG), protein and cells in the urine. Findings indicate castration diminishes, but does not abolish, TMP-induced nephrotoxicity in the male rat.

696 HYDROCARBON NEPHROPATHY INDUCTION IN MALE RATS BY CRUDE TRICYANOHEXANE. J.W. Barnett, Jr., F.R. Johannsen, and C.J. Levinsky. Monsanto Company, St. Louis, MO; A.E. Bothe and D.F. Johnson, International Research & Development Corporation, Mattawan, MI.

A complex mixture composed primarily of 1,3,6-tricyanohexane (crude TCH) was administered in the diet of CD® rats of both sexes to provide dose levels of 15, 50 and 150 mg/kg/day for 13 weeks. In females, the only sign of toxicity was a slight decrease in body weight at the highest dose level. The kidney was the target organ in males. Microscopic changes included proximal tubule degeneration with hyaline droplet formation, granular casts at the proximal convoluted tubule, proximal tubular dilatation, tubular epithelial regeneration and chronic inflammation. The incidence and severity of effects were dose related. Clinical chemistry and urinalysis parameters showed no significant changes, but kidney weights were elevated at the two highest doses. Although the etiology of the kidney lesions was not established, hydrocarbon nephropathy may be unique to male rats and directly related to altered reabsorption of alpha-2-microglobulin in the proximal tubule. Structural analogies between the branched hydrocarbon TCH and 2,3,4-trimethylhexane, a model nephrotoxin for the male rat, suggest that a common biochemical mechanism may underlying the induction of the observed nephropathy.

695 METABOLISM OF 2,2,4-TRIMETHYLPENTANE (ISOCOTANE). A NEPHROTOXIN IN MALE RATS. C.T. Olson, Y. Yu, D.W. Hobson*, M.P. Serve, Harry G. Arm.ong Aerospace Medical Research Laboratory, *NMBR/TA, Wright-Patterson AFB, OH. Sponsor: Dr. B. O. Stuart

2,2,4-Trimethylpentane (2,2,4-TMP), a principal component of gasoline, when used in gavage studies with male Fischer 344 rats produced kidney lesions indistinguishable from those seen in male rats in acute gasoline inhalation studies. Female rats exposed to 2,2,4-TMP in the same study demonstrated no kidney lesions. To ascertain whether a sexual difference in metabolism of 2,2,4-TMP could account for the nephrotoxicity, groups of male and female Fischer 344 rats were given 2,2,4-TMP by gavage and urine collected for 48 hours. The following metabolites were identified, in similar proportion, in the urine of both male and female rats: 2,2,4-trimethyl-1-pentanol; 2,4,4-trimethyl-1-pentanol; 2,4,4,trimethyl-2-pentanol; 2,2,4-trimethyl-1-pentanoic acid; 2,4,4-trimethyl-1-pentanoic acid; 2,4,4,4-trimethyl-2-hydroxy-1-pentanoic acid; 2,2,4-trimethyl-5-hydroxy-1-pentanoic acid; and 2,4,4,4-trimethyl-5-hydroxy-1-pentanoic acid. Kidney extracts from both sexes demonstrated the presence of 2,4,4-trimethyl-2-pentanol, 2,2,4-trimethyl-5-hydroxy-1-pentanoic acid, and 2,4,4-trimethyl-1-pentanoic acid. Kidney lesions were evident only in male rats.

697 EFFECT OF 2,2,4-TRIMETHYLPENTANE ON PROTEIN AND DNA SYNTHESIS IN MALE FISCHER 344 RATS. D.R. Peterson, N.M. Hallock, R.D. Phillips. EXXON Corp., East Millstone, NJ. Sponsor: G. F. Egan

2,2,4-Trimethylpentane (TMP) produces kidney lesions in male rats characterized by hyaline droplet formation, proximal tubular cell degeneration and intratubular casts. Three to four weeks of dosing appear to be required for development of the full complement of changes. We set out to determine whether a simple biochemical test, such as DNA or protein synthesis, presages or parallels development of the kidney lesions. Male F-344 rats were given daily doses (1.0 ml/kg, p.o.) of TMP for up to 21 consecutive days. Control rats were given daily doses of distilled water. 24 hrs after 1, 3, 7, 14 and 21 days of dosing, groups of 5 TMP-dosed and 5 control rats were injected with either thymidine [methyl-3H] or leucine[14C] (0.3 mcg/kg, i.p.). A significant increase in kidney DNA synthesis was observed after three days of TMP dosing and all subsequent intervals. A maximum stimulation of DNA synthesis of about 400% was observed after 7 and 14 daily doses, declining to 280% at 21 days. Liver DNA synthesis showed a non-significant stimulation of about 375% after one day of dosing, but no apparent treatment-related effect at subsequent intervals. No significant treatment-related effects on protein synthesis were detected. Incorporation of thymidine into kidney DNA appears to be a useful marker for early onset of hydrocarbon induced nephrotoxicity.

Two-dimensional electrophoretic analyses of rat kidney samples have shown that the spontaneous hyaline droplet formation in the adult male rat is due to the accumulation of the male rat specific protein, alpha 2u globulin, in the lysosomes of the proximal tubules. These studies have also shown that oral exposure to decalin exacerbates these hyaline droplets by interfering with the normal metabolism of alpha 2u globulin causing increased accumulation of this particular protein. Infusion of alpha 2u globulin or leucyze into control or decalin treated female rats demonstrated that the only factor specific to the male rat necessary for hyaline droplet formation and exacerbation by decalin is the presence of alpha 2u globulin and that the hydrocarbon does not cause general lysosomal dysfunction, but may specifically inhibit alpha 2u globulin catabolism. The distribution of radiolabelled decalin indicates that binding of the hydrocarbon or a metabolite to proteins in the kidney is responsible for the nephrotoxicity. Our conclusion is that this male rat hydrocarbon nephrotoxicity is not predictive of the response expected in man, even in some subpopulations which are proteinuric.

699 DIFFERENTIAL TOXICITY OF ISCHEMIA AND HgCl2, IN RABBIT RENAL CORTICAL SLICES. C.E. Rueger, A.J. Gandolfi, K. Brendel, R.B. Nagle. Arizona Health Sciences Center, Univ AZ, Tucson, AZ.

Ischemia and HgCl2 exposure in vivo produce necrosis to the S3 segments of renal proximal tubules. To examine if the lesion produced by HgCl2 is caused by the membrane lesion, placement of proximal cortical slices were exposed to HgCl2, or ischemic conditions. Cortical slices (300 micron) were prepared from male New Zealand kidneys and incubated in vitro with HgCl2, at 10^-6M for 12 hr in a DME/F12 media. Ischemic injury was produced in cortical slices incubated in DME/F12 media or buffered salt solution gassed with N2 (100%) for 0, 45, 90, or 180 min followed by a reoxygenation period (95% O2, 5%) of 0, 2, 4, or 8 hr. Histopathology of slices incubated in HgCl2 showed selective S3 necrosis. Slices incubated in DME/F12 media and exposed to N2 followed by 0, showed no changes from controls incubated continually with 02. However, slices incubated in buffered salt solution and exposed similarly developed a necrosis after 90 min of N2 exposure involving the S1 and S2 proximal tubular cells, leaving the S3 cells intact. N2 exposure for 180 min resulted in necrosis of all areas of the slice. Although HgCl2, and ischemic insults affect the same cell types in vivo, the in vitro induced lesions affect different cell types, suggesting independent mechanisms of injury. (University of Arizona Graduate Fellowship).


These experiments were designed to determine the influence of age on the response of the kidney to ischemia. Renal ischemia was induced in female Fischer-344 rats, 3-4 or 37-38 months old, by renal arterial and venous occlusion and was followed by 0, 1, 24 or 96 hr of reflow. Controls were sham operated. A transient post-ischemic increase in blood urea nitrogen (BUN) and serum creatinine (Scr) was observed in young rats. In old rats, BUN and Scr remained markedly elevated through 96 hrs post-ischemia. In vitro renal cortical slice accumulation of organic ions was inhibited to a greater extent in old rats than in young rats 96 hr post-ischemia. Histologically, renal tubular damage was more severe in old than in young rats 24 and 96 hr post-ischemia. Tubular regenerative activity was similar in old and young rats at 96 hr, but tubular repair was more complete in young rats. Organic ion accumulation by renal slices from untreated rats was inhibited by in vitro acute but to a greater extent in old rats than in young rats. These data suggest that old rats are more susceptible to renal ischemia than young rats and that this may be related to intrinsic age-related differences in basal renal metabolism.

701 RABBIT PROXIMAL TUBULE SUSPENSIONS FOR THE STUDY OF EARLY NECROTIC EVENTS. L.A. Rylander, J.S. Phelps, K. Brendel, and A.J. Gandolfi. Dept. Pharmacology and Toxicology, Univ AZ, Tucson, AZ.

Suspensions of rabbit renal proximal tubules were used to study the short term effects of a specific neoprotocin. Changes in intracellular K+, ATP, and glutathione dehydrogenase (LDH) release along with alterations in organic ion accumulation and histopathology can be used to determine the chronology and concentration dependency of toxic events caused by in vitro exposure to proximal tubule toxins. 1,2-dichlorovinyl sulfide (DCV) and GdCl2 were chosen as model renal proximal tubule toxins. Loss of intracellular K+ and inhibition of organic ion accumulation were the most sensitive indicators of tubule cell compromise with respect to both concentration and length of exposure. At equimolar concentration (10^-6M), both DCV and GdCl2 caused declines in K+ levels and organic ion accumulation to by 30% and 60% respectively, at 1 hr. No effects on these parameters were produced by 10^-6M of either compound, even after 6 hrs. Increased LDH release, decreased GSH content, and histopathological changes were also time and concentration-dependent but required a latency period. ATP content was the least reliable indicator of tubule cell injury. These findings in the proximal tubule suspension system to be useful for the study of early toxic events in the proximal tubule cells. (Johns Hopkins CAAT).
Chloroform (CHCl₃) and 1,1,2-trichloroethane (TCE) were chosen as model nephrotoxins to study the in vitro responsiveness of rat proximal tubules. Tubules were isolated from male Sprague-Dawley rats by perfusion with collagenase and found to be primarily proximal, as evidenced by high alkaline phosphatase (ALP)/hexokinase ratios (46.5±16.1) and reactivity to periodic acid-Schiff stain. Viability, assessed by trypan blue exclusion and NADH penetration, was initially 90-95%. Incubations were conducted in gas-tight glass flasks at 37°C under 5% CO₂, 95% O₂, with tubules suspended in culture medium. Test chemicals volatilized from flask center wells to result in final concentrations of 2, 7, and 12 mM CHCl₃ and 1, 2, and 7 mM TCE. Under these conditions, both CHCl₃ and TCE increased the release of lactate dehydrogenase (LDH), N-acetylglucosaminidase (NAG), ALP, and p-aminophenylphosphate uptake in a concentration-related manner. Cytotoxicity indicators, in order of sensitivity, were PHA uptake, LDH, NAG, and ALP release. At the highest concentration of both chemicals, leakage of LDH was nearly maximal at 2 hr, but at lower concentrations, LDH release was linear for the 4 hr incubation. These results suggest that isolated proximal tubules are potentially useful for studying the subcellular effects and the nephrotoxic mechanisms of halogenated hydrocarbons.

Little information exists on vancomycin (VAN)-induced nephrotoxicity in animals. The purpose of this study was to characterize VAN nephrotoxicity in female Sprague-Dawley rats. VAN was injected (i.p.) at 0, 75, 225, 375, 450, 600 and 750 umol/kg. Animals were killed 48 hr later and kidney weight, blood urea nitrogen (BUN) concentration and renal cortical slice organic anion (p-aminophenylphosphate, PHA) and organic cation (tetraethylammonium, TEA) accumulation were used to estimate renal dysfunction. VAN increased kidney weight and depressed slice PHA accumulation at doses ≥ 375 umol/kg; slice TEA accumulation was depressed at doses ≥ 450 umol/kg. BUN was elevated by 600 and 750 umol/kg VAN. VAN (5 and 10 mM) also depressed gluconeogenesis, PHA and TEA accumulation when incubated with renal cortical slices from naive rats. E-aminocaproic acid, an inhibitor of renal protein reabsorption, had no effect on VAN (750 umol/kg) toxicity when administered at dosages of 100, 500 or 1500 umol/kg.

The objective of this study was to characterize VAN-induced nephrotoxicity in female mice. Mice were treated with VAN (0, 150, 450, or 750 umol/kg, ip) and kidney injury was determined 3 to 168 hr later. VAN increased kidney weight within 3 hr; the magnitude and duration of the increase was dose-related. BUN concentration was elevated 3 hr after VAN (450 or 750 umol/kg) and reached a maximum (dose-related) at 48 hr. BUN concentration returned toward normal by 168 hr. Renal cortical slice organic ion accumulation was depressed at 3 hr (750 umol/kg). Maximum depression of slice organic ion accumulation occurred at 168 hr and was dose-related. Severe distal nephron damage was observed at 3 hr; relatively few proximal tubules showed morphologic change at 3 hr. The distal nephron had regenerated by 48 hr but most proximal tubules had extensive vacuolation of their apical cytoplasm; the vacuoles were filled with electron dense material. The brush border of proximal tubules remained intact. Tubular casts composed of cellular debris and strongly basophilic, amorphous material were commonly seen both at 3 and 48 hr. Thus, VAN produced a rapid and dose-related degree of nephrotoxicity in mice. The distal nephron, as well as the proximal nephron, was a target for VAN toxicity.


Attempts to avoid gentamicin-induced toxicity in the presence of renal dysfunction include altering dosage or dosing interval to achieve a therapeutic serum concentration that is similar to one in a normal patient. These protocols assume the nephrotoxicity threshold is unchanged in the presence of renal dysfunction. This study used exponentially declining infusions based on the preinfusion pharmacokinetics to achieve identical serum gentamicin(G) concentration profiles in control (C) and subtotal nephrectomized (NE) dogs and compared serum chemistries and histopath to control (C) and nephrectomized (NN) untreated dogs. For C and NN dogs resp, infusion steady-state serum concentrations were 5.25±0.30 vs 5.54±0.51 (mcg/ml) and elimination rates were 0.19±0.02 vs 0.20±0.01 (hr⁻¹) (mean±SEM). Post-infusion histopath scoring of renal lesions (0-30, with 5 being most severe) were 11±5 (C), 4±2 (NN), 2±2 (C) and 0±0 (NN). Among the significant differences in chemistries were decrease in creatinine clearance between NG and NN and increase in seric urea nitrogen between NG and NN and between NG and C. This supports increased sensitivity in the NG dogs. (Supported by NTR NIAADM-AM 31862 and NIBHS-ES 07046).

Laboratory rats are usually assumed to be homogenous populations within each strain; however, previous studies in our laboratory suggested that there may be a subgroup of Sprague-Dawley rats which are highly sensitive to aminoglycoside nephrotoxicity. This study clearly identified a subgroup which was highly sensitive to supratherapeutic doses of gentamicin (25 mg/kg tid, for 7 days). Statistical analysis of post-treatment serum creatinine (SCR) and urea nitrogen (SUN) concentrations demonstrated two distinct populations: normally responding rats (SCR = 1.92 ± 0.54, SUN = 71.5 ± 18.4, N=67) and highly sensitive rats (SCR = 4.10 ± 0.83, SUN = 146.4 ± 24.9, N=12) (mean ± SEM mg/dl). Comparison of pre-dosing blood and serum chemistries between these two populations revealed statistical differences only in initial serum osmolality, oxygen tension and total protein. Since there is a subpopulation of humans which are at risk for developing aminoglycoside nephrotoxicity due to unknown host factors, these highly sensitive Sprague-Dawley rats may provide an animal model for investigating this human clinical problem. (Supported by NIH NIEHS - AM 31862 and NIBRS - ES (07046).


Effects of dose reduction method and length of dosing on gentamicin(G) nephrotoxicity were studied in subtotally nephrectomized(Nx) Sprague-Dawley(SD) rats. Previous work indicated that SD rats with prior renal insufficiency were resistant to G while dogs were sensitive. Control (C) and Nx rats received 75 and 30mg/kg/d, resp. for 7,14 or 21 days. Adjusted serum creatinine (SCR) and urea nitrogen (SUN) ([(post-pre)/pre] declined in C over time (3.3 to 0.7, 3.1 to 1.5, resp.), while increasing in Nx rats (0.4 to 0.7, 0.6 to 1.3). Although cumulative histopath scores (0-20) were similar between treatments, interstitial nephritis decreased in C (5.0 to 1.5) and increased in Nx rats (3.0 to 3.0). In a second study, C was given for 7 to C (n=10) or Nx (n=20) rats. A fixed interval (FI) or fixed dose (FD) regimen compensated for renal dysfunction in Nx. Adjusted SCR and SUN, and histopath scores, were greater in FI than FD (1.7 vs 0.5, 0.5 vs 0.0, and 14.6 vs 8.5, resp.) higher rates of increase in Nx rats over time suggests the need for multiple evaluations during drug administration. Results of the PD vs. FI rat study agree with those in dogs, indicating that dose reduction method may significantly lower nephrotoxicity.


PCBC has been implicated as the metabolite responsible for hexachlorobutadiene-induced nephrotoxicity. To determine the mechanism of PCBC-induced toxicity, PCBC (0.02-1.0 mM) was added to a suspension of RPT and incubated for 15-60 min, while oxygen consumption (QO2), GSH content, and LDH content were monitored. At 2 min, ouabain-insensitive QO2 (01-02) increased 17-63%, while maximally-stimulated ouabain-sensitive QO2 via nystatin, NYS-02), and GSH and LDH content were unaffected. To probe mitochondrial function in situ, ADP, oligomycin, or atracurium was added to digitonin permeabilized tubules. PCBC had no effect on ADP-stimulated QO2 (state 3), but increased oligomycin- and atracurium-insensitive QO2 (state 4), a good indication of uncoupling. At 60 min, 01-02, NYS-02, uncoupler-stimulated 02, GSH content, and LDH content increased 30-38, 31-59, 45-63, 14-28%, and 13-19%. These results show that: 1)PCBC is a potent tubular- and mitochondrial-toxicant, 2)PCBC uncouples oxidative phosphorylation as an early event in toxicity and 3)PCBC inhibits the electron transport chain and causes plasma membrane damage at later time points. (Supported by ES03529 and AM28616).

709 GLUTATHIONURIA INDUCED BY MERCURIC CHLORIDE AND HEXACHLOROBUTADIENE. P.M. Silber, A.J. Gandolf, K.K. Bredel, Dept. Pharmacology, University of Arizona, Tucson, AZ.

Our previous studies revealed the progression of renal functional changes following acute 1,2-dichlorovinylsulthine-induced tubular injury. Early indicators of kidney damage included increased levels of glutathione (GSH) and gamma-glutamyl transferase (GGT), an enzyme involved in renal GSH metabolism. Yet, there is little information about the effect of other nephrotoxins on renal handling of GSH in vivo. Female S-D rats (N=4) received ip injections of HgCl2 (2 mg/kg) or hexachlorobutadiene (HCB, 300 ppm). Urine was continuously collected for 12 hr. By 8 hr after HCB dosing, urinary GSH excretion (216 mg/hr) was 18 times that of control levels. HgCl2-induced glutathionuria (83 mg/hr) was less pronounced, and only seen 12 hr after dosing. Urinary excretion of GGT was elevated (20 U/mg creatinine) in HCB dosed animals 4 hr after dosing, and by 8 hr after HgCl2-treated animals, but renal GSH levels were unaffected by both treatments. Thus, nephrotoxin-induced urinary glutathionuria may result from impaired renal GGT activity, leading to depressed GSH re-uptake from the proximal tubule lumen. (NIEHS-ES-070-91).

Unilaterally nephrectomized (NFX) and sham-operated (SO) male long Evans hooded rats (175-200g) were injected with radioactive 203HgCl₂, i.p. (100 µg Hg/kg b.w., 5 µCi/kg b.w.) 10 days after surgery. Forty eight h after the injection of HgCl₂, the content and distribution of mercury in the kidneys were determined by X-counting. Twelve days following surgery the remnant kidneys (1.60±0.10g) from the NFX rats (n=5) weighed significantly more (p<0.05) than the corresponding kidneys (1.25±0.72g) from the SO rats (n=5), indicating compensatory growth. Forty eight h following the injection of HgCl₂, a greater amount of Hg (% of dose) went to e.g. tissue in the remnant kidneys from the NFX rats (28±5%) than in the kidneys from the SO rats (19±4%). Interestingly, though, the same fraction of the dose of Hg that accumulated in the remnant kidneys of the NFX rats (44±8%) accumulated in the combined 2 kidneys of the SO rats (46±11%). The enhanced accumulation of Hg in the remnant kidneys could be primarily accounted for by a 2-fold increase in the amount of Hg taken up by each g of outer medulla (41±3%), relative to that found in the kidneys of SO rats (19±7%). Therefore, in the remnant kidney that has undergone compensatory growth, the accumulation and distribution of Hg is significantly different from that in the normal kidney.

**712** EFFECTS OF SOMAN AND DIISOPROPYLFLUOROPHOSPHATE (DFP) ON RENAL ATPase IN FISCHER 344 (F) AND SPRAGUE-DAWLEY (SD) RATS. J. McG. Baggett and W.O. Brecut. Dept. Pharmacol., Col. Med., Univ. NE Med. Ctr., Omaha, NE.

Soman and DFP have been reported to disrupt renal function in the rat. However, the sensitivity of the F and SD rats to these organophosphate compounds differed; the SD rats responded less well to soman. The effects in either strain on renal function could not be correlated with inhibition of renal cholinesterase. This study was undertaken to examine the effects of soman and DFP on renal ATPase. Renal cortical microsomes were prepared and NGH and Na,K-ATPase assayed by standard methods. Two hours after the agents, Na,K-ATPase from SD rats was depressed significantly with the greater response observed with DFP (25% reduction vs 19% for soman). F rats showed a modest 10% reduction at 2 h, but a significant 25% inhibition of ATPase at 6 h after DFP. Soman inhibited the enzyme in F rats by 31% at 6 h, but only 11% at 2 h. The greater response of SD compared to F rats at 2 h after DFP correlates well with the changes observed in renal function. The effects of DFP and soman in the F rats do not correlate with the disruption of renal function. (Supported by U.S. Army Contract DAMD 17-82-G-2220.)

**711** THE EFFECTS OF CHROMATE ON CITRININ-INDUCED RENAL DYSFUNCTION IN THE RAT. F. Haberman, J. Baggett and W.O. Brecut, Dept. Pharmacol., Univ. NE Med. Ctr., Omaha, NE.

Previous studies in this laboratory revealed an effect of chromate to potentiate the nephrotoxic effects of mercuric ion. Citrinin, an organic anion, is a known nephrotoxin. The present study was to assess the possible interaction of chromate and citrinin. Male Sprague-Dawley rats were used. Chromate at a non-effective dose (10 mg/kg) enhanced the depression of p-aminophenyl and tetraethylammonium accumulation caused by citrinin (35 and 50 mg/kg). With intact animals housed in metabolism cages, citrinin (35 mg/kg) increased urine output significantly, but the combination of chromate (10 mg/kg) and citrinin produced a response significantly greater than the sum of the individual actions. A similar response was observed with urinary glucose concentrations and glucose excretion. These data indicate that chromate can potentiate the nephrotoxic action of citrinin in the rat. (Supported by NIH grant 1R01DK37186.)

**713** EFFECT OF PROBENECID ON ACUTE N-(3,5-DICHLOROPHENYL)SUCINIMIDINE (NDPS) INDUCED NEPHROTOXICITY. G.C. Rankin, D.J. Yang, V.J. Teets, H.H. Lo, and P.L. Brown. Deps. of Pharmacology and Anatomy, Marshall University School of Medicine, Huntington, WV.

Results from previous studies with the selective nephrotoxin NDPS have suggested that a metabolite of external origin might be responsible for NDPS-induced renal toxicity. This study examined the effect of probenecid on NDPS-induced nephrotoxicity. Male Fischer 344 rats (4 rats/group) were pretreated (i.p.) with probenecid (60 or 120 mg/kg, i.p.) or saline (2.5 ml/kg, i.p.) Renal function was monitored at 24 and 48 hr. Probenecid 60 mg/kg did not alter NDPS-induced nephrotoxicity. However, probenecid 120 mg/kg blocked or reduced the diuresis, increased proteinuria, decrease in tetraethylammonium (TEA) uptake, elevation in BUN concentration and increased kidney weight produced by NDPS (0.6 mmol/kg) alone. The increased kidney weight and blood urea nitrogen (BUN) concentrations, and decreased p-aminophenyl (PAH) uptake produced by NDPS (1.0 mmol/kg) treatment were blunted by probenecid (120 mg/kg) pretreatment. These results suggest that at least one nephrotoxic metabolite of NDPS is an organic acid. (Supported by NIH grant AM 31210).
STUDIES OF NEPHROTOXICITY DUE TO MIXED INHALATION EXPOSURE TO STYRENE AND TOLUENE IN RATS. 
L. Dahlström-King, S. Chakrabarti, J. Brodeur, and B. Tuchweber. 
Dép. méd. trav. hyg. mil. and Dép. nutrition, Fac. méd., Université de Montréal, Québec, Canada.

Previously we have shown the nephrotoxic potential of styrene (S) or toluene (T) due to inhalation exposure (The Toxicologist 5: 57, 1985). Here we have evaluated the changes in nephrotoxicity due to mixed inhalation exposure to S and T. Groups of rats were simultaneously exposed to a minimum toxic dose (1000 ppm) of S and T for 6 h. Then, urine was collected for 24 h and the animals were sacrificed. An additive response was observed with regard to urinary excretion of either N-acetyl-β-D-glucosaminidase (NAG) or glucose. Light microscopy showed no modification of renal proximal tubules compared to those in S- or T-alone treated groups, except an infiltration of mononuclear cells in the interstitial tissue was noted due to such mixed exposure. Ultrastructural studies did not show any modification of renal tubules of rats exposed to either S or T. But numerous and large lysosomal bodies as well as cytoplasmic vacuoles were observed in the renal proximal tubules due to mixed exposure. These results indicate that mixed exposure to S and T leads to increased nephrotoxicity compared to exposure to either solvent. Such exposure produced increased urinary excretion of mandelic and phenylglyoxyllic acids and thioethers. (Supported by IRSS, Québec).

STUDIES OF ACUTE NEPHROTOXIC POTENTIAL OF CARBON DISULFIDE IN RATS. S. Caron and S. Chakrabarti. 
Dép. méd. trav. hyg. mil., Fac. méd., Université de Montréal, Québec, Canada.

This study was designed to evaluate the acute nephrotoxic potential of carbon disulfide (CS₂) in male and female Sprague-Dawley rats. Either male or female rats were divided into 6 groups. The first three groups were treated s.c. in corn oil with 0, 250 or 500 mg CS₂ per kg; the second three groups were first pretreated i.p. with 3-methylcholanthrene (3-MC) (20 mg/kg) for 2 days and then treated s.c. with 0, 250 or 500 mg CS₂ per kg. Prior to injection of CS₂ or vehicle, all the groups were subjected to fasting for 18 h. Urines were collected for 24 h after injection of CS₂ and the rats were then killed. Various biochemical parameters of renal injury and the urinary thioethers were measured. The results showed no impairment of renal tubular function. But blood urea nitrogen was significantly increased only at the highest dose level of CS₂ and only in male rats pretreated with 3-MC, although such increase, but not significant, was also noted at 250 kg, suggesting a possibility of producing renal glomerular damage at high acute dose level. These data further suggest that metabolic activation of CS₂ is necessary for acute glomerular injury. Male rats were not affected by such treatments. Urinary thioethers were increased with increasing dose of CS₂. (Supported by Institut de recherche en santé et en sécurité du travail du Québec).

STUDIES OF ACUTE NEPHROTOXIC POTENTIAL OF TRICHLOROETHYLENE IN FISCHER-344 RATS. S. Chakrabarti and B. Tuchweber. 
Dép. méd. trav. hyg. mil. and Dép. nutrition, Fac. méd., Univ. Montréal, Québec, Canada.

Previous studies from our laboratory have shown that trichloroethylene (TRI) can produce a dose-dependent liver injury in rats (The Toxicologist 4: 182, 1984). Limited information is available about the nephrotoxic potential of TRI. The present study was therefore undertaken to reexamine the nephrotoxic property of TRI in male Fischer-344 rats. Groups of rats, after pre-treatment with phenobarbital (80 mg/kg, i.p., 3 days), were treated i.p. in corn oil with 0, 0.5, 1.0 and 2.0 ml TRI per kg b.w. Urines were collected for 24 h after the treatment and the animals were then killed. Both the nephrotoxicity and the metabolism of TRI were studied by measuring several biochemical parameters characteristic of renal injury and the principal urinary metabolites of TRI respectively. Significant increase in urinary level of N-acetyl-β-D-glucosaminidase and blood urea nitrogen was observed during 0-24 h only at the highest dose level of TRI. Urinary excretions of both trichloroethanol and trichloroacetic acid reached an apparent saturation at the highest dose level of TRI. These results demonstrate that TRI at high acute dose levels has the potential to produce renal injury both at the tubular and glomerular regions in Fischer-344 rats. (Supported by Institut de recherche en santé et en sécurité du travail du Québec).

STUDIES OF ACUTE NEPHROTOXIC POTENTIAL OF CARBON DISULFIDE IN RATS. S. Caron and S. Chakrabarti. 
Dép. méd. trav. hyg. mil., Fac. méd., Université de Montréal, Québec, Canada.

This study was designed to evaluate the acute nephrotoxic potential of carbon disulfide (CS₂) in male and female Sprague-Dawley rats. Either male or female rats were divided into 6 groups. The first three groups were treated s.c. in corn oil with 0, 250 or 500 mg CS₂ per kg; the second three groups were first pretreated i.p. with 3-methylcholanthrene (3-MC) (20 mg/kg) for 2 days and then treated s.c. with 0, 250 or 500 mg CS₂ per kg. Prior to injection of CS₂ or vehicle, all the groups were subjected to fasting for 18 h. Urines were collected for 24 h after injection of CS₂ and the rats were then killed. Various biochemical parameters of renal injury and the urinary thioethers were measured. The results showed no impairment of renal tubular function. But blood urea nitrogen was significantly increased only at the highest dose level of CS₂ and only in male rats pretreated with 3-MC, although such increase, but not significant, was also noted at 250 kg, suggesting a possibility of producing renal glomerular damage at high acute dose level. These data further suggest that metabolic activation of CS₂ is necessary for acute glomerular injury. Male rats were not affected by such treatments. Urinary thioethers were increased with increasing dose of CS₂. (Supported by Institut de recherche en santé et en sécurité du travail du Québec).

INFLUENCE OF HYPERTENSION ON THE HEPATORENAL TOXICITY OF STYRENE. S. Décarie and S. Chakrabarti. 
Dép. méd. trav. hyg. mil., Univ. de Montréal, Québec, Canada.

The toxicity of styrene (S) is believed to be due to the formation of reactive styrene oxide mediated by microsomal oxidative enzymes. It is also known that hepatic microsomal drug metabolism is significantly increased due to hypertension. Groups of adult male normotensive (WKY) and spontaneously hypertensive (SHR) rats were exposed by inhalation to 0, 800 and 1600 ppm S for 5 h. After the exposure, the urines were collected for 22 h and the animals were then sacrificed. The various biochemical parameters of hepatorenal toxicity were measured. Exposure of rats to 800 ppm S showed significant increase in the urinary excretion of γ-glutamyl transpeptidase (γ-GT) and proteins as well as an increase, although not significant, in serum glutamic pyruvic transaminase (SGPT) due to hypertension. Metabolism studies showed a significant increase in the urinary thioethers due to hypertension. Exposure of rats to 1600 ppm S produced significant reduction of urinary sorbitol dehydrogenase as well as a decrease, but not significant in the urinary γ-GT due to hypertension. The metabolites of S were also decreased due to hypertension. These data suggest that the hepatorenal toxicity may be modified, worsened or ameliorated due to the presence of hypertension. (Supported by CAFIR, Université de Montréal).
Mixed acute exposure to styrene (S) and toluene (T) produced an increased nephrotoxicity compared to that of either solvent (unpublished results). Such information, with regard to mixed subchronic exposure to S and T is not available. So, adult male Sprague-Dawley rats were treated simultaneously with 4 mmole S per kg i.p., twice a day at an interval of 4 h and 10 mmole T per kg orally once a day, 5 days a week for 4 consecutive weeks. After the last day of treatment, the rats were placed in metabolism cages for collection of urines for 24 h and then were sacrificed. Hepatotoxicity, measured by serum transaminases activities, was not modified due to such mixed exposure to S and T. But such mixed exposure produced significant increases in the urinary excretion of y-glutamyl transpeptidase and proteins as compared to those in either solvent. An increase, but not significant, in the urinary excretion of N-acetyl-β-D-glucosaminidase and glucose was also noticed due to such exposure. Metabolism studies showed only a significant increase in hippuric acid due to mixed exposure. These data indicate that mixed subchronic exposure to S and T may have the potential to increase the nephrotoxic response as compared to that of either solvent. (Supported by Institut de recherche en santé et en sécurité du travail du Québec).

Aspirin (A) is one of the more popular over-the-counter analgesic-antipyretic-antiinflammatory drugs in current use. At high supratherapeutic dose, A is known to be nephrotoxic in both humans and laboratory animals. Such toxicity is believed to be due to reactive metabolite(s) mediated by oxidative micromosomal enzymes. Acute ethanol (E) consumption is known to inhibit hepatic micromosomal drug metabolism. So acute consumption of E could modify the nephrotoxicity due to A. Separate groups of 5 adult male Sprague-Dawley rats (180-220 g b.w.) were treated orally with 0, 5.7 g E, 600 mg A or 5.7 g E plus 600 mg A per kg b.w. The animals were then fasted for 16 h and the urines were collected for 24 h after the treatment and they were then sacrificed. A showed significant nephrotoxic potential as verified by significant large increase in the urinary excretions of γ-glutamyl transpeptidase (γ-GT), N-acetyl-β-D-glucosaminidase (NAG), proteins, glucose and blood urea nitrogen (BUN) compared to control or E-treated rats. On the other hand, all these nephrotoxic parameters were significantly reduced when rats were treated simultaneously with A and E. These results suggest that coadministration of ethanol could protect against nephrotoxicity due to aspirin. (Supported in part by Institut de recherche en santé et sécurité du travail du Québec).

Previous studies from our laboratory have shown that toluene (T) is nephrotoxic at 20 mmole/kg but non toxic at 10 mmole/kg b.w. in male Fischer-344 rats. Such toxicity is thought to involve the formation of a metabolic reactive intermediate through a micromosomal oxidative enzyme system. Chronic or repetitive ethanol (E) consumption is known to increase the activity of the hepatic micromosomal mixed-function oxidation system and such, could increase the toxicity of T. Therefore, separate groups of rats were given either drinking water or drinking water containing 10% alcohol along with their food for three consecutive weeks. At the end of three weeks, they were given orally in corn oil 0, 10 mmole or 20 mmole T per kg b. w. After the administration of T or vehicle, urines were collected for 24 h and the animals were then sacrificed. Various biochemical parameters characteristic of renal injury were measured. A significant increase of urinary proteins and glucose at 10 mmole T per kg due to association with alcohol was observed, but not so at 20 mmole T per kg. Neither blood urea nitrogen nor urinary creatinine was modified due to association of T with E. These results indicate that subchronic consumption of ethanol for a longer period of time than presently studied may have the potential to increase the renal toxicity due to toluene. (Supported by THS72C).

Subchronic exposure to 0.3 and 0.5 mmole of bromobenzene (BB) per kg b.w. has been shown to increase certain hepatic micromosomal enzyme activities (Toxicol. Letts. 20: 79, 1984). BB exerts its acute toxicity due to its metabolic activation to a reactive intermediate mediated by hepatic micromosomal mixed function oxidation system. Groups of adult male rats were given 1. p. with 0, 0.1 and 0.5 mmole BB per kg in corn oil for 3 weeks. Thereafter, half of each of these groups was treated i.p. with a single acute dose of 2.5 mmole BB per kg. Urines were collected for 24 h before the animals were sacrificed. The hepatotoxicity (as measured by serum glutamic oxaloacetic transaminase and sorbitol dehydrogenase) induced by an acute dose of BB was significantly reduced by prior subchronic exposure to BB at 0.5 mmole/kg, but not so at 0.3 mmole/kg. The renal toxicity induced by acute exposure to BB, as measured by increased urinary level of N-acetyl-β-D-glucosaminidase was significantly reduced in rats pretreated with 0.3 mmole BB per kg but not so with 0.5 mmole BB. Such protection of BB toxicity was not due to an increased metabolism of BB as verified by its urinary metabolic profile. These data suggest an apparent protection of acute hepatorenal toxicity of BB due to prior subchronic exposure. (Supported by Institut de recherche en santé et sécurité du travail du Québec).
INHIBITION OF THE ACUTE RENAL TOXICITY OF METHYL CHLORIDE IN MICE BY GLUTATHIONE (GSH) DEPLETION. S.J. Cheliman, R.M. Norton, and J.S. Bus. Chemical Industry Institute of Toxicology, Research Triangle Park, NC

Previous data from this laboratory have demonstrated the importance of GSH conjugation in the metabolism and toxicity of methyl chloride (MeCl). Its possible role in the acute renal toxicity of MeCl was investigated in the present report. Groups of 5 male B6C3F1 mice were exposed to 1500 ppm MeCl 6 hr/day 5 days/week for 2 weeks, with and without daily pretreatment with the GSH synthesis inhibitor L-buthionine-S-R-sulfoximine (BSO). 2 mmol/kg i.p. 1.5 hr prior to MeCl exposure. Incorporation of H-thymidine (TdR) into renal DNA was used as an index of MeCl-induced kidney cell necrosis and compensatory regeneration. TdR was administered in osmotic minipumps implanted s.c. at the start of week 2 exposure. Mice were killed 24 hr after the last exposure and the specific activity of renal DNA was determined. Exposure to MeCl caused a three-fold increase in the incorporation of TdR into renal DNA (337 ± 40 dpm/μg DNA vs. 105 ± 40 control). BSO, which depleted renal nonprotein sulfhydryl (NPSH) by 60% and hepatic NPSH by 28% at the start of MeCl exposure, prevented MeCl-induced increases in DNA specific activity (60 ± 15 dpm/μg). These results suggest that conjugation of MeCl with GSH is an important step in the metabolism of MeCl to intermediates toxic to the kidney.


In order to investigate the stereospecificity of lysine nephrotoxicity, rats were infused with 3.0 g/kg of D- or L-lysine (over 20 min into a caudal vein). Kidney function was evaluated by measuring several constituents of serum and urine. Structure and ultrastructure were assessed by light and electron microscopy. L-lysine caused functional changes, indicated by increases in serum urea and creatinine, and a reduction in creatinine clearance. In addition, urinary protein was increased, urine volume was slightly increased, pH was decreased and kidney weights and kidney/body weight ratios were increased. L-lysine also caused cellular necrosis of the proximal tubules. Some cells were severely injured, as indicated by altered mitochondria (often containing flocculent densities), margination of nuclear chromatin, and irregular nuclear membranes. The only effects of D-lysine were vacuolation and enlargement of cells in scattered small groups of tubules, and dilation of the interstitial space between the basilar processes of adjacent cells in some proximal tubules. Thus, L-lysine was clearly nephrotoxic, while D-lysine caused only some minor changes at comparable dosage.

NEPHROTOXIC POTENTIAL OF ACRYLONITRILE IN FISCHER-344 RATS. L. Rouilse(b), S. Chakraborti(c) and B. Tuchweber(b). Dép. méd. trav. hyg. mil. (c) and Dép. nutrition(b). Fac. méd., Université de Montréal, Québec, Canada.

In view of its very limited information, we have evaluated the acute nephrotoxic potential of acrylonitrile (ACN) in male Fischer-344 rats. ACN was given i.p. in saline to groups of 6 rats at doses 0, 10, 20, 40, 60 and 80 mg/kg. Urines were collected for 48 h and the animals were sacrificed at 24 h and 48 h after the exposure. Significant increased levels of urinary volumes, glucose as well as increased levels (not significant) of N-acetyl-D-glucosaminidase (NAG), and proteins were observed at higher dose levels of ACN at 24 h. Light microscopy showed an increase in size and number of renal lysosomes or dense bodies at 60 mg ACN per kg after 48 h. Ultrastructural studies showed increase of dense bodies and endocytotic vacuoles at the same higher dose of ACN at 48 h. Autophagic vacuole formation as well as dilatation of endoplasmic reticulum membranes was also noted at the higher dose level. Acute inhalation exposure to ACN at 200 ppm for 4 h produced increases in the urinary volumes, proteins, glucose and NAG 24 h after the exposure. These results demonstrate that ACN could produce acute nephrotoxic insult preferably at the proximal tubular region of the rat kidney. (Supported by Institut de recherche en santé et en sécurité du travail du Québec.)

ACUTE TOXICITY OF BIOPTERIN IN MICE. A.R. Comb, Young-Ja Park, and H.K. Polkens. Div. of Pharmacol. and Tox., College of Pharmacy and *Inst. for Biomed. Res., Univ. of Texas, Austin, TX 78712

Tetrahydrobipterin (BH4) is a cofactor for synthesis of many neurotransmitters and clinical interest in its use exists. We recently reported the acute toxicity of BH4 in mice (The Toxicologist 3, 239, 1986). Because of synthetic and stability considerations, and because of the probability that it can be a biological precursor to BH4, we studied the acute toxicity of bipterin (B) in BALB/c mice. The group 14-day LD50 in female mice was 879 mg/kg (95% CI, 797-970 mg/kg). Oral doses of B up to 3000 mg/kg were not toxic. As with BH4, the target organ for intoxication of B is the kidney. Kidney weights (g ± SD) were: controls, 0.245 ± 0.022; vehicle controls, 0.243 ± 0.022, and for B-treated animals, 0.329 ± 0.022 (p < 0.001). Heart and liver weights were increased, and spleen weights were reduced. Pretreatment with diethyl maleate (DEM) 0.4 ml/kg (dil. in corn oil) significantly increased the toxicity of B. The LT50's and 95% CI's were 6.3 days (4.0 - undefined) for control w + B, 700 mg/kg, 2.6 days (1.8 - 3.3) for DEM + B, 700 mg/kg, 5.5 days (3.5 - undefined) for DEM + B, 1400 mg/kg, and 2.3 days (1.6 - 2.8) for DEM + B, 750 mg/kg. Reduced glutathione (GSH) levels in the liver were reduced 2 h after B, 850 mg/kg, but had recovered by 4 h. On the other hand, the decline in GSH levels approached significance in the kidneys and was highly significant at 4 hours (g ± SD, 1.042 ± 0.167 mg/gm tissue in controls, vs. 0.584 ± 0.048 in mice given B.)

Rubratoxin B dissolved in DMSO and administered to Syrian hamsters by the i.p. route had a LD50 of 0.4 mg/kg body weight. Gross alterations consisted of congestion of the liver, spleen and kidneys. Histopathologic alterations were congestion of the spleen and congestion and mild degenerative changes of hepatocytes. The morphopathogenesis of lesions following a single i.p. LDL50 dose was evaluated. Histopathologic alterations were limited to the kidney and were characterized by renal tubular degeneration and necrosis. The renal lesions were first observed at 2 hours after administration, increasing in severity to a maximum at 20 hours after administration. Tubular regeneration was observed beginning at 24 hours and continued to the end of the test period. Clinicopathologic alterations were an increase in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which reached a maximum at 8 hours after administration and declined to control levels at the end of the test period. Renal and hepatic ultrastructural alterations were also evaluated.

INFLUENCE OF Zn ON Pb INCLUSION BODY FORMATION IN RAT KIDNEY. Peter L. Goering, Prakash Mistry, and Bruce A. Fowler, NIH, Research Triangle Park, NC.

The formation of intranuclear inclusion bodies associated with chronic Pb exposure is altered in rat kidney proximal tubule cells following concurrent in vivo exposure to metals such as Cd. This study was undertaken to examine the acute effect of Zn treatment on Pb inclusion body formation. Male CD rats were pretreated with Zn (12 mg/kg, sc) 48 and 24 hr prior to injection of Pb (3 mg/kg, iv). Kidneys were removed 20 hr later. Zn pretreatment increased both the size and number of inclusion bodies in the S, proximal tubule segment compared to the Pb-only group. X-ray microanalyses revealed that in Zn-pretreated rats, both Pb and Zn were associated with the inclusions. The mechanism of Zn effects on Pb inclusion body formation may involve the high-affinity Pb,Zn-binding proteins present in kidney cytosol. Zn displaced Pb from the isolated proteins in vitro but increased the protein-mediated uptake of Pb into cell-free kidney nuclei by 145%. These results suggest that the Pb,Zn-binding proteins are involved in Pb-induced inclusion body formation by translocating Pb into the nucleus and that other metals, such as Cd and Zn, which influence the binding of Pb to high-affinity proteins may facilitate or reduce inclusion body formation. (Supported in part by NIRA 1F32 ES-05307-01A1).


Ochratoxin A, a fungal contaminant of cereal grains, has produced renal abnormalities in swine and poultry. Groups of eighty rats per sex were administered gavage doses of 21, 70, 210 µg/kg or vehicle (corn oil) for 2 years (5 d/wk) to evaluate toxicity from chronic exposure to Ochratoxin A. After six months, renal function was decreased, and after nine months, changes in renal epithelium of high dose rats were evident (Toxicologist 4:12, 1984). Subsequent evaluation after 12 months revealed an inability of high dose rats deprived of water to concentrate urine. Rats at the 70 and 210 µg/kg level, when allowed access to water, exhibited an increase in urine volume and decrease in specific gravity preceding the 15-month interim termination. Microscopic examination revealed a multifocal and bilateral degeneration of renal epithelial cells in all high dose rats. Nephropathy and karyomegaly of renal tubular epithelium occurred in rats at the 70 mg/kg level and above. Rates of both sexes receiving 70 and 210 µg/kg terminated at either 9 or 15 months exhibited a dose-related diminution in absolute kidney weight and in kidney-to-brain weight ratios. Supported by NTP Contract No. NO1-ES-95653-01.


The chronic toxicity of Quinapril (CI-906), a non-sulfhydryl angiotensin converting enzyme inhibitor, was assessed in 52 week studies in the dog and rat. Groups of 4 beagle dogs of each sex were given 0, 10, 50 or 100 mg/kg/day orally. Gastric erosions occurred in 2 males at 100 mg/kg and 1 female at 50 mg/kg. In 2 high dose males, leukocytosis and increased serum AST, ALT and alkaline phosphatase levels reflected chronic active inflammation in the liver. Groups of 15 Wistar rats of each sex received no treatment or daily gavage doses of vehicle, 10, 50 or 100 mg/kg. Renal changes of focal tubular basophilia, hyperplasia and atrophy with thickened basement membranes and interstitial mononuclear cell infiltrates were noted at 50 or 100 mg/kg, but BUN was slightly increased in high dose males only. Reduced heart weight in all drug-treated male rats, increased plasma renin activity in high dose male rats and in drug-treated dogs of both sexes, and hypertrophy/hyperplasia of the juxtaglomerular apparatus in drug-treated animals of both species were expected pharmacological effects of this class of compounds. No clinical or pathological manifestations of toxicity occurred in either species at a dose of 10 mg/kg/day.

This study examined the functional significance of prenatal ethylene glycol exposure in rats. Pregnant rats were given 20, 40 or 60 mg/kg ETU on gestation day 11. This caused a low frequency of hydronephrosis (HN) in the offspring of the 40 and 60 mg/kg groups. Renal function of the pups was assessed for the first five weeks after birth. Medullary function was examined by measuring urine osmolality and volume after DDAVP challenge. PAH and TLA uptake by renal cortical slices in vitro, and clearance of lithium were used as indices of cortical function. Sodium, potassium and creatinine clearance were measured, along with other serum and urine parameters. Rats with HN were most severely affected in ability to produce concentrated urine. This deficit became more pronounced with maturity. Creatinine clearance (CER) was markedly decreased in HN rats, resulting in decreased clearance of other solutes. In rats without HN, body weight was decreased by ETU and uptake of PAH by renal cortical slices was increased for the first two weeks after birth. Sodium clearance, normally low in sucklings, increased in controls after weaning but remained low in ETU exposed rats. Prenatal exposure to ETU caused persistent HN and decreased medullary function in a fraction of offspring. It had minimal or no effects on renal function in rats with morphologically normal kidneys.


Weaning female rats maintained on a low calcium to phosphorus ratio diet (Ca, 0.4%; P 0.5%) rapidly develop cortico-medullary nephrocalcinosis whereas no lesion develops in male rats. In a comparative pharmacokinetic investigation of the fate of 45Ca following i.v. administration to male and female rats over the period in which the lesion develops in female animals showed no significant sex-related differences. Despite the markedly greater calcium intake by the male rats, plasma calcium levels were similar to those in females. The rapidly and slowly exchangeable calcium pools declined with age in both sexes, the relationship between the pools remaining constant during the experimental period. The rate of exchange between the pools (vE) was generally higher in female rats, whereas the rates of entry and loss from the total pool were similar. No clear trend with age was seen, the rate constants for calcium metabolism (kE and k(t)) being similar in both sexes at all times. Thus this kinetic analysis in male and female rats before, during and after the period in which females develop nephrocalcinosis, suggests that changes in calcium metabolism in the whole animal are not associated with the induction of the kidney lesion. (Supported by UK Ministry of Agriculture, Fisheries and Food)


Weaning female Sprague-Dawley rats fed a semisynthetic diet with a low calcium to phosphorus ratio (Ls: Ca 0.4%; P 0.5%) developed cortico-medullary nephrocalcinosis within 4-6 weeks, whereas animals fed a high calcium to phosphorus ratio diet (Hs: Ca 0.6%; P 0.5%) did not. Mineral balance studies during weeks 2, 4, 6 and 8 showed that, although intake and excretion of calcium was greater at all time in the HS diet animals, the "true" absorption and retention of calcium was not significantly different from that in the LS diet animals. Phosphorus intake and total excretion was also similar for both groups during this period, although urinary excretion was markedly different. Bioavailability of phosphorus decreased with age in both groups. Recovery of injected 45Ca which increased during the experimental period for both groups was greater in the HS diet group at week 2. Recovery of injected and orally administered 32P was substantially greater in the low calcium diet animals at the earlier times, but only after injection at the later time. Thus the development of nephrocalcinosis did not appear to be related to gross disturbances in calcium or phosphorus metabolism (supported by UK Ministry of Agriculture Fisheries and Food).

EFFECT OF CORN OIL VEHICLE ON SUBCHRONIC HEPATOXICITY OF CARBON TETRACHLORIDE (CT) IN CD-1 MICE. L.W. Condie, R.D. Earle, M. Robinson, and J.P. Berecz. Toxicology and Microbiology Division, HERL, USEPA, Cincinnati, OH.

This study was conducted to evaluate the effect of corn oil gavage on the subchronic hepatotoxicity of CT. Male and female CD-1 mice were gavaged with 0, 1.2, 12, and 120 mg/kg CT in either corn oil or 1% Tween-60 vehicles (10 mL/kg) once daily for five consecutive days per week for 13 weeks. The study revealed that the hepatotoxicity of CT was enhanced in the corn oil vehicle groups of mice. Increases in serum enzyme activities (alanine and aspartate aminotransferases and lactate dehydrogenase) were detected in the 12 mg/kg CT corn oil male and female groups but not in the corresponding Tween-60 groups. When comparing the serum enzyme activities in the high dose groups, there were increases in both the male and female corn oil groups as compared to the corresponding Tween-60 groups. Histopathological findings indicated that hepatocellular changes occurring during the administration of CT at the 12 mg/kg and 120 mg/kg dose levels were more frequently observed when CT was given in corn oil. The experimental findings indicate that the corn oil vehicle lowered the no-observed-adverse-effect level from CT exposure by an order of magnitude (from 12 to 1.2 mg/kg) and also enhanced the hepatotoxicity of CT in the high dose treatment groups. (This abstract does not necessarily reflect EPA policy.)

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The dose-response and time-action characteristics of CCl₄'s toxic effects were investigated in young adult C57BL/6 mice, Hartley-Albino guinea pigs, F₃432 × BXD1 (pig) rats, and Sprague-Dawley rats. One male (M) & female (F) mouse was randomly assigned to each of the following combinations of CCl₄ doses & sacrifice (sac.) times: 1.24 & 0.48 cc/kg, s.c., & 12, 24 & 48 hrs. One M & F g. pig were similarly assigned with CCl₄ doses of 0.12 & 0.24 cc/kg. Five M & F rats were randomly assigned to sac. times of 12, 24, 48, 72 & 96 hrs. post 1 cc/kg, s.c., CCl₄. Controls were administered 0.9% NaCl, s.c., & sacrificed as before. Most animals lost weight in the 24 hrs. post CCl₄ (M rats = -3.3 ± 2.1%, F rats = -3.3 ± 3.6%, X ± S.D.). Most controls gained weight. All species developed extensive & severe hepatocellular vascular degeneration, & focal or massive necrosis which was often surrounded by inflammatory cells in 24 hrs. post CCl₄. By 48 hrs. these hepatocellular alterations were resolving, mitotic figures were prominent & most of the liver parenchyma was returning to within normal morphological limits. By 96 hrs. the rats' livers appeared histomorphologically to be within normal limits. Controls did not display hepatotoxic changes.

THE ROLE OF THE BILIRUBIN COMPONENT IN MANGANESE-BILIRUBIN INDUCED CHOLESTASIS
P. Ayotte and G.L. Plag, Dép. de pharmacologie, Univ. de Montréal, Montréal, Québec, Canada, H3C 3J7.

We previously showed that an alteration of the bile canalicular membrane permeability occurs following a regimen composed sequentially of manganese (Mn) plus bilirubin (BR). This study was designed to investigate whether unconjugated or conjugated bilirubin is involved in this cholestatic interaction. Male Sprague-Dawley rats were given the following (iv): a) Mn (4.5 mg/Kg); b) unconjugated bilirubin (UCB; 25 mg/Kg); c) bilirubin ditaurate (BDT; 30 mg/Kg); d) Mn-UCB; e) Mn-BO7. Bile flow was measured and bile assayed for manganese, bilirubin (total), bile salts, cholesterol and phospholipid content. The results show: 1) the Mn-UCB treatment induced a cholestasis (60% reduction in bile flow); the Mn-BO7 treatment was ineffective; 2) bile salt excretion rate was not modified during the cholestatic phase (Mn-UCB group), as billary bile salt concentration was increased (40%). These results suggest that bilirubin involvement occurs prior to conjugation and that the MnBR-induced cholestasis is not related to a defect in bile salt excretion. This further supports our contention that a diminution of canalicular membrane permeability is likely to be the key factor in this form of experimental cholestasis. (Supported by the MRC).

EVALUATION OF THE HEPATORENAL TOXIC POTENTIAL OF 1,2-DICHLOROPROPAINE (DCP): ACUTE, SUBACUTE AND SUBCHRONIC ORAL EXPOSURES IN RATS. R. Ramakrishan, S. Murallidhara, C.K. Dallas, J.J. Kim and J.V. Bruckner, Department of Pharmacology and Toxicology, College of Pharmacy, University of Georgia, Athens, GA.

The objective of this research was to assess the oral toxicity and the renal toxicity of DCP, a DCP which was given orally in corn oil to male S-D rats daily at doses of 0, 100, 250, 500 and 1,000 mg/kg bw for 10 consecutive days. Hepatic centrilobular necrosis and increased serum enzyme levels were seen 24 hr after a single dose of 500 or 1,000 mg/kg. There were no increases over controls in serum or urinary enzyme levels after 3 and 10 days of administration of 100, 250 or 500 mg/kg. There were, however, dose-dependent increases in serum bilirubin and kidney glutathione (GSH) levels and decreases in liver GSH, cytochrome P-440 and glucose-6-phosphatase activity during this period. Substantial mortality occurred in the 1,000 mg/kg group. In the subchronic study, rats received oral doses of 0, 100, 250, 500 and 750 mg/kg. 5 days per week, for up to 12 weeks. Dose-related mortality occurred in response to 500 and 750 mg/kg. Although serum and urinary enzyme levels were not substantially affected by these or by lower doses, serum bilirubin levels were significantly elevated. Our findings indicate that both short- and long-term ingestion of DCP can cause liver injury in the rat. (Supported by U.S. FDA Coop. Agreement CR 811215).

ASSESSMENT OF THE EFFECTS OF 3,4,3',4'-TETRABROMOMOBIPHENYL (3,4-TBB) ON HEPATIC GLUTATHIONE (GSH) CONCENTRATIONS IN RATS. D. Dixon, S.D. Sleight, S.D. Aust, and K. Maruishi, Deps. of Pathol., Biochem., and Ctr. for Env. Toxicol., Michigan State University, East Lansing, MI.

Metabolism of many aromatic hydrocarbons by the hepatic microsomal monooxygenase system results in formation of epoxide intermediates. Since hepatic GSH may detoxify these electrophiles, the cytotoxicity of these metabolites may be increased during GSH depletion. 3,4-TBB is a minor component of Firemaster BP-6. It is metabolized, binds to the TCCD receptor, induces AHH activity and is hepatotoxic. Griffith's enzymatic recycling assay was used to determine the effects of 3,4-TBB on hepatic GSH levels and to determine if induction of cytochrome P-448 microsomal enzymes by pretreatment of rats with 3,4,5,3',4',5'-hexabromobiphenyl (3,4,5-2HB) would alter the effects of 3,4-TBB. Groups of 3 male 160-180 g rats were given a single oral dose of corn oil, 3,4-TBB (17 mg/kg) or 3,4,5-2HB (1 mg/kg) 24 hr before administration of 3,4-TBB. Rats were killed at 2, 4, 8, 24 or 48 hr after dosing. Hepatic GSH levels in treated rats were not significantly different from the controls (control levels ±SD: 2.55±7.8 nmol/GSH g of liver, n=3). Therefore, it is unlikely that hepatotoxicity associated with 3,4-TBB is related to GSH depletion. Supported by NIHES Grant ES-02781.
The toxicity and carcinogenicity of transplacental and/or chronic exposure to polybrominated biphenyls (PBB) were examined. Mouse dams were exposed to PBB in feed for 60 days prior to breeding and throughout breeding, gestation, and lactation. Indirect exposure of F1 pups via the dams continued until chronic dose group assignment at 8 weeks of age. F1 mice were exposed perinatally and/or chronically to 16 months of age. Following 14 months of chronic adult exposure to PBB in feed, selected mice were killed and their livers (a principle target organ) were examined by transmission electron microscopy. A spectrum of ultrastructural changes existed in the livers of mice exposed chronically to PBB. Architectural and marked hepatocellular proliferation, pleomorphism and anaplasia, were characteristic of preneoplastic and neoplastic changes. Expansive nodular regeneration, hepatocyte compression and loss of cellular adherence were frequent findings. Widespread irregularities in cell size and shape, redistribution of organelles and duct cells, and numerous aberrant organelles, and wide variations in cytoplasmic densities and organelle composition were additional features of the toxic process. (Supported by Contract N01-ES-8-2151)

Our previous studies have demonstrated that BHT is metabolized to quinone methide by a cytochrome P-450-linked monooxygenase system and that the metabolite binds to the SH group of protein. In the present study, we investigated whether BHT induces hepatic injury. Administration of BHT (500 or 1000 mg/kg; oral) to rats caused a rapid and dose dependent depletion of hepatic glutathione within 6 hrs. The content of lipid peroxide was not markedly changed by BHT. Administration of high dose of BHT (1000 mg/kg) caused elevation of SGOT and SGPT 6 hr post dosing and reached a maximum at 24 hr. Hepatic centrilobular necrosis was evident 24 hr after dosing. Pretreatment with cobaltous chloride (CC) (60mg/kg, s.c.; 2 days) completely inhibited the elevation of SGPT produced by the high-dose of BHT. Further CC caused depletion of cytochrome p-450 and induction of hepatic glutathione. The results indicate that a single large dose of BHT to rats induced an acute hepatic injury accompanied by necrosis. Furthermore, these results suggest that the hepatic damage was associated with prolonged depletion of glutathione rather than with lipid peroxidation in the liver, and that the activated metabolite of BHT rather than the parent compound induced the tissue damage.

A triazolopyrimidine with antiallergic properties was administered by gavage to adult beagle dogs (5/sec/dose) at doses of 0, 40, 200 or 400 mg/kg in equally divided daily doses for 1 month. After 21 days of dosing, there were marked increases of cholesterol, alkaline phosphatase, SGOT and SGPT in the high dose group. Dosing was subsequently discontinued in this group on Day 27. The dogs given 200 mg/kg were dosed for 32 days and they had similar elevation of the aforementioned parameters. Some recovery was seen in the dogs given 400 mg/kg 5 days after cessation of dosing. At necropsy the liver weight was increased, and grossly, irregular, rough surface and margins were seen on the livers of the 200 mg/kg or 400 mg/kg groups. Microscopically, liver changes consisted of bile duct proliferation in association with portal, periportal and interlobular fibrosis in a dose related fashion in all treated groups. Focal areas of hepatocellular necrosis and occasionally cholestasis were also seen in the 200 and 400 mg/kg groups. When comparable doses were given to rats for a month, changes observed in dogs were not seen. This study indicated that the beagle dog is a more appropriate species than the rat to monitor the potential hepatotoxic effect particularly on the biliary system for this class of compounds at comparable drug levels.

Serum ALT has long been used as a qualitative indicator of hepatocyte necrosis. However, a direct relationship between the extent of hepatocytic necrosis and elevations in serum ALT activity, particularly with values in excess of 5000 IU/L, has not been reported. In these studies the quantitative relationship between histopathological changes and increases in serum ALT was investigated in male F344 rats. Hepatocyte necrosis was induced by administration of acetaminophen (APAP) at doses of 500, 600, 750, or 1000 mg/kg (p.o.) Serum ALT activity and histopathological changes were evaluated 24 hr after treatment. Sections of liver from the left, median, and right anterior lobes were examined histologically. Hepatic necrosis was assessed in each lobe on a 6-point numerical scale of severity; these histologic scores were summed to obtain a score for each animal. Serum ALT values ranged from 40 to 50,000 IU/L. A linear, dose-related, correlation between log-transformed ALT values and histologic scores was observed (r=0.95, n=40). These data indicate that serum ALT activities can be utilized as a quantitative measure of hepatic damage over an extended range of values in the rat.
INFLUENCE OF EXTRACELLULAR CALCIUM ON THE TOXIC EFFECTS OF ALCOHOL IN THE PERFUSED RAT LIVER. O. Strubelt, H. Younes, and R. Pentz. Institute of Toxicology, Medical University of Lübeck, D-2400 Lübeck, FRG

An increase of intracellular Ca²⁺ has been implicated as a possible mechanism of hepatotoxic cell injury. Previously (Arch. Pharmacol. Suppl. 329, 91; 1983), we have shown that hepatic Ca²⁺ accumulation occurs in rats treated with allyl alcohol (AA). We now tested the role of hepatic Ca²⁺ overload in AA-induced hepatotoxicity using the isolated rat liver perfused with a medium containing Ca²⁺ in concentrations between 0 and 5 μM. At medium Ca²⁺ concentrations (1.25 or 2.5 mM), AA (0.59; 1.17; 11.7 mmol) produced the following signs of hepatotoxicity in a dose-dependent manner: a release of GOT, GPT and SDH, an increased oxygen consumption and bile flow, an increase of hepatic Ca²⁺ (30-90 %) and of hepatic malondialdehyde. Removal of Ca²⁺ from the medium prevented AA-induced hepatic Ca²⁺ accumulation but did not influence AA-induced hepatotoxicity.

High extracellular Ca²⁺ (5 μM) per se led to a threelfold increase of liver Ca²⁺ but only produced slight hepatotoxicity. AA-induced hepatotoxicity was somewhat increased at high extracellular Ca²⁺ but the effect was mainly additive. In conclusion, AA-induced hepatotoxicity injury does not depend upon an influx of Ca²⁺ from extracellular sources.

EFFLUX OF OXIDIZED (GSSG) AND REDUCED (GSH) GLUTATHIONE FROM PERFUSED LIVER FROM NAFENOPIN (W)-TREATED RATS. J. G. Conway, L.K. Garvey, D.A. Nepton, and J.A. Popp. CITT, F.I.O. Box 12137, Research Triangle Park, NC. Sponsor: R.E. Rickert

It has been hypothesized that H₂O₂ produced by peroxisomal fatty acyl-CoA oxidases may diffuse through the cytoplasm and cause DNA damage in liver cells treated with xenobiotic proliferating compounds, e.g., N. Efflux of GSSG into bile was used as an indicator of H₂O₂ and/or hydroperoxide metabolism by the cytoplasmic enzyme glutathione peroxidase. Male F-344 rats (5-200g) treated with methylcellulose vehicle (control) or 20 mg of N by gavage for 7-12 days were fasted 20 h and livers perfused in situ with Krebs-Henseleit buffer containing 50 μM ascorbate and 0.75 μg albumin. In livers from control rats, basal rates of GSSG efflux were 97 ± 14 SE (N=8) nmol/g/hr and increased to 286 ± 40 nmol/g/hr during subsequent infusion of linoleic acid (LA) (350 μM), a substrate for fatty-acyl CoA oxidase. In livers from N-treated rats, basal rates of GSSG efflux were 95 ± 15 nmol/g/hr and increased to 934 ± 142 nmol/g/hr with LA infusion. GSSG efflux in bile was similar in livers from control (174 ± 15) and N-treated (129 ± 15 nmol/g/hr) rats and decreased about 25% with LA infusion. These data are consistent with higher concentrations of H₂O₂ and/or hydroperoxides in livers from N-treated rats both in the absence and presence of exogenous LA.

GLUCOSE-6-PHOSPHATASE AS A MARKER OF FREE RADICAL INJURY IN DIFFERENT REGIONS OF THE LIVER. M.T. Smith, H.J.C. Wirt and S. Thompson, School of Public Health, University of California, Berkeley, CA

Most toxic agents damage cells of a specific type in a target organ. For example, carbon tetrachloride and bromochloromethane (BrCCL₁) mainly damage hepatocytes in the centrilobular region of the liver. The reason(s) why these compounds produce such localized damage remains unclear. Quantitative cytochemical techniques may provide the answers to such questions because biochemical activity can be directly related to tissue morphology. Using these techniques we have developed an in vitro system to look at the susceptibility of different hepatocytes to free radical-mediated damage caused by toxic agents and UV irradiation in the absence of O₂ and nutritional gradients. This system is based on measurements of glucose-6-phosphatase (G6Pase) activity in single cells located in different regions of the liver sections. We have found that G6Pase activity is selectively decreased in centrilobular hepatocytes by BrCCL₁ and cumene hydroperoxide but that UV irradiation causes an even loss throughout the liver. These results show that liver cells are equally susceptible to damage by free radicals but that metabolic activation of BrCCL₁ occurs to a far greater extent in centrilobular hepatocytes. Supported by the National Foundation for Cancer Research.

HEPATIC RESPONSE OF RATS TO BROMOBENZINE, CARBON TETRACHLORIDE AND ETHIONINE. R.R. Marenpet, R. Wilson, D.M. Lariviere, and H.J. Esber. MTF, NIEHS, Research Triangle Park, NC and E&G Mason Research Institute, Worcester, MA

Assessment of the hepatotoxic response of rats to bromobenzene (BrB), carbon tetrachloride (CCL₄) and ethionine (Eth) was made by evaluating liver histopathology and determining serum liver enzymes. A single treatment of BrB at 60, 60, and 90 mg/kg and CCL₄ at 2400, 240, and 24 mg/kg by gavage in corn oil was given. Eth was administered i.p., at 1000, 500 and 50 mg/kg in saline. Animals were bled at 24 hours or 7 days after treatment. AST, ALT, alkaline phosphatase (AP), sorbitol dehydrogenase (SDH) and 5'-Nucleotidase (5'-N) were quantitated on a Gemini centrifugal analyzer. Enzymic changes were observed only at hours post administration and only in the high dose groups. BrB and CCL₄ increased serum AST, ALT and LDH. In addition, BrB produced elevations in AP and 5'-N. Eth increased serum ALT and LDH levels only, whereas serum SDH was decreased in all treated groups. Marked to severe coagulation necrosis of perilobular hepatocytes was observed in BrB and CCL₄ treated rat livers with Eth causing only minimal single cell necrosis. The data suggest acute hepatic toxicity with high degree of repair by day 7 as manifested by histopathologic evaluation and liver enzyme analysis.
Isolated perfused rat (IPRL) and hamster (IPHRL) livers, obtained from naive animals, were exposed to dantrolene sodium (P-440, 10 μg/ml). IPRL and IPHRL preparations removed approximately 32% of the F-440 from recirculating perfusate within 1 hr. Aminodantrolene (P-405) and acetylamiodanatrolene (P-490) were the sole metabolites observed in perfusate. In a second set of experiments, rats and hamsters were dosed with 300 mg F-440/kg/day, p.o., on each of 4 consecutive days in an attempt to saturate metabolic pathways without producing overt hepatotoxicity. The striking difference in the metabolic profiles in IPRL preparations obtained from pretreated rats was the absence of F-405 indicating an apparent increase in acetylation of F-405 to form F-490. Aside from an apparent doubling of the rate of extraction of F-440 in IPHRL preparations, there were no qualitative differences in the metabolic profiles of livers obtained from naive or pretreated hamsters. The presence of histologic evidence of hepatotoxicity (triaditis) only in pretreated hamster livers may be associated with the presence of F-405 or a subsequent metabolite.

Groups of Charles River CD rats were similarly exposed for 4 weeks to daily oral doses of either 1,2,3-trichloropropene (TCP) or 1,1,2,3-tetrachloropropene (TECP) for comparative determination of subchronic toxicologic effects. Test groups consisted of 5 male and 5 female rats each and were exposed to zero, 3, 10, 30, 100 or 300 mg/kg/day TRCP or TECp by daily gavage. A vehicle (corn oil) of similar volume was used for each group. One of the females exposed to 300 mg/kg TECP (wk 2) and all males and females administered 300 mg/kg TRCP died (wk 1). Body weight and food consumption were reduced in males dosed with 100 mg/kg TRCP and dose-related reductions were observed in both sexes receiving 100 mg/kg and 300 mg/kg of TECp. There were no discernible treatment-related effects in biochemical parameters (GLU, BUN, Na+, K+, Cl−, Total Prot., ALB., SGPT, SAP) evaluated after 4 weeks of exposure. Definitive necrotic/degenerative lesions related to TECp treatment were observed in the centrilobular region of the livers of male and female rats given 300 mg/kg/day. Slight to moderate fatty changes in liver of rats, which died during study week 1 after repeated exposure to 300 mg/kg/day TECp, were probably related to treatment.

Despite only rare clinical occurrences of Dantrium® associated hepatotoxicity (DH) after excessive doses and chronic exposure and failure of acute/chronic studies involving rats, mice, dogs, rabbits, squirrel and Rhesus monkeys to predict DH, efforts have continued to identify a sensitive species. SPF Syrian Golden hamsters were administered Dantrium by gavage for 10 or 20 days (0, 35, 75, 150 and 300 mg/kg/day). Dosage-related increased urinary levels of urobilinogen and decreased body weights occurred on days 10 and/or 20 in males (M) and females (F). DH characterized by triaditis (3/5 M, 150 mg/kg/day; 1/5 F and 5/5 M, 300 mg/kg/day), bile obstruction (3/5 M, 300 mg/kg/day) and hepatocellular necrosis (2/5 M and 1/5 F, 300 mg/kg/day) occurred on day 10. Triaditis (4/5 M) and bile duct proliferation (3/5 M) occurred day 20 in high dose M only. Increased serum levels of ALT (SGPT) and SDH occurred on days 10 (F, 300 mg/kg/day; F, 75 mg/kg/day) and 20 (M, 300 mg/kg/day). Increased serum levels of LDH occurred on day 20 (M, 300 mg/kg/day). DH affects primarily M hamsters at a threshold of 150 mg/kg/day (ca. 30x the maximum recommended clinical dose) with increased incidence and severity above threshold, suggesting saturation and/or alteration of critical metabolic path(s).

To form the biologically useful heme precursor uroporphyrinogen III, uroporphyrinogen I synthase (URO-S), the 3rd enzymatic step in the heme pathway, requires the protein uroporphyrinogen III cosynthase (EC 4.2.1.75). Without the cosynthase the biologically useless isomer uroporphyrinogen I is formed. Recent reports have indicated that the cosynthase may function as a folate-binding protein thus providing tetrahydrofolate for uroporphyrinogen III synthesis. The purpose of the study was to investigate the ability of sulfamerazine to inhibit the activity of uroporphyrinogen III cosynthase in terms of its ability, in conjunction with URO-S, to direct the formation of uroporphyrinogen III. Utilizing purified preparations of URO-S and Uroporphyrinogen III synthase, sulfamerazine inhibited total uroporphyrinogen formation and decreased the ratio of uroporphyrinogen III formed to uroporphyrinogen I. Both effects were reversible with tetrahydrofolate. In vivo administration of sulfamerazine (1 g/kg) resulted in elevated hepatic levels of the uroporphyrin I isomer. These results suggest that sulfonamides may impair the biosynthesis of the uroporphyrinogen III isomer. (Supported by NIH Grant ES-02424)

Galactosamine and phalloidin hepatotoxicity in isolated hepatocytes require extracellular Ca++, whereas acetaminophen-induced bleb formation in hepatocytes is independent of it (Science 212:338 (1981); HLA 215:1257 (1981)). This raises the question whether or not extracellular Ca++ is universally required for chemical cytotoxicity. We obtained evidence that the acetaminophen hepatotoxicity as measured by the acetaminophen-induced irreversible inhibition of O2 uptake in isolated perfused rat liver either requires extracellular Ca++ or not, depending on whether the rat is pretreated with ethanol (4 g/Kg) or starved, respectively. Livers from control rats show a rapid respiratory inhibition upon infusion of acetaminophen (25 mM) and the inhibited rate remained stable for 20–30 minutes. However, livers from alcohol-pretreated or starved rats showed a biphasic respiratory inhibition by acetaminophen—the fast phase followed by a slow phase. The slow phase is abolished by removing Ca++ from the perfusate in the ethanol-pretreated rat livers but not in starved livers. This difference may result from the fact that ethanol-pretreated rats are flooded by neutrophils but perhaps not starved rats. (Supported by AAM848.)

EVIDENCE FOR POLYMORPHONUCLEAR LEUKOCYTE INVOLVEMENT IN ACETAMINOPHEN HEPATOTOXICITY. S.J., S. Ray, A. Pilaro and D. Laskin, Dept. of Pharmacol. & Toxicol., Rutgers University, Piscataway, N.J. 08854

Hemoglobin-free perfused rat livers have been utilized to study acetaminophen hepatotoxicity by monitoring the rate of hepatic O2 uptake and tissue fluorescence (366+450 nm). Pretreatment of rats with ethanol (4 g/kg) potentiated the acetaminophen-induced irreversible inhibition of hepatic O2 uptake and the decrease in tissue fluorescence. These changes were blocked by 2 mM ethanol in the perfusate. Alcohol pretreatment for 4 hrs increased the hepatic content of neutrophils from 0.5x10^9 to 6.7x10^9 cells/g liver and increased the peroxidase activity of the liver homogenate (an indicator of polys) from 2 to 20 Units/g. Alcohol treatment of rats for 1 to 6 hours also increased hepatic O2 uptake. Alcohol-induced increases in hepatic peroxidase activity and respiratory rates correlated positively (r = 0.921). These data suggest that (1) acute alcohol treatment of rats causes an infiltration of polymorphonuclear leukocytes into the liver, (2) the alcohol-induced increase in hepatic O2 uptake is due to the activation of hepatic neutrophils, and (3) acetaminophen is metabolized into its reactive intermediate by peroxidases in neutrophils leading to the inhibition of hepatic respiration. (Supported by AA-05848 and GM34310.)

INHIBITION OF MITOCNDRIONAL RESPIRATION BY ACETAMINOPHEN AS A POSSIBLE MECHANISM FOR GLUCONE DEPLETION IN THE PERFUSED RAT LIVER. R. Esterlina, and S.J. Dept of Pharmacol. Toxicol., Rutgers University, Piscataway, N.J.

The association between acetaminophen (AA) hepatocellular hepatotoxicity and hepatocellular glycolysis depletion is well known, but the mechanism(s) causing this depletion has not yet been determined. NADH-linked (state 3) respiration in isolated mitochondria can be totally inhibited by 25 mM AA. Succinate supported respiration is not affected by this dose indicating that the mitochondrial respiration is inhibited by AA at coupling site 1. Furthermore, in the isolated perfused rat liver treated with AA (25 mM), respiration can only be inhibited by 44%, most likely due to uninhhibited site 2 and 3 respiration. Isolated perfused rat livers treated with 2 - 25 mM AA showed a dose-dependent increase in lactate output and a delayed increase in glucose output. This suggests that AA-induced alterations in cellular ATP, ADP and AMP have stimulated glycolysis and glycogenolysis. The glycogenolysis can be explained by allosteric alterations of phosphorylase a activity due to lowered glucose and increased AMP levels or by an increase in cytosolic calcium secondary to the inhibition of mitochondrial respiration. A similar mechanism may be operating in vivo to stimulate glycolysis depletion in AA-treated rats. (Supported by AAM848.)

HEPATOTOXICITY OF 2,4- AND 2,6-DIMETHYLANILINE IN THE DOG AND RAT. M.L. Hardy, C.R. Short, R.W. Taylor. Department of Veterinary Physiology, Pharmacology, Toxicology, LSU School of Veterinary Medicine, Baton Rouge, LA.

The isomers 2,4- and 2,6-dimethylaniline (DMA) have been reported to produce divergent hepatic lesions in the dog and rat. These changes were investigated following a 10 day treatment period with 2,4- or 2,6-DMA. Male Beagles were dosed orally at 25 mg/kg with either 2,4- or 2,6-DMA. Male Fischer 344 rats were gavaged with 2,6- or 2,4-DMA at 25% of their respective LD50's (262.5 mg/kg; 117 mg/kg). A second and third group of rats also received phenobarbital (PB) or 3-methylcholanthen (3-MC). 2,6-DMA induced hepatic fatty degeneration in all treated dogs; 2,4-DMA caused no detectable lesions on light microscopy. In rats 2,6-DMA produced no significant liver lesions other than a decrease in minuscudal size. 2,4-DMA induced a subtle but distinct change characterized by swollen cells with voluminous homogeneous cytoplasm and segregation or clumping of cyttoplasmic substructures. A mild diffuse accumulation of fat, arranged in fine vacuoles, was visualized using Oil Red O. These results were in contrast to reported hepatocytic vaculization and bilirary hyperplasia, but were consistent with the reported hepatomegaly. Pretreatment with PB in 2,4-DMA treated rats resulted in death of 50% of animals by day 5. 3-MC pretreatment in the 2,4-DMA group produced little change.
TOXICITY OF ETHYLENE DICHLORIDE (EDC) FOLLOWING INHALATION EXPOSURE. R.J. Francovitch, P.D. Siegel, C.J. George, N.A. Schor, and W.J. George. Tulane University School of Medicine, New Orleans, LA. Sponsor: R.F. Ochillo

Toxicity of EDC was evaluated in mice following inhalation of vapors for four hour periods in a specially designed exposure chamber. A dose dependent increase in mortality to EDC was observed over a concentration range of 200-2000 ppm. Mortality at post-exposure periods of 24 or 48 hours was exacerbated by pretreatment with phenobarbital (80 mg/kg/day for 3 days). SKF-525-A pretreatment at 50 mg/kg one hour prior to EDC exposure decreased lethality as well as renal damage. The administration of diethylmaleate (DEM), a glutathione depleting agent (0.64 mg/kg), one hour prior to EDC exposure, potentiated lethality of EDC at 500 and 1000 ppm. Conversely, N-acetyl cysteine (NAC), a sulfhydryl compound administered prior to exposure to EDC, produced fewer deaths than observed with untreated animals. Acetaminophen, when administered alone at a dose of 250 mg/kg, produced no apparent toxicity, but when given prior to EDC (1000 ppm) exposure resulted in enhanced hepatotoxicity as determined by elevations in serum enzyme activities. These data indicate that lethality and associated organ damage following EDC exposure occur via a mechanism involving a depletion of hepatic GSH.

CONCENTRATION DEPENDENCE AND REVERSIBILITY OF GASTRIC LESIONS INDUCED BY REPEATED GAVAGE OF ACRYLIC MONOMERS.

Foregut hyperplastic lesions have been induced in rats by repeated gavage of local irritants such as acrylic monomers. This study examines concentration-response and reversibility of gastric lesions induced by methyl acrylamidoglycolate methyl ether (MAGME) and ethyl acrylate (EA). Two concentrations (22, 200) of MAGME at 500 and 1000 mg/kg BW or 2% ethylacrylate (EA) at 200 mg/kg BW were administered daily by gavage to 15 rats/sex for 4 weeks. Rats were sacrificed at 0, 3 or 6 weeks after termination of dosing. Ethylacrylate and MAGME induced foregut hyperplasia, hyperkeratosis and submucosal edema. These lesions were grossly visible as diffuse white thickening with EA and as raised spots with MAGME. The incidence and severity of the lesions were dose but primarily concentration dependent. With 2% MAGME at 500 mg/kg, there were no gross lesions and mild hyperplasia. Lesions were reduced after 3 weeks recovery. Only minimal microscopic changes remained after 6 weeks recovery for 2% MAGME at both 500 and 1000 mg/kg. These results indicate that rat foregut hyperplasia depends primarily on local irritant concentration not total body dose and the lesions are completely reversible.

HYPERPLASIA OF THE GASTRIC GLANDULAR EPITHELUM IN B6C3F1 MICE TREATED WITH 15(R)-15-METHYL PGE2. D.O. Brentsatter and R.C. Piper. Pathology and Toxicology Research, The Upjohn Co., Kalamazoo, MI 49001

B6C3F1 mice were exposed once per day by oral intubation to 15(R)-15-Methyl PGE2 in saline for 14 days, 90 days or 2 years. Hyperplasia of the glandular mucosa of the stomach was observed in treated mice after each period of exposure. The degree of hyperplasia was dose and time dependent and the slope of the dose-effect curve was greatest in mice exposed for 2 years. Morphologically there was a selective mucus cell hyperplasia characterized by a mild increase in acidic mucus. A unique feature of the hyperplasia was the development of epithelial cysts beneath the muscularis mucosa. The extension of hyperplastic gastric glandular epithelium beneath the muscularis mucosa is characteristic of the mouse and has been reported in gastric hyperplasia caused by a variety of stimuli in this species.
760 EVIDENCE THAT PYRROLE FORMATION IS A PATHOGENETIC STEP IN γ-DIKETONE NEUROPATHY. M.B. Center, Gy. S. Quinn, C.W. Anderson, D.C. Anthony and D.G. Graham. Duke University Medical Center, Durham, NC

The d,1 and meso diastereomers of 3,4-dimethyl-2,5-hexanedione (DMHD), 3,4-diethyl-2,5-hexanedione (DEHD), and 3,4-diisopropyl-2,5-hexanedione (DIPHD) were synthesized and purified. The rate of pyrrole formation for each diastereomer was compared to that of 2,5-hexanedione (2,5-HD), and each was administered to rats to determine relative neurotoxicity. The rate of pyrrole formation were in the order d,1 DMHD > meso DMHD > 2,5-HD > d,1 DEHD > meso DEHD > d,1 DIPHD > meso DIPHD. Cyclic voltammetry showed that the pyroles derived from DMHD, DEHD, or DIPHD autoxidized more readily than that from 2,5-HD. Daily ip injections of these compounds into rats produced hind-limb paralysis after a cumulative dose of 1.6 mmol/kg of d,1 DMHD or 5.9 mmol of meso DMHD. Paralysis was not achieved with either diastereomer of DEHD or DIPHD, although significant systemic toxicity resulted. Peripheral nerves from rats treated with the diastereomers or DMHD revealed the characteristic pattern of swellings, neurofilament-filled axons, while sections from DEHD- or DIPHD-treated rats showed no abnormalities. The strong correlation between rate of pyrrole formation and neurotoxicity is seen as evidence that this is a necessary step in the pathogenesis of γ-diketone neuropathies.

761 MECHANISMS OF PYRROLE AUTODESTRUCTION IN 2,5-HEXANEDIONE-TREATED PROTEIN. A.P. DeCaprio, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY. Sponsor: L.S. Kaminoff

Current evidence implicates pyrrole adduct formation in axonal protein as a critical step in γ-diketone neurotoxicity. This study examines aspects of pyrrole formation and the secondary antioxidative phenomena of covalent crosslinking in 2,5-hexanedione (2,5-HD)-treated protein. Pyrrole concentrations apparently decreased when pyrrolylated bovine serum albumin (pyrrole-BSA) was incubated under air, but not under N₂. Pretreatment of pyrrole-BSA with nonspecific protease increased detectable pyrrole. Crosslinking of pyrrole-BSA was pH-dependent, with increased intermolecular and intramolecular bridging at pH 7.4 and 9.5, respectively. Crosslinking was inhibited by a free radical scavenger (ascorbate) or by incubation under N₂, but was enhanced by K⁻-peroxalate, a free radical initiator. SDS-PAGE of mixtures of BSA, ribonuclelease (RN), pyrrole-BSA, and pyrrole-RN revealed that intermolecular crosslinking was mediated solely by pyrrole-pyrrole bridging. These findings demonstrate that autocatalysis following pyrrole formation proceeds via free radical mechanisms. The results also suggest that crosslinking of pyrrolylated protein in vivo may be less widespread and more specific than previously thought. (Supported by NIH/NINDS grant MH-01972)
Microtubule assembly properties were examined for tubulin purified from 2,5-hexanedione treated rats and compared with rat brain tubulin modified by in vitro reaction with 2,5-hexanedione. DEAE-purified twice cycled rat brain tubulin was incubated in 1 M sodium glutamate at 37°C for 16 h alone (in vitro control) or with 100 mM 2,5-hexanedione (in vitro treated) followed by a post-incubation cycle of warm and cold ultracentrifugations. In vitro treated rat brain tubulin assembled with a shortened nucleation phase, a more rapid elongation phase, at lower temperatures and with less inhibition by added calcium when compared with in vitro control.

Tubulin purified from the brains of rats treated for 4 weeks with 1% 2,5-hexanedione assembled with kinetic, temperature and calcium sensitivity characteristics comparable to a mixture of 90% in vitro control and 10% in vitro treated tubulin. Tubulin purified from the testes of 2,5-hexanedione treated rats also displayed altered assembly. Both in vitro and in vivo treatment with 2,5-hexanedione resulted in the induction of assembly component cross linked tubulin which was likely responsible for the altered assembly behavior.

The effect of an oral dose of 750 mg/kg TOCP on endogenous phosphorylation of specific brain cytosolic proteins and spinal cord neurofilaments has been studied in chickens following the development of delayed neurotoxicity. In vitro phosphorylation assay using [γ-32p] ATP was carried out. Proteins were resolved on one-dimensional 8% SDS-PAGE for brain proteins, 7% for neurofilaments, and two-dimensional gel electrophoresis, stained with coomassie blue, and autoradiographed. The amount of proteins, as well as the amount of 32P incorporation, were quantified by microdensitometry. TOCP administration enhanced the Ca⁺⁺-calmodulin phosphorylation of brain cytosolic proteins of Mr 52-59K, 70K and 300K by as much as 155%, 199% and 166%, respectively. Two-dimensional gel electrophoresis confirmed the 52-59K proteins as α and β tubulin and the 300K protein as MAP-2. Furthermore, TOCP treatment also increased Ca⁺⁺-calmodulin dependent phosphorylation of the three spinal cord neurofilament subunits: i.e. 70K (139%), 160K (138%) and 210K (123%), as shown by 1-D and 2-D gel electrophoresis.

Supported in part by NIOSH Grant No. OH02003.
Brain dialysis was used to determine extracellular potassium (K⁺) changes in the piriform cortex of awake rats during kainic acid (KA)-induced seizures. The impact of brain dialysis on local cerebral glucose use (LCGU) during seizures was assessed with a qualitative [14C]-2-deoxyglucose (2-DG) method. A dialysis fiber loop was stereotaxically implanted into the piriform cortex. About 24 h later the fiber was perfused (1 μl/min) with Krebs-Ringer medium. Samples were collected for 1 h each and analyzed by flame photometry for K⁺. After two control samples were collected, KA (16 mg/kg, i.p.) was injected; convulsions ensued about 1 h later. The 2-DG method was performed 3 h post KA injection. K⁺ increased from 3.4 to 4.2 μM during the first hour and returned to 3.6 μM during the third hour post KA injection. LCGU was greatly decreased adjacent to the fiber, and, in some areas more removed, was greater ipsilaterally than contralaterally to the fiber. The results indicate a loss of K⁺ homeostasis during early stages of KA-induced seizures. Also, the 2-DG method shows how brain dialysis affects the piriform cortex seizure. This may be important in interpreting data from seizure experiments using brain dialysis. Supported in part by U.S. Army DAMD17-83-C-3242.


Kainic acid (KA) is a potent neurotoxic agent that is thought to act at a specific subtype of neuronal excitatory amino acid receptors. KA neurotoxicity may be due to the opening of membrane channels which allows the accumulation of intracellular Ca²⁺. We have examined the effects of Ca²⁺ and other cations on the specific binding of [³H]KA to rat forebrain to ascertain whether Ca²⁺ channel occupancy might alter the kinetics of the receptor binding. [³H]KA binds to rat forebrain synaptosomal membranes with a dissociation constant (Kd) of 5.6 μM with a Bmax of 571 fmol/mg protein and a Hill coefficient of 0.97 for the high affinity binding site. 1 mM CaCl₂ inhibits KA binding by 50% and increases the Kd to 11.1 μM with no significant change in Bmax. [³H]KA binding is inhibited by Ca²⁺ ≥ Mn²⁺ > Sr²⁺ > Co²⁺ > Ba²⁺. These data demonstrate that physiological concentrations of Ca²⁺ modulate the kinetics of [³H]KA receptor binding and suggest an association of cation channels with the receptor, consistent with neurophysiological findings. (Supported in part by USPHS Grant NS 13584).

THE EFFECTS OF DFP ON STRAPTOPHICAL UPTAKE OF ⁴⁵Ca²⁺. J.A. Wisler and R.B. Forney. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46223.

It has been suggested that calcium channel blockers may protect against acute organophosphorus (OP) toxicity by blocking OP-induced influx of Ca²⁺ into axon terminals thereby preventing an increase in neurotransmitter release. The present study investigates the effects of DFP on ⁴⁵Ca²⁺ uptake in synaptosomes prepared by the differential centrifugation of male Long Evans rat cortex on 0.32M sucrose gradients. Triplicate aliquots of synaptosomes (2mg/ml protein) were incubated for 5 minutes at 37°C with saline or 10⁻⁴M to 10⁻⁴M DFP. The calcium ionophore A23187 and the calcium channel blocker nifedipine were used as positive and negative controls respectively. ⁴⁵Ca²⁺ uptake was assessed by incubating the synaptosomes for 10 seconds with 5mM K⁺ or 75mM K⁺. Net ⁴⁵Ca²⁺ uptake was determined as K⁺ stimulated calcium uptake (75mM K⁺) less basal uptake (5mM K⁺). No significant changes in net ⁴⁵Ca²⁺ uptake were observed in the presence of DFP. These results suggest that DFP does not increase calcium influx in cortical neurons.

Postnatal exposure to lead (Pb) results in increased tissue levels of Pb and decreased tissue concentration of the essential element zinc. Accordingly, we investigated the effects of preweaning Pb exposure on the activity of the zinc dependent enzyme alkaline phosphatase and carbonic anhydrase in kidney, liver, and brain tissue. Neonatal rats were intragastrically given 50 mg Pb acetate/Kg-BW on days 6, 9, 12, 15 & 18 postpartum. Animals were sacrificed on day 13 & 21 for determining tissue zinc & Pb concentration and activity of the two enzymes.

The concentration of Pb increased in all tissues. A decrease in liver zinc was observed on day 13, (C=50.72 ± 4.42 vs Pb=37.78 ± 0.90 ppm). Kidney zinc was decreased on day 13 (C=24.02 ± 0.95 vs Pb=18.68 ± 1.25 ppm) & 21 (C=21.05 ± 0.72 vs Pb=18.31 ± 0.38 ppm) in the Pb treated animals, whereas no effect on brain zinc was observed.

Alkaline phosphatase activity was increased only in kidney at day 13 in the Pb exposed animals. In comparison, carbonic anhydrase activity was lower in liver at day 13 and brain at day 21. The results of this study indicate that low-level Pb exposure has little impact on the activity of alkaline phosphatase and carbonic anhydrase in the developing rat.


Amiodarone hydrochloride is an antiarrhythmic agent widely used in the treatment of cardiac disorders. Several untoward effects have been recognized and neuropsychology following amiodarone therapy has recently been reported. No information is available on the mechanism of amiodarone neuropsychology in the published literature. The present studies were carried out to study the effect of amiodarone on rat brain synaptosomal ATPases in an effort to understand its mechanism of action. Na+, K+-ATPase and Mg2+-ATPase activities were inhibited by amiodarone in a concentration dependent manner with IC50 values of 50 μM and 10 μM, respectively. 3H-ouabain binding was also decreased in a concentration dependent manner with an IC50 value of 12 μM. Kinetics of 3H-ouabain binding studies revealed that amiodarone inhibition of 3H-ouabain binding is of competitive nature. K+-activated p-nitrophenyl phosphatase activity decreased to a maximum inhibition of 32% at 200 μM amiodarone. These results suggest that amiodarone induced neuropathy may be due to its interference with sodium dependent phosphorylation of Na+, K+-ATPase reaction, thereby affecting active ion transport phenomenon and also with oxidative phosphorylation, resulting in low turnover of ATP in CNS. (Supported by HL-20622.)


In the CNS Na+, K+-ATPase exists as two molecular forms with different sensitivities to the cardiac glycoside, ouabain (α+, high affinity; α−, low affinity) but hitherto undefined functions. Since we are interested in the pre- and perinatal effects of hormones and neurotoxins on the Na+, K+-ATPase isoenzymes we have first investigated their development in rat brain. Foetal (P16) and perinatal (P19-L1) enzyme activity was measured as was that in reaggregate cultures from Pb rat brain. Brain regional differences were noted perinatally where forebrain α(+) and α− activities rose more slowly than in cerebellum. In general agreement with previous work foetal whole brain (around P16) contained predominantly the α enzyme form whereas on day P19 both forms were present in equal proportions in both regions. The α(−) form showed predominance postnatally at L1 mirroring the adult profile. In reaggregate cultures (from P16 brain) development of the α form relative to α(−) was greatly retarded by the absence of serum factors. Serum-supplemented aggregates at 14 DIV displayed adult proportions of the two isoenzymes. The differential development of the two Na+, K+-ATPase forms in culture provides a useful model to study xenobiotic effects on function.

SENSITIVITY OF SYNAPTOSOMAL Ca2+-ATPase TO CYCLODIENE PESTICIDES. B. D. Mehrotra, S. R. McIlroy and D. Desai, Department of Chemistry, Togoloo College, Togoloo, MS. 38174, Department of Neurology, Univ. Miss. Med. Ctr., Jackson, MS 32126

Cyclodiene pesticides such as aldrin (A), dieldrin (D), endrin (E) are potent neurotox-icants. There have been some reports showing that these pesticides produce neurotoxicity by altering Ca2+ regulated events. The present work was initiated to study the effect of these compounds on Ca2+ pump activity in rat brain synaptosomes (RBS). RBS were prepared by ficoll-sucrose gradient. The in vitro effects of these pesticides on RBS Ca2+-ATPase were assessed by preincubation. All three compounds inhibited Ca2+-ATPase in vitro. A 50% inhibition occurred at 10, 73, and 100 μM of A, D and E respectively. Reduction of Ca2+-ATPase in RBS by these pesticides suggests that they may exert their neurotoxicity by altering Ca2+ pump activity. (Supported by NTH/MBRGS Grant No. RR-0811).
Activation of muscarinic cholinergeric receptors (MR) leads to several biochemical events including an increased turnover of phosphoinositides (PI). In this study we have investigated whether repeated administration of the organophosphorus insecticide disulfoton (DS) would affect PI metabolism in rat brain. Basal and carbachol-stimulated PI metabolism were measured in cerebral cortex slices, by measuring the accumulation of inositol phosphates (IP) in the presence of lithium. In control animals carbachol caused a 60% increase in IP accumulation with an EC50 of 100 μM. Maximal effect occurred with a LiCl concentration of 7.5 mM and required the presence of calcium. Administration of DS for 10 d (2 mg/kg/d, po), decreased the number of MR in cortex from 1.1 to 0.7 pmol/mg of protein without changing the affinity of the receptors (both measured by binding of [1H]QNB). Acetycholinesterase was inhibited by 85%. Basal IP accumulation was unchanged in DS-treated rats, while carbachol-stimulated IP accumulation decreased by 18%. No changes of noradrenergic-stimulated IP formation and of alpha-1-adrenoceptors were present in cortices from DS-treated rats. A recovery study indicated that MR binding and carbachol-stimulated IP accumulation recovered within 2 weeks after the end of the treatment. (Supported in part by ES-03424.)

Inhibition of Protein Synthesis by Trime thyltin
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Trime thyltin (TMT) is a potent animal and human neurotoxin which produces neuronal lesions particularly in the limbic system. Morphological studies have suggested that protein synthesis (PS) may be affected by TMT (Ann. J. Pathol. 97, 237, 1979). We have investigated whether TMT inhibits PS (measured as incorporation of [1H]valine into TCA-precipitable material) in mice, after ip administration. TMT (30mg/kg) decreased PS in brain by 47%, 1 h after administration. However, it also caused a profound hypothermic effect. When mice were kept at 35°C to prevent hypothermia, TMT still decreased PS in brain by 20%. In further experiments we measured PS 24 and 48 h after a single injection of TMT. In brain, PS was reduced by 23% at 24 h and 19% at 48 h. On the other hand, in liver PS was decreased by 27% at 24 h but did not differ from controls 48 h after TMT administration. A regional study in brain indicated that TMT decreased TMT in the hippocampus and in the cerebral cortex, but not in cerebel um, 24 h after administration. TMT also inhibited PS in vitro, with an IC50 of 100μM. Neither SnCl2 nor Dl- or mono-methyltin had any effect on PS in vitro. These results suggest that disruption of the protein synthetic process by TMT might be involved in its neurotoxic action. (Supported in part by a grant from the Dept. of Environ. Health, Univ. of Washington.)

Effect of ClO2 on Cell Proliferation and Protein Synthesis in Developing Rat Brain

Since chlorination of drinking water has been shown to result in production of toxic by-products (e.g. chloroform), chlorine dioxide (ClO2) is being considered as an alternative drinking water disinfectant. Previous studies (Taylor and Pfohl (1985), Orme et al. (1985)) showed that ClO2 retards behavior and forebrain growth and decreases thyroxine levels in rats. We have examined the effect of direct ClO2 exposure on brain regional cell proliferation and protein synthesis in developing rats. Animal weights were decreased at postnatal days 21 and 35, while forebrain weights were decreased at day 35 after ClO2 treatment. Cerebellar weights of treated animals were unchanged. Incorporation of [3H]-thymidine into DNA was decreased in the forebrain at day 21. Otherwise, no significant changes in [3H]-thymidine and [14C]leucine incorporation into DNA and protein, respectively, were seen in forebrain or cerebellum relative to controls. No significant change in serum thyroxine levels was evident. This result, along with the lack of a transient depression in cerebellar cell acquisition characteristic of hypothyroid developing rats suggests that changes measured in brain maturation parameters are not the result of an antithyroid effect of ClO2. This abstract does not necessarily reflect EPA policy.


TMT & TET induced biochemical changes in the rat CNS that were studied 24h after daily dosing of TMT 4 mg/kg, TET 20mg/kg or one dose of TET 10mg/kg. Brain vs., lysozyme enzyme (LE), DNA, CNT, AChE & Chat levels, muscimol and QNB binding were assayed. TMT & TET induced LE changes that were most marked in the hippocampus with TMT & in medulla and cortex with TET. TMT induced decreases in hippocampal DNA, AChE & Chat levels. DNA decreased weeks 2-5, whereas the decrease in Chat & AChE was most marked at week 2, returning to normal by week 5. A mg/kg dose of TMT decreased by 40% cerebellar muscimol binding within 2 days. This decrease was maintained throughout the study. A biphasic response in CNP activity was seen following one 10mg/kg dose of TET—a marked increase at week 2 in the medulla and a marked decrease in the forebrain at weeks 3-5. The results are discussed in relation to the known morphological CNS effects induced by the two organo-metals.
779 DELAYED NEUROTOXICITY OF TRIPHENYL PHOSPHITE (TPP) IN THE HEN. C. O. Carrington and M. B. Abou-Dagga. Duke University Medical Center Durham, NC 27710

Many organophosphorous compounds are able to induce a neuropathy one to two weeks after a single exposure known as OPIDN. While many organic phosphoric acid esters have been tested for OPIDN, very few phosphorous acid esters (organophosphites) have been examined. Although (TPP) has been found to produce a neuropathy in several species, some differences from what is usually known as OPIDN have been noted. Consequently, we conducted a study on the effect of a subcutaneous dose of 1000 mg/kg TPP on the adult hen, the test animal for OPIDN. TPP induced a clinical and neuropathological condition which is typical of OPIDN, although the signs tended to develop a few days earlier. TPP was an effective inhibitor of neurotoxic esterase (NTE), the putative target site for OPIDN, both in vivo and in vitro. In vivo, over 70% inhibition of NTE was observed in brain and over 95% inhibition in sciatic nerve. In vitro, TPP had a K_i for NTE of 1.1 x 10^5 which is close to that of the most potent organophosphate inhibitors. Although the administration of phenylmethylsulfonyl fluoride (PMSF; 30 mg/kg administered 24 hours prior) prevented DFP [1.7 mg/Kg s.c.] induced delayed neurotoxicity, PMSF did not protect any of eight animals dosed with TPP. Supported by NIH grant no. HO2003.

780 HEAT INACTIVATION OF PHENYL VALERATE HYDROLASES FROM HEN AND RAT BRAIN. E. Reiner, C.S. Davis, B.W. Schwab, L.M. Schopfer*, and R.J. Richardson. Toxicology Program and Department of Biological Chemistry*, University of Michigan, Ann Arbor, MI.

Previous work in our laboratory indicated that hen brain neurotoxic esterase (NTE) is more thermostable than rat brain NTE and that the kinetics of heat inactivation is biphasic in both cases. In order to determine whether NTE activity is comprised of 2 enzymes whose relative amounts may be species dependent, heat inactivation plots were determined for all of the phenyl valerate hydrolases including total (PVAse), NTE, and inhibitor-resistant (IRE) activities in homogenates of hen and rat brain at 45, 50, 55 and 60°C. Each activity for each species and temperature exhibited biphasic first-order inactivation kinetics. Apparent rate constants and corresponding relative amounts of activity were calculated for the fast (k_f; a_f) and slow (k_s; a_s) components for each plot. For a given preparation and temperature, the activities of PVAse, NTE and IRE decreased at nearly the same rate with similar k_f and a_f values and k_s = 50%. The intercepts a_f and k_f were a function of temperature. These results do not indicate the presence of 2 forms of NTE, but reflect heat-induced structural changes common to all PVAse in both rat and hen which lead to loss of activity. This work was supported, in part, by NIH research grants ES 02770 and ES 01611.

781 THE EFFECTS OF SOMAN AND SARIN ON PHOSPHATIDYLCHOLINE DISTRIBUTION IN THE ELECTROPLAX FROM ELECTROPHORUS ELECTRICUS. C. Supernovich and P. Rosenberg. Univ. of CT, School of Pharmacy, Section of Pharmacology and Toxicology, Storrs, CT.

Effects of organophosphates not due to cholinesterase (CHE) inhibition may be caused by alterations in cellular membranes. Phosphatidylcholine exchange protein (PCEP) was used to monitor phosphatidylcholine(PC) distribution after electric eels were bathed in organophosphate for 1 week. Single cells were then incubated with PCP and 14C-PC small unilamellar vesicles for 105 minutes at 37°C. PCP catalyzes exchange of 14C-PC from the vesicles to the outer leaflet of the plasma membrane phospholipid bilayer. CHE was inhibited 30% to 45% after 1 week exposure to 10-9m soman and sarin. PC labeling (control 26%) was decreased after 1 week exposure to sarin (13%) which then returned to control levels 2 weeks post sarin. Labeling increased after 1 week exposure to soman (from 23% to 42%) which increased further (54%) in the 2 week post soman period. These results correlate with effects of in vitro (10-4M) soman where CHE was inhibited 100% and PC labeling was increased from 20% to 32%. Soman and sarin do not increase permeability to PCP, suggesting that they alter the percentage of PC in the outer bilayer of the unmodified plasma membrane of the electroplax. (Supported by Department of Army, DAMD 17-82-C-2086)

782 SPINAL CORD CHOLINESTERASE ACTIVITY PREDICTS BRAIN REGIONAL CHOLINESTERASE ACTIVITY IN SOMAN (GD)-POISONED RATS. V. Jimerson. T.-M. Shih, M. Pannella, T. Kovnik, O. Smith, F. Cowan, and R. Mallman. USAMRICD, APG, MD 21010 and University of North Carolina, Chapel Hill, NC 27514.

Concurrent measurement of cholinesterase activity (ChE) and acetylcholine concentrations (ACh) in discrete rat brain regions following GD poisoning is not feasible, since ACh measurements require total enzymatic inactivation using head-focused microwave. The present study investigated whether measuring ChE in distal spinal cord (SC) could be used to predict brain regional ChE [in brainstem (B), cortex (C), hippocampus (H), midbrain (M), cerebellum (CB), and striatum (S)]. Male rats (200-275g) were injected s.c. with 0.6 LD50 of GD, evaluated for toxic signs, and killed (without microwave) subsequently at selected times. ChE was assayed in SC and brain regions by a colorimetric method. Toxic signs were apparent in 591 (41/69) of the animals. The correlations between SC and brain regional ChEs were analyzed. For sign-free animals, the coefficients of correlation (r) were .95, .90, .93, .94, and .83 in B, C, H, M, CB, and S. For all animals the r's were .98, .94, .93, .96, .96, and .94 in the same respective areas. Thus, in rats poisoned with u. 0.6 LD50 of GD, measuring SC ChE offers a means of predicting GD-induced depression of brain regional ChE when direct measurement of ChE is not feasible.

Organicfluorophosphate hydrolases (DFPases) have been previously thought of as consisting of two basic types: Mazur-type and Squid-type. Recently, a Mazur-type activity was discovered in Tetrahymena thermophila. Tetrahymena-DFPase activity was purified using a Sephacryl S-200 column. One ml fractions were collected and examined for hydrolysis of DFP and soman using a F' electrode in a buffered salt solution, pH 7.2, at 30°C. 1 mM MnCl₂ was added to the buffer to examine possible stimulation. Five DFPases have been identified at present. Molecular weights range from 87,000 to 96,000. None of the enzymes corresponds to the Mazur-type or Squid-type DFPases already characterized. For example, DFPase-5 hydrolyzes DFP faster than soman, but is stimulated by soman hydrolysis by the addition of Mn²⁺. DFPase-2 is the most stable of the 3 enzymes ranging from 80,000 - 72,000 molecular weight. DFPase-1 is labile at 35°C. Since organic fluorphosphates are hydrolyzed by T. thermophila, perhaps we are sampling parts of the metabolic system of these materials.


Aluminum (Al) has been implicated as the etiological agent in several clinical disorders including dialysis encephalopathy and osteomalacia. In animal studies, toxic amounts of Al-NTA caused liver and kidney necrosis, brain atrophy and death. The mechanism(s) of AI toxicity in the CNS is still not known. To further investigate the effects of AI on the CNS, 2 groups of weanling rats (12/group) were injected with either 8mg NTA/kg (control) or 2mg Al-NTA/kg (AI-treated). Six rats from each group were sacrificed by the near-freezing method after 1 and 3 weeks of daily injection. The dissected brain parts (cerebral cortex (CC), hippocampus (HI) and striatum (ST)) were prepared for the determination of cAMP, biogenic amine and AI levels. Significant increases were observed in cAMP content in CC and HI of the 1-week AI-treated group while the level of dopaminergic cell bodies was decreased in ST. As for the 3-week Al-treated rats, serotonin was significantly increased in HI but decreased in CC. In addition, midbrain level in CC of Al-treated rats was significantly higher than the control rats. It appears that chronic treatment of rats with Al could possibly have effects on neurotransmitter synthesis, release, uptake or breakdown. AI could hypothetically affect on either CA specificity, breakdown or both.

THE USE OF CHOLINESTERASE INHIBITORS, ETHOPROPAZINE AND Bw284c51, TO DISSOCIATE THE ACTIVITIES OF ACETYLCHOLINESTERASE (AChE) AND BUMETALCHOLINESTERASE (BChE) OF MUSCARINIC MUSCLE OF BUFALO MARINUS. K. Bu and R.F. Ochillo, Laboratories of Pharmacology and Toxicology, Xavier University of Louisiana, New Orleans, LA 70125.

We previously characterized cholinesterase activities in the muscular muscle of Bufo marinus (Pharmacologist 26: 224, 1983). Since cholinesterase activity is due to the combined activities of AChE and BChE and, because separation of the two enzymes is difficult, we have used specific inhibitors to inhibit one enzyme while studying the activities of the other. Ethopropazine (10^{-5}M) is a specific inhibitor of BChE and Bw284c51 (10^{-5}M) is a specific inhibitor of AChE. Each inhibitor was preincubated with enzyme preparation for 15 min before adding the substrate, acetylcholine (ACh). The specific activities of each enzyme were determined by spectrophotometric techniques. AChE was characterized by excess substrate inhibition (bell shaped curve) while BChE had a characteristic sigmoid curve. The Km for hydrolysis of ACh by AChE was 0.67x10^{-4}M which was much smaller than the corresponding Km for BChE(3.57x10^{-3}M). According to our data, selective inhibitors of AChE and BChE can be used successfully to study the activities of each of the enzymes in a mixture without prior separation. (Supported by Grant 3506 RRO8008 from NIH).

EFFECTS OF MERCURY (Hg^{2+}) ON NEUROTRANSMITTER RELEASE (NT) IN RAT STRIATAL SYNAPTOSOMES & FROG SKELETAL NEUROMUSCULAR JUNCTION (NMJ). M. Hate, G. Cooper and D. Minnema, Dept. Environ. Hlth., U. Cinti, Cinti, OH. Sponsor: P.B. Hammond.

Electrophysiological studies have shown that Hg in vitro produces an increase in both spontaneous and action-potential-evoked NT; however, the mechanism of action remains unclear. The present study examines the effect of Hg in vitro on dopamine (DA) release from rat striatal synaptosomes and on acetylcholine (ACh) release in the frog NMJ. Purified synaptosomes preloaded with 3H-DA were superfused with physiological HEPES buffer. Hg (3 μM) caused a dose-dependent increase in spontaneous DA release that was not substantially influenced by extracellular Ca²⁺. The total amount of DA release evoked by a 1-sec pulse of 61 μM K⁺ (depolarization) was increased in the presence of 3 μM Hg, which could be accounted for by the Hg-induced increase in spontaneous release. In the frog NMJ bathed in Ca²⁺-free Ringer's solution, tetanic nerve stimulation during Hg exposure greatly accelerated the increase in spontaneous ACh release whereas the increase in ACh release was depressed or delayed in Ringer solutions in which Na⁺ was entirely or partially replaced by Li⁺ or sucrose. These results suggest that Hg has negligible effects on Ca²⁺ channels, but rather, increases the probability of NT. These effects of Hg on release might be a consequence of Na⁺, K⁺-ATPase inhibition. (Supported by ES-01390).
Carbon disulfide (CS₂), tetraethyl lead (Et₄Pb), tetraethyl tin (Et₄Sn), dibutylthreitol (DBT), and gossypol acetic acid (GAA), significantly decreased brain norepinephrine (NE) in rats. The central dopamine increased after IP administration of CS₂, Et₄Pb, and GAA, but decreased after DBT and GAA. The brain serotonin decreased only after DBT. Two doses of DBT decreased the NE longer than 24 hours but did not increase dopamine. L-DOPA, SC, given with DBT delayed the decrease in NE by 24 hours. The similar behavioral and autonomic effects of each of these compounds suggests a central sympatholytic effect and an antipsychotic type of sedation and rigidity. A possible mechanism is reversible inhibition of dopamine beta hydroxylase through the reduction of the copper ion of the enzyme. Each of these reducing agents, together with the boranes previously studied, have similar behavioral and autonomic effects and a common effect on NE concentration, suggesting that the agents act through a physicochemical property rather than by combination with a cellular component. These data have implications to the toxicity of the single agents. They also provide an index of activity previously lacking, of systemic antioxidant effect.

Reduced PRO levels have been correlated with feces toxicity in cattle. Reduced levels of PRO and dihydroxyphenyl acetic (DOPAC) and homovanillic (HVA) acids occur in rats administered extracts from toxic feces seed. To study preventive measures for this ergot-like disorder in cattle, bromoergokryptine (CB-154 = DA agonist) was used as a model compound in rats. Animals were administered (p) MET (DA antagonist) and QP (SHT agonist) prior to CB-154. Rats were anesthetized (ether), bled (for PRO analyses), and whole brains removed for DA, DOPAC, HVA, 5HT, 5-hydroxyindoleacetic acid (5-HIAA), and norepinephrine (NEpi) analyses. The combination of MET-QP more effectively inhibited CB-154 suppression of PRO (P<0.05) than the individual drugs. CB-154 had no apparent effects on the measured catecholamines. The combined MET-QP effects significantly (P<0.05 increased (43%) the HVA/DA ratio and decreased (26%) the 5-HIAA/5HT ratio. DA, 5HT and NEpi levels were unchanged. These results suggest that MET-QP thwarts CB-154 suppression of PRO by increasing DA turnover (PRO inhibiting factor) and slowing 5HT turnover (PRO stimulating factor). Also, the data suggest that MET-QP may be useful for combating feces toxicity.

Regional concentrations of biogenic amines and their metabolites have been used as neurotoxicology endpoints in research in our laboratory. To our knowledge, the daily variations of these amines and their metabolites have not been studied. Four male CD-1 mice were decapitated and the brains dissected every four hours for 48 hours. Perchloric acid extracts from the hypothalamus, medulla oblongata, cerebellum, midbrain, corpus striatum, and cortex were analyzed on a high performance liquid chromatograph with an electrochemical analyzer for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), serotonin (5HT), and 5-hydroxyindole-3-acetic acid (5-HIAA). Rhythmic alterations were seen in the concentrations of the following over time: VMA, NE, DA, DOPAC, 5HT, and 5-HIAA in the hypothalamus and striatum; DA, 5HT, and 5-HIAA in the medulla oblongata; DA, DOPAC, HVA, and 5-HIAA in the cortex and midbrain; DA, DOPAC, HVA, and 5-HIAA in the cortex. Concurrent rhythmic patterns were seen in most of the remaining amines and metabolites in each region. These data will allow for more accurate interpretations of results using these endpoints.

High doses of ionizing radiation produces marked hypotension, often accompanied by decreased regional cerebral blood flow (rCBF). Humoral mediators such as serotonin (NT) and histamine (HA) have been suspects in radiation-induced hypotension and decreased rCBF. To investigate biochemical evidence of humoral mediation the postradiation plasma levels of NT and HA were determined simultaneously with hippocampal rCBF (via hydrogen clearance) and systemic blood pressure (BP). Compared to controls, primates exposed to 100 Gy, whole-body, gamma radiation exhibited a 77% decline in BP, a 68% decrease in rCBF, a 20% rise in NT, and a 150% increase in HA within 10 min postradiation. The HA returned to preradiation levels by 45 min and the NT by 15 min. However, the NT level steadily increased again by 16% in the following 45 min. The BP and rCBF never returned to preradiation levels. These findings do not confirm a causative relationship between the postradiation release of NT and HA and the decrease in BP and rCBF, but they do indicate a definite temporal relationship.
NEUROTOXICITY OF A NEW STRUCTURAL ANALOG OF MPTP

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) produces a selective destruction of nigrostriatal dopaminergic neurons in several species. We have synthesized an analog of MPTP, 1-methyl-4-(2-methylphenyl)-1,2,3,6-tetrahydropyridine (2′-CH3-MPTP) which produces even more dopaminergic toxicity in mice than MPTP itself. Under conditions in which MPTP was relatively ineffective in C57 black mice (i.e., 2 injections per day of 0.113 mmol/kg at an interval of 6 hrs for 2 days), 2′-CH3-MPTP caused large decrements in the neostriatal content of dopamine (DA) and its metabolites and a corresponding decrement in the capacity of neostriatal synaptic transporters to take up 3H-DA. 2′-CH3-MPTP also produced a virtually complete loss of neurons in the zona compacta of the substantia nigra. 2′-CH3-MPTP is oxidized in mouse brain mitochondrial preparations by monoamine oxidase B at 2 to 5x the rate of MPTP. As with MPTP, the potent DA uptake inhibitor maxima and the monoamine oxidase inhibitor deprenyl, protect mice against the toxic effects of 2′-CH3-MPTP. Mice treated with 2′-CH3-MPTP became aphagic, adipic, and akinetic and demonstrated gait abnormalities and tremor. 2′-CH3-MPTP should prove to be a valuable research tool.

INTERACTIVE EFFECTS OF LEAD (Pb2+) AND 6-AMINO-L-EVULINIC ACID (ALA) IN VITRO ON SYNAPTOSOMAL 1-AMINOBRUTYRIC ACID (GABA) RELEASE. D.J. Mannema and I.A. Michaelson, Dept. Envr. Hlth., Univ. Cinti., Cinti., OH. Sponsor: P.B. Hammond

Previous studies have shown a decrease in GABA release from synaptosomes prepared from Pb-exposed rats. It has been suggested that the decrease in GABA release is an indirect Pb effect mediated through increases in ALA accompanying chronic Pb exposure. This suggestion is based on reports that Pb added in vitro has no effect on synaptosomal GABA release, and that ALA added in vitro reduced depolarization-evoked release. In the present study, rat cortical synaptosomes preloaded with 3H-GABA were superfused (2 ml/min) with a physiological HEPES buffer. Superfusate fractions were collected at 15 sec intervals. Addition of Pb (1-30 μM) to the buffer resulted in a dose-dependent increase in spontaneous release. Pb (3 and 10 μM) reduced GABA release evoked by a 1-sec exposure to 61 μM K+. ALA, at relatively high concentrations (30-300 μM), did not alter high-K+ depolarization-evoked release but did slightly increase spontaneous GABA release. The effect of a combination of 3 μM Pb and 100 μM ALA on GABA release was additive. These data show that Pb can directly produce alterations in GABA release evoked by increased ALA produced de novo following chronic Pb exposure on GABA release has still to be defined. (Supported by ES-03399)


Previous in vivo behavioral data has suggested differential effects of Type I and II pyrethroids on the GABA receptor-ionophore complex. The present study was conducted to determine the interactions of Type I and II pyrethroids with the three major binding sites on the GABA receptor-lonophore complex using [3H]-flunitrazepam (FLU), [3H]-muscimol (MUS), and [35S]t-butylcyclophosphorochlorinate (TBPS) binding. Competition experiments with [3H]-FLU and [3H]-MUS indicate a lack of competition for binding by the pyrethroids. Type I pyrethroids failed to displace [3H]-FLU at concentrations as high as 50 μM. Type II pyrethroids inhibited [35S]-TBPS binding to rat brain synaptosomes with IC50 values ranging from 5 - 10 μM. The data presented here indicate an interaction of Type II pyrethroids with the GABA receptor-ionophore complex. The lack of competition with the other two major binding sites (i.e., GABA and benzodiazepine) suggest that the interaction of the Type II pyrethroids with the GABA receptor-ionophore complex is restricted to the domain of the TBPS/picrotoxin binding site(s).

RELATIONSHIP BETWEEN cAMP-DEPENDENT PROTEIN KINASE AND SYNAPTOSOMAL UPTAKE OF GABA IN MOUSE BRAIN REGIONS. EFFECT OF MEBUX. J. F. Lenoch, A.K. Charles and C. Timchalk, Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, N.Y.

Recent evidence indicates that protein kinase-mediated phosphorylation is involved in brain neurotransmitter functions. Since organochlorine pesticides like kepone and mirex (MX) are known to modify neuronal functions, effects of MX on synaptosomal cAMP-dependent protein kinase (cAMP-PK) activity and GABA uptake were investigated as a function of MX concentration (0-1.5 mM) in mouse cerebral cortex (CC), brain stem (BS) and cerebellum (CB). Although the -cAMP and +cAMP activities were stimulated by MX in CC, across-PK and cAMP-PK ratio showed no apparent difference from control. While BS and CB exhibited significantly higher cAMP-PK base level activity than CC (3.5 and 5.3-fold, respectively), only BS FK ratios were enhanced by MX. In contrast, activity ratios in BS were consistently lower than CC and BS. Conversely, GABA uptake by both BS and CB regions was increased at all concentrations of MX compared to CC which showed no major changes in GABA uptake pattern except an increase at lower concentrations (50-200 μM). The results reveal that although GABA uptake and synaptosomal phosphorylation activity by cAMP-PK were modified by MX at different levels in a region-specific manner, there was no direct correlation between the changes in cAMP-PK ratios and GABA uptake in these brain regions.
The dopamine and glutamate theories are potential mechanisms in schizophrenia and amphetamine induced psychosis. Alterations in glutamate tissue levels have been observed. Recently it has become possible to measure the uptake capability of synaptic vesicles for glutamate (Glu), and approach directly the effects these agents have upon the storage mechanism. Cortical synaptic vesicles were prepared from rats chronically administered amphetamine, haloperidol, or both agents. Uptake from the amphetamine treated group was increased 47% over control values. In contrast, haloperidol treatment did not affect vesicular uptake or reverse amphetamine's effect when both agents were administered together. As a control, direct effects of amphetamine and several antipsychotic agents upon vesicular Glu uptake were evaluated in vitro. Amphetamine was found to increase Glu uptake above control values in a concentration dependent manner (11% @ 100 μM). In contrast, antipsychotic's generally decreased Glu uptake. IC₅₀'s ranged from approximately 217 μM for Chloropromazine to no effect for Haloperidol. This suggests that increases in tissue glutamate levels by amphetamine may be caused by alterations in vesicular storage which are not mediated by dopaminergic function.

Lindane (LND), a chlorinated hydrocarbon, is used in the treatment of ectoparasite infections in humans, produces toxicity which takes the form of convulsive activity. Mice were treated with 80 mg/kg LND and challenged 1 or 24 hrs after LND with convulsants having different mechanisms of action: pentylentetrazol (PTZ), 3-mercaptopropionyl acid (MPA), bicuculline (BCG), pilocarpine (PTX), or strychnine (STR). Convulsions were scored using a seizure severity rating scale. GABA turnover was also estimated 1 and 24 hrs after LND by measuring GABA concentrations in the cortex, striatum, hippocampus and cerebellum fluorometrically. 30 and 60 minutes after inhibition of GABA metabolism by AODA. The results of the behavioral experiments and the potentiation of all convulsants by a 1 hr LND pretreatment. In addition, the 24 hr pretreatment decreased the effects of PTZ or PTX while having no effect on MPA, BCG, or STR. LND had no effect on GABA concentrations or turnover at either of these time points. The differential response of certain convulsants 1 day after LND in conjunction with a lack of effect of LND on GABA turnover suggests modulation of a specific site in the CNS. The mechanism of this effect is undergoing further investigation.

Lack of induction of brain synaptic somal release of acetylcholine and glutamate by chlorinated alicyclic compounds. J.E. Chambers, Mississippi State University, Mississippi State MS.

Chlorinated alicyclic compounds, such as the insecticide dieldrin, elicit hyperexcitability and, in electrophysiological experiments cause enhanced release of acetylcholine from peripheral nerve terminals. These experiments investigated the in vitro release of [3H]acetylcholine or [3H]glutamate from synaptosomes isolated from mosquito brain or rat cerebral cortex. Both spontaneous release and release evoked by elevated K- levels were studied in crude and purified synaptosomal preparations. Tests were performed in the presence or absence of Ca++. The following compounds were studied at various concentrations up to 10-6: dieldrin, endrin, heptachlor epoxide, heptachlor, γ-chlordane, α-chlordane, aldrin, aldrin trans-diol, lindane, isodrin, chlordene and ketocendrin. None of the compounds enhanced the spontaneous or evoked release of either neurotransmitter in either species. Despite contradictions in the literature, it appears that these compounds do not elicit symptoms of poisoning by enhancing excitatory neurotransmitter activity in brain cholinergic or glutaminergic nerve terminals.


Clonidine (CLON) and its structural analogue, lofexidine (LOF) are potent adrenergic alpha-2 agonists. Currently, CLON is a widely used antihypertensive drug and LOF is an investigational compound being readied for the market place. Recently, CLON and LOF have been shown to alter the opiate withdrawal syndrome in rats and CLON has been suggested as a treatment modality for pregnant women undergoing opiate detoxification. Orinitine decarboxylase (ODC) activity, a measure of polyamine synthesis capacity, was measured in rat pups exposed to either 0, 0.15 or 0.60 mg/kg/day CLON or 0, 0.60 or 2.4 mg/kg/day LOF s.c. on days 8-20 of gestation. On postnatal day (PND) 1, litters were culled to 8 pups, and brains from 8 male and 8 female randomly culled pups were collected for neurochemical analysis. PND 1 body weight was reduced in a dose-dependent fashion, with LOF reductions twice that of CLON. Whole brain ODC activity reflected these weight reductions; ODC activity was significantly reduced in both sexes after LOF but not CLON. Subsequent behavioral evaluation of offspring produced no evidence for deficits, despite the early drug effect on body weight and brain ODC.
The role of inhibition of Cyt Ox activity in the etiology of acute cyanide poisoning remains unclear. To further study this phenomenon, we compared the effects of CN, in vitro, on brain MitoOx activity and respiration in MitoOx activity. Brain MitoOx, from male CD-1 mice, were isolated by differential centrifugation. 4,4' dN was added to all of MitoOx suspensions such that the final concentrations were 10^{-6} to 10^{-3} M. Cyt Ox activity was determined spectrophotometrically and MitoOx respiratory activity polarographically in the presence (state 3) and the absence (state 4) of ADP. Cyt Ox activity was inhibited in a linear fashion, the IC50 was approximately 4 x 10^{-3} M. States 3 and 4 respiratory rates were slightly inhibited at up to 10^{-5} M and 80% inhibited at 10^{-3} M. Thus, large effects on respiration required greater than 50% inhibition of Cyt Ox activity. This suggests that 50% of MitoOx Cyt Ox activity may be functional reserves. Additional studies in vivo are required to test this further. (JCP was supported by a Staufer Chemical Co. Fellowship in Toxicology.)

MCA produces death in mice within 12 hr. after acute oral or dermal exposure. Surviving mice may exhibit a myotonia of the front paws with difficulty in walking. Histopathology of the brains and mice exhibiting myotonia reveal RBC's in various regions as late as 6 wks. following MCA (380 mg/kg, po). Based on these findings, it was undertaken to examine the integrity of the BBB in MCA treated mice. Two mice. 3 hrs. after MCA (380 mg/kg, po), entry of [14C]-nulin or [3H]-dopamine into all mouse brain regions is significantly increased compared to controls. The amount of [14C]-nulin allowed across the BBB is maximum at 5 hrs. after dosing, coinciding with the onset of signs of toxicity, and remains higher than control at 8 and 16 hrs., but falls to control levels or below at 24, 48, and 72 hrs. [14C]-nulin entry into mouse brain does not occur to an appreciable extent below lethal doses of MCA. At an LD50 (380 mg/kg, po), mice which are moribund but not those which are unaffected 4-6 hrs. after treatment show significantly increased entry of [14C]-nulin or [3H]-dopamine into the brain compared to controls. Myotonic mice also show an entry greater than control at 24 hrs. Damage to the BBB appears to be associated with MCA lethality and myotonia in mice.

We have previously observed elevated blood pressure in rats after exposure to high concentrations of high boiling coal liquid (SRC-II heavy distillate, HD). The purpose of this study was to investigate the dose-response relationship for this effect and determine whether HD causes permanent or reversible changes in cardiovascular function. Male Fischer rats were exposed to 0, 0.24, or 0.70 mg/L HD via inhalation for 6 weeks (6hrs/day, 5 days/week). Half of the groups were evaluated 10-15 days after completion of exposure, whereas the remaining were evaluated after a 6-week recovery period. Blood pressures and heart rates of rats from both the low and high exposure groups were significantly elevated compared to controls. Blood and plasma volumes were elevated in the high exposure group. HD also produced a dose-related decrease in plasma angiotensin levels, whereas plasma aldosterone was unaffected. These cardiovascular measurements were not different from control values after the 6-week recovery period. Results of this study indicate that short term exposure to these concentrations of HD produced several transient changes in cardiovascular function, but further studies are needed to identify the mechanisms and chemical components involved in producing this effect. Work supported under DOE contract DE-AC06-76RL01830.


Monocratine (MCT) given to rats via drinking water produces right heart hypertrophy and pulmonary hypertension. Male rats (60g) were kept on MCT drinking water (20 mg/L) for up to 20 days. Ring segments of right pulmonary artery taken below the 3rd bifurcation were placed in 37°C tissue baths filled with oxygenated Krebs-Henseleit buffer. Contractions produced by stepwise addition of norepinephrine or serotonin were expressed as percentages of KC1 (120 mM) induced contraction. Responses to serotonin were not altered by MCT treatment. Mean ED50 and maximum contraction for all vessels exposed to serotonin were 3.2 uM and 74%, respectively. Norepinephrine-induced contractions were altered by MCT treatment. There was no significant difference (p>0.05) between ED50s for each treatment (mean ED50 12.6 mM). However, mean maximum contraction ranged from 50% at 15 days of MCT to 80% at 5 days of MCT. KC1-induced tension was also significantly altered by MCT treatment (p<0.05); KC1 producing 162 ± 53 mg tension in the control group and 75 ± 36 mg in the 20 day group. Changes in contraction responses were apparent before right heart hypertrophy. Supported by USPHS HL 25258 and NIEHS ES0 7091.


A series of 15 anesthetized and 18 unanesthetized Sprague-Dawley rats (300 gms) were used to study the effectiveness of Carboxylase in preventing or reversing the lethal effects of cyanide poisoning. The lethal intraperitoneal dose of cyanide which killed 12 of 13 animals was 4.0 mg/kg. Time to death was approximately 8 to 12 minutes. Four of nine anesthetized rats given cyanide and treated prior to or simultaneously with 2-3 ml of Carboxylase survived at least 30 to 40 minutes; with 2 surviving 72 hours. In contrast 11 unanesthetized rats treated with Carboxylase at 5-7 minutes after cyanide exhibited an extension of time to death (30 minutes +) and had a survival population of 55% (6/11). (These 6 animals were all alive and appeared normal 72 hours after poisoning.) The effectiveness of Carboxylase in treating cyanide poisoning appears dose dependent. Rats treated with 1 ml of the compound showed a lower survivor population (1/4) than those treated either 2.0 ml (2/4) or 2.5 ml (3/3). These results indicate that animals given Carboxylase prior to or simultaneously with a lethal dose of cyanide had extended survival times. However rats treated after cyanide showed both increased survival times and an increase in survival rate.


Chemically-induced vascular cell injury may play an important role in the development of cardiovascular disease. The present studies were conducted to assess the effects of acute acrolein exposure in primary cultures of vascular endothelial cells (VEC) and smooth muscle cells (SMC). Confluent cultures of both cell types were exposed to initial acrolein concentrations of 2, 5 or 50 ppm for 4 and 24 hr. Final acrolein concentrations were monitored at designated time points using a hexyresorcinol assay. Cellular glutathione levels, lactate dehydrogenase (LDH) release and morphological alterations were used as indices of toxicity. Exposure to acrolein caused a dose- and time-dependent increase in LDH release and morphological damage. Acrolein-induced cytotoxicity was more pronounced in SMC than VEC. Total glutathione levels in VEC exposed to acrolein (50 ppm) for 24 hr were lower than those of respective controls. In contrast, the glutathione levels of treated SMC were higher than those observed in control cultures. These results indicate that SMC and VEC differ in their responses to acute acrolein exposure.
Allynamine toxicity in perfused rat hearts
F.J. Boor, J.L. Sklar, F.G. Anderson, Department of Pathology, University of Texas Medical Branch, Galveston, Tex. Sponsor: A.E. Ahmed

The cardiovascular toxin allylamine (AA) was perfused for 2 hr in two types of isolated rat heart preparations. (1) 27 hearts perfused with AA (0.3 to 30 μM) at constant flow were assessed for heart rate, blood pressure, and loss of intracellular enzymes (CPK, LDH, GOT) in effluent. Hearts treated with a concentration of 3 mM or higher AA showed extensive contraction band necrosis and massive loss of enzymes; morphologic lesion grades and enzyme loss (60-120 min) appeared dose-dependent. Controls (n=6) showed no lesions or enzyme loss. (2) Rat hearts (n=6) perfused with 10 mM AA at constant pressure (100 mm Hg) also showed necrosis morphologically; physiologic parameters were measured in these hearts by means of a latex balloon in left ventricle (LV). Compared to control (n=4) hearts perfused with AA showed an abrupt (10 min) increase in peak LV pressure, followed by a later (120 min) decrease. End diastolic pressure rose, indicating a marked increase in LV compliance; coronary flow showed little change. Thus, AA has a positive inotropic and a necrotizing effect on myocardium, resulting in marked loss of compliance. These effects are independent of coronary flow. (Supported by NIH Grants HL 26189 and HL 00929).

Ornithine decarboxylase (ODC) is the rate limiting enzyme in the synthesis of the polyamines. ODC activity is highest during cell replication and growth, it is stimulated by a variety of tumor hormones, and has been used to assess cardiac response to catecholamines. ODC activity was measured in cultured heart cells from neonatal rats. Hearts were obtained aseptically from 2-4 day old Sprague-Dawley rats, and placed in crude 0.1 M trypsin solution for sixteen hours at 4 °C. Hearts were then transferred to 37 °C incubation medium for 25 min and disrupted by rapid repipetting. Plated at a density of 4 X 10^6 cells per 35 mm petri dish, heart cells become confluent within 48 hrs and beat spontaneously, rhythmically and in synchrony at 150 to 250 beats per minute. Basal ODC activity was between 10 and 25 pmol CO2/30 min/mg protein during the first 5 days. Addition of fresh medium to cells caused a transient 10-fold increase in ODC activity that peaked 4 hrs after medium change. ODC activity did not increase in beating heart cells treated with 10^-12 to 10^-3 M isoproterenol. The findings suggest that the receptor mediated stimulation of ODC activity by isoproterenol is impaired in cultured cardiac myocytes.

Prevention of allylamine (AAM)-induced vascular cell injury by benzylamine oxidase inhibitors (BZAO).
S.L. Grossman, M.S. Stefanick, K. Ramos, Dept. of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, Phila, PA

AAM is a selective cardiovascular toxin utilized in the synthesis of sedatives and anesthetics. Several studies have suggested that activation of AAM by BZAO results in the formation of a toxic metabolite. The present studies were conducted to assess the effects of the BZAO, semicarbazide (SC) or diethyliothiocarbamate (DDC), on AAM-induced toxicity in primary cultures of rat aortic endothelial and smooth muscle cells. Lactate dehydrogenase (LDH) release and morphological alterations were used as indicators of toxicity. Confluent cultures were exposed to AAM (2 to 200 μM) for 4-24 hr. The effects of AAM in the presence or absence of SC (200 μM) or DDC (2 μM) were evaluated 4 hr after exposure. AAM alone produced a dose- and time-dependent increase in LDH release and morphological alterations. In contrast, cultures treated with SC or DDC were protected against the toxicity of AAM. These results suggest that BZAO may play an important role in AAM-induced vascular cytotoxicity.

Spontaneous disseminated panarteritis in laboratory beagle dogs used in toxicity studies.

Disseminated panarteritis was found in 17 (9 males and 25 females) of 49 laboratory beagle dogs (25 males and 24 females). The dogs, which came from a Czechoslovakian breeding kennel, were used in a 6-month oral toxicity study and were 15-18 months old at necropsy. Panarteritis was not associated with clinical or gross abnormalities. The incidence was similar in the control and test article-treated groups. Mainly medium arteries throughout the body, particularly branches of the aorta, and coronary, epigastric and testicular vessels, were affected. Chronic mononuclear cell panarteritis was the predominant feature. Mixed cellular inflammation of the wall, proliferation or degeneration of muscle cells, focal fibrinoid deposit in the tunica media, fragmented elastic interna and intimal thickening associated with cellular proliferation also occurred. Round, worm intestinal infiltration and visceral larval migrans granularas in several organs were common. Histologic changes are compatible with those of immune arteritis. The pathogenesis including the contributions of genetic or round worm antigenic factors is being investigated. This panarteritis is spontaneous and may complicate the interpretation of test-article effects in toxicity studies.
In order to investigate the mechanism of acute toxicity of CPA, its inotropic effect was tested in vitro using 3cm long segments of jejunum, ileum, vas deferens, uterus, chain-linked tracheal rings (smooth muscles), and phrenic-nerve-diaphragm preparations from rat and isolated frog ventricles. Isometric contractions were recorded with force-displacement transducer and Grass polygraph. CPA (200ug/ml) had negative inotropic effect on frog ventricles, and no effect on neuromuscular junction, or on vas deferens contractility. CPA did not block the inotropic effects of epinephrine, dopamine and serotonin (5HT) in vas deferens, indicating that CPA was not an adrenergic blocking agent as chlorpromazine. CPA produced a tonic contraction of tracheal rings, and this may explain the previously reported dyspnea preceding death of mice treated with CPA. It has a strong mycaminic effect on small intestines and esophagus which could be counteracted by atropine. CPA, which has a structural similarity with 5HT and ergotamine, shared their positive inotropic and chronotropic properties on various systems. This finding suggests that CPA found in feeds contaminated with Aspergillus or Penicillium fungi could have an adverse effect on nidation and on the reproductive processes in animals consuming moldy feeds.

The cardiotoxicity of SK&F 94120, a novel inotropic/vasodilating compound on the dog heart at toxicological doses

E C Joseph, R J Eden, J H Harlean and P Bathem

The cardiotoxicity of SK&F 94120, a novel inotropic/vasodilating agent has been investigated in the beagle dogs following intravenous and oral administration of up to 6 months duration (i.v. doses 5, 15, 45mg/kg, p.o. doses 500mg/kg). Cardiovascular parameters were assessed in these studies together with histologic evaluations. A further hemodynamic study (BP, HR, carotid dp/dt max, pre-injection period) following intravenous administration at toxicological doses was also performed. Cardiac Pathology of varying severity was observed (i.v. studies at 15 and 45mg/kg, p.o. studies 25mg/kg and above). The changes consisted of increased incidence of endocardial and valvular fibroplasia and haemosiderin deposition in the endocardium of the left ventricle and a low incidence of focal necrosis of the ventricular papillary muscle. Hyperplasia and hypertrophy of the media of the intramural arteries and a mild periarthritis of medium sized arteries in the right atrium were also observed at high doses. Tachycardia occurred at high doses in all studies and hypotension was also observed. The cardiotoxicity is considered to be related to these haemodynamic effects.


The antiplatelet drug, oxagrelate, was administered orally to Beagle dogs (6/kg dosage) for one year at dosages of 300mg/kg/day. Toxicity was assessed by clinical laboratory analyses, electrocardiographic (ECG) examinations, and gross and microscopic pathology. There were two deaths in the high dose group; one each during Weeks 29 and 41. Reduced motor activity and rectal temperatures were seen in medium and high dose animals. Alkaline phosphatase was significantly increased in high dose animals. There was sinus tachycardia (200-270 beats/min.) in some animals from all drug treated groups. ECG evidence of myocardial damage was not present in high dose animals until Weeks 30 and 52 and consisted of electrical alternans, ventricular extrasystoles, and ST segment depression. Microscopic findings in high dose animals were valvular endocardiosis, and myocardial degeneration and fibrosis of the left ventricular wall and papillary muscle. Oxagrelate causes peripheral vasodilation and reflex and direct cardiac acceleration. The hearts of dogs surviving to the end of the study were compared to those of normal dogs. The results are suggestive of a direct toxic effect on the myocardium, rather than primary cytotoxicity. This study is an example of delayed cardiac damage in the hyperdynamic heart caused by heart rate dependent myocardial ischemia.


CGS 15337, a novel benzodiazepine receptor-binding agent, was suspended in 3% cornstarch and administered by gavage to Sprague-Dawley CD rats at daily doses of 25, 100, 300, or 500 to 1000 mg/kg for 36 weeks. The groups of 25 and 100 mg/kg were asymptomatic, while compound-related effects were noted at doses > 300 mg/kg and included increased serum GPT and/or GOT levels and cardio-myopathy, which was characterized by focal subacute lymphocytic inflammation. In addition, vacuolation of myocardial cells and focal Zender's necrosis were observed in the 500 -1000 mg/kg group. In a separate study, CGS 15337 in 3% cornstarch was administered by gavage to beagle dogs at daily doses of 30, 90, and 300 mg/kg for 13 weeks. Dose-related effects were noted in all treated groups and included: morbidity, hematological and biochemical alterations, electrocardiographic changes, organ weight increases, and cardiomyopathy. Hematological evaluations revealed vacuolation, myolysis, and/or necrosis of the myocardial fibers as well as passive congestion, focal necrosis, hemorrhage and/or central cirrhosis in the liver. In summary, these data indicate that repeat oral doses of CGS 15337 are cardiotoxic at doses > 300 mg/kg for a 2-week duration in the rat and a 13-week duration in the dog.
816 CARDIOVASCULAR EFFECTS OF PRENATAL RESERPINE EXPOSURE IN RATS. C.A. Kimmel*, P.A. Sullivan and G.L. Kimmel*. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR, and *Reproductive Effects Assessment Group, U.S. Environmental Protection Agency, Washington, DC.

The cardiovascular effects of prenatal reserpine exposure were studied in rat pups from dams dosed with 0, 0.375 or 0.75 mg/kg reserpine sc on gestation days 12-15. High dose offspring were significantly reduced in weight on postnatal days (PN6) 0-60 but not thereafter. ECG recordings were made on PN6a 30, 60 and 342 in conscious animals and direct anesthetized blood pressure (BP) was measured at PN342 before and after a noradrenaline (NE) challenge. Heart rate was unaffected by reserpine. In males, R wave amplitude, S wave amplitude, and QRS intervals were decreased with dose. Females showed a reduction in the PR interval only. Baseline BP was not changed, but peak pulse pressures after NE challenge showed a significant dose-related increase in females, while males were increased at the low but decreased at the high dose. These data show subtle but sex-specific changes in cardiovascular function following in utero reserpine exposure which may be related to sex-specific alterations in behavioral and neurochemical parameters reported previously in animals from these same studies.

817 EFFECT OF PRENATAL EXPOSURE TO ISOPROTERENOL, PROPRANOLOL OR RESERPINE ON BETA-ADRENERGIC RECEPTORS IN RATS. G.L. Johnson, F.R. Alley, S.J. Ehrehreich, X. Joseph, P.K. Seth and T. Balazs. CDB, FDA, Washington, DC.

These studies were performed to determine if prenatal exposure to isoproterenol (I), propranolol (P) or reserpine (R) alters beta-adrenergic receptors in adulthood. Pregnant Sprague-Dawley (Holtzman) rats received s.c. either saline (S), 2 ml/kg; I, 0.1 mg/kg; P, 20 mg/kg or R, 0.75 mg/kg. S, I and P were administered b.i.d. on days 9-16 of pregnancy; R was administered once daily on days 12-14 of pregnancy. When pups (from 4-8 litters/treatment group) were 2 to 4 months of age, beta-receptors were assessed: 1) pharmacodynamically by measuring basal heart rate (HR) and HR responses to s.c. I in 6 pentobarbital anesthetized rats/group, and 2) biochemically by specific 3H-dihydroalprenolol binding to crude myocardial membrane preparations from 4 rats/group. In rats exposed to S in utero, neither basal HR (344 ± 24 bts/min) nor HR responses to I at 0.1-300 μg/kg, s.c. (±432 ± 174 ±10 bts/min) were significantly different than in rats exposed to I, P or R in utero. In addition, neither the affinity (Kd = 0.0770 ± 0.05) nor number of receptors (Bmax = 36.4 ±2.6 fm/mg protein) in rats exposed to S in utero were significantly different than in rats exposed to I, 10 R in utero. In conclusion, prenatal exposure to I, P, or R did not alter cardiac beta-adrenergic receptors in adulthood.


The toxicity of a novel cardiotonic agent 4,5-dihydro-6-[4-(1H-midazol-1-yl)phenyl]-2H-5-methyl-3(2H)-pyridazone, monohydrochloride (CI-930) was evaluated following oral administration to rats and dogs. Rats were given CI-930 at doses of 0, 5, 25 and 50 mg/kg/day in the diet for 13 weeks. Male rats given 50 mg/kg showed hepatomegaly and male rats given doses of 25 or 50 mg/kg enlarged salivary glands. Microscopically, the enlarged livers had fatty change and decreased hepatocellular cytoplasmic basophilia, while the enlarged salivary glands showed acinar cell hypertrophy. Slight dose-related changes in BUN, serum cholesterol and blood glucose levels were also observed in these animals. These changes were reversible after a two week period of drug withdrawal. Dogs received 0, 0.5, 1.5 and 3.0 mg/kg of the drug in gelatin capsules, daily for 13 weeks. Slight to moderate increases in the heart rate with concomitant decreases in PR and QT intervals were noted in some animals at all dose levels. There were no major clinical signs, electrocardiographic, biochemical, hematologic or gross pathologic changes observed in the dogs. Principal histopathologic changes included medial hyperplasia of coronary arteries in females given doses of 1.5 mg/kg or higher and in males given 3 mg/kg doses.

819 EVALUATION OF ANTIDOTES FOR THE TREATMENT OF LETHAL NIFEDIPINE OVERDOSAGE. O. Strube, and K.-W. Blederich. Institute of Toxicology and Clinic of Cardiology, Medical University of Lübeck, D-2400 Lübeck, FRG.

Nine potential antidotes were investigated with respect to their efficacy in reversing the acute toxicity of the calcium entry blocker nifedipine (N). Anesthetized rats infused with N 0.5 mg (all doses per kg/min) exhibited a sharp decline of blood pressure, heart rate, cardiac output and peripheral resistance. In the ECG, sinus bradycardia followed by AV-dissociation and an escape rhythm originating from the AV-node or the bundle of His occurred. Without antidotal treatment, these rats died after 62.3 ± 5.3 min of N infusion. Survival time increased by 100 % and more upon additional infusion with CaCl2 5 mg, calcium gluconate 15 mg, isoprenaline 10-20 μg or dopamine 50-100 μg and by 50 % after preanesthetic 200 μg. Epinephrine, norepinephrine, angiotensin and the plasma expander haemaccel were ineffective in this respect. All drugs prolonging the survival time also reversed the cardiac output. Ca++ and dopamine also increased the blood pressure and isoprenaline accelerated the escape rhythm. In rabbits infused with N 0.2 mg, CaCl2 more than doubled the survival time, too, but isoprenaline and dopamine proved to be ineffective. In conclusion, Ca++ is the drug of choice for the antidotal treatment of nifedipine intoxication.
Suicidal and accidental intoxications with β-adrenergic blocking drugs have been frequently observed during the last years. From these observations it can be concluded that β-blockers with cholinergic-like activities (propranolol, oxprenolol, alprenolol, acebutolol) cause more severe complications and often fatal outcomes. Bradycardia, hypotension, low cardiac output and cardiogenic shock are the main clinical features of β-blocker poisoning. Therapeutic management of intoxicated patients with β-adrenergic stimulants, dopamine, dobutamine, glucagon or aminophylline has been unsuccessful in severe cases, as the heart becomes refractory to pharmacological and electrical stimulation. In an own case of suicidal intoxication with acebutolol after ingestion of about 8 g of the drug the lethal outcome could not be prevented by treatment with isoprenaline, dopamine, glucagon and electrical stimulation. High concentrations of acebutolol and its main pharmacologically active metabolite have been found in blood and different organs and are compared to the data found in the literature. From experimental studies in rats (Drug Research 34, 1265, 1985) early treatment of acebutolol intoxications with isoprenaline and dopamine is recommended.


Fisher 344 male rats have a dose and time dependent proximal tubular degeneration, induced by certain petroluem-based fuels. This degeneration may be associated with a low molecular weight alpha globulin termed alpha-2U globulin. A new method using dialfiltration, ion-exchange and hydroxylapatite chromatography was developed to obtain monospecific immunologic reagents for alpha-2U globulin. This technique effectively removes contaminant alpha globulins and pre-albumins which occur with standard precipitation and ion-exchange methods. The purity of the immunologic reagents was determined by SDS-polyacrylamide gel electrophoresis, and two-dimensional immunoelectrophoresis. The corrected reference range for alpha-2U globulin purified by the above technique is age-dependent. In adult male Fischer 344 rats, urinary alpha-2U globulin levels range from 1-3 mg/ml. (Supported by AFOSR grant# 84-0283A)

APPETITE SUPPRESSION, BEHAVIORAL, AND CARDIOVASCULAR EFFECTS OF PHENYLPROPANOLAMINE (PPA) IN DOGS. L.R. WEISS, R.H. BROWN, and J.A. VICK. Food and Drug Administration, Washington, D.C.

In earlier reports, we compared oral pharmacological and toxicologic doses of PPA on behavior (Toxicologist 2, 114, 1982), food intake (Toxicologist 4, 984), and heart rates (Toxicologist 5, 112, 1985) in rodents. These studies showed that with increasing doses, behavioral depression, reduced food intake rates, and tachycardia occurred. This paper extends our work to dogs pretreated with PPA orally for 30 min, monitoring mean arterial pressure (MAP), systolic (SP) and diastolic (DP) pressures, and heart rates by tail-cuff digital methods. Food ingestion was measured following a challenge meal (25 g food/kg) and behavior was assessed by video-taped camera (15 min). PPA doses (3.1 mg/kg and above) caused piloerection, vagal bradycardia, increased MAP, SP, and DP for 30-60 min, while 6.25 mg/kg intensified these effects lasting for 2-3 hrs, and higher doses (12.5 and 25 mg/kg) caused severe and long-lasting bradycardia with elevated MAP, SP, and DP for 4-6 hrs. Food intake was unchanged for doses up to 12.5 mg/kg but marked reduction occurred at 25 mg/kg lasting for 4-6 hrs. Mixed agitation, depression and decreased activity were noted at 25 mg/kg. These data suggest that cardiovascular responses are the most important changes of toxicological significance associated with the use of PPA.


The oculocardiac reflex (a vagal maneuver) can be used when recording electrocardiographic tracings and may allow the investigator to differentiate between certain cardiac arrhythmias (i.e., atrial flutter, atrial fibrillation, atrial tachycardia, AV junctional tachycardia, and sinus tachycardia). By applying gentle pressure on the eyeball(s), a stimulus causes an impulse to move along the afferent pathway via the ophthalmic branch of the trigeminal nerve to vagal centers and the efferent pathway to the heart along the vagus nerve. The vagus nerve innervates the SA node, the atria, the AV node, and the ventricles. This reflex slows the rate, AV conduction, and myocardial contractility. The decrease in AV conduction induced by the stimulation of the oculocardiac reflex results in a reduction in the ventricular rate, thus allowing differential diagnosis of supraventricular tachyarrhythmias. The electrocardiograph should always be running while this maneuver is being performed in order to record the response. The oculocardiac reflex should not be stimulated for more than 5 to 10 seconds. It is our contention that this maneuver can be used diagnostically when obtaining electrocardiograms in larger species during safety evaluation studies.
IN VIVO AND IN VITRO SAFETY EVALUATION STUDIES OF FACTOR VIII:C. R. Chay, B.S. Brar and L.C.K. Wong, Department of Drug Safety Evaluation, Research and Development Division, Revlon Health Care, Tuckahoe, N.Y.

Factor VIII is a commercially available anti-hemophilic agent. An improved product, Factor VIII:C having higher potency as compared to Factor VIII is being developed. The purpose of these studies was to evaluate the safety of Factor VIII:C following intravenous administration in rats and rabbits. In acute studies, Factor VIII:C was found to be well tolerated after a single intravenous administration of 200 IU/kg in rats and 120 IU/kg in rabbits at a rate of 0.5 to 1.0 ml/min. In five day repeated dosing studies in rats (6/group) and rabbits (3/group), the intravenous administration of Factor VIII:C at dosages of 0, 40 or 120 IU/kg/day produced no drug-related changes in clinical observation, body weight, organ weight, hematologic and clinical chemical parameters. Histomorphologic evaluation of organs and tissues of animals treated with repeated doses of Factor VIII:C also showed no drug induced changes. No irritation was observed at the injection site. In a four compatibility study of Factor VIII:C with human whole blood from donors revealed no evidence of hemolysis. In conclusion, Factor VIII:C was well tolerated by rats and rabbits and in in vitro study, it was compatible with human blood.

Interaction of Dietary Protein Level on Dose Response Relationships during Aflatoxicosis in Young Chickens. K. E. Richardson, L. A. Nelson, and P. B. Hamilton, Depts. of Poultry Science and Statistics, North Carolina State University, Raleigh, NC

Dietary protein level has been reported to influence sensitivity to aflatoxin, but the interaction of protein level on dose-response relationships of aflatoxin has not been investigated. Aflatoxin at 12 dosages ranging from 0 to 2.34 µg/g of feed was fed to eight groups of 10 young chickens consuming a 10.00 or 12.75% protein diet for 3 weeks. The body weights, liver relative weights and liver lipid contents were determined. Mathematical models were fitted to the data and the dose-response curves were predicted. A quadratic polynomial fit body weight on 12.75% protein diet whereas a plateau-linear model fit body weight on the low protein diet. This suggests that in a low protein diet aflatoxin affects only one of the factors controlling growth. Plateau-linear models fit liver relative weight and liver lipid contents on both low and normal diets. For both variables the low protein diet decreased the apparent minimum effective dose and increased the positive slope of the linear response. The apparent minimum effective doses (µg aflatoxin g feed) using this experimental approach were calculated to be 1.2 and 2.00 for body weight, 1.08 and 1.65 for liver lipids and 1.45 and 2.34 for liver relative weight in low and normal protein diets, respectively.

Inhibition of Uridine Uptake in Cultured Vascular Smooth Muscle Cells as a Rapid, Sublethal Cytotoxicity Assay for Cardiovascular Toxins. R.M. Hysmith, T.K. Welch and F.J. Boor. Chemical Pathology Division, The University of Texas Medical Branch, Galveston, TX 77550. Sponsor: Ahmed E. Ahmed

A sensitive assay for sublethal damage to primary cultured cells was developed, utilizing uridine uptake as the indicator of injury. Primary cultures of porcine aortic smooth muscle cells were exposed to varying concentrations of the cardiovascular toxins monomethylamine and 8-amino-propionitrile for different time periods up to 8 hrs. After exposure to the toxin, the capacity of cells to uptake 5.6 [3H]uridine (largely into trichloroacetic acid soluble pools) over a 20 min period was determined. The concentration of toxin necessary to inhibit [3H]uridine uptake by 30% (compared to control cells) was used as an endpoint for cytotoxicity; at this endpoint, cells fully recovered when free of toxin. This [3H] uridine uptake assay was compared with the standard cell culture methods of vital stain exclusion (trypan blue) and cytocytotoxic response (specific release of [3H]uridine or [3H]thymidine from prelabeled cells). This assay was 20-fold more sensitive than vital stain exclusion or cytotoxic assay methods. Supported by NIH Grants 1R 56189, HL 00929.


Serial blood sampling for clinical pathologic evaluation is currently not done on our rodent toxicity studies due to weight loss and the use of an anesthetic produced adverse effects in untreated rats, e.g., a decreased body weight gain, which may interfere with proper safety evaluation. After investigating other bleeding sites, the tail vein was selected for testing. A method was developed for serial sampling without the need for an anesthetic and which appeared to cause no adverse effects. Ninety-two male rats were assigned to three groups: (1) 40 rats for terminal bleeding (TB), (2) 26 for anesthetized (40% O2/60% CO2) serial orbital sinus bleeding (OSB) and (3) 26 for serial tail vein bleeding (TVB). Samples were collected during 1, 2, 4, and 6, and evaluated by our Clinical Pathology section. Blood volumes from TVB were comparable to OSB and sample quality was good. The TVB procedure took 2.5 hours/26 rats compared to 0.5 hours/26 rats for OSB. Although TVB rats did not struggle or show discomfort during the bleeding sessions, there was a significant reduction in body weight gain for both TVB and OSB rats. Therefore, serial sampling is not recommended for rats being evaluated for toxicity in parameters other than clinical pathology.
AN EVALUATION OF BLOOD SAMPLING METHODS FOR RAT TOXICITY STUDIES - II. CLINICAL PATHOLOGY.


This study compared hematologic and clinical chemistry test results performed on rat blood collected from 3 different sites: tail vein, orbital sinus and posterior vena cava. Groups of Sprague-Dawley rats were bled terminally (TB) at weeks 1, 2, 4, and 6. Additional rats were serially bled at the same intervals either by the tail vein (TVB) or orbital sinus (OSB). Body weight gain, quality and quantity of blood obtained, and clinical pathology tests were measured. The only hematology and chemistry results which differed between TVB and OSB were a 37% decrease in glucose, 23% decrease in carbon dioxide content and 4% increase in hemoglobin, hematocrit and RBC count for TVB groups. Significant differences between both TVB and OSB as compared to TB were seen for most tests. These differences may be related to anesthesia, stress, and bleeding site. Incidences of hemolysis and clotted specimens were low. TVB is recommended as a serial blood sampling technique to monitor Clinical Pathology parameters, since it can be done without anesthesia, avoiding possible interactions with the test article. Reference ranges must be established for each bleeding method.

BEAGLE PAIN SYNDROME: A LATENT DISEASE OF DOGS POTENTIALLY COMPLICATING TOXICITY STUDIES.


Instances of a little known, incompletely characterized disease of dogs, beagle pain syndrome, have been observed in our laboratory. The distinct clinical, laboratory, and pathologic features of this disease are described here to alert others involved in toxicity testing of chemicals in the existence of this latent disease of dogs. Clinically affected dogs showed pain, arched backs, stiff necks, reduced appetites, and fever (104-106°F). Progressive loss of physical condition occurred. Neutrophilic leukocytosis (30-60 thousand) and thrombocytosis (> 600 thousand) were present. Sera were positive for rheumatoid factor. Necrotizing polyarteritis and amyloidosis were observed histologically. The syndrome showed a periodicity of both clinical and laboratory parameters and has been observed for periods of 7 to 30 weeks. It is considered that this idiopathic syndrome can be non-specifically precipitated in predisposed dogs by treatment and its incidence can be dose-related. As such, its occurrence can complicate interpretation of toxicity studies in dogs; recognition of the syndrome should obviate this possibility.

DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR PESTICIDES.

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This laboratory has developed enzyme-linked immunosorbent assays (ELISA) for a variety of pesticides and proteins including benzoxyphenylureas, paraquat, bioallethrin and the Bacillus thuringensis var. israelensis (Bt) toxins. This report deals with the thiocarbamate herbicide molinate (5-ethyl-1-carboxiobutane). Each of three hapten was coupled to a variety of carrier proteins by either a mixed anhydride or diazoation method. Selected antigens were used to immunize rabbits. Serum from these rabbits had high titers to the immunizing antigen as well as to the carrier protein alone. More importantly, there is cross reactivity with hapten coupled to other than the carrier, indicating specificity for the hapten. The serum does not cross react with any other carrier protein. At least one coating antigen and serum combination has been utilized to yield a standard curve for the detection of molinate with sensitivity below 8 ng/ml. Once validated against classical methods, this ELISA assay for pesticides is a rapid, economic tool for screening large numbers of environmental or biological samples. (Supported in part by California Department of Food and Agriculture and NIHES 2 R01 ES02710-03).

CONTROL OF SPILLAGE FROM MOUSE FEEDERS CONTAINING GROUND FOOD.


Food spillage was measured for 4 weeks with CD-1 mice (20/group) fed ground food in 3 different types of feeders: feeder 1) a 6 x 7 cm glass jar with a wire basket insert, feeder 2) a 6 x 7 cm glass jar with a wire basket insert and a stainless steel ring measuring 26 mm O.D., 10 mm I.D., 8 mm thick, and feeder 3) a commercially available 7.5 x 5.8 cm glass jar with a thick metal insert and a lid. Ground food (fresh weekly) and water was provided ad libitum and food spillage was recorded three times a week.

The mean spillage of food per mouse per week was 3, 0.1 and 0.9 gm for male mice and 10, 0.8, and 2.2 gm for female mice with feeders 1, 2, and 3, respectively. The mean frequency of spillage was 24, 0, and 10 percent for male mice and 46, 8, and 25 percent for female mice with feeders 1, 2, and 3, respectively. The smaller size of feeders 1 and 2 resulted in less contamination with urine and feces and the use of a metal insert in feeders 2 and 3 markedly reduced spillage. It was concluded that the use of feeder 2 resulted in minimal spillage, minimal contamination and a marked reduction in workload and time to obtain a more accurate estimate of food consumption.
COMPARATIVE DOSED FEED AND GAVAGE SUBCHRONIC STUDIES OF TRICRESYL PHOSPHATE (TCP) IN F344 RATS AND B6C3F1 MICE. M. Heitmancik, B. Deskin, M. Ryan, B. Carlson, B. Herger, A. Peters, and R. Irwin. Battelle, Columbus, OH and National Toxicology Program, Research Triangle Park, NC.

TCP is a tri-aryl-phosphate ester that contains <0.1% of an ortho isomer which is neurotoxic. Comparative 13-week studies were conducted to determine effects of vehicle and administration route on toxicity. TCP was administered by gavage in corn oil at doses of 800, 400, 200, 100, and 50 mg/kg or in mash feed (NIN-07) at concentrations of 4200, 2100, 1000, 500, or 250 ppm (mice) or 13,000, 6600, 3300, 1700, or 900 ppm (rats). Equivalent levels of oral exposure and a dose-related inhibition of serum cholesterolesterase activity were produced. Hindlimb weakness and tremors occurred consistently in mice in the gavage study at the 800 mg/kg level. Preliminary results indicate cytoplasmic vacuolization of adrenal cortex, degeneration of seminiferous tubules (males), interstitial ovarian cell hyperplasia or vacuolization (females) were consistent microscopic changes. Axonal degeneration and demyelination also occurred in mice (high dose) in both studies. Lumbar degeneration of spinal cord in both species occurred only in the gavage study, suggesting the corn oil vehicle may affect absorption and distribution of the test chemical.


Twenty-four male Sprague-Dawley rats (274 ± 3 g) were adapted for 10 days to 25 ± 2°C and 4 ± 1°C, respectively. Thereafter, 12 rats at each ambient temperature received a non-tolsh dose (15 μg/kg) of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) ip in corn oil, whereas 12 control rats were injected with vehicle alone. Three control and 3 TCDD-treated rats of each temperature group were killed 1, 3, 7, and 14 days after dosing. Liver and IBAT were removed and processed for light (H.E., PAS and Sudan Black B) and electron microscopy. In liver, a centrilobular hypertrophy, fatty infiltration and cell necrosis followed by formation of regenerating islets were observed. Initially, number of fat droplets decreased whereas size of fat droplets increased in brown adipocytes. This was followed by an increase in number and a decrease in size of fat droplets (day 7). By day 14 IBAT was largely depleted of fat. Mitochondrial swelling was accompanied by umbrella-like transformation of cristae. Cold adaptation accelerated the time course of changes in both liver and IBAT.

METABOLISM OF PALMITIC ACID IN TCDD-DOSED TREATED RATS. K. Ruzman and H. Grein. Abt. für Toxikologie der Gesellschaft für Strahlen- und Umweltforschung mbH München, Neuherberg, F.R.G. and Dept. of Pharmacology, Toxicology & Therapeutics, Univ. of Kansas Medical Center, Kansas City, KS U.S.A.

Twelve male Sprague-Dawley rats (200 ± 20 g) were administered i.p. 2 mg/kg C-13-palmitic acid (20 μCi) in olive oil (5 ml/kg). One day later 4 rats received i.p. 400 μg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in corn oil (5 ml/kg), whereas 8 rats were injected i.p. with vehicle. Four of these 8 rats had free access to feed, whereas the other 4 rats were pair fed with the 4 TCDD-dosed rats. Body weight and feed intake were monitored daily. Urine and feces were collected daily. Exhalation of C02 was measured daily for 30 min in a suitably equipped respirometer. Radioactivity was determined in a scintillation spectrophotometer. Exhalation of total C02 was calculated from the RLU. Data show that body weight loss in TCDD-dosed rats cannot be accounted for by reduced feed intake. Urinary and fecal excretion of 13C02 was not different among the groups. However, TCDD-treated rats exhaled 10 and 30% more 13C02 than ad libitum and pair fed controls, respectively. Since exhalation of 13C02 in this experimental design reflects total fat utilization, it is apparent that TCDD-treated rats burn fat at a higher rate than expected for their nutritional status. This increased fat utilization seems to at least partially responsible for TCDD-induced "wasting syndrome".

BAP METABOLISM IN THE PRESENCE OF SIMPLE AND COMPLEX ORGANIC MIXTURES. D.L. Springer, D.A. Dankovic, B.L. Thomas, and R.E. Bean. Pacific Northwest Laboratory, Richland, WA.

Previous studies have shown that the mutagenic and carcinogenic activities of Bap were decreased when co-administered in complex organic mixtures. The mechanism of this interaction was evaluated under in vivo conditions in which 5.5 or 50 μg of H-Bap or 50 μg/ml of a simple mixture (H-1 containing 5.5 μg/ml H-Bap) were incubated with rat liver S9. The samples were taken over a 60 minute period and extracted first with hexane and then ethylacetate. At low Bap concentrations, the effective rate of disappearance of Bap was rapid, whereas with H-1 the effective rate for Bap was slower than with Bap (5.5 μg/ml) alone but more rapid than at 50 μg/ml of Bap. There were also large differences in the rates of disappearance in the presence of components of H-1, with the order of disappearance as follows: dibenzanthracene > Bap > perylene > BeP > benzo(a)perylene > chrysene. Bap metabolites extracted into ethylacetate increased with increasing incubation time. In the presence of another complex mixture, the metabolite profile was qualitatively similar to that of Bap alone; however, the effective rate of degradation of Bap was also decreased. These data suggest that the differences in mutagenic and carcinogenic activities for Bap, when it was co-administered with the complex mixture, were due to decreased rates of metabolism. Work supported by DOE Contract DE-AC06-76RL0-1830.

Compound A and Compound B were evaluated for toxicity in a battery of acute tests (acute oral, guinea pig maximization, photosensitization, dermal irritation, Ames and multiple genetic end point) and a two week oral fetotoxicity study. Compound A was found to have a lethal LD50 of 0.25 mL/kg, and was an extreme sensitizer, a mild dermal irritant (PDII of 1.7), and not mutagenic or fetotoxic in the tests employed. Compound B had an oral LD50 greater than 4 mL/kg, was a moderate dermal sensitizer and mild irritant (PDII of 1.4), was not mutagenic in the Ames test but weakly increased the incidence of SCE's and gene mutations, and was not fetotoxic.

Neither compound was found to be a photosensitizer, but during the course of the photosensitization study Compound A was found to cause neuromuscular signs (hind limb paralysis) and a bilateral necrosis of the medulla oblongata in female guinea pigs. This same lesion was found in female rats receiving a single oral dose of 0.25 mL/kg and in nonpregnant females dosed for two weeks at 0.03 mL/kg. Compound B was not found to produce any of these neurologic effects.


PD (CAS No 123–54–6), widely used as a chemical intermediate, was investigated for potential hazards by single exposure to the liquid or vapor. The acute peroral LD50 (ml/kg) for undiluted PD to rats was 0.78 (0.66-0.91) for males and 0.59 (0.51-0.70) for females, with deaths occurring within a few hours of dosing. By 24 hr occluded contact on rabbit skin, the acute percutaneous LD50 (ml/kg) was 1.41 (0.80-2.49) for males and 0.81 (0.59-1.12) for females; deaths occurred within 1 to 24 hr. The LT50 for rats exposed to a saturated PD vapor atmosphere was 52 min (7060 ppm) for males and 55 min (7912 ppm) for females. The 4-hr LC50 was 1224 (1063–1049) ppm. At 919 ppm no deaths occurred and signs of toxicity were minimal, indicating a steep slope for the concentration-response curve. Minimal erythema and edema were produced on rabbit skin by 4 hr occluded contact, and marked erythema and edema, with necrosis, by 24 hr contact. Ocular instillation of 0.1 ml PD produced minor conjunctivitis and iritis of less than 24 hr duration, but no corneal injury. These findings indicate PD to be of moderately high acute peroral and percutaneous toxicity. It is hazardous by acute exposure to around 1000 ppm vapor, minimally irritating to the eye, and depending on the duration of contact, mildly to moderately irritating to skin.


Trace element (TE) interactions are an important aspect of trace element nutrition and toxicity. Sensitive, multi elemental analysis on a microscopic scale is provided by the synchrotron radiation x-ray microprobe. Rates were adapted to a T replete (purina) or TE adequate (AIN) diet and provided drinking water containing 0 or 500 ppm lead acetate for 30 days. At sacrifice, brain, liver, kidney, and aorta were frozen in liquid nitrogen. Frozen sections of 20 μm were cut and mounted on a thin polyamide support film for analysis. TE were quantitated by the fluorescent x-rays from selected microscopic regions and analyzed by multivariate analysis. These observations illustrate the complex interactions between essential and toxic TE in a wide variety of organ structures.

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The relationship of skeletal lead to blood lead and toxicity is not clearly understood. Noninvasive measurements of lead concentrations in human bone are needed. This can be accomplished using the x-ray fluorescence (XRF) method. Here we used a 50-mCi $^{109}$Cd source to irradiate the leg (tibia) for 2000 s. The lead K-x rays and elastically-scattered photons emitted at an angle of about 150° to the incident photons were measured with a Ge(Li) detector. The observed ratio of lead K-x rays/elasitically scattered photons allows calculation of the Pb/Ca atomic ratio from known photon cross sections. Lead concentration in bone ash is derived from known values of tibia composition. Comparison of XRF results for phantoms and bone samples with values found by other methods provided validation of the procedures. Preliminary analysis of results from 13 hospital patients indicate an average lead concentration of about 40 ppm relative to bone ash.

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A study was conducted in female Wistar rats assessing the relative lethality and gross behavioral effects induced by the neopentyl, ethyl, benzyl and diethylaminomethyl (DEAE) esters of (R)-1-(phenylethyl)-imidazole-5-carboxylic acid. The LD$_{50}$ values obtained for the neopentyl, ethyl, benzyl and DEAE esters were 8.6, 17.8, 31.6 and 56.2 mg/kg, respectively. Hypnotic activity was observed in surviving animals exposed to the neopentyl (3.6-6.5 mg/kg) or ethyl (3.6-31.6 mg/kg) esters. While for the benzyl analog, sedative hypnotic activity was seen in the higher doses (31.6-10 mg/kg), the low dose group (3.16 mg/kg) exhibited ataxia only. In contrast to these effects, surviving DEAE-treated rats were moderately ataxic at the higher doses but exhibited hyperactivity and hyper responsiveness to acoustic stimuli at the lowest dose (3.16 mg/kg). These results indicate that the lethality and pharmacological activity induced by esters of (R)-1-(phenylethyl)-imidazole-5-carboxylic acid are affected by changing the ester alky group. (Supported by Contract No. DAAG29-81-D-0100-D01427).

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Research in many areas of toxicology requires instrumentation which can determine trace elements in biological materials with high sensitivity and good spatial resolution. The National Synchrotron Light Source at Brookhaven is a facility which produces X-rays up to 20-Kev energy with unprecedented brightness. Synchrotron radiation can be exploited in toxicology by using it to produce characteristic K- and L-X rays in samples of biological interest and thus to determine the elemental composition of the material. The method is nondestructive, can use wet samples at atmospheric pressures, has multielement detection capability from about 5f to U, can be used to probe areas around ten microns or less in diameter, and can determine elemental concentrations corresponding to about 10$^8$ atoms. The new NIH BNL X-Ray Microprobe Research Resource has been set up to make it possible for biologists to make use of the instrument for various research topics. Principles of the methods will be presented and arrangements for use will be given.

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A quantitative, automated colorimetric assay to determine cell viability following in vitro exposure to toxicants: K. E. Bogers and C. E. Ware, Division of Biomedical Sciences, University of California, Riverside, CA 92521.

With an increase in the number of toxicological systems involving in vitro treatment, there is a need for a technique that can rapidly assess cell viability following in vitro exposure to a toxicant which is efficient and sensitive. Here we describe an assay that utilizes a tetrazolium dye, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT), which is reduced to a deep blue formazan by mitochondrial dehydrogenases. This assay is done in microtiter plates and the amount of dye reduced is determined by an automatic multwell scanning spectrophotometer. This assay has been modified to incorporate a NADH fortified S-9 liver fraction to determine the effect of metabolism on the toxicity of a compound. The concentration of a compound that causes 50% mortality has been compared with the assay, trypan blue dye exclusion for a number of toxicants on three cell lines. For each treatment group tested, the MTT assay was a highly sensitive indicator of cell viability. The rapidity of this assay allowed for a complete quantitative dose titrations and kinetic analyses of the toxicant. Supported by PHS ES03105.
Control data from 13 chronic dog studies were compiled and divided into one-year segments covering a seven year period. The data base included 244 female dogs with a mean age of 9.2 ± 2.4 months at the initiation of the various studies. Compilations included body weights, ophthalmology examinations and results from hematology, serum chemistry and urinalysis determinations. Histopathology data from 119 dogs were available for compilation. Spontaneous neoplastic lesions were reported in the following tissues: adrenal, bone, kidney, liver, lung, lymph node, mammary gland, ovary, pituitary, skin thyroid/parathyroid, tongue, and vagina.

The mechanism by which ochratoxin causes pale bird syndrome, failure of chickens to utilize dietary carotenoids for carcass pigmentation, was investigated. Graded doses of pure ochratoxin A (0, 0.5, 1.0, 2.0, and 4.0 µg of toxin per g of feed) were incorporated into a white corn-soy diet supplemented with an efficiently used oxy-carotenoid (110 µg of lutein/g diet) and fed to broiler chicks from hatching to 3 weeks of age. The concentrations of free lutein and its metabolites, lutein diester, lutein monoester and oxo-lutein, in the jejunal contents, jejunal mucosa, serum, liver, and toe web from these birds were measured by high pressure liquid chromatography. Based on the threshold level of ochratoxin required for an effect on these carotenoids and on the severity of the effect, five separate loci for the action of ochratoxin in pale bird syndrome were detected: 1) dilution of carotenoids in intestinal contents, 2) depressed uptake by intestinal mucosa, 3) depressed transport in serum, 4) altered accumulation in liver and 5) altered acylation steps in the integument.

Solvent-extraction of lubricant base oils using N-methyl pyrrolidone (NMP) is an effective refining technique that is becoming more widely used in the petroleum industry. Acute, subchronic, and chronic studies were conducted to assess the potential toxicity of a typical NMP-extracted oil. The acute oral LD50 and inhalation LC50 in rats, and dermal LD50 in rabbits were greater than 5 g/kg, 5.7 mg/l and 2 g/kg, respectively. The refined oil produced minimal primary dermal and ocular irritation in rabbits and no dermal sensitization potential in guinea pigs. No clinical signs of overt toxicity and no significant findings in clinical pathology, histopathology or organ and body weight changes were seen in rats following 3-week and 90-day dermal toxicity studies, or a 3-week aerosol inhalation study. No developmental toxicity was noted in a Segment II dermal teratology study in rats and no tumorigenic potential was detected in a lifetime skin-painting study in mice. These studies demonstrate that a typical oil extracted with NMP produced essentially no evidence of toxicity in any of the parameters monitored.
IN VIVO PERCUTANEOUS ABSORPTION OF AROMATIC SOLVENTS IN HAIRLESS MICE. A.S. Susten, B.L. Danes, and K.W. Niemeier, National Institute for Occupational Safety and Health (NIOSH), DBES, Cincinnati, OH. Sponsor: T.R. Lewis

Information relating to dermal absorption of chemicals is becoming of increasing interest to occupational health professionals involved in risk assessment. Percutaneous absorption values have been determined for a series of single-ring aromatic solvents, using a recently described direct method for studying volatile chemicals in hairless mice (Susten et al., Amer J. Ind. Med. 7:323, 1985). Total dermal absorption was summed from the radioactivity found in the animal carcass, excreta, and expired breath and no correction factors were required. Results, reported as percentages of the applied dose absorbed (means and standard errors), were 0.89 ± 0.3, 2.1 ± 0.5, 3.4 ± 0.9, and 4.5 ± 1.0, for benzene, toluene, ethylbenzene, and aniline, respectively. Dermal absorption values were reviewed in relation to physical properties of the compounds (e.g., vapor pressures, octanol/water coefficients) to determine if such properties could be used to estimate absorption potential. The results were also compared to existing dermal absorption values derived by other procedures and in other species. For this series of related aromatic compounds, volatility appeared to be one factor affecting absorption.

CONTINUOUS INTRAVENOUS INFUSION STUDY WITH PIBENZIMOL (NSC-327921) IN BEAGLE DOGS. J.C. Page1, L. Malapei2, L. M. Kastelle1, Batelle Columbus Laboratories1, Columbus, OH; Ohio State University2, Columbus, OH; and National Cancer Institute3, Bethesda, MD (NCT Contract No. N01-CM-17365)

Pibenzimol, an antineoplastic agent, was implicated in the deaths of two cancer patients. Pancreas may have been the target organ of pibenzimol-induced toxicity. Beagle dogs were given one or two 120 hour, continuous intravenous infusions of pibenzimol (0.208 mg/kg/hour) or vehicle. Pibenzimol caused occasional episodes of emesis, lower levels of serum potassium and an apparent delay in recovery from surgery-induced platelet reductions. Blood glucose levels were not affected by treatment. Inflammation and necrosis noted in the pancreas of one dog and thymic lymphoid depletion noted in three dogs were the only lesions attributed to pibenzimol. From 1.0 to 1.5 percent of an infused dose was eliminated as unchanged compound in the urine. Plasma steady state levels were achieved within 24 hours after the start of an infusion. Higher plasma levels during the second infusion resulted from a decrease in total body clearance of pibenzimol.


Repeated delivery of drug solutions to the lungs of unanesthetized animals is difficult and often results in physical trauma. We have developed a drug delivery system employing a surgically implanted tracheal catheter that permits the repeated administration of test substances to the lungs of rats and ferrets. With practice, this simple technique can be performed in less than 15 minutes. The surgical procedure in an anesthetized animal is as follows: 1) The trachea is exposed after making a ventral midline incision between the suprasternal notch and the cricoid cartilage; 2) Polyethylene catheter is sutured to the sternohyoid muscle and is threaded subcutaneously from the trachea, around the foreleg to the dorsal neck surface where it is exteriorized and securely fastened; 3) A small needle is used to puncture the trachea and the catheter is inserted to an appropriate depth; 4) The incisions are closed and the animal allowed to recover for several days. Employing this method, tracheal catheters have remained in place and patent for at least one month and have been extremely well tolerated by both rats and ferrets. We have found this technique to be an excellent method for the repeated administration of solutions to the lungs of unanesthetized animals.

DIETARY INDUCED PERIODONTITIS AND ORO-NASAL FISTULATION IN THE RAT. M. Robinson, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Macclesfield, Cheshire, SK10 4U, UK. Sponsor: J.F.H. Purchase

A recent long term bioassay in specific pathogen free Alph/Apr (Wistar-derived) rats revealed an unusually high incidence of periodontitis with associated oro-nasal fistulation. The lesions were either unilateral or bilateral and were present in the region of the first molars tooth. There was no marked rhinitis with destruction of the turbinate bones and some rats developed squamous metaplasia of the nasal epithelium. The disease was considered to be caused by a change in diet at one year from a pelleted Porton combined diet to a steam expanded Porton combined diet. The difference in the manufacturing processes of the two diets resulted in the presence of long sharp fibres of oats or barley in the PCD expanded diet. These fibres probably initiated the periodontitis which eventually progressed to oro-nasal fistulation.
QUANTITATION OF HYMOXOXON IN BITTERWEED BY HPLC: H.L. Kim, Department of Veterinary Physiology and Pharmacology, College Station, TX 77843. Sponsor S.H. Safe

Bitterweed (Hyemoxyx odorata DC) is an economically important toxic range plant that grows in the Edwards' Plateau of Texas where approximately 25 million sheep and 1.4 million goats graze. A sesquiterpene lactone, hyemoxon, has been isolated and the toxic symptoms elicited by hyemoxon in sheep are essentially identical to those of bitterweed, however no reproducible and reliable assays for this toxin are currently available.

Two HPLC assays were developed: hyemoxon was extracted into ethyl acetate from the initial hot water extract of a bitterweed sample and analyzed by reversed phase HPLC; the mobile phase consisted of 45-60% methanol in water and the elution was monitored at 225 nm. The preliminary results indicate that the hyemoxon content of bitterweed varied from 0.2 to 1.3% as determined by HPLC analysis compared to that of 1.0-4.9% determined by gas chromatography. A second method utilizes the facile conversion of hyemoxon into more stable diazotones, piloropin and 

UTAKE OF MERCURY COMPOUNDS IN MOUSE BRAIN. Michiaki Aihara and Raghubir P. Sharma, Center for Environmental Toxicology, Utah State University, Logan, UT and Institute of Whole Body Metabolism, Shiroi, Inba, Chiba, Japan.

Adult male CD-1 mice were injected ip with various doses of either methylmercuric chloride (MMC) or mercuric chloride (MC) and their blood and organs were collected at specified periods. Mercury concentration was determined in brain, blood and other tissues. After appropriate calculations for mercury in brain tissue and plasma, the rate of mercury uptake in brain was computed. A first-order rate independent of the dose, was apparent for the first 24 hr, after which the uptake did not follow first-order. Mercury peaked in brain after 7 days for both chemical forms; the brain:blood ratio for mercury progressively increased during this period. The rate constant (k) for entry of mercury into brain was 0.06 hr^-1 for MMC, the rate was 50 times lower for MC. Until 3 days after administration MC was equally distributed between erythrocytes and plasma, whereas MMC was located mainly in erythrocytes. Coadministration of L-cysteine influenced the distribution of MC in several organs but not in blood or brain. Glutathione depletion caused an increase in blood and brain MMC concentrations. Late retention of mercury in organs can be ascribed to binding with macromolecules.

ULTRASTRUCTURAL CHANGES IN ISOLATED HEARTS ASSOCIATED WITH ETHANOL AND TWEE 80 INFUSION. A.R. Watson, B.R. Nanno, R.P. Misra, H.R. Fowler, C.A. Dempsey and J.E. Manno. La State Univ. Med. Ctr., Depts. of Pharmacology, (Sec. Toxicology) and Pathology, Shreveport, LA.

The morphological effects of ethanol (E), Tween 80 (T) and their combination were evaluated in isolated rat hearts in a modified Langendorff procedure. These compounds have been used as vehicles for in vitro and in vivo administration of cannabinoids. The combination of E and T has been reported to alter cardiac contractility and coronary vascular resistance of isolated hearts. The possibility that structural changes may be related to responses was investigated. Spontaneously beating rat hearts were perfused via the aorta with a modified Krebs-Henseleit buffer (pH 7.4, 37°C, 10 ml/min), and infused with either buffer or treatment compound (0.2 ml/min/3 min). Hearts were fixed by 3% glutaraldehyde perfusion at 1.5, 3 and 10 min. post infusion. H & E stained sections of left ventricle showed few changes for the frequent finding of wavy myocardial fibers associated with T infusion. Electron microscopy revealed slight mitochondrial and subcellular edema in buffer-perfused hearts, and similar changes with some z-band abnormality in E-treated hearts. However, the T and T-E treated hearts showed the most severe changes: mitochondrial swelling and disruption, myofibrill disorganization and sarcoclemmal disruption.

BLOOD CHROMIUM LABELING OF ERYTHROCYTES. Michiaki Aihara and R.P. Sharma. Center for Environmental Toxicology, Utah State University, Logan, UT and Institute of Whole Body Metabolism, Inba, Chiba, Japan.

In various toxicokinetic studies it is important to determine the residues of a toxicant in organs and relate these to the concentration of that chemical in blood. However, adjustments are rarely made to account for the presence of chemical in blood contained within the tissue. To calculate the accurate chemical content of an organ, its blood volume should be precisely determined. Male CD-1 mice (30 g) were injected iv with mouse erythrocytes labeled with "Cr. Groups of animals were killed 20 min later either by decapitation or pentobarbital anesthesia. Radioactivity was determined in blood and major organs and the blood volume computed. Brain blood volume was similar (10 ml/g) after the two methods of euthanasia. In other organs decapitation yielded less blood volume in tissues compared to that calculated after anesthesia. The values (ml/g) for the two modes (decapitation and anesthesia) were 131 and 156 for lung, 28 and 49 for liver, and 52 vs. 71 for kidney, respectively. Spleen showed significantly different blood volumes (47 vs. 245) for the two methods of killing. The results emphasize the importance of accurately determining blood volume in tissues employing the euthanasia used in toxicology studies.
AN EVALUATION OF THE DECISION TREE APPROACH FOR ASSESSING PRIORITIES FOR SAFETY TESTING OF FOOD ADDITIVES. J.C. Phillips, R. Purchase, P. Watts and S.D. Cangelosi. BIBRA, Careshalton, Surrey, UK

Large numbers of food additives and contaminants require toxicological evaluation to establish their safety-in-use. As resources to undertake this task are limited a rational mechanism for assigning priorities for testing is required. One possible approach, the Cramer, Ford & Hall "decision tree", has been revised and extended to allow many more chemical structures to be considered and to take into account recent toxicological information. The revised "tree" has been evaluated using a large number of permitted food additives, and a satisfactory separation of compounds into the three toxicity classes obtained. In addition more than 1000 food flavour and 400 plastics monomers have been classified. The assessment of flavours suggested that priority should be given to the safety evaluation of allyl esters, derivatives of eugenol, anthranilates and specific groups of heterocyclic compounds. The majority of plastic monomers (34%) were assigned to the presumptively most toxic class, and a number of structural features likely to be associated with enhanced toxicity highlighted. The decision tree approach appears to be a useful method for suggesting priorities for the safety evaluation of large groups of compounds. (Supported by the UK Ministry of Agriculture, Fisheries and Food)


A technique was developed/evaluated for the repeated or intermittent collection of blood from the rat tail utilizing commonly found lab. materials, and without the need for anesthesia. Rats were individually restrained and tail vein intimated by aid of a tourniquet. The vein was punctured with a heparinized 21 G, 1" hypodermic needle and blood (up to 2 ml) was collected into heparinized tubes by vessel pressure. Male Sprague-Dawley rats with initial wt. (mean ± SD) of 192 ± 9.1 g were studied over a period of 16 weeks. Comparing naive rats (N, 6 rats) to animals sampled once every two weeks (X, 11 rats) there was no difference in general hematology (Hct = 0.44 ± 0.02, R and 0.44 ± 0.03, X) including reticulocyte counts (176 ± 0.29%, N and 1.3 ± 0.16%, X). Biochemical values were as follows: Plasma ascorbate (µg/mL), 1.3 ± 0.18 (N) and 1.5 ± 0.12 (X), p = .05; plasma corticosterone (ng/µg), 275 ± 19 (N) and 253 ± 106 (X), p = .05. The advantages this method has over others are that it requires no cannulation or surgery, is easy to perform and has no minor effects on blood chemistry and can be applied to pharmacokinetic or bioavailability evaluations.


For the establishment of testing priorities, gaps in toxicity data have been identified and structure/activity relationship (SAR) analyses performed on chemicals used solely or uniquely by the U.S. Army. Use categories of 60 selected chemicals include explosives, propellants, obsecurants, pyrotechnics, intermediates, and antimoldew agents. Nitrates and nitro compounds comprise the largest chemical classes. SAR analyses were performed for 20 (33%) chemicals since, for these chemicals, little or no toxicity data exist. Carcinogenicity data were located for 9 (15%) chemicals; 5 (56%) of these have been found to have carcinogenic activity. Mutagenicity data exist for 28 (47%) chemicals; 19 of the 28 (68%) are mutagenic. Data on structural analogues suggest possible mutagenic activity for 4 of 6 (67%) chemicals lacking mutagenicity data. Of 11 (18%) chemicals for which teratogenicity data exist, positive results have been found for 9. Data on primary eye or skin irritancy exist for 35 (58%) chemicals; 32 of 35 (91%) are irritants. Based on the activity of analogues and chemical classes, 11 of 11 (100%) additional chemicals may be irritants. (This work is supported in part by the U.S. Army Medical Acquisition Activity. Contract No. DAMD17-84-C-4133.)


As a start in developing information for industrial hygiene purposes, the acute toxic properties of QL were investigated. Various routes of administration were examined in rats, mice and rabbits including: inhalation, oral, intraperitoneal and dermal. The rates of decomposition of QL aerosols were measured in air and nitrogen at various relative humidities. Decomposition products were identified. Acute inhalation LD50 values and oral and intraperitoneal LD50 values were determined in rats and mice. Rabbit dermal LD50 values were also estimated. The material was found to be a skin irritant but not a skin sensitizer or eye irritant. Acute administration of QL to rats and mice by various routes inhibited both erythrocyte and plasma cholinesterase. The only rat urinary metabolite of QL detected was diisopropylaminoethanol.

A search for a noninvasive method to repeatedly monitor the stomach content pH in the conscious rat was conducted. Current methods require anesthesia, a surgical procedure or killing the rat to excise the stomach. Such procedures are unsuitable for subchronic and chronic drug safety evaluation studies. A method developed in our laboratory uses a specially modified flexible pH electrode from Microelectrodes Inc. of Londonderry, NH. This electrode is compatible for use with any standard laboratory pH meter using a U.S. Standard lead hookup. In vivo use requires a silver-silver chloride reference electrode. Similar pH electrodes are currently used to monitor pH in the esophagus and stomach of both pediatric and adult human patients. Results of developmental studies up to 6 weeks' duration show this method can be used to reliably monitor pH changes in the stomach of the conscious rat. Changes in stomach content pH have been successfully monitored in untreated rats and in rats treated both orally and parenterally with chemicals that inhibit acid secretion. Repeated measurements can be made with no detectable adverse effects.

A M ETHOD TO ASSESS ACUTE ORAL TOXICITY AND ESTIMATE AND APPROXIMATE LD₅₀ IN RATS. D.J. Ball, M. Evans, M.H. Davies, and R.E. Stoll. Sandoz Research Institute, E. Hanover, NJ.

An alternative method using from 14 to 25 rats has been developed to evaluate acute oral toxicity and obtain an estimated LD₅₀ in rats. Fifteen drug substances were tested by this method and the results were compared to classical LD₅₀ values previously obtained for each respective substance. In each study, three rats/dose group were given a single oral administration of test substance at 50, 500 and 1500 mg/kg, respectively. An additional two animals/group were dosed at any concentration that produced lethality at 48 hours postdose. Also, one or two additional groups of 5 animals/group were placed on study based on the lethality observed in the initial dose groups at 48 hours postdose. All animals were carefully observed for overt signs of toxicity and lethality at four time points on Day 1, then once daily to the termination of the study. All animals were sacrificed 14 days after administration of the test substance. An approximate LD₅₀ was obtained by the method of Litchfield and Wilcoxon or Spearman and Karber. Of the 15 substances tested, 14 had estimated LD₅₀ values within the 95% confidence limits established with the classical LD₅₀. More important, with the use of fewer animals, this method allows one to assess the incidence, degree and duration toxicity for the substance tested, and in most cases, will establish a no effect and maximum tolerated nonlethal dose level.

C ONTROVERSY OVER THE ETIOLOGICAL ROLE OF AFLATOXINS IN HUMAN CANCER. D.P.H. Paiieh. Department of Environmental Toxicology, University of California, Davis, CA.

Aflatoxins are potent carcinogenic mycotoxins frequently detected in food. Epidemiological studies in several regions of Asia and Africa have demonstrated a positive association between dietary aflatoxin intake and liver cancer rate. Flaws in the methodology used have undermined the significance of the results and conclusions of these studies. A recent study in the United States, which minimized these flaws, showed that the association between cancer and aflatoxin was very weak. This study, together with the flaws in previous studies, poses a serious question for the aflatoxin-cancer hypothesis. Epidemiological evidence is available supporting an etiological role for hepatitis B virus in liver cancer. However, when geographical and temporal distributions of the disease and the suspect causative agents are considered, the incidence of liver cancer seems to correlate more strongly with exposure to aflatoxin than to hepatitis B virus infection. Despite the controversy between the aflatoxin and the hepatitis B virus hypotheses, they offer together a multifactorial etiological explanation for the occurrence of liver cancer.


In 1967, it was reported that induction of three metabolizing enzymes occurred in liver microsomes of mice and rats on softwood bedding of either red cedar, white pine or ponderosa pine, which was reversed when the animals were placed on a hardwood bedding composed of a mixture of beech, birch and maple. This study was performed to compare the two types of bedding. Groups of 10 male CD-1 mice were housed a) without bedding, b) on softwood bedding and c) on hardwood bedding. Two further groups housed either on softwood or hardwood bedding and receiving 500 mg/ml phenobarbital in their drinking water acted as positive controls. After one week, hexobarbital sleeping times were performed on half the mice in each group, following which they were killed, the liver weight and cytochrome P450 and microsomal protein levels were measured. The remaining mice were treated similarly except that sleeping times were not performed. Results showed no significant differences in sleeping times, cytochrome P450, microsomal protein levels or liver weight between mice without bedding and mice on softwood bedding. Shorter sleeping times and higher cytochrome P450 levels were recorded in comparison with controls for mice on hardwood bedding although no differences in microsomal protein levels or liver weight were recorded.
Before exposure to TMT, macaque monkeys were trained to perform a 3-choice, variable-delay matching-to-sample task. This test can register changes in cognitive as well as non-cognitive functions. Compared to vehicle-treated monkeys, acute oral exposure to 0.5 or 1.0 mg/kg TMT caused a selective decrement in accuracy of delayed matching, while simultaneous matching was relatively spared. This implicates a loss in short-term memory independent of any changes in attention or motivation. The deficit gradually emerged and recovered within 21 days of exposure; maximal impairment occurred 5-7 days after exposure. TMT-induced hyperactivity appeared and subsided more rapidly (Graefe et al., this meeting) suggesting different mechanisms for effects on cognition and activity. Repeated weekly exposure to an ascending series of doses (0.5 to 3.0 mg/kg) produced very small gradual changes, qualitatively similar to acute effects. Brains of monkeys given 0.5 mg/kg were normal. Higher doses caused overt neurological signs and pathological damage focused in the limbic system, notably CA3 and CA4 (Reuhl et al., this meeting). Supported by ES-00260, ES-07065 and ES-03461.

Most learning/memory studies with nicotine have used conditioned avoidance tasks which rely on negative reinforcement. We tested the hypothesis that nicotine (NIC) improves memory and learning in 3 positively-reinforced tasks using male S.D. rats food-deprived to 60% orig. wt. Scopolamine (SCOP), which causes memory deficits in humans, was used as a control. In an AutoShaped Lever-Touch task, in which rats learn to associate lever presentation with food-pellet delivery, both NIC and SCOP (0.25-0.8 mg/kg) impaired acquisition in a dose-related manner when injected either 15 min before or immediately after the daily sessions. In a Single Spatial Alternation task, which requires that responding be alternated between 2 retractable levers, SCOP (0.25-0.8 mg/kg) decreased responding and accuracy while NIC-induced impairment was seen only after the 0.8 mg/kg dose. In an 8-arm Radial Maze, SCOP (0.25-0.8 mg/kg) increased errors and maze-running time, while 0.8 mg/kg of NIC increased running time but did not alter maze performance. Since NIC had major effects on the autochasing task and not in the 2 "memory" tasks, our data suggest that NIC primarily impairs acquisition/learning while SCOP impairs both learning and memory. (Supported by the Kentucky Tobacco Research Board and the U.K. Graduate School)
Alcohol, barbiturates, and benzodiazepines share discriminative stimulus properties and all are subject to abuse. Previous work in our laboratory demonstrated that mice could be trained to discriminate between i.p. toluene and vehicle. The present experiment sought to evaluate if the rat could also learn the same discrimination and to further characterize toluene’s discriminative stimulus properties. Rats were trained to discriminate i.p. toluene (100 mg/kg) from vehicle in a two-lever operant task in which responding was under the control of a fixed-ratio 20 (FR20) schedule of food presentation. Acquisition of the discrimination required a minimum of 100 training days. Injections of either methohexital or oxazepam generally produced toluene-lever responding in a dose-dependent fashion in most animals. The discriminative stimulus properties of toluene were not found to generalize to those of chlorpromazine despite response rate suppressor. These results are consistent with those obtained in the mouse and provide further evidence that toluene has stimulus effects similar to CNS depressants. (Supported by NIDA Grant DA-03112 and NIEHS Training Grant ES-07087)

Abuse of organic solvents remains a significant public health problem. Abuse potential may be related to an agent’s ability to produce a barbiturate-like intoxication. Previous work in our laboratory demonstrated that inhaled toluene produced pentobarbital-like discriminative stimulus effects (Rees, et al., Life Sci. 37: 1319-1325, 1985). The present work examined if halothane, 1,1,1-trichloroethane (TCE), ethanalysis, fluoroethyl, and isomyl nitrite administered via inhalation and oxazepam given i.p. could produce pentobarbital-like effects. A drug discrimination procedure was used in which different groups of mice were trained to discriminate a training dose of either 15 or 20 mg/kg i.p. pentobarbital (PB) from saline in a two-lever operant task in which responding was under the control of a fixed-ratio 20 (FR20) schedule of milk presentation. Drug-lever responding increased in a concentration-related manner following inhalation exposure to TCE or halothane but not following fluoroethyl or isomyl nitrite; oxazepam also produced PB-lever responding. Ethanol produced PB-lever responding in only a few mice. (Supported by NIDA Grant DA-03112 and NIEHS Training Grant ES-07087)

The styrlyoxazine 813U (Burroughs-Wellcome), an irreversible inhibitor of choline acetyltransferase (ChAT) does not impair working memory in rats. Group I rats received 25.50, or 100 mg/kg 813U i.p. and were then sacrificed at various times. Group II received 50 or 100 mg/kg 813U i.p. on days 1, 10, and 18 after reaching criterion (11 correct choices on a 12-arm radial maze). Working memory was evaluated for 4 weeks before rats were sacrificed. In Group I 813U produced dose dependent decreases in ChAT in the neocortex, hippocampus, and caudate one hour after injections. By day 20, ChAT had nearly recovered to pre-injection levels. In Group II, ChAT in the neocortex and hippocampus was reduced to 50 and 40% of control levels. Despite significant reductions in ChAT, rats still performed at criterion levels. The data suggest 813U does not impair working memory in rats perhaps because either acetylcholine stores in the neuron were not depleted enough in 4 weeks to alter behavior, or reductions in ChAT alone were not sufficient to impair memory. Supported by EHS 65-07141.
The most common cause of fatalities in fires in the United States is smoke inhalation. The common toxic element of smoke in different fires is carbon monoxide (CO). In order to determine why human victims are unable to escape from burning buildings, a complex maze was built as a model of a dwelling. Adult Long Evans rats 22 hr water deprived and after a 15-min holding period in the start box were released in the maze. The maze had 8 blind alleys. Following entry into the maze, each animal had 15 min to reach the goal box and receive 5 cc of water. If it did not reach the goal, it was removed from the maze. One trial per day was given to each rat. Entries into blind alleys (errors) and total distance and total time to reach the goal were recorded. The animals were exposed to 2000, 3000, 3500 and 4000 ppm of CO during the 15-min holding period, as well as in the maze during the next 15 min. As the CO concentration increased, a greater proportion of the animals failed to reach the goal. At 3000 and 3500 ppm CO, the animals that entered the maze traveled greater distances than necessary to reach to goal, indicating that the effect of the CO was to disorient the animals. At 3500 and 4000 ppm, no animals reached the goal and the total distance traveled decreased. (Supported by NBS Contract 60RAM4406039).

To evaluate the nature of behavioral deficits produced by organophosphate exposure, albino rats were injected s.c. with 50 μg/kg or 85 μg/kg 1,2,2-trimethylpropyl methylphosphonofluoridate (Somam) or with saline. Three procedures were used to evaluate behavior, beginning 4 weeks after injection. One group was tested in 1) open field activity and 2) reactivity to tactile stimuli. A second group was tested in 3) the Stone maze, a multiple T-maze with 14 choice points. Rats exposed to Somam showed increased activity in the open field and increased reactivity to tactile stimuli; behavior on these 2 tests was positively correlated. Somam produced learning deficits in the Stone maze; all rats made similar numbers of errors at the start of testing, controls had reduced the number of errors by the end of training but exposed rats had not. Responses of rats receiving Somam were more variable than those of saline-injected rats in all tests; some rats in both Somam groups showed behavior similar to that of controls while others showed very different behavior. These results show that organophosphate can produce behavioral changes that persist for at least 4 months after exposure. (Supported by ES 07141 and DAMD 17-C-2225).


RDX is a military explosive that, based on laboratory animal studies and reports of poisoning episodes, may have neurotoxic properties. The present series of experiments was undertaken to evaluate broadly the behavioral effects of acute RDX exposures in male CD rats. When compared to vehicle-treated (2% carboxymethylcellulose in water) rats, RDX (12.5-50 mg/kg), p.o., 2-hr pre-session produced: 1) dosage-related decreases in figure-eight maze motor activity; 2) decreases (not dosage-related) in landing speed; 3) dosage-related conditioned flavor aversions; 4) dosage-related decreased incidence of schedule-controlled responding (VR 50-response and VI 90-second); and 5) dosage-related decreases in startle response amplitude and dosage-related increases in startle response latency. In most instances these effects were substantial and apparent after even the smallest dosage, which is perhaps about 10% of the LD 50. In addition, in some experiments behavioral disruptions were observed on the days after acute RDX administration. Plasma levels of RDX produced by this regimen reached 2-4 μg/ml, and brain levels ranged from 6-9 μg/g. Acute RDX exposures are therefore likely to be associated with multiple deficits in neurobehavioral integrity. Research supported in part by U.S. Army MEDC.

Influence of perinatal-exposure on the behavioral effects of polybrominated biphenyls (PBB) in adult rats and mice. G.C. Haggerty, F.J. Kurtz, B.D. Carlton, A.C. Peters and R.S. Chhabra. Battelle, Columbus, OH and *National Toxicology Program, NIEHS, RTP, NC.

Subchronic exposure to PBB has been shown to produce neurobehavioral changes in rats. The present studies examined whether PBB exposure during early development modified the behavioral effects (motor activity, grip strength, hot plate, acoustic startle and conditioned avoidance) induced by subsequent PBB dosing in adult rats and mice. Animals were exposed to dosed or control feed for 60 days before breeding, and throughout gestation and lactation. At 2 months of age, the young were assigned to chronic dose groups. Behavioral testing took place at 2 months (prior to adult dosing) and at 11 months (9 months of adult dosing). While motor activity scores were increased at 2 months in rats perinatally exposed to PBB, behavioral effects at 11 months were relatively minor. For mice, a marked depression in forelimb grip strength was observed in PBB-treated animals both at 2 and 11 months. Data analysis indicated that the grip strength impairment seen at 11 months was greater in mice pre-exposed to PBB during early development. These results suggest that in mice susceptibility to the chronic effects of PBB on behavior may be altered by perinatal exposure. (Supported by NIEHS Contract No. N01-ES-8-2151).
Several early postnatal behaviors are used to evaluate central nervous system effects of gestational exposure to toxic agents. Neonatal "test batteries" often include body weight (BW), surface righting (SR), negative geotaxis (NG) and reflex suspension (RS). At weaning, tests of exploratory activity include open field (OF), spatial maze (SM) and continuous corridor (CC). The uniqueness or sameness of the information obtained from the tests has not been established. To examine relations between these tests correlations of performance on the tests were determined in control rats and rats exposed gestationally to morphine. Morphine-exposed rats had reduced body weight at birth and showed developmental delays in performance. Positive correlations between tests (P < 0.025) were: RS with NG, RS with BW, and CC with SM for both control and morphine-exposed rats. BW correlated with OF for morphine-exposed rats only. The small number of positive correlations (4 out of 12 possible) suggests that most of the tests measure different components of behavior. (Supported in part by USPHS Grant DA 03237).
THE EFFECT OF A GANGLIOSIDE MIXTURE ON THE DEVELOPMENT OF TRI-O-TOLYL PHOSPHATE-INDUCED DELAYED NEUROTOXICITY. M.M. Berry, B.K. Trosko, D. Tanaka, Jr. and S.J. Bursian, College of Veterinary Medicine, Department of Anatomy, Department of Animal Science, and Center for Environmental Toxicology, Michigan State University, E. Lansing, MI. Sponsor: D. Polin

The effect of a beef-brain ganglioside mixture on the development of tri-o-tolyl phosphate (TOTP)-induced delayed neurotoxicity was examined. Three groups of 10 adult White Leghorn hens were administered in a single oral dose 250 or 500 mg TOTP/kg body weight. Additionally, half the birds in each group were administered, by intraperitoneal injection, a beef brain ganglioside mixture (10 mg/kg body weight) for 21 consecutive days beginning at the time of TOTP administration. Birds were observed daily from day 8 through day 21 for development of ataxia and paralysis. Brains, spinal cords, and sciatic nerves were processed for histological examination at the end of the observation period. Birds in the 250 mg group receiving ganglioside displayed only mild ataxia (score 1.4) at day 21 while untreated birds were severely ataxic (score 5.4). Ganglioside administration had no effect in the 500 mg group. Results suggest that gangliosides may offer a degree of protection against organophosphate-induced delayed neurotoxicity.


Organophosphate-induced delayed neurotoxicity was studied in the European ferret (M. putorius furo) to show species sensitivity, assess clinical signs and histopathologic lesions, and to evaluate the use of the ferret as a model species. In dermal and oral tests, adult male ferrets were given a single dose of tri-o-tolylphosphate (TOTP). In each test, 10 ferrets per group were treated with 0, 250, 500, or 1000 mg TOTP/kg body weight. At 48 hours post treatment, half the animals in each group were sacrificed to assess whole brain neurotoxic esterase activity. The remaining ferrets were observed and received neurologic examinations for 58 days. All ferrets treated dermally with 1000 mg TOTP developed clinical signs of delayed neurotoxicity ranging from ataxia to partial or complete paresis. Three out of the five ferrets treated dermally with 500 mg TOTP showed some degree of ataxia. None of the animals dosed orally with TOTP developed progressive delayed neurotoxic signs. These results suggest that the ferret may be a suitable model for delayed neurotoxicity tests.

BEHAVIORAL EFFECTS OF DERMALLY APPLIED HERBICIDES, J. A. Mitchell and M. C. Wilson, Dept. of Pharmacology, Sch. of Pharmacy, Univ. of Mississippi, University, MS. Sponsor: W. M. Davis

Male Swiss mice, 25-30 g, were utilized to define the behavioral effects of dermally applied herbicides. The herbicides Lasso (alachlor, 45.5% (AI), Basalan [Dichloralin, 43% (IP), Premerge 3 [Dinozob, 51% (DI), Basagran [Bentazon 42% (BI), Chlorelonil [PCNB 24% (PI)], and Maneb 80 [maneb, 80% (MI)], were tested for their effects on locomotor activity and for their ability to establish conditioned taste aversion. Group activity measures were assessed in an actometer immediately following the application (0.15 ml) of the compound. The total number of movements made by each group (N=5) was measured over a 4 hr session. A, F, D, and M treatments increased activity over that induced by the vehicle control (xylene). Sixty animals were tested in a conditioned taste aversion paradigm using a normally preferred 0.3% saccharin solution. Following acclimation and conditioning to the test procedure, the animals were allowed 30 min access to a syringe containing saccharin followed immediately by the application of the substance or control solution. Twenty-four hrs later, the animals were given the choice of 2 syringes, one containing water and the other, saccharin. The percent saccharin consumed and total fluid intake were calculated for each group (N=8). A, F, D, and M produced a significant aversion to the saccharin. Total fluid intake, however, was not significantly altered. (Supported in part by the Research Inst. of Pharm. Sciences.)

BEHAVIORAL RESPONSES TO DERMALLY APPLIED PYRETHROIDS, J. A. Mitchell, M. J. Kallman, and M. C. Wilson, Depts. of Pharmacology and Psychology, Sch. of Pharmacy, Univ. of Mississippi, University, MS. Sponsor: W. M. Davis

Male Swiss mice, 25-30 g, were used to assess the behavioral effects of the pyrethroids, fenvalerate [Pydrin (P)] and permethrin [Amkush (A)]. Eighty animals were subjected to a conditioned taste aversion paradigm utilizing a normally preferred 0.3% saccharin solution. Following acclimation and conditioning to the test procedures, the animals were allowed 30 min access to a drinking syringe containing saccharin followed immediately by the dermal application of the pyrethroid or control solution. Twenty-four hr later, the animals were given the choice of two syringes, one containing water and the other, saccharin. The percent saccharin intake and total fluid intake were measured for each group. P (1800, 600, and 60 mg/kg) and A (300 and 30 mg/kg) significantly reduced the % saccharin intake without affecting total fluid intake. Fifty mice were then tested for the effects of the pyrethroids on locomotor activity. Each group (N=5) was placed in an activity chamber immediately after exposure to the test compound. The total number of movements were measured over a 4 hr session. Activity was increased by A (300 and 30 mg/kg) and P (1800 and 600 mg/kg). (Supported in part by the Research Inst. of Pharm. Sciences.)
THE NEUROTOXICITY OF ACETYL ETHYL TETRAMETHYL TETRALIN (AETT): A CORRELATIVE BEHAVIORAL AND MORPHOLOGICAL STUDY
J. Saphe, L.B. Trombette, M.F. Young
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AETT, a primary demyelinating agent, was used to correlate behavioral deficits with morphological abnormalities. The behavioral tests included open field running, balance beam, tail flick, dorsal limb retraction, and grasping reflex tests. Subsequently, the animals were anesthetized and perfused with glutaraldehyde. Morphological studies consisted of observations of the peripheral and central nervous system by light and electron microscopy. Male Sprague-Dawley rats were orally intubated with AETT in corn oil (100 mg/kg) 3 times a week for 5 weeks. Changes were seen in rearing frequency, locomotion, and balance. These changes correlated with cellular abnormalities. Onion peeling of the myelin sheath from sections of tibial nerve, spinal cord, and medulla were seen. Cytoplasmic inclusion bodies in neurons of the medulla and spinal cord were also found. A direct relationship between behavioral defects and morphological changes was observed.


Homecage diurnal activity of adult female cynomolgus monkeys and male F-344 rats was studied. Monkeys were given acute or repeated (weekly for 14 weeks) doses (0, 0.5-4.0 mg/kg, p.o.) of trimethylen chloride (TMT), and rats received acute doses of TMT (0, 3.0-7.0 mg/kg, p.o.). Activity of individually caged animals was continuously monitored by infrared photo-detectors. A 12:12 hr light/dark cycle was automatically maintained with lights on at 0600 hr for monkeys and 1500 hr for rats. Dose-related hyperactivity 2-3 days post-TMT was observed in both species during the active phase of the diurnal cycle; smaller activity increases in treated animals occurred in the quiescent part of the diurnal cycle. Monkeys were hypoactive during the light phase 6-7 days after each TMT dose. TMT also altered the diurnal pattern of activity in both species. These results suggest that 1) TMT alters the amount and diurnal pattern of activity in monkeys and rats in a similar manner and, 2) that monkeys are more sensitive than rats to TMT. Neuropathological correlates are described at this meeting by Reuhl et al. Supported by grants ES-07065, ES-00260 and ES-03461.

A MULTIVARIATE ANALYSIS OF THE BEHAVIORAL RESPONSE TO PARATHION EXPOSURE. F.O. Risinger and W.M. Bourn, Northeast Louisiana University, School of Pharmacy, Monroe, LA. Sponsor: T.H. Eickholt.

Measures of locomotor activity have been widely used for characterization of chemical induced behavioral changes. In this study the behavioral response to parathion exposure was examined using a multivariate analysis of open field behavior. Rats were dosed orally (.25 - 1.25 mg/kg) and observed for 15 minutes in an open field chamber. These observations were recorded 2 hours after dosing, which was found to be the peak interval for behavior. Cholinesterase changes. The animals were tested under either high light (750 lux) or low light (5 lux) conditions. Multiple discriminant analysis of the open field data indicated the greatest separation of dose groups occurred in the low light condition. Two significant discriminant functions were derived with a group classification rate of 83%. In the high light condition 1 significant discriminant function was derived with a group classification rate of 50%. Variable profiles indicated initial ambulation (first 3 min.) as the most important discriminating variable in the low light condition while ambulation during the latter portion of testing was important in the high light condition. Rearing was also important in the high light condition.


There are few careful studies of the effects of toluene on unlearned animal behavior during acute exposure. We divided a plastic vacuum desiccator into 6 wedges with diffusing plena above and below, yielding an internal volume of 9.5 l and ventilated with 40 lpm of clean compressed air. An overhead circular fluorescent lighted photocells 2 cm from the apex of each wedge; each mouse could serve as its own control. The flat vertical surfaces of each wedge were lined with screening that increased activity. Performance stabilized rapidly for most mice. Six groups of six mice were exposed to each of five concentrations of toluene (500-3000 ppm) or air in a Latin-square design for 1 hour on Tuesdays and Fridays.

Activity increases were obvious at 560 ppm, and decreases at 3000 ppm. The concentrations at which these reversible activity increases occurred are the lowest reported to date; nonetheless, this preparation is less sensitive than studies in the laboratory of the effects of toluene on learned behavior. Support: DA00623, ES01247, and CONACyT sabbatical fellowship No. 40370 to VAC.

The appearance of trichloroethylene (TCE) in ground and surface water supplies represents a potential hazard to public health. To examine the long-term effects of TCE on the developing central nervous system, rats were exposed to TCE throughout gestation until 21 days postpartum via their dams' drinking water (312, 625 and 1250 mg/l). Control dams received distilled water. A navigational swim test was used to test for learning anomalies in older animals (100-150 days old). Rats that were exposed to 312 mg/l of TCE early in life exhibited significantly depressed navigational learning when tested several months after cessation of exposure to TCE. This indicates that exposure to TCE during the period of active brain development may produce long-term behavioral anomalies. (This does not necessarily represent EPA policy; Support by U.S. EPA Coop. Agreement CR800961B).


Trichloroethylene (TCE), a major industrial solvent, is a widespread contaminant of drinking water and is of increasing concern from a public health perspective. Seventy-day-old Sprague-Dawley female rats were randomly assigned to one of five exposure groups: 0 ppm (controls), 156 ppm, 312 ppm, 625 ppm, or 1250 ppm TCE. Exposure began fourteen days prior to breeding and continued until pups were weaned at 21 days postparturition. Locomotor activity of pups, measured from days 36 to 41 post-conception, was significantly depressed in those pups exposed to 312 ppm and 1250 ppm TCE during the dark cycle on day 37 and light cycle on day 38. Pups exposed to 625 ppm TCE also exhibited depressed activity, although it was not a significant effect. Circulating levels of corticosterone measured on day 42 post-conception were not significantly different between exposure groups. However, those pups exposed to 312 ppm TCE exhibited markedly depressed corticosterone levels which approached significance from controls. These data indicate effects of TCE at a low dose, but apparent lack of effects at higher doses. (This does not necessarily represent official EPA policy; Supported by EPA Coop. Agreement CR800961B).

CATECHOLAMINERGIC MODULATION OF 1,1,1-TRICHLORO-2,2-BIS(p-CHLOROPHENYL)ETHANE (DDE)-INDUCED TREMOR. D.W. Herr* and H.A. Tilson. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Tremor is a symptom of various diseases and exposure to some insecticides such as DDT. Neurochemical studies report a dose-and-time related increase in turnover of biogenic amines after DDT administration in rats. DDT (75mg/kg;po) was given to male Fischer-344 rats and tremor was measured 8 hr later by placing the rat on a free moving platform secured to a load cell transducer. Analog output was transformed into a power spectrum by a Fourier transformation. The α-1 antagonists phenoxybenzamine (5mg/kg;sc;30 min prior to DDT) and prazosin (0.5mg/kg;sc;3.5 hr after DDT) decreased tremor intensity. The α-2 antagonist yohimbine (1.5mg/kg;sc;7 hr after DDT) increased the toxicity of DDT causing lethalities. The β antagonist propranolol (2.5mg/kg;sc;30 min prior to DDT) and the dopamine (DA) antagonist haloperidol (0.1mg/kg;sc;30 min prior to DDT) increased tremor intensity. The DA agonist apomorphine (1mg/kg;sc;7.5 hr after DDT) altered the power spectra due to stereotypy. Since noradrenergic has a facilitatory action on spinal motoneurons, the data indicate that α-1 receptors have an excitatory role in the causation of tremor. Also, the β adrenergic and DA systems may tonically inhibit tremor. *Toxicology Curriculum, University of North Carolina, Chapel Hill, funded by ES 07126.

The in vivo- in vitro technique for assessment of DNA repair and S-phase in mouse hepatocytes has potential as a short-term test for detecting both genotoxic and epigenetic hepatocarcinogens. Epigenetic hepatocarcinogens; i.e., carcinogens that are negative in short term tests for genotoxicity, elevate the number of cells in the DNA synthesis phase (S-phase) of the cell cycle. This elevation in S-phase is believed to reflect regenerative hyperplasia but the relationship between hepatocyte toxicity and induction of S-phase is not known. The objective of the present study was to examine the relationship between hepatocyte toxicity, measured as the leakage of hepatocellular enzymes into the serum, and induction of S-phase following a single dose of carbon tetrachloride (CCL4). The enzymes evaluated were gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). S-phase was assessed by quantitative autoradiography. Doses of 1 or 10 mg CCL4/kg did not induce S-phase in the liver and did not elevate serum GGT, ALP, GPT or GOT above control levels. Doses of 30 to 100 mg CCL4/kg elevated S-phase above control levels in a dose-dependent manner and also elevated GGT and GPT, but not GOT and ALP, above control levels in a dose-dependent manner. This data supports the hypothesis that S-phase induction in the mouse liver following administration of CCL4 is related to hepatocyte toxicity and probably reflects regenerative hyperplasia. Studies with additional hepatocarcinogens are in progress.


The nucleoside 5-bromo, 2'-deoxyuridine (BrdUr) competes with endogenous thymidine for incorporation into newly synthesized DNA. Using anti-BrdUr Ab and fluorescein isothiocyanate (FITC)-or peroxidase-conjugated second Ab, BrdUr-containing cells can be labeled. Human fibroblasts (HF), treated with N-methyl-N-nitro-N-nitroso-guanidine (MNG, 10 \( \mu \)g/ml) and subsequently with 25 \( \mu M \) BrdUr-DME medium were then exposed to anti-BrdUr Ab, FITC-conjugated second Ab were added. Control HF received no MNG. Inhibition of replication in MNG-treated cells was indicated by diminished fluorescence of nuclei. Nuclei exhibiting a small amount of fluorescence may indicate DNA repair. 30 rats were exposed 24 hours to an I.p. injection of CCl4 (1.5 ml/kg). Fifty \( \mu M \) BrdUr was injected 3 hours before sacrifice. Livers were quartered in isopentane (-150°C) and freeze-dried. Immunohistochemical studies with tissue sections indicate hepatocyte regeneration and/or DNA repair. This protocol of replication/repair assessment is much faster and more sensitive than traditional autoradiography and eliminates the use of radioactive materials. Results are achieved under 4 hours. The use of tissue sections allows maintenance of morphological detail while permitting localization of toxic response.


The host-mediated assay (HMA) is designed to take advantage of the total metabolism of the intact animal for both activation and detoxification of a given chemical. Bacteria which can survive when introduced into the treated animal can be recovered for subsequent genetic analysis. Mutation frequencies (MF) are determined by comparing the number of mutants over the number of surviving colonies compared to control. A modification of the Salmonella typhimurium microsome preincubation assay was used to include measurements of cell viability to predict potential effect of toxicity on MF. Lower cell concentrations were used to mimic in vivo toxicity. At higher mutagen concentrations the MF compared to controls was inversely proportional to the number of cells plated. It is recommended that toxicity of a compound to bacteria be assessed in addition to consideration of the LD50 when selecting doses in the HMA.
DNA ADJUG FORMATION BY THE SESQUITERPENE LACTONE HYMNEXON, V.L. Sylvia, H.L. Kim, J.O. Norman & D.L. Busbee, Dept. of Anatomy and of Physiol. and Pharm. College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

Hygmenoxon (HYM), a toxic sesquiterpene lactone found in bitterweed, binds deoxyguanosine in a cell-free system and forms adducted guanine residues in sheep lymphocyte DNA. Mitogen-stimulated DNA synthesis in lymphocytes is inhibited by HYM at concentrations greater than 0.10 mM. DNA repair is initiated by concentrations exceeding 0.05 mM and inhibited above 0.10 mM. The reactive dialkyl-form of HYM produces stable Schiff base products with deoxyguanosine which are separable from unreacted HYM and deoxynucleosides by reverse-phase HPLC. These procedures allow quantitation of tritiated nucleoside adducts in HYM-treated sheep lymphocytes and mouse sarcoma cells. Proton nuclear magnetic resonance spectra of purified HYM-deoxyguanosine adducts reveal a less of signals for hydroxyl groups in the bis-hemiacetal of HYM. Spectral analyses show the major adduct to have 33 carbons and a molecular weight of 734, suggesting an interaction of at least two guanine residues per HYM molecule. This adduct configuration is consistent with the high degree of cross-linking observed in HYM-treated DNA. The capacity of HYM (0.01 mM) to produce DNA cross-linking is comparable to that of mitomycin C (0.0025 mM). HYM-induced DNA cross-linking has also been shown in UV4, cross-link sensitive, CHO cells. Supported in part by HL31973, CTR #1448, TAES, and the United States Department of Agriculture.

SENsitIVITY ANALYSIS OF RyDBERG'S MODEL FOR ALKALINE UNWINDING OF DNA. J. Cramer and R.B. Conolly, Toxicology Pro-Chem., School of Public Health, The University of Michigan, Ann Arbor, MI 48109

Ryberg (Rad. Res. 61:274, 1975) proposed a model of DNA strand separation in alkali which has since been widely used to quantitate single strand breaks in alkaline unwinding-based DNA damage assays. This model has the form:

\[ \text{In } F = (K/Mn) \text{tb} \]

where F is the fraction of DNA that is double stranded, k is a friction coefficient for strand rotation, Mn is the average molecular weight of DNA, and b is an unwinding rate constant. We report here a sensitivity analysis of Ryberg's model using a computer-based simulation language and parameter ranges from Ryberg (op. cit.). Ryberg's estimates of b ranged from 0.5 to 0.67. Changes in b of this magnitude require doubling k or halving Mn to fit the data and radically affect predictions of the degree of unwinding. For example after 12 hr 14% of the DNA is double stranded when b = 0.66 and 26% when b = 0.5. Analysis of how parameter variability affects simulation accuracy increases our understanding of both the model and the data. (Supported by USEPA CR812556. Does not necessarily reflect EPA policy.)


SK&F 8542 (trimetereen), a diuretic/antihypertensive agent with weak antifolate activity, was investigated for dominant lethal mutations in germ cells, Male mice (50/group) received SK&F 8542 at 5, 25 or 100 mg/kg/day (p.o.) for 5 days, cyclophosphamide at 100 mg/kg on day one; and, male reproductive performance was evaluated over 48 days with untreated females. There were no adverse effects on mating and fertility in any drug-treated group. Cyclophosphamide induced dominant lethal mutations in early spermatid to mature sperm stages of germ cell maturation as indicated by decreased implants and/or live embryos and increased early resorptions from matings within the first 20 days after treatment, while SK&F 8542 did not. A biochemical assay for activity of the de novo pathway for thymidine synthesis indicated that SK&F 8542 has no antifolate effect on the testes while methotrexate had pronounced effects. Thus, the maximum tolerated dose of SK&F 8542 (20X multiple of maximum human therapeutic dose) did not produce dominant lethal mutations in germ cells of male mice and had no antifolate effect on the testes.
METABOLISM OF 2,4-DIAMINOTOLUENE TO MUTAGENIC AND DNA-BINDING FORMS BY S9 AND ISOLATED RAT HEPATOCYTES, B.B. Purlong, R.P. Weaver and J.A. Goldstein, NIHs, Research Triangle Park, NC

Primary cultures of adult male F344 rat hepatocytes (RHC) were used to investigate the metabolism, DNA binding and mutagenesis of 2,4-diaminotoluene (2,4-DAT). Binding of 2,4-DAT to DNA in RHC increased linearly over 24 hours. Treatment of rats with a P450 inducer, 3,5-benzofurane (BNF; 2 days, 80 mg/kg) had no effect on binding of 2,4-DAT to DNA. Binding of DNA of control RHC was inhibited 30% by metyrapone, and 70% by pentachlorophenol. Metyrapone (MT), a P450 inhibitor, inhibited binding of 2,4-DAT to DNA 30 and 70%, respectively, in RHC from control and BNF treated rats. Two inhibitors of sulfation, pentachlorophenol (PCP) and 2,6-dichloro-4-nitrophenol, inhibited DNA binding by 91 and 85% in RHC from BNF treated rats, while PCP inhibited binding by 70% in control RHC. 2,4-DAT was a more potent mutagen than 2,5- or 2,6-DAT in the Ames Salmonella mutagenesis assay using S9 from control F344 rats. BNF treatment of rats increased mutagenesis of the DAT isomers. These studies indicate that 2,4-DAT binds to DNA in RHC, and binding can be inhibited by a P450 inhibitor and by sulfotransferase inhibitors and is not influenced by BNF induction. Mutagenesis and DNA binding may involve different metabolic pathways.

TRANSFORMATION OF NIH/3T3 CELLS BY DNA FROM 7,12-DIMETHYLBENZANTHRACENE-INDUCED RAT MAMMARY TUMORS. A.E. Aust. Departments of Biochemistry and Microbiology and Public Health, Michigan State University, East Lansing, MI

Recent findings suggest that the genomic targets for mutagenic chemical carcinogens are cellular proto-oncogenes (e.g. ras). Experiments were designed to examine the specificity of activation of oncogenes using various chemicals which induce rat mammary adenocarcinomas. Female Sprague-Dawley rats were treated with a single intragastric administration of 7,12-dimethylbenzanthracene (DMBA) (5 mg/animal). After 20 weeks the animals were sacrificed and the tumors removed. The tumors were frozen and pulverized on dry ice-cooled mortars. The high molecular weight DNA samples, purified using a phenol procedure, were examined for transforming activity in NIH/3T3 mouse cells using a calcium phosphate transfection procedure with 190 µg of tumor DNA per 5.4 x 10⁶ cells. The DNA from 8 of 11 tumors successfully transformed the NIH/3T3 cells at a potency of 0.01-0.02 foci/µg DNA. These results suggest that a cellular proto-oncogene (e.g. ras) has been activated as a result of treatment of the rats with DMBA. Cells from the transformed foci have been expanded in culture so that isolated DNA can be examined for specific oncogene activation. Supported by the Biochemistry Department and the Colleges of Natural Science and Human Medicine, Michigan State University.


In this study six strains of fungi were surveyed for their ability to metabolize and detoxify 7,12-dimethylbenz(a)anthracene (DMBA), a well-known mammalian carcinogen. Experiments with (14C)-DMBA indicated that the extent of metabolism ranged from 5.8 to 28.1% after five days of incubation. The biological activities of the organic-soluble metabolites of DMBA produced by various fungi were subsequently determined in the Salmonella typhimurium reverse assay. The mutagenicity of DMBA was reduced by 63.4 to 75.9% depending on the organism surveyed. DMBA metabolites were isolated from batch cultures of Syncephalasium racemosum and Cunninghamella elegans by HPLC and identified by their UV-visible, mass, and nuclear magnetic resonance spectra. Results indicate that DMBA was metabolized primarily at the methyl groups to form hydroxymethyl derivatives of DMBA. Further oxidation resulted in the formation of three optically active hydroxymethyl trans-dihydridiol isomers. CD spectra revealed that most of these fungal trans-dihydridiols are mirror images of mammalian trans-dihydridiol metabolites, suggesting that there are differences in the regio- and stereoselectivity of the cytochrome P-450 of fungi and rat liver microsomes.

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INDUCTION OF DNA REPAIR AND REPLICATION IN RAT HEPATOCTYES AFTER IN VIVO TREATMENT WITH 2-ACETYLBENZAMINOFUORENE AND 4-ACETYLBENZAMINOFUORENE. C.B. Seiblock and G.M. Deed, IBM Corporation, H60/282, San Jose, CA.

The structural isomers 2-acetamidofluorene (2AAF) and 4-acetamidofluorene (4AAF) were investigated for activity in the in vivo-in vitro hepatocyte DNA repair assay. Adult male Fischer-344 rats were administered the compounds by oral gavage at 2, 12, or 24 hr before hepatocyte isolation. The cells were cultured with 3H-thymidine, and induction of unscheduled DNA synthesis (UDS) was measured by quantitative autoradiography. 2AAF produced a dose-related increase in UDS at 10 and 50 mg/kg, with maximal response occurring at 12 hr post-treatment. At all time points, the fraction of replicating cells in these cultures was similar to that of controls, typically <0.1%. 4AAF produced a slight increase in UDS at 200 and 500 mg/kg, and maximal response also occurred at 12 hr post-treatment. Furthermore, 4AAF produced marked increase in DNA replication at 24 hr post-treatment: at the low and high doses, 8.5% and 34.3% of the cells were undergoing replication, respectively. In a separate experiment, the hepatotoxicity of 4AAF was assessed in vivo by measuring serum concentrations of several marker enzymes, diagnostic of hepatocellular damage. A 24 hr after 4AAF administration. No evidence of hepatotoxicity was detected, suggesting that 4AAF induces DNA replication by a direct mechanism.

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The genetic toxicity of TCM, a plasticizer for poly(vinyl chloride), was evaluated in the Unscheduled DNA Synthesis (UDS) and Chinese Hamster Ovary/Hypoxanthine-Phosphoribosyl-Transferase Forward Mutation (CHO/HFR) Assays for the UDS assay, TCM was suspended in ethanol at concentrations from 250 to 5000 nL/mL. For the CHO/HFR assay, TCM-ethanol suspensions ranged from 5 to 5000 nL/mL. Although cell toxicity was seen in either assay system even at the 5000 nL/mL concentration, higher doses were not used because of TCM insolubility. TCM did not induce significant changes in the nuclear labeling of primary rat hepatocytes and was evaluated as inactive in the UDS assay. TCM did not induce dose-related increases in the mutant frequency at the HFR locus in CHO cells and was considered inactive in the CHO/HFR assay.

(Supported by the DNA Trimeillitante Eaters Panel)

Overproduction of $H_2O_2$ has been implicated in hepaticarcinogenesis caused by chronic exposure to peroxisome proliferators. The effect of $H_2O_2$ on DNA of intact RH was evaluated by determining single-strand DNA breaks (SSDB) using alkaline elution. Addition of $H_2O_2$ (0.05-1 mM) to 18 hr monolayer cultures of RH caused linear concentration-dependent increases in SSDB which were maximal within 30 min. LDH release was 10% of total LDH and no double-strand DNA breaks or DNA-protein crosslinks were observed at any $H_2O_2$ concentration. Experiments with mixed untreated and $H_2O_2$-exposed RH suggested that SSDB occurred in intact RH, not by post-lysis DNA damage. Incubation of RH at 4°C for 30 min prior to and during $H_2O_2$ treatment had little effect on catalase activity but increased SSDB 1.2-2 fold (0.05-1 mM $H_2O_2$) and increased the elution rate detected with $H_2O_2$ concentrations as low as 0.01 mM. The kinetics of SSDB repair were estimated by incubating RH with 2 mM $H_2O_2$ at 4°C, then switching to 37°C $H_2O_2$-free medium for various periods. Disappearance of SSDB was linear (initial $T_{1/2} = 9$ min) only for the first 15 min at 37°C, resulting in repair of about 65% of SSDB. Rejoining of SSDB was maximal (85%) at 60 min of repair.

Aldicarb is a widely used carbamate insecticide, which has been found as a contaminant in ground water in 15 states. The purpose of this study was to examine the potential genotoxicity of aldicarb and its metabolites. Nitrososodicarb was included because the formation of N-nitroso derivatives from nitrite and carbamate pesticides has been reported previously. The membrane retention method (Hasa et al., 1983, Mutat. Res. 122: 177) was used for the quantitation of unscheduled DNA synthesis (UDS). Rat hepatocytes obtained via the iso-density Percoll centrifugation procedure were used. Cytotoxicity was determined by trypan blue exclusion and the leakage of glutamic-oxaloacetic transaminase. DNA concentration was determined fluorometrically by a slight modification of the procedure of Vytasek. Aldicarb, aldicarb sulf-oxide, and aldicarb sulfone were found to be incapable of eliciting any detectable UDS response at all concentrations tested. In contrast, dose-dependent UDS responses were observed with nitrosodcarb. Thus, it may be advisable to include nitrosodcarb in the risk assessment analysis of the agricultural use of aldicarb. (Supported in part by the College of Agricultural & Life Sciences, Univ. of Wisconsin-Madison)

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Urinary bladder is a target tissue for aromatic amine carcinogens. The intrinsic capacity of this tissue to form DNA damaging products was investigated in an in vitro model. Organ cultures of urinary bladder, isolated from New Zealand white rabbits, were exposed to aromatic amines, and DNA repair, measured by autoradiography, was used as an indicator of DNA damage. 2-Aminofluorene (2-AAF), 2-acetylamino-fluorene (2-AAF) or N-hydroxy-2-AAF induced DNA repair. The response was concentration dependent with maximum repair observed at 10^{-3} M 2-AAF or 2-AAF and at 10^{-4} M N-OH-2-AAF. A positive response was also observed with benzidine (BZD); however, no DNA repair was seen after exposure to acetylBZD or diacetetylBZD. Although acetylation of 2-AAF did not decrease its genotoxicity acetylated BZD derivatives were not genotoxic. Thus, in urinary bladder, acetylation of BZD seems to be a detoxification pathway, contrary to what has been observed in liver. These results indicate that rabbit urinary bladder has the ability to biotransform aromatic amine carcinogens to DNA damaging products and has the capacity to repair damaged DNA.
909 MUTAGENICITY OF OXONIZED AND NITRATED PYRENES AND PHENANTHRENES GENERATED IN VITRO. Shane, D.V., Giamalva D.H. and Pryor, W.A. Institute for Environmental Studies and Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803. Sponsor: C.R. Short

Pyrenes (Py) and phenanthrenes (Phe), which themselves are not mutagenic, as well as their nitrated and oxonized derivatives occur in urban air. To simulate environmental conditions, Py and Phe were exposed to NO, and O_3 in vitro. The mutagenicity of the mixtures were evaluated in the Ames assay using TA 100 and TA 98 in the absence and presence of a rat microsomal preparation (59). All the Py and Phe derivatives caused both direct-acting base pair and frameshift mutagenicity, except for oxonized Phe that caused only frameshifts. In general, these compounds were metabolized to less mutagenic compounds in the presence of S9. Fractionation of the nitro-Py (Mpy) mixture showed that the major component is 1-nitropyrene (68%), a potent mutagen. Fractionation of nitro-Phe (Nphe) mixture indicates that 60% is 9-nitrophenantherene (9Nphe) and the remainder consists of equal amounts of the 1 and 3 isomers of Nphe. The 9Nphe is more toxic and less mutagenic than the nitrated mixture. More than 50% of the oxonized Phe (Ophe) consists of diphenaldehyde (DPhe) which is inactive in the Ames assay.


The genotoxicity of Selenazofurin, a selenium analog of the anticancer drug candidate Tiazofurin, was evaluated in a battery of short term tests. This potent antimitabolite is chemically defined as: 2-B-D-ribofuranosyl-4-selenazole-carboxamide. Mutagenicity was evaluated in five strains of Salmonella and in SCE and point mutation assays using V79 Chinese hamster lung cells. All assays were performed with and without metabolic activation. No increase in mutant frequency was observed in the Salmonella tester strains at concentrations up to 10000 ug/plate. Mammalian cell mutagenicity was assessed by the induction of 6-chioguanine (6-TG) resistance at the NOSPT locus in V79 cells.

Survival of mammalian cells was reduced to 13% with 50 ug/ml Selenazofurin, although no increase in mutant frequency was observed. Sister chromatid exchange (SCE) in V79 cells was analyzed following two rounds of DNA synthesis in the presence of 5-bromo-2-deoxyuridine. In the SCE assay, concentrations of drug from 0.01-10 ug/ml increased the incidence of exchanges to a maximum of 2.42-fold over background with evidence of a dose-response trend. Significant induction was apparent in both the absence and presence of S9. The mechanism of SCE induction has not been established although the compound appears to interact with DNA.

910 EVALUATION OF IN-VIVO MUTAGENICITY OF METHYLENE CHLORIDE IN MICE BY DOMESTANT LETHAL TEST. R.R. Bajie, M. Greening, and T. Tolen. A & M Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, New York 11201

Methylene chloride, a widely used industrial solvent, has been shown to be mutagenic by Ames test. This study was undertaken to determine its in-vivo mutagenicity. Groups of ten male s/w mice were injected subcutaneously tri-weekly with 5 ml/kg of 5% v/v or 10% v/v solution of methylene chloride in corn oil; control groups were injected with 5 ml/kg corn oil. Injections were given for four weeks, followed by a week's rest. Each male mouse was then 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 and the fetuses removed. Fetuses were dried, counted for each uterine horn; average weight per litter determined. Half were placed in 12 KOH solution followed by 1% alizarin-s reagent, and the others in 10% formalin. Testes of the male mice from each group were removed and preserved in 10% formalin. No significant difference in any of the mutagenicity parameters was found between control and treated groups. No significant abnormalities were found in the testes, but significant abnormalities were observed in ribs and skull of the fetuses from the experimental groups.

912 FORMATION OF CYCLIC ADDUCTS OF DEOXYGUANOSINE BY REACTION WITH TRANS-4-HYDROXY-2-HEXENAL AND TRANS-4-HYDROXY-2-NONENAL IN VITRO. D.K. Winter, M.J. Small and W. F. Maddon, Veterinary Medicine Pharmacology and Toxicology, University of California, Davis, CA and USDA/WRRC, Albany, CA

Trans-4-hydroxy-2-hexenal (t-4-HH), a reactive metabolite isolated from the pyrrolizidine alkaloid senecione, and trans-4-hydroxy-2-nonenal (t-4-HN), a lipid peroxidation product, reacted nonenzymatically with deoxyguanosine at pH 7.4 in vitro with each compound yielding four covalently-bound adducts. Adducts were isolated using reverse-phase, high-performance liquid chromatography and characterized by their mass spectra and proton magnetic resonance spectra. From the spectral data, it was determined that saturated six-membered rings were generated by reaction at the 1- and 2-N2 positions of deoxyguanosine, forming four diastereomeric adducts. These results demonstrate that t-4-HH and t-4-HN possess the ability to alkylate deoxyguanosine in vitro physically, and suggest similar mechanisms for pyrrolizidine alkaloid and lipid peroxidation toxicity. (Supported by NIH ES03343.)
Recently our laboratory isolated trans-4-OH- hexenal from the hepatic microsomal metabolism of the macrocyclic pyrrolizidine alkaloid (PA) seneconine and demonstrated in vivo that hepatic necrosis occurred following injection into the hepatic portal. To demonstrate similarities in the toxic effects of these compounds, as well as additional macrocyclic PAs and alkenals, genotoxicity and cytotoxicity were examined in primary cultures of rat hepatocytes. A positive cytotoxic response was exhibited by seneconine, retorosine, seneciphylline, (19-OH seneconine), trans-4-OH-hexenal, trans-4-OH- alkenal, and nonenal as measured by the release of LDH. A weaker response was elicited by hexenal. Doses used of each of these compounds ranged from 30-600 nmole/10^12 cells, with each compound exhibiting a linear dose response within this range. All eight compounds exhibited a positive, dose-related genotoxic response as measured by autoradiographic detection of unscheduled DNA synthesis. These results would predict a carcinogenic role for both the PAs as well as the alkenals. This would suggest similarities in the mechanisms of action of the PAs and the alkenals, lending support to the proposed role of trans-4-OH- hexenal as an important toxic metabolite of the PAs. Supported by NIH ES03343.

**MUTAGENIC ACTIVITY AND POLYCYCLIC AROMATIC HYDROCARBON (PAH) LEVELS IN URINE OF HUMANS EXPOSED TO THERAPEUTICAL COAL TAR.** R.C. Confero, M. Zordan, P. Vianer, and A.C. Lesia. Istituto di Medicina del Lavoro and Dipartimento di Biologia, Università di Padova (Italy). Sponsor: M. Motti.

The urinary mutagenicity and excretion of PAH were monitored for 3-4 days in three non-smoking patients treated for psoriasis with applications of crude coal tar.

Total PAH urinary levels after treatment ranged from 9.9 to 507 μg/g of creatinine. Low w.w. PAH represented 60-90% of total. Benzo(a)anthracene and benzo(a)pyrene were present in low amounts. Mutagenic activity after metabolic activation on Salmonella typhimurium TA 100 and TA 98 was detectable in all extracts. Activity increased after addition of beta-glucuronidase; the highest values were in each patient: 37, 6007, 74, 500.

129, 300 induced revertants/g of creatinine. The contribution of conjugated metabolites was about 60%. A weak direct mutagenic activity on both strains was present in one subject. Indirect mutagenic activity was correlated with total PAH concentration (r=0.87, p<0.01).

(Supported by C.N.R., P.F. "Oncologia")
Mathematical model of data from the alkaline elution assay. S. Ruangwiswes and R.B. Connolly. Toxicology Program, School of Public Health, The University of Michigan, Ann Arbor, MI 48109

The alkaline elution assay indirectly detects DNA single-strand breaks and alkali-labile lesions by quantitating the filtration rate of single-stranded DNA after alkaline unwinding. We have found that alkaline elution data for DNA damage caused by X-rays (Kohn et al., Biochem. 13:4134) and by CaCrO (Christie et al., Biochem. Pharmacol. 33:1661) can be accurately simulated by a model describing the challenged DNA as comprised of two subpopulations, DNA₁ and DNA₂. This model has the form:

\[ F = DNA₁ e^{-k₁t} + DNA₂ e^{-k₂t} \]

where \( F \) is the fraction of DNA remaining on the filter. \( k₁ \) and \( k₂ \) are first-order elution rate constants and \( t \) is time. DNA₁ and DNA₂ elute independently and simultaneously and are distinguished by \( k₁ \) and \( k₂ \). These constants and the proportion of DNA in each subpopulation can be estimated from the experimental data. Mathematical models of data, like the one described here, can be used to stimulate development of mathematical "biological models" of biochemical events comprising a toxic response. (Supported by Dow Chemical Co.)

A battery of mutagenicity tests was performed with nafarelin, an agonist analog of LH-RH containing tryptophan (Trp) and histidine (His). Included were the Ames assay and the gene conversion assay with yeast strain D7. Both tests were negative without S9 activation, and the Ames test was negative with S9, but the yeast test was positive with S9. Since the yeast test is based on conversion of cells to Trp independence, we hypothesized that release of Trp by metabolism of the drug could account for the positive result. The test was repeated using Trp instead of drug. The result was positive even at the lowest Trp concentration. In another experiment with the drug, amino acid analysis of the incubation mixture revealed the presence of Trp but no detectable His. Since the Ames test is based on mutation to His-independent cells, these data are completely consistent with the negative result in the Ames test and the false "positive" result in the yeast test. These data suggest the need for caution in interpreting the results from mutagenicity assays with peptide drugs.


Several over-the-counter cough-cold preparation active ingredients or formulations were tested in vitro for mutagenicity in the Ames Salmonella/microsome assay. Brompheniramine maleate, doxy- lamine succinate, ephedrine sulfate, guaifenesin, and phenylephrine were evaluated individually. The marketed product Quelidine® Cough Syrup (Abbott) containing ammonium chloride, chlorpheniramine maleate, dextromethorphan, ephedrine, ippecac, and phenylephrine; and a formulation modeled after Diametane® decongestant tablets (Robbins) containing brompheniramine maleate and phenylephrine were also tested. Five strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA1538) were exposed to each test article both in the absence and presence of a rat liver homogenate (S-9) metabolic activation system. Negative and positive controls were included in each assay. Strain characteristics were verified using ampicillin resistance, and crystal violet and UV sensitivity tests. Each test article was subjected to duplicate assays at four concentrations. Based on standard criteria, all compounds tested proved to be non-mutagenic.


Bisphosphonates (BP's) are structural analogs of pyrophosphate which regulate bone metabolism and are used therapeutically for inhibition of heterotopic ossification, in the treatment of Paget's disease and as bone scanning agents. New members of this class are continuously being investigated. Mutagenicity test (unscheduled DNA syntheses, Ames, Drosophila and in vitro cytogenetic assays) results with BP's have been uniformly negative, except in the mouse lymphoma assay (MLA). This assay has produced apparent positive results for all BP's tested with a heterocyclic aromatic side group structurally similar to pyridine. Two BP's were tested for differential toxicity to the two cell types present in the MLA cultures. The wild-type cells (Tk+/-) were approx. 10-fold more sensitive to BP than are the mutants (Tk/-). This differential toxicity to the surviving cell population (primarily Tk+/-) will produce an artificial increase in the apparent mutation frequency. Thus, the Tk locus does not appear appropriate for evaluating BP's with an heterocyclic aromatic side groups and these compounds show no potential for mutagenicity.

EFFECT OF METHYLCHOLANTHRENE ON INDUCTION OF ATHEROSCLEROSIS IN MICE WITH VARYING SUSCEPTIBILITY FOR TUMORS. B.J. Paigen, P.A. Holmes, and A. Morrow. Children's Hospital Medical Center, Oakland, CA

It has been suggested that atherosclerotic plaques arise from a single mutational event. If so, carcinogens or mutagens would be expected to enhance the development of atherosclerosis. And, animals that are genetically most susceptible to a compound's carcinogenic effect would also be expected to be most susceptible to its effect on atherosclerosis. This was tested in two congenic mouse strains that differ only at the Ah locus, a gene which controls inducibility of the P-450 enzymes. The Ah+ strain is more susceptible to tumor induction by 3-methylcholanthrene (MC) than the Ah- strain. Both strains and their F1 progeny were fed an atherogenic diet for 14 weeks. MC was administered to half of each of these groups during the first week. Aortas were evaluated by microscope for lesions. Without MC all three groups responded equally to the diet in terms of lesion number and size. MC caused an increase in total lesion area in all groups, but this increase was significantly greater in the Ah- and F1 strains than in the Ah- strain. The same experiment in backcross progeny (F1 x Ah-) demonstrated that the increased sensitivity to MC segregates with the Ah gene.

Hepatic nodules may form spontaneously in susceptible strains of mice (e.g., C3H/He) and the numbers of nodules can be influenced by treatment with many compounds including PB and other inducers of xenobiotic metabolism. In this study PB was administered to male C3H/He and C57BL/6 mice at a dose of 85 mg/kg/day for the diet for 91 and 100 weeks respectively. PB treatment resulted in liver enlargement which was associated with a sustained induction of hepatic xenobiotic metabolism in both mouse strains. The metabolism/clearance of PB was also induced. In the livers of control and PB treated C3H/He mice basophilic nodules were observed from 30 weeks onwards and in PB treated mice eosinophilic nodules were also present. Comparatively few lesions were present in the livers of either control or PB treated C57BL/6 mice. These results demonstrate a marked strain difference in PB-induced liver nodule formation in the mouse. Clearly the formation of liver nodules in PB treated C3H/He mice is not a consequence of the failure to sustain induction of hepatic xenobiotic metabolism. (Supported by U.K. Ministry of Agriculture, Fisheries and Food)


A number of chemicals, including certain enzyme inducers, have been reported to produce liver nodules after prolonged administration to mice. In this study we have compared the properties of spontaneous basophilic nodules (BN) present in untreated male C3H/He mice and eosinophilic nodules (EN) induced by administration of 85 mg/kg/day PB in the diet for up to 91 weeks. DNA synthesis was increased in both BN and EN compared to normal liver tissue. Mixed function oxidase enzyme activities in BN were similar to or below those present in the surrounding host tissue from untreated mice, whereas activities in EN and the surrounding host tissue from PB treated mice were greatly induced. After 91 weeks the activity of glucose-6-phosphate dehydrogenase was elevated in BN whereas little effect was observed in EN. In contrast, y-glutamyltransferase was markedly elevated in EN but not in BN. These results demonstrate that PB administration results in the formation of different nodules to those which occur spontaneously in C3H/He mice. Whether or not the properties of the spontaneous BN resemble those produced by classical carcinogens awaits elucidation. (Supported by the UK Ministry of Agriculture, Fisheries and Food)

927 QUANTITATION OF NUCLEAR PORES IN NORMAL HEPA TOCYTES AND HEPATIC NEOPLASTIC NODULES IN AN INITIATION/PROMOTION HEPATOCARCINOGENESIS SYSTEM IN RATS. M.G. Evans and S.D. Sleight, Dept. of Pathol., Mich. St. Univ., E. Lansing, MI 48824

Changes in subcellular organelles are associated with the transition to neoplasia. The hepatic neoplastic nodule in the rat is considered a "preneoplastic" lesion. We hypothesized that numbers of nuclear pores may differ between cells from these nodules and normal liver. Liver tissue from female Sprague-Dawley rats used in an initiation/promotion hepatocarcinogenesis study was obtained at necropsy. Rats weighing 180-200 g were injected ip with initiator (diethylnitrosamine, 10 mg/kg bw) 24 h after 2/3 hepatectomy; 30 days later rats were fed a diet containing a tumor-promoting dose (100 ppm) of 2,2',4,4',5,5'-hexabromobiphenyl (HBB) for 150 days. Controls were not hepatetctomized, initiated, nor fed HBB. Hepatic neoplastic nodules seen grossly were confirmed by light microscopy and were only seen in some HBB-treated rats. Nuclei and control liver sections were fixed, freeze-fractured, and photographed at 24,700X using a transmission electron microscope; numbers of nuclear pores per 9 cm² were counted. Statistically, no significant difference was found between nodules and control livers. We concluded that the number of nuclear pores in normal rat hepatocytes does not differ from those in cells of hepatic neoplastic nodules. (Supported by Ctr. Env. Tox., MSU)


A semi-quantitative analysis of the sequential development of hepatic nodules in two strains of mouse given a standard laboratory diet or one containing PB to allow a daily intake of 85 mg/kg/day has been made. Basophilic nodules were observed in control C3H/He mice from 30 wks. These increased both in number and size to term at 91 wks. Treated animals showed a marked enhancement of nodule numbers at all time intervals. The additional nodule burden comprised a second nodule type formed of large eosinophilic cells. Treated C57BL mice showed a few eosinophilic nodules at 60 wks and these also increased in number and size to term at 100 wks. Treated C3H/He mice reversed to control diet at 60 wks showed a reduction of nodule numbers at 91 wks while C57BL, similarly treated, showed no increase in nodule numbers at 100 wks. In neither strain was there an increase in the incidence of carcinomas among treated animals. (Supported by the UK Ministry of Agriculture, Fisheries and Food)
Phenobarbital (PB), when administered to weaned male mice after an initiating dose of carcinogen as newborns, promoted the liver tumor response in BALB/c and Swiss mice, but inhibited this response in newborn male C57BL/6 x C3H F1 hybrids (B6C3F1) initiated with diethylnitrosamine (DEN). When initiated as young adults PB had a promotional effect in the male B6C3F1. This phenomenon has not been examined in females but it is known that the hormonal environment during the neonatal period is an important determinant of hepatic tumor formation in later life. Because PB induces various cytochrome P-450-mediated hydroxylases in mouse liver, initial experiments were performed to examine various basal hydroxylases in liver microsomes from B6C3F1, C57BL/6 and C3H/HeJ mice using testosterone (T) as a substrate. Hydroxylated T metabolites were separated by HPLC. Both sexes of B6C3F1 and C3H mice formed 6α-, 7α-, 16α-, and 16γ-hydroxy-T as well as significant quantities of androstenedione (A) and several unknowns. In both strains amounts of 6α- and 7α-hydroxy-T formed were approximately 50% higher in females than in males. Microsomes from female B6C3F1 mice also formed significantly more A than those from the males. There were no sex differences in the formation of other T metabolites. It is concluded that the sex-difference in the expression of certain hepatic mixed-function oxidases, which is possibly induced neonatally, may be related to sex differences in tumorigenic response in later life.

TUMOR INITIATING ACTIVITY OF STRUCTURAL ANALOGS OF ACRYLAMIDE. R.J. Bull, M. Robinson, and R.D. Laurie. College of Pharmacy, Washington State University, Pullman WA, and Toxicology and Microbiology, USEPA, Cincinnati, OH.

Acrylamide (AA) is a tumor initiator in the mouse skin (Bull et al., Cancer Res. 44:1077 1984). To determine structural requirements for this activity, we tested N-methylacrylamide (MA), N-hydroxyacrylamide (HMA), acrylonitrile (AK), propionitrile (PN), propionamide (PA), and methyly carbamate (MC) in Sencar mice. Acrylamide and vinyl carbamate are known to be tumor initiators in the mouse skin. Since AA was most active orally, compounds were given by that route. Subsequent promotion with TPA used the topical route (1 μg 3× per week for 30 weeks). MA and HMA were weakly active as tumor initiators. The other chemicals were without significant effect. All compounds with a vinyl group except ACN proved positive. The lack of activity by PA suggests that the double bond is important for activity. It remains to be determined whether the liver activity resulting from substitution on the amide group is due to the masking of this group or to an indirect modification of the reactivity of the vinyl group. [This abstract does not necessarily reflect EPA policy.]

PROMOTION OF CARCINOGENESIS BY TRICHLOROACETIC ACID (TCA), C. Mather, N.J. Parnell, J.H. Exon and I.D. Kolter. Veterinary Medicine, University of Idaho, Moscow, ID 83843.

Trichloroacetic acid is a major non-volatile byproduct formed during chlorination of water that contains organic material. The initiating and promoting effects of TCA were investigated using a rat altered foci bioassay. The experimental protocol used has been shown to induce gamma-glutamyl transpeptidase (GGT)-positive foci in hepatic tissue following an initiating dose with a genotoxic carcinogen. Initiation Protocol: 24 hours following 2/3 partial heptectomy, rats were treated either a single oral dose (1500 mg/kg) or 5000 ppm TCA in drinking water for 10, 20, or 50 days. Two weeks after the end of TCA exposure, the rats were exposed to TCA for up to 12 months with 500 ppm phenobarbital in drinking water. Promotion Protocol: groups of 2/3 partially hepatectomized were initiated with a single oral dose of diethylnitrosamine (10 mg/kg) and then administered 20, 50, or 500 ppm TCA in drinking water. Exposure to TCA resulted in a significant induction of GGT-positive foci only in the promotion protocol. Stimulation of peroxisome-dependent palmitoyl-CoA oxidation was also significantly elevated in rats promoted with TCA. The immune responsiveness, including natural killer cell-mediated cytotoxicity, antibody production, delayed-type hypersensitivity reaction, interleukins 1 and 2, and prostaglandin activity, of the experimental animals in both the initiation and promotion protocols was tested. Significantly altered NK cell response and antibody production were observed in TCA-treated rats. The findings suggest that TCA is a weak promoter of hepatic carcinogenesis in the rat.
Phenobarbital (PB) treatment of male mice previously initiated at 15 days of age produces a variable response in liver tumor formation that appears to be strain related; enhancing (promoting) liver tumors in some strains while inhibiting liver tumors in others. The present study assessed liver tumor promotion by PB in the B6C3F1 hybrid and its parental strains (C3H and C57BL/6). Mice were initiated with dimethylnitrosamine (5 μg/gbw) at 15 days of age. PB was administered in the drinking water from weaning until sacrifice. Control groups included untreated, initiated only, and PB treated only mice. Mice were sampled 16 wks (10 mice) and 24 wks (15 mice) postweaning. Liver tumors were quantitated for each mouse. Initiated/promoted C3H mice displayed a significant increase in both tumor incidence and liver tumor number over initiated only mice after 16 wks and 24 wks of treatment. Both B6C3F1 and C57BL/6 mice sampled at 24 wks exhibited an increase in tumor incidence and liver tumor number in initiated/promoted mice compared to initiated only mice. The number of liver tumors produced by initiation/promotion was strain dependent (C3H > B6C3F1 > C57BL/6).

INHIBITION OF INTERCELLULAR COMMUNICATION BETWEEN PRIMARY CULTURED MOUSE HEPATOCYTES BY LIVER TUMOR PROMOTERS. R.J. Ruch, J.E. Klaunig, and M.A. Pereira. Dept. of Pathology, Medical College of Ohio, Toledo, OH 43699 and HERL, U.S. EPA, Cincinnati, OH 45268

Tumor promoters have been shown to inhibit gap junction-mediated intercellular communication (IC) in cultured cells. Since IC is thought to be important in normal cellular growth control, this effect by promoters may be an important mechanism of tumor promotion in vivo. We have evaluated IC between primary cultured B6C3F1 male mouse hepatocytes and the effects of the rodent liver tumor promoters phenobarbital (PB), lindane, DDT, and a PCB (Arochlor 1254). Precultured donor hepatocytes were labelled with 4 μCi/ml of [5-3H]uridine for 4 h. Non-labelled recipient hepatocytes were plated on the washed, labelled donors at a 10-fold greater density; tumor promoters or DMSO solvent were also added at this time. After 2-24 h, the cells were fixed and processed for autoradiography. IC could be detected as an increased grain distribution over recipients in contact with donors indicating the passage of label from donors to recipients. Non-donor-contacting recipients were not labelled. In DMSO-treated cultures maximum IC (i.e., 78% of the donor-contacting recipients) was detected at 8 h. At non-cytotoxic concentrations (μg/ml), PB (20-100), lindane (0.5-1), DDT (0.1-5) and PCB (0.1-5) decreased IC.


Several organochlorine insecticides appear to increase rodent liver tumors through an epigenetic mechanism of tumor promotion. The biochemical changes associated with promotion are not well understood. In this promotion study, groups of mice were exposed to diethylmaleate (20ppm) in the drinking water for 14 weeks, and dietary chlordecone (50ppm), heptachlor (10ppm) or DDT (50ppm) for 25 weeks. At termination the livers were analyzed for neoplastic lesions and induction of three drug-metabolizing enzymes. Glutathione transferase activity toward two substrates was increased by both chlordecone and heptachlor. Aryl hydrocarbon hydroxylase activity was significantly increased by exposure to all three organochlorine insecticides. UDF glucuronyl transferase activity toward 4-nitrophenol was elevated to a lesser extent by the insecticide exposures. The rank order correlation for tumor incidence and enzyme induction for the three exposures was the same, although the two effects are not necessarily mechanistically related. Nevertheless, liver enzyme induction by these insecticides may be an index of their rodent liver promoting activity.

HEPATIC TUMOR PROMOTERS PHENOBARBITAL AND POLY- BROMINATED BIPHENYLS INHIBIT METABOLIC COOPERA- TION BETWEEN RAT LIVER EPITHELIAL CELLS. M.S. Rezebeck, J.E. Troso, C. Jones, S.D. Sleigh, Depts. of Pathology and Pediatrics/Human Dev., Center for Environmental Toxicology, Michigan State University, E. Lansing, MI 48824.

FireMaster BP-6 (FM), a mixture of polybrominated biphenyls, and phenobarbital promote hepatic carcinogenesis in rats. Dietary vitamin A levels do not significantly affect the promoting activity of FM. Since liver is the target tissue, these compounds were tested in vitro in a metabolic cooperation (MC) assay using a rat liver epithelial cell line (WB-F344). Inhibition of MC is a possible mechanism of tumor promotion. In this assay, cells able to metabolize 6-thioguanine (HGPRT cells) transfer the resultant lethal product via gap junctions to mutant cells lacking the enzyme needed for toxic conversion (HGPRT cells). Compounds which block the transfer of the lethal product allow HGPRT cells to survive. At nontoxic concentrations, phenobarbital caused a 2-fold increase in FM and FM caused a 2.5-fold increase in HGPRT cell survival. Thus these hepatic tumor promoters inhibit MC of hepatic epithelial cells in vitro. Nontoxic concentrations of retinyl acetate caused a 2-fold increase in HGPRT cell survival, and did not affect blockage of MC by phenobarbital or FM. Thus retinyl acetate does not antagonize the promoters' effects on MC, and in fact inhibits MC. Supported by NIEHS Grant ES-07146.
We reported that 3,3',4,4',5,5'-hexamobromobiphenyl (355-HBB) potentiated tumor promoting effects of 2,2',4,4',5,5'-hexamobromobiphenyl (245-HBB) as determined by enhancement of gamma glutamyl transpeptidase enzyme-altered foci (GCT-EAF). To determine effects of 345-HBB and 245-HBB on persistence of EAF and on formation of hepatic tumors, a sequential study using the Peto protocol was done. Thirty days after partial hepatectomy and diethylintrisamine administration, groups of 24 female Sprague-Dawley rats were fed a basal diet or basal diet containing 0.1 ppm 345-HBB, 10 ppm 245-HBB, or 0.1 ppm 345-HBB plus 10 ppm 245-HBB for 140 days. Rats were killed on day 170 or 240, respectively. The xSE of tumor-promoting effects of liver for groups of 6 rats killed on day 170 and 240, respectively, was: control, 144±12, 140±13; 345-HBB, 126±29, 114±24; 245-HBB, 664±84, 575±129; 345-HBB plus 245-HBB, 2293±193, 1708±98. The xSE of tumor-promoting effects of liver for groups of 6 rats killed on day 480 was: control, 1.0±0.6; 345-HBB, 1.8±0.9; 245-HBB, 5.6±0.9; 345-HBB plus 245-HBB, 16.0±1.1. Results indicate that 345-HBB potentiated tumor promoting effects of 245-HBB. Supported by NIHS ES-02781 and Michigan Agricultural Experiment Station.

ENHANCEMENT OF NASAL TUMORS BY CONGENERS OF POLYHALOGENATED BIPHENYLS AND PHENOBARBITAL. K.K. Jensen and G.D. Sleight. Department of Pathology and Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824

Nasal tumors rarely occur in laboratory rats. We report the increased incidence of nasal tumors in rats in an exposure experiment designed to assess hepatic tumor promotion by congeners of polybrominated biphenyls (PBB). Thirty days after partial hepatectomy and diethylintrisamine administration, groups of 12 female Sprague-Dawley rats were fed a basal diet or the basal diet containing 500 ppm phenobarbital (PB), 0.1 ppm 3,3',4,4',5,5'-hexamobromobiphenyl (355-HBB), 10 ppm 2,2',4,4',5,5'-hexamobromobiphenyl (245-HBB) or 0.1 ppm 345-HBB plus 10 ppm 245-HBB. After the 140 days of dietary treatment, all rats were fed the basal diet for 310 days. The number of rats having nasal carcinomas was: control, 2; PB, 1; 345-HBB, 2; 245-HBB, 2; 345-HBB plus 245-HBB, 1. The average time until death due to the nasal carcinomas in rats treated with PBB or PB was 378±18 days. Carcinomas in control rats were found at necropsy on day 480. The number of rats having polyploid adenomas on day 480 was: control, 0; PB, 4; 345-HBB, 1; 245-HBB, 1; 345-HBB plus 245-HBB, 3. Results suggest that PBB and PB decreased the latency time but did not alter the incidence of nasal carcinomas. PBB and PB also increased the incidence of polyploid adenomas.
We determined that HA and HE when administered by gavage to rats and mice induced NA. These substances are found in drinking water so that the GI tract would be a major site of distribution. Feulgen stained sections were evaluated for NA consisting of pyknosis, karyorrhexis and micronuclei. In rats, NA were induced by 1,2-dichloroethane (1,2-DCE) in the forestomach, duodenum, jejunum and ileum and by 1,2-dichloroethane (1,2-DCE) in the duodenum, jejunum, ileum and proximal colon. 1,1-Dichloroethane was inactive which corresponds to its lack of carcinogenic activity in the GI tract. 1,2-DCE increased the mitotic activity in the forestomach while 1,2-DCE decreased the mitotic activity throughout the GI tract. In mice, none of the 3 HE affected the incidence of NA or mitotic figures. This corresponds to their lower carcinogenicity in the GI tract of mice than in rats. Dibromo- and trichloroacetone induced NA only in the duodenum in mice. This would suggest carcinogenic activity in proximal small intestine but not in the other portions of the GI tract. It is concluded that the NA assay might be used to screen genotoxic chemicals for carcinogenic activity and to determine species and tissue sensitivity. This abstract does not necessary reflect EPA policy.

Several chlorinated acetones have been identified in drinking water and these as well as a number of chlorinated acroleins are produced by chlorination of humic acid solutions. Many of these compounds were positive in the Ames assay in this laboratory. To determine if carcinogenic activity was associated with these chemicals, acetones-1,1-dichloro (1,1-DCA), 1,1-dichloro (1,1-DCA), 1,1,1-trichloro (1,1,1-TCA), 1,1,3-trichloro (1,1,3-TCA), and acroleins-2-chloro (CAC), 3,3-dichloro (DDCA), and 2,3,3-trichloro (TCAC) were applied topically to SENCA mice (40/group) at doses of 50, 75, 100 mg/kg (1,1-DCA, 1,1,1-TCA [50 only]); 100, 200, 400 mg/kg (CAC, DDCA, TCAC); 400, 600, and 800 mg/kg (1,1-DCA, 1,1,1-TCA). Doses were applied 6x over a 2 week period in 0.2 ml ethanol per application. After two weeks, 10 µg TPA in 0.2 ml acetone was applied 3x weekly for 10 weeks. After 24 weeks 2 animals with tumor for respective groups above were: 1,3-DCA (48, 45, 30); 1,1,1-TCA (10); 2-CAC (30, 26, 38); 3,3-DCC (3, 0, 0); 2,3,3-TCC (10, 5, 0); 1,1-DCA (0, 5, 3); 1,1,1-TCA (10, 5, 0); controls (5). These data suggest that 1,3-DCE and related compounds induce tumors in the mouse skin. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

Toxicology and carcinogenesis studies of two structurally-related, semi-permanent hair dyes: HC Blue 1 [2,2'-((4-(Methylamino)-2-Nitrophenyl)-Imino) Bis-Ethanol; CAS No. 2784-94-3] and HC Blue 2 [2,2'-((4-(2-Hydroxyethyl)Amino)-3-Nitrophenyl)Imino] Bis-Ethanol, CAS No. 33229-34-4] were conducted by administering the test chemicals in feed (0.15 to 2.02) for 103 weeks to male and female F344/N rats and B6C3F1 mice. In male and female mice, HC Blue 1 produced dose-related increases in the incidences of hepatocellular neoplasms. HC Blue 1 produced a positive trend in the occurrence of hepatocellular neoplasms in male rats and a dose-related increase in pulmonary proliferative lesions in females. There was no evidence of carcinogenicity for HC Blue 2 in either species. Evidence suggests that both chemicals are absorbed from the gastrointestinal tract and are excreted, in part, in the urine. The marked difference in the carcinogenicity of these closely related hair dyes might be due to differences in biotransformation. It is hypothesized that HC Blue 2 is conjugated and predominately excreted as such, while HC Blue 1 may be metabolized to a primary aromatic amine.
ACRYLIC ACID: SKIN CARCINOGENESIS IN ICR/HA MICE. Ilia L. Cote, Anne Hochwalt, Irving Seidman, Gleb Budzilovich, Jerome J. Solomon, and Alvin Segal. Department of Environmental Medicine, Pathology & Neuropathology, New York University Medical Center, NY, NY 10016. Sponsor: C. Snyder.

Acrylic acid (AA) is a compound commonly used in the production of plastics. The present study was initiated to investigate the effects of chronic dermal application of AA and its potential as a tumor promoter in two stage carcinogenesis. Female mice were exposed to 25 μl AA (4% v/v in acetone), applied to dorsal skin, 3 times per week for 1.5 years. This dose was determined in acute and subchronic (6 weeks) exposure trials. Mice in another group were first initiated with 20 μg 7,12-dimethylbenz(a)anthracene (DMBA). Control animals received acetone alone or DMBA followed by acetone. Each group contained thirty mice. Tumors were observed in four animals in the DMBA/AA group (one squamous cell carcinoma and 3 papillomas). Two tumors, both squamous cell carcinomas, were observed in the AA alone group. No skin tumors were observed in controls. This suggests that AA is acting as a complete although weak carcinogen. Supported by USPHS Grants ES00260, ES03043 and CA13343.


The pharmacokinetics of the esophageal carcino- gen methyl-n-amylnitrosamine (MNAN) were studied after a single i.p. injection of 25 mg MNAN/kg to 4 male Wistar rats. Nitrosamine blood levels were measured at intervals by analysis of 1C3, of acidified 75 ml blood samples, by gas chromatography (GC) with Thermal Energy Analysis. Maximum MNAN level was 11.2 μg/ml at 15 min. MNAN elimination from blood followed first-order kinetics, with a half-life of 21 min. The data fitted best with a 1-compartment model. Urinary MNAN metabolites included 2- 3- and 4-hydroxy-MNAN (Mirvish, Cancer Res. 45: 577, 1985), and 3- and 4-oxo- MNAN, which were identified by capillary GC-MS of HPLC fractions, with HPLC performed as before. Metabolites in blood included at least traces of all these compounds. Major blood metabolites were 4-oxo-MNAN (maximum, 6.1 μg/ml) at 1-2 h, with disappearance over next 2 h) and 4-hydroxy-MNAN (maximum, 1.9 μg/ml at 30 min). Of urine metabolites in the same experiments, 80% was excreted in first 6 h. MNAN half-life was less than that of 41 min for chethyl nitros- amine (25 mg/kg) in rats (Wischnok, Tox. Appl. Pharmacol. 43: 391, 1978). Support: NIH grant RO1-CA-35623.

INDUCTION OF OVARIAN TUMORS IN B6C3F1 MICE BY CHRONIC ORALLY ADMINISTERED 4-VINYLCYCLOHEXENE. J.J. Collins and A. Manus. NIEHS, NTP, Research Triangle Park, NC; SoRI, Birmingham, AL. Sponsor: R. Tang.

4-Vinylcyclohexene (VCH), a dimer of 1,3-butadiene present in significant quantities in the gases discharged during tire curing, was administered orally in corn oil (gavage) to female B6C3F mice for 10 weeks at doses of 0, 200 or 400 mg/kg body weight. Survival of the high dose female mice was lower (PKO-001) than that of the vehicle controls, whereas survival of low dose female mice was comparable to that of the controls. Oral administration of 4-VCH to female B6C3F mice for 2 years by gavage was associated with an increased incidence of a number of nonneoplastic lesions, including mild, acute inflammatory lesions and epithelial hyperplasia of the forestomach, congestion of the lungs and adrenal glands at the high dose, and cytological alteration of the adrenal cortex at both doses. However, the most striking finding was the markedly increased (PCO-01) incidences of rare ovarian neoplasms, including mixed benign tumors, granulosa cell tumors, and granulosa cell tumors or carcinomas (combined), in both groups of dosed female mice. In addition, the increased incidence of adrenal gland adenomas in high dose female mice may have been compound related.

SQUAMOUS METAPLASIA IN MOUSE BLADDER EPITHELIUM INDUCED BY DIETARY VITAMIN A DEFICIENCY. R.E. Bagdon, C.J. Molloy, and J.D. Laskin, UMDNJ-Rutgers Medical School, Piscataway, NJ.

CF-1 mice fed a diet deficient in vitamin A for 12-16 weeks developed characteristic signs of retinoid deficiency including body wasting, poor hair coat, altered gait, and xerophthalmia. Histologic examination of bladder tissue from these animals revealed that the normal transitional epithelium had been replaced by a stratified squamous epithelium similar to epidermis. Spinous and granular layers of cells containing keratohyaline granules were visible in the tissue as well as a prominent cornified layer of keratinized cells which lined the lumen. Extracts of vitamin A-deficient bladders when analyzed by gel electrophoresis and immunoblotting with antikeratin antisera were found to contain 6 keratins that were similar to those produced in normal epidermis. Four of these keratins were not present in bladders from untreated mice. Thus, vitamin A deficiency in the mouse produces a squamous metaplasia in the bladder epithelium that resembles skin. Adequate supplies of vitamin A appear to be essential for normal bladder epithelial development.

The nicotine-derived nitrosamine NNK, present in tobacco products and smoke, induces lung adenomas in mice, tracheal papillomas in hamsters and esophageal and nasal cavity tumors in rats. Like other nitrosamines, it is postulated to be metabolically activated by \( \alpha \)-hydroxylation. CB of [14C]-NNK to male SD rat lung microsomal protein was assessed to determine if cytochrome P-450 mediated the activation. Incubations (37°C) included 5 mM MgCl\(_2\), 1 mM EDTA, a NADPH-generating system, 1 mM [14C]-NNK and protein in 0.1M Tris pH 7.5. Blanks (–NADP) were determined for each time, treatment and protein concentration. Protein was washed 6x with hot methanol. GSH (1 mM) was necessary for consistent detection of NADPH-dependent CB by decreasing nonenzymatic CB. NADPH-dependent CB of [14C] was linear up to 45 min and up to 2.0 mg/ml protein and was 3.8 pmol bound/mg protein/min compared to 2.8 pmol/mg protein/min for rat liver microsomes. NADPH could not substitute for NADPH. CB was decreased (p<0.05) by 31% under 5% and 9% under CO:O\(_2\) (4:1) atmospheres. KCN (1 mM) and metyrapone (1 mM) decreased CB (p<0.05) by 12% and 15% respectively. SKF-525A (1 mM) however significantly decreased (p<0.05) NADPH-dependent CB binding by 79% suggesting involvement by P-450. (Supported by Kentucky Tobacco and Health Research Institute)

DNA SYNTHESIS AND ADDUCT FORMATION IN METHYLENZYL NITROSAMINE (MBN) ESOPHAGEAL CARCINOGENESIS: EFFECT OF ZINC DEFICIENCY T.F. Schrag, P.M. Newberne, S.A. Brultman. Boston University School of Medicine. Boston, MA.

Zinc deficiency significantly increases esophageal DNA synthesis before and after exposure to MBN. This may contribute to the enhanced esophageal tumor incidence caused by zinc deficiency since levels of \( \Delta ^{2} \)G and \( \Delta ^{3} \)G at the start and end of dosing are also significantly increased. By switching control and zinc deficient diets after dosing, esophageal tumors are between that of the unswitched diets. Adduct levels in the switched diet groups do not change but post dosing DNA synthesis does. Compared to control diet throughout, rats switched to zinc deficient diet after dosing show a longer period of esophageal DNA synthesis inhibition, and two rather than one subsequent peak of significantly increased DNA synthesis. Rats switched to control diet after dosing show a shorter period of significantly inhibited DNA synthesis than do rats that remain on zinc deficient diet. There is also a period of normal DNA synthesis prior to a significant enhancement which is not seen in rats given only zinc deficient diet. These results suggest that post dosing esophageal DNA synthesis may be involved in or indicative of dietary effects on subsequent esophageal tumor incidence induced by MBN.


Recently the nasal passages have been recognized as an important target site in inhalation toxicology. To better understand the role of host defense mechanisms in the molecular dosimetry of inhaled chemicals, a model compound was developed for studies of chemical-induced DNA damage. ENEC was synthesized from \( \alpha \)-ethylurethane by nitrosation with dinitrogen tetroxide. The resulting volatile nitrosocarbamate, a known direct acting carcinogen which spontaneously decomposes to yield ethylidiazohydroxide, had a \( t_{1/2} \) in FQ buffer (pH 7.4) of 222 min. Isolated rat nasal mucus or albumin added to the solution decreased the \( t_{1/2} \) to 181 and 152 min, respectively, suggesting that ENEC reacts with nasal secretions. In addition, ENEC was a substrate for rat nasal carboxyl-esterase, a potential site of enzymatic detoxication. To determine if inhalation of ENEC vapor results in quantifiable DNA adducts 16 rats were exposed nose-only for 2h x 2d to \( \approx 80 \) ppm ENEC and sacrificed immediately after the second exposure. Competitive RIA for O'-ethyldeoxyguanosine revealed 4.6 ± 0.7 and 0.1 ± 0.8 (xSEM) moles O'Etdeox/mole DNA in respiratory and olfactory nasal mucosa, respectively. These results suggest that ENEC will be a useful compound to study molecular dosimetry of DNA adducts in the rodent nasal passages.
955 A SIMPLE ALGORITHM TO PREDICT EYE IRRITATION
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Dallas, TX. Sponsors: N. A. Kune

A preliminary algorithm was developed to predict the eye irritant potency of chemicals from simple, readily-available physico-chemical data. Reference eye irritation data were supplied by Union Carbide Corporation (from whom details of the scoring system are available). The algorithm is a linear regression equation employing octanol-water partition coefficients (P), zero- and first-order "basic connectivity" indices (C), and zero- and first-order "valence connectivity" indices (X). Partition data and connectivity indices were gathered from published and/or commercial sources.

Study of an initial group of 49 chemicals yielded the following equation:

I = 0.817 + 7.17(logP) + 1.62C + 0.12X

The correlation between the observed and predicted scores was 0.86.

A simple algorithm to accurately predict the outcome of in vivo experiments could significantly reduce the number of animals used in routine testing. Additional classes of compounds are under study for further algorithm development.

956 INFLUENCE OF REDUCED NUMBERS OF RABBITS ON THE ADEQUACY OF EYE IRRITATION TESTS. N.S.
Rogers, and R.A. Carvin. J.T. Research Institute and
Amoco Corporation, Chicago, IL.

The Draize eye irritancy test in rabbits has been the focus of recent efforts to reduce the use of live animals in toxicity testing. Although a suitable alternative is not yet available, we have studied the adequacy of reducing the group size. Data generated from 6-rabbit eye irritation tests of 155 various materials were used to determine the ability of irritation scores from all possible combinations of 2-, 3-, 4-, or 5-rabbit subsets to predict the Draize score derived from 6 rabbits. There were 2325, 3100, 2325, and 930 possible combinations of 155 studies for the 2-, 3-, 4-, and 5-rabbit subsets, respectively. We classify materials using a four-level adjuvant rating system based on (among other factors) the Draize score. Comparisons indicated that 2-, 3-, 4-, and 5-rabbit scores were in 91, 94, 96, and 98% 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 0.84, 0.99, 0.99, and 0.99, respectively. This study confirms the findings of an earlier report by DeSousa et al. (TAP, 76:234-242, 1984), and indicates that a high level of accuracy can be obtained with reduced numbers of animals per test group.

953 EFFECTS OF SODIUM CYCLAMATE AND SODIUM CHLORIDE ON METABOLIC COOPERATION BETWEEN CHINESE RAMSTER V79 LUNG FIBROBLASTS. A.R. Malcolm, U.S. E.P.A., and

Current evidence suggests that the nonnitrative sweetener, cyclamate, is not a complete carcinogen. There is, however, evidence that cyclamate possesses cocarcinogenic and tumor-promoting activity in the urinary bladder of mice and rats, respectively. We are testing the hypothesis that tumor promoters inhibit metabolic cooperation (MC) based on the transfer of toxic 6-thiouguanine metabo-

lites from hypoxanthine-guanine phoshothioyl transferase competent wild-type cells to co-cul-
tured, enzyme-deficient, mutants. Sodium cycla-
mate inhibited MC in a dose-related fashion at high (1-5 mg/ml) concentrations. Tests with sodium chloride alone failed to show inhibition of MC at concentrations of 1-2 mg/ml NaCl. These results support the hypothesis that sodium cyclamate has tumor-promoting potential and that such potential is not associated with sodium. Because some tumor promoters may produce effects through metabolites, selected metabolic products (cyclenehexylamine, cyclohexanol, and trans-cyclohexan-1,2-diol) are being tested for effects on MC. This abstract of a scientific meeting presentation does not necessarily reflect U.S. E.P.A. policy.

Human and rabbit eye irritation responses to four prototype household products have been compared under identical test conditions. After establishing a no-effect concentration in rabbits, dosing was initiated in humans starting at 1/2 of the rabbit no-effect concentration. Dose volumes of 0.01 ml and 0.1 ml were instilled into the test eyes and the equivalent amount of sterile water into the control eyes. Concentrations were gradually increased until a predetermined "cut-off" reaction was observed. Grades were assigned by a technician trained in evaluating rabbit eye irritation responses and by a MD Ophthalmologist. Two to three groups of 8 subjects each were treated at or below the cut-off concentration. The same concentrations were then instilled into rabbit eyes for a direct comparison of responses. Results from the study indicate that 1) the lower volume dose in animals (0.01 ml) correlates better with human response than did the conventional Draize/FHSA dose (0.1 ml); 2) rabbit tests overestimate human response with 0.01 ml in animals producing more irritation than the 0.1 ml in humans and 3) the human test data closely approximates consumer experience from accidental exposures.


DMAEE (CAS No 3303-62-3), a liquid used as a catalyst in the manufacture of polyurethane foams, may come in contact with skin, and was therefore investigated for its percutaneous and local dermal toxicity by single and repeated exposure in rabbits. Single occluded contact for 3 min or longer with undiluted DMAEE caused severe local inflammation and necrosis. Acute percutaneous LD50 values (ml/kg) for undiluted DMAEE were: 4 hr contact 0.41(0.30-0.54) for males and 0.63(0.54-0.7) for females; 24 hr contact 0.37(0.25-0.55) for males and 0.37(0.20-0.67) for females. For a 20% (v/v) aqueous solution, LD50 (ml/kg) values were 2.16(1.45-3.17) with males and 2.83(1.66-4.77) for females. With 9 daily applications of 1 ml aqueous solution containing 2.5 to 10% (v/v) DMAEE, there was a concentration-related decrease in food consumption and body weight. Renal tubular epithelial swelling (collecting ducts) and moderate to marked local erythema and necrosis were also present. Subchronic recurrent applications of DMAEE, 1 ml of 0.25 to 2.0% (v/v aqueous), did not produce any evidence of nephrotoxicity or other systemic toxicity, but a cumulative moderate to marked dermatitis was present. A potential for local cutaneous inflammation and systemic toxicity exists by skin contact with aqueous solutions containing DMAEE.

A SIX MONTH OCULAR SAFETY EVALUATION STUDY OF CELIPROLOL HCL OPHTHALMIC SOLUTION IN RABBITS. B. Brar, R. Chau and L.K. Wong, Dept. of Drug Safety Eval., Revlon Health Care, Tuckahoe, NY.

Celiprolol Hcl is a cardioselective beta-adrenoceptor antagonist. When applied topically this compound is effective in decreasing intraocular pressure in laboratory animals and is a potentially useful agent for the treatment of glaucoma. This study was undertaken to evaluate the ocular and systemic toxicity of the ophthalmic solution. Celiprolol Hcl ophthalmic solutions 0.5%, 1.0%, 2.5% and placebo vehicle 0.1 ml were instilled into the right eyes of New Zealand White rabbits 5/sex/group BID for six months. The eyes were scored for irritation at 24, 48 and 72 hrs. after the first instillation and weekly thereafter. A complete ophthalmological examination with indirect ophthalmoscopy and slit lamp biomicroscopy was conducted once during the quarantine period and approximately at one month intervals. One rabbit/sex/group was sacrificed at 3 months. There were no mortality, body weight, hematological, clinical chemical or ophthalmological changes that could be attributed to treatment. Gross and microscopic examination of ocular and other tissues revealed no drug induced effects. It was concluded that Celiprolol Hcl ophthalmic solution up to 2.5% was non-irritating to the eye and produced no systemic toxicity in rabbits.

A METHOD FOR RABBIT DERMAL TOXICITY TESTING EMPLOYING VETRAP® BANDAGING TAPE. R. C. Myers, K. R. Hufford, and N. S. Bellich, Bushy Run Research Center, Union Carbide Corporation, Export, PA. 15632 Sponsor: E. R. Homan.

The 24-hour occluded rabbit skin test is the common procedure for evaluating dermal toxicity. Several methods of ensuring suitable dermal contact with the test material, while preventing its removal and ingestion, were investigated. The most successful technique involved wrapping layers of gauze and polyethylene sheeting over the dosed skin of the animal's trunk. To secure these wrappings, a layer of Vetrap® Bandaging tape (3M) was used. Vetrap® is a highly elastic and self-adhesive material which contains no glue-like substances. It does not adhere to the skin or fur and is easily removed. Without any additional restraining devices, all wrappings remained securely in place throughout the 24-hour contact period. Dosed animals suffered no apparent discomfort or limitation of movement, and access to feed and water was not restricted. In limited comparisons, dermal toxicity test results were similar for chemical samples dosed by a method using animal restraint and retested by using the Vetrap® method.
DERMAL TOXICITY OF ALCIDE ALLAY® IN RABBITS. M. Abdel-TahAIM, G. Skowronski, S. Gerges, R. Turkall and A. Abu-Hadeed, Pharmacology Dept., NJ Medical School, Newark, NJ

Alcide is a germicidal drug which is highly effective in killing a wide range of bacteria and fungi. 2.0 g/kg Alcide gel (containing 1.21 or 0.32 g % NaClO2 as active ingredient) or placebo were applied once per day, while 3.0 g/kg Alcide liquid (containing 0.43 or 0.26 g % NaClO2) or placebo were administered three times per day for 30 days. Maximum erythema was observed only in the high dose gel group after 7 days of treatment, but skin appeared normal by day 18. Histologically fixed skin at day 30 showed inflammatory changes in the high and low dose gels and hyperkeratosis in all gel groups. After 30 days treatment, hematologic and clinical chemistry tests and necropsy were performed. MCHC decreased significantly in the Alcide gel compared to the control group. In all gel treatments, BUN/creatinine levels decreased significantly. Pancreas/body weight ratios were significantly increased in all gel groups while the pituitary/body weight ratio was significantly increased in the placebo and high dose gel groups. Hematology and clinical chemistry parameters were within the normal range of values for the Alcide liquid groups. These results were supported by the histology data. Spleen, pancreas, brain and ovary/body weight ratios were significantly higher in both liquid dosage groups compared to the control group.

EFFECT OF MONOETHYL GLUTAMATE ESTER (GEE) ON TOTAL INTRACELLULAR GLUTATHIONE (GSH) LEVELS OF ACETAMINOPHEN (APAP) AND BUTHIONINE SULFOXIMINE (BSO)-TREATED RAT HEPATOCYTES. R.F. Frick and J. Jorge, Pathophysiology Division, US Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, MD.

GEE, unlike GSH, is thought to be transported intact and deesterified to increase intracellular GSH levels. GEE has been shown to be effective in vivo against the hepatotoxic effects of APAP (Puri and Meister, PNAS, 80, 5258). In primary cultures of rat hepatocytes, the effects of GSH, GEE, and the GSH produg, L-2-oxothiololidine-4-carboxylate (OTC), on total intracellular GSH levels were studied. Cells were exposed to APAP (5 μM) or BSO (1 μM), a specific inhibitor of GSH synthesis, followed by the addition of either GSH, GEE (1 μM each), or OTC (5 μM). After 4 hr, total GSH levels (nmol/10^6 cells) were: 44.1 (control), 102 (GSH only), 78.1 (GEE only), 83.4 (OTC only), 19.2 (APAP only), 56.7 (GSH + APAP), 54.7 (GEE + APAP), 53.8 (OTC + APAP), 32.0 (BSO only), 35.4 (GSH + BSO), 36.8 (GEE + BSO), and 36.3 (OTC + BSO). GSH, GEE, and OTC all increased GSH levels (p<0.001) and prevented the decrease by APAP (p<0.001). BSO inhibited GSH synthesis in all cases (p < 0.001 vs control), including GEE, an unexpected observation. The GEE data might be explained by: (a) BSO inhibits the uptake of GEE, (b) GEE is broken down extracellularly, the products transported, and resynthesized to GSH intracellularly.

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A monoclonal antibody directed against the major isozyme of cytochrome P-450, which is isolated from the hepatic microsomes of 3-methylcholanthrene-induced adult rats, inhibited the aryl hydrocarbon hydroxylase (AHH) activity of those microsomes >90% and of benzo[a]pyrene (BaP)-induced epidermis, >87%. However, when basal cells were isolated from the epidermis of 3-day-old rats, cultured under conditions that allow differentiation and stratification, and subsequently induced in vitro by BA, the induced AHH of those cultured cells was not inhibited by the monoclonal antibody. Immunohistochemical staining of uninduced epidermis from newborn rats using this monoclonal antibody indicated the absence of this P-450 isozyme, but after in vivo induction by BA, distinct staining of the spinous cell layer was observed. No positive staining was seen for either control or BA-induced cultured keratinocytes.

LECTIN BINDING AS A PROBE OF IRRITANT-INDUCED CHANGES IN KERATINOCYTE PROLIFERATION AND DIFFERENTIATION IN VITRO. W. Ku and I. A. Bernstein. Toxicology Program, Dept. of Env. and Ind. Health, Univ. of Michigan, Ann Arbor, MI.

A cutaneous keratinocyte culture system has been developed to model the effects of a chemical irritant and alkylating agent, bis -( 8-chlороethyl) sulfide (BCES), on cell proliferation and early differentiation. Lectins are used to reveal cell surface carbohydrate changes as the keratinocytes differentiate. In the newborn rat epidermis, the isolectin, Griffonia simplicifolia 1-B4 (GS 1-B4), binds to basal cell surfaces. Ulex europaeus agglutinin I (UEA) binds to the surfaces of spinous and lower granular cells. Monolayer cultures of rat keratinocytes in low Ca medium (0.08 mM) exhibit a unimodal pattern in the ratio of UEA to GS 1-B4, bound over a 7 day period as determined by a fluorometric dual-lectin binding assay. Autoradiographic and biochemical evidence suggest that the variation in the UEA/GS 1-B4 ratio during this culture period reflects proliferative (low ratio) and early differentiative (high ratio) phases. Cultures exposed to 1 μM CBCES at Day 1 show later perturbations in the UEA/GS 1-B4 ratio that are consistent with 1) a decreased growth fraction and 2) an arrest of further keratinocyte differentiation and a failure to desquamate. Differential lectin binding in monolayer cultured keratinocytes appears useful in studying irritant-induced changes in the cutaneous epidermis.
THE EFFECT OF TPA AND DIFFERENTIATION ON CELLULAR RETINOIC ACID BINDING PROTEIN (RABP) IN PRIMARY RAT EPIDERMAL KERATINOCYTE CULTURES. R. Kohoe, R.S. Mitra, R.A. Brown, J.A. Bernstein. Toxicology Program, University of Michigan, Ann Arbor, MI

Retinoids demonstrate anti-tumor promoting capacity and the ability to direct differentiation in epithelia. The molecular mechanism of this action remains unknown, but may be related to intracellular binding proteins. Primary rat keratinocyte cultures responsive to retinoic acid (RA) were used to investigate the expression of cellular RABP during differentiation and after treatment with phorbol acetate (TPA). Keratinocytes were grown to confluence in medium containing 0.1mM Ca (lowCa). These were treated with either 2.0mM Ca (nor Ca) to induce stratification or 1nm TPA for 24 hours to study tumor-promoting effects. Cytosolic samples of the cells were incubated with 3H-all trans-11,12-RA with and without a 200 fold excess of cold RA. RABP was implicated when analysis of S-20S sucrose gradient centrifugation demonstrated a peak of radioactivity that was reduced by the 200x cold RA. Nor Ca cultures showed increased amounts of RABP in terms of molcule/mg total cytosolic protein. TPA exposed cultures demonstrated altered RABP compared to lowCa samples in both amount and sedimentation profile. These studies suggest that the level of RABP may be influenced by cellular differentiation and by compounds (TPA) that affect this process. Supported by NIH-CA32470 and ES07062

A SENSITIVE ASSAY FOR THE DETERMINATION OF LOW LEVELS OF DNA DAMAGE IN PRIMARY CULTURES OF RAT EPIDERMAL KERATINOCYTES. F. L. Ribeiro, R. S. Mitra and J.A. Bernstein. Toxicology Program, Dept. of Env. and Ind. Health, University of Michigan, Ann Arbor, MI.

Nucleoids are structures containing supercoiled DNA associated with a small amount of protein, prepared from intact cells by gentle lysis at neutral pH with a nonionic detergent. Sedimentation rates of these structures in neutral sucrose gradients containing 2 M NaCl are affected by the presence of DNA single strand breaks (SSB). This method, modified for application to epidermal keratinocyte cultures, was employed in an effort to investigate the effect of bis(+)-chloroethyl) sulfide (BCEs), an alkylating agent, on the integrity of DNA. Rat epidermal keratinocytes, grown as monolayers in low Ca++ medium (0.06mM), were exposed to BCEs for 1 hr. Formation of SSB, determined by this method, is dose dependent, with the maximal effect occurring at 10 uM BCEs. SSB were detected in cultures exposed to as low as 0.05 uM. This represents the most sensitive effect attributed to BCEs yet to be determined with this system. Exposure to 1 uM BCEs results in an inhibition of 3H-thymidine incorporation and a 20% loss of DNA from the cultures. After the removal of BCEs, followed by 4 hr incubation, cells exposed to 1 uM BCEs exhibited normal nucleoid sedimentation rates.


A and MA esters are the subject of 1 in 40 of New Chemical Notices submitted to EPA by manufacturers and importers as required by the Toxic Substances Control Act. EPA is concerned with their possible toxic threat to man and environment, which, because of the unavailability of experimental data on these chemicals, must be predicted by analogy with which there is data. Review of the literature shows that some A esters when painted on mice for life, caused squamous cell carcinomas and sometimes lymphomas. Both A and MA esters are rapidly hydrolyzed in vivo, and the free acids catabolized to CO2. A and MA esters react with glutathione (GSH); alkylating the S atom by Michael addition; the free acids do not. The acute toxicities of A/MA esters are qualitatively similar, although MA esters are less toxic. A and MA esters are generally inactive in the Ames test, but are positive in the mouse lymphoma assay. Certain A/MA esters have been shown to cause neurotoxic effects, but for most substances, carcinogenicity is the major concern. Since MA derivatives have not been shown to be qualitatively different in expression of their biological activity, it must be assumed that dermal contact with either A or MA esters may be a carcinogenic hazard.


Subchronic toxicity studies of petroleum stocks were conducted by dermal application to rats. No significant irritation of skin or effects on body weight gain, hematology, serum chemistry, organ weights, or histology were seen when several solvent-refined petroleum lubricant base oils were administered for 13 weeks at daily doses of 2000 mg/kg/day. In contrast, marked toxicity was seen with Clarified Slurry Oil (CSO, CAS #64741-62-4), the heavy residual fraction from the Fluidized Catalytic Cracker, which contains more than 50% PAHs. CSO was applied to rats 5 days/week for 13 weeks at dose levels of 8, 30, 125, 500, or 2000 mg/kg/day. Dose-related mortality and depression of weight gain occurred at 30 mg/kg/day or greater. Other dose-related effects included hypoplastic anemia and thymic depletion at 30 mg/kg/day or greater, liver necrosis and cholangiolitis at 125 mg/kg/day or greater. Liver effects included: increased serum conc. ALT, AST, APase, triglycerides, and bilirubin; decreased serum glucose and chloride; bile duct inflammation; extensive hepato cellular necrosis progressing toward fibrosis similar to postnecrotic cirrhosis.

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SUBCHRONIC DERMAL TOXICITY OF INY-6202 IN THE RABBIT. S.E. Loveless, H.C. Chen and G.L. Kennedy, Jr., E.I. du Pont de Nemours & Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P.O. Box 50, Newark, DE.

The subchronic dermal toxicity of INY-6202 (Propanoic acid, 2-[5-(6-chloroquinolin-2-yloxy)ethylene ester] 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 treated areas were wrapped with flexible bandages for 6 hours a day and then washed off with water. Rabbits were weighed and observed daily during the test. Hematologic and clinical pathologic studies were conducted on all rabbits 3 days prior to the first dose, 1 day following the 21st dose and after a 15-day recovery period. Six rabbits per group (3 of each sex) were given a complete pathologic examination the day following the 21st dose; the remaining 3 per group were similarly examined after a 15-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. Histopathologic and clinical pathologic examinations revealed no compound-related effects. INY-6202 at doses up to 2,000 mg/kg failed to elicit any signs of toxicity following 21 consecutive days of dermal exposure.


Finishes impart physical characteristics such as flexibility and processability to fibers such as Dacron® and nylon. These finishes are mixtures of esters of fatty acids and alcohols, sulfated or ethoxylated fatty glycerides, amine or alkanol amine soaps, polyethylene oxide polymers, alcohol or nonylphenol alkylates among others. Finishes have the potential for contacting skin during production of the fiber and in the final end product which could be a finished fabric. A series of 11 representative finishes were tested for their skin irritancy potential, applied as a liquid to rabbit skin and as contained on the fiber surface to human skin. On rabbit skin, the finishes ranged from slightly to severely irritating. The degree of irritation could not always be predicted from the irritancy of the individual chemicals and could be reduced by lowering the concentration tested. When present on fibers at usual concentrations (0.3 to 0.8% finish on yarn) none of the 11 finishes tested produced irritation (or sensitization) of human skin.

REPRODUCIBILITY OF ACUTE TOXICITY TESTS - ORAL TOXICITY, EYE, AND SKIN IRRITATION. G.L. Kennedy, Jr. and O.L. Dashiell, E.I. du Pont de Nemours & Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P.O. Box 50, Elkon Road, Newark, DE.

To evaluate the variability of standard acute toxicity tests, the oral LD50 of an experimental insecticide, the eye irritation potential of 4-nitrobenzenepropanoic acid and the skin irritation potential of 2-bromo-4-nitrobenzenepropanoic acid were determined several times between 1980 and 1982. The oral LD50 values (8 replicates) ranged from 190 and 69% mg/kg. The mortality dose-response curves differed significantly but no single experiment stood out as being significantly different from the rest when compared by logistic regression. The skin irritation test was repeated 7 times with no significant differences seen with regard to erythema at 24 hrs or edema at 24 or 48 hrs. At 48 hrs, the degree of erythema remaining (none to mild) was greater the first time the test was conducted. Six replicates of the eye irritation test failed to reveal any significant differences. The reproducibility of the 3 tests studied was good with the general ranking of toxicity being the same for each experiment. This reflects the reduced number of variables for a particular study type and, in the case of the irritation tests, the use of the same individual investigators for irritancy readings.

USE OF ARAHED SKIN TO ENHANCE THE SENSITIVITY OF IN RHEER GUINIA PIG SENSITIZATION METHOD. M. Uekensky & H. Szilveszter, Horion Products Western Hemisphere Research, Parsippany, N.J., U.S.A.

Two studies were conducted to determine if abrasing the skin at the application sites will increase the sensitivity of the Fluehler method to potential sensitizers that are too weak to detect because of their low dermal penetration ability. Isoeugenol, a known sensitizer in petroleum, was used as the test material. In the first study, three groups of 10 Hartley strain albino guinea pigs were induced using nine (3/wk) occluded topical applications. Two groups were induced with minimally irritating concentrations (1-4%) of isoeugenol. The first group was induced on intact skin and the second was induced on abraded skin. The skin was abraded using a hypodermic needle. The third group was induced on intact skin with a more irritating concentration (5%) of isoeugenol. A fourth control group did not receive induction applications. Fourteen days after the 5th induction application, the animals were challenged on a naive intact site with 1% isoeugenol which was determined to be the highest non-irritating concentration. The group induced with 30% isoeugenol on intact skin showed the highest incidence and severity of sensitization reactions. The group induced with 1% on intact skin showed the least response. The fourth non-induced control group showed no reaction at challenge. In the second study, 3 groups of 10 animals were induced by 3 applications (1 week). One group of animals was induced with 3% isoeugenol on sites abraded using stainless steel needles. A second group was induced with 3% isoeugenol on abraded skin. A third group was induced with 30% isoeugenol on intact skin and a fourth control group received no induction applications. All groups were challenged 14 days after the last induction application on naive sites with 1% isoeugenol. The incidence and severity of reactions were comparable between the 3% abraded and 30% intact groups and both were greater than the 3% intact group.

The results of both these studies indicate that abrasing the skin at the application sites enhances the sensitization of the Fluehler method, probably by enhancing dermal penetration of the sensitizer. This may be useful for identifying potential sensitizers that do not readily penetrate the intact skin.
Based on the MEST test design developed by this laboratory, a number of miscellaneous materials and design variables were evaluated, including: (1) The sensitization potential of 13 cationic ionophores, (2) the sensitivity of Swiss Webster mice of ages ranging from 4 to 40 weeks as a test model, (3) the effects of a two week (vs. the traditional one week) induction period on test sensitivity, and (4) the efficacy of two different ear pretreatments in increasing test system sensitivity. In addition, several compounds tested in the MEST were observed to have unrelated interesting biological effects, such as stimulating hair growth and causing treated ears to lengthen.

Percutaneous absorption and cutaneous toxicity of xenobiotics would best be assessed in a viable in-vitro skin preparation which allows sampling of the intact arterial and venous cutaneous circulations. We report the perfusion of 30 isolated perfused porcine skin flaps (IPPSF) supplied by the caudal superficial epigastric artery. A single pedicle axial pattern island tubed skin flap created on Yorkshire weaning pigs in one surgical procedure was harvested 2-6 days later, and transferred to a computer-controlled temperature-regulated perfusion chamber for 10-12 hrs. Perfusate was a Krebs-Ringer bicarbonate buffer (pH 7.4) containing albumin and glucose(G). Viability was characterized by maintenance of constant perfusate pressure, flow (1-2 ml/min/IPPSF), pH and osmolality, as well as linear G utilization and lactate production in the absence of perfusate lactate dehydrogenase (<10 IU/IPPSF). Cumulative G consumption was approx. 200 mg/IPPSF. Morphologic criteria will be described. The IPPSF has been useful for studying NaF-induced cutaneous toxicity and melathion and caffeine percutaneous absorption. (Supported by US Army Medical Research and Development Command DAMD-17-84C-4103)

Five laboratories independently evaluated eight coded samples in a double blind investigation of the performance of the MEST. A standard protocol (previously published by Allied) was utilized. Each material was evaluated using a group of 15 test and 5 control female CF-1 mice, six to eight weeks old. Test animals were induced on days 0, 1, 2 and 3, challenged on day 10 and rechallenged on day 17.

All five laboratories correctly identified three as strong sensitizers (TCSA; N,N-Dimethyl-P-nitrosoaniline, and DNCB) and three as non-sensitizers (phenol, benzoic acid, and aluminum chloride). The three nonsensitizers were dermal contact irritants used as negative dermal sensitization controls. A moderate sensitizer (eugenol) was correctly identified as such by three of the five laboratories, while a weak sensitizer (neomycin sulfate) was also correctly identified as such by three of the five laboratories.

Acute toxicity of the skin may be studied using an in vitro isolated perfused porcine skin flap (IPPSF). To assess IPPSF viability, one must differentiate lesions which occur as a result of cell death, surgical procedures, in vitro perfusion, and xenobiotic-induced toxicity. This study characterizes the morphology of these lesions using enzyme histochemistry, light and transmission electron microscopy (TEM) and correlates them to previously described biochemical parameters. TEM was the most sensitive discriminator. Cell death in normal nonperfused tissue was marked by single vacuoles at 8hrs. 24-72hrs postoperatively, nuclear clumping, intracellular edema, and hypertrophy of the epidermis occurred. Perfusion was associated with enhanced nuclear pleomorphism in viable IPPSF. In biochemically abnormal IPPSF (low glucose utilization and flow) mitochondrial alterations and vacuoles were noted at 4hrs. NaF induced toxicity caused multiple vacuoles in strata basale and spinosum within 1hr. Light microscopy was relatively insensitive. Changes occurred with enzyme histochemistry. These studies suggest that TEM of the IPPSF is a powerful tool for cutaneous toxicology. (Supported by US Army Medical Research and Development Command DAMD-17-84C-4103)
The IPPSF is a unique model whereby the absorption and metabolism of topically applied xenobiotics may be examined in an in vitro system. Surgical procedures, perfusion techniques, and methods for assessing cutaneous viability are presented elsewhere. This report describes the percutaneous absorption of $^{13}$C-malathion and caffeine. Following 2 hrs of stable perfusion, 200 µL of a topical solution, sp. act. = $10^{-6}$ Ci (200 µg total dose), was applied to a 5 cm² area on the epidermal surface of each IPPSF. Absorption profiles of both compounds were marked by a steady accumulation of $^{13}$C in the arterial reservoir during an 8 hr absorption period. The venous fluxes were low for 1-2 hrs, increasing linearly thereafter at a rate of approximately 3.8 DPM/min² for caffeine. The amount of compound in perfusate was predictable from the area under the venous flux-time curve. Net absorption was slightly higher for caffeine, averaging 1.2 % of the total dose, vs 1.0 % for malathion. These results suggest that the IPPSF is a useful model for the mixed absorption of xenobiotics. This work was supported by the U.S. Army Medical Research and Development Command (DAMD 17-84-C-4103).

Hydrazine, an animal carcinogen, is an important hypoglycemic fuel used in space shuttle auxiliary power units and in the F-16 emergency power unit. The TWA-TLV® is 0.1 ppm with a skin notation, yet there is scant quantitative information on dermal absorption of hydrazine vapors. This study determined the permeability constant of hydrazine vapors in Fischer 344 rats. Animals were exposed in a dermal vapor absorption chamber and serial blood samples were obtained from a jugular cannula. Time course blood hydrazine curves were analyzed with a two compartment model to determine the rate of dermal absorption of hydrazine. Kinetic constants were determined from the best fit of the model to blood concentrations after intravenous exposure. The first order elimination rate was 2.0 hr⁻¹; the rate constant for transfer from the blood to the deep compartment was 0.8 hr⁻¹, and the rate constant for transfer from the deep to the blood compartment was 0.08 hr⁻¹. The total amount absorbed and the rate of metabolism were estimated by requiring the model to match the observed blood concentrations for each experiment. The skin permeability constant for hydrazine vapor calculated in this manner is very low, 6 x $10^{-5}$ cm/hr, a value similar to that of water vapor.

The purpose of this research was to determine which species of laboratory rats was the best in vitro model of percutaneous penetration and metabolism of T-2 in humans. Discs of full-thickness abdominal skin were mounted on Teflon diffusion cells. The dermal surfaces were bathed by phosphate buffered saline. Each epidermal surface was dosed with 79 or 581 ng [³H]-T-2/cm², in either methanol or dimethylsulfoxide (DMSO). The [³H]-T-2 which penetrated after 48 hr (expressed as percent of dose; 581ng) was 1.0, 9.7, 2.8 and 1.4% for the human, rat, guinea pig and rabbit, when the vehicle was methanol. The penetration was 29.2, 52.6, 51.9, and 19.6% for the human, rat, guinea pig and rabbit, when the vehicle was DMSO. The percent penetrated was similar for both dose levels. Metabolism was extensive in the human, rat, and rabbit with the main metabolite being HT-2 toxin. Previous studies comparing human to monkey indicated penetration in these 2 species was different when methanol was the vehicle. The above results indicate the rabbit is the best model of percutaneous penetration by [³H]-T-2 in humans.
DERMAL PENETRATION OF SELECTED LIPOPHILIC PESTICIDES THROUGH SKIN. R.E. Grissom, Jr. and P.E. Guthrie. North Carolina State University, Toxicology Program, Raleigh, NC.

Dermal penetration of permethrin, fenvalerate, and DDT was determined in vivo and in vitro in female mice. In the in vivo phase of the study, the amounts of labeled pesticide were measured at the sites of application and in the blood, liver, kidney, fat, excreta, and carcass. In the in vitro phase of the study was performed using a Franz diffusion cell system. The amount of labeled pesticide was measured in the skin and in the bathing medium. The three compounds were found to penetrate similarly in vivo and in vitro.


Absorption, distribution and excretion of dermally applied 14C-chloreconone was studied in young and adult female Fischer 344 rats at 6, 24, 48, 72 and 120 hours. A physiological pharmacokinetic model was fit to the data in order to determine absorption and excretion rate constants, tissue/blood ratios, and blood-tissue permeability rates. The dermal absorption rate constant was found to be time dependent having a maximum in the 6 to 24 hr period for young and in the 24 to 48 hr period for adults. The untreated skin had a resistance to uptake in adult but not in the young, thus producing a higher skin burden in the young. Urine kinetics required a variable excretion rate constant as excretion occurred quickly but then subsided even as the body burden increased. This may imply dermal metabolism or nonlinear kinetics. Dermal absorption and distribution in the body was not proportional to dermal dose. Content in urine and feces increased more rapidly with dose than did dermal absorption. The calculated dermal absorption rates were compared with in vitro skin absorption data. Mathematical modeling enabled the prediction of absorption and distribution at longer times and determination of equilibrium levels following continuous exposure.

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

DERMAL PENETRATION OF CARBOFURAN IN YOUNG AND ADULT FISCHER - 344 RATS. P.V. Shah1, H.L. Fisher2, M.R. Sumler1, and L.L. Hall1. Northrop Services Inc., RTP, NC 27709, USEPA, HERL, DBD, Reproductive Toxicology Branch, RTP, NC.

Dermal penetration of carbofuran was determined in young (33 days) and adult (82 days) female Fischer 344 rats by in vivo and in vitro methods. The dermal penetration at 120 h was 43% for young and 18% for adult rats. The young to adult ratio was greater than one at all time points (average 2.9) and had a maximum of 4.2 at 24 h. Urinary excretion of the absorbed dose was about 95% in young and adult at 120 h. The whole-body retention was slightly higher in adults. Kidney showed the highest tissue to blood ratio (4.6 in adult, 2.3 in young). The ratio for carcass was 2.8 in adult and 2.4 in young. The urine/blood concentration ratios were very high, 453 in adult and 573 in young. The feces/blood ratio were 64 in adult and 65 in young. Skin absorption by the in vitro continuous flow system was 41% for young and 11% for adult, compared to 36% and 13% by in vivo methods. The static in vitro method gave consistently lower skin penetration values of 12% for young and 8.8% for adult. Dermal absorption of carbofuran appears to be higher in young by a factor of about 2.5. Differences in kinetics of excretion and retention were observed between young and adult.

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

AGE RELATED PERCUTANEOUS PENETRATION OF CARBARYL, DETERMINED BY IN VIVO AND IN VITRO METHODS. L.L. Hall1, H.L. Fisher1, M.R. Sumler2, and P.V. Shah1. USEPA, HERL, DBD, Reproductive Toxicology Branch, RTP, NC. Northrop Services Inc., RTP, NC.

Radiolabelled carbaryl (CAB) was applied to the previously clipped back skin of 53 and 82 day old female Fischer 344 rats. The radioactivity in the treated skin, the rest of the body, urine and feces was determined at 6, 24, 48, 72 and 120 hours after dosing. Skin from the same age rats was placed in continuous flow and static diffusion cells and the viability maintained with Minimal Essential Media containing 4% fetal calf serum. The in vivo system indicated young and adult dermal penetration at 72 hours was about equal at 38.5% and 38.4% respectively. The static cells gave lower results of 20.9% and 20.3% for young and adult. The flow system was the most divergent method giving 20.2% absorption in the young and 18.1% in adults. Following in vivo application, about 37% was found in urine, 2 to 3% in feces, and 1% in the body at 72 hours for both young and adult. Kidney had the highest concentrations at 72 hours as indicated by a tissue/blood ratio of 3:8 for adults and 4.1 for young. The tissue/blood ratios for liver and other soft tissues ranged from 1.6 to 1.8. Young and adult rats appeared similar in their dermal absorption and in the retention and excretion of CAB radioactivity. The in vitro dermal penetration of CAB was lower than in vivo absorption. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.
EFFICACY OF A NON-OCCLUSIVE BARRIER MODEL TO STUDY TOXIN PHARMACOKINETICS AFTER DERMAL EXPOSURE. R.W. Mennemacher, Jr., D.L. Bunner, J.G. Pace, R.E. Dinterman. US Army Medical Research Institute of Infectious Diseases, Frederick, MD. Sponsor: R.F. Fricke.

A barrier model was developed to evaluate dermal toxicity of trichothecene mycotoxins. To study pharmacokinetics following dermal exposure, we had to establish the efficacy of this model. Guinea pigs' backs were shaved and, 24 h later, a solution containing 0.1 mCi of [14C]-insulin (a non-absorbed marker) was applied to the skin. A 6 x 6 cm, 0.5-in-thick, surgical foam pad with a 4 x 4 cm hole was placed on the skin. The opening was covered with a plastic screen, then a brass screen; these were held in place by elastic bandages. Guinea pigs were placed in metabolism cages. Guinea pigs with the barrier were killed on day 1 and day 28, while control animals, without a barrier, were killed on day 28. The barrier model showed negligible loss of radioactivity from day 1 to day 28, with 97% being retained on the skin or barrier and only 3% in the carcass. In contrast, the controls retained less than 1% in the skin by day 28 and even by day 7 lost 36% of the radioactivity in the urine and cage washing. Since the barrier prevented any significant loss of radioactivity, the model is valid for studying the pharmacokinetics of dermal exposure.


Petroleum fractions produced by refinery processes are complex mixtures containing chemical classes such as paraffins, diaromatics, polycyclic aromatic hydrocarbons (PAHs) and heterocyclic PAHs (e.g. carbazoles, benzo[c]carbazoles). Since many compounds found in these classes are of toxicological significance, we investigated their systemic bioavailability through skin, the most likely route of human exposure. Chromatographic fractionation and GC-MS identification were used to establish chemical composition. From in vitro and in vivo percutaneous absorption studies using radiolabeled surrogates, relative dermal bioavailability of various chemical classes was determined. In three refinery streams (clarified slurry oil, light cycle oil and heavy coker gas oil), carbazole derivatives were found to be the most bioavailable compounds. In rat subchronic studies, repeated skin applications of clarified slurry oil (CSO) produced adverse liver effects. Based on the content and high systemic bioavailability of carbazole and benzo[c]carbazole derivatives (some of which are known hepatotoxins), these heterocyclic nitrogen-PAHs appear to play a role in mediating the observed toxic effects.

PREPARATION OF THE BARRIER LAYER FOR IN VITRO PERCUTANEOUS ABSORPTION STUDIES. R.L. Bronaugh and R.F. Stewart. Division of Toxicology, Food and Drug Administration, Washington, DC.

The skin membrane for in vitro skin penetration studies was prepared so that it was similar in thickness to the in vivo barrier layer. A dermal section from the surface of the skin contained the epidermis and the upper papillary dermal tissue where the blood capillary loops reside. Improved absorption measurements of hydrophilic compounds were obtained with this membrane using a polyethylene glycol 20 oleyl ether (1) receptor fluid in the diffusion cells. With the haired rat, the preparation of a 300 um thick section (and screening for barrier damage with tritiated water) resulted in a membrane that gave values in good agreement with in vivo results for cinnamon anthranilate and benzo(a)pyrene absorption. When sparsely haired fuzzy rat skin was used, a 200 um section of skin could be prepared without the need for screening for barrier damage. Good agreement was obtained between in vivo and in vitro values for a fragrance ingredient (acetyl ethyl tetramethyl tetralin, AETT) and DET using 0.5% (1) as the receptor fluid. The skin of the fuzzy rat seemed more similar in permeability properties to human skin than did the hairless mouse when the absorption of 6 compounds was compared. Fuzzy rat skin appears to be useful for the preparation of membranes for diffusion cell studies.

SKIN DEPOSITION OF 3,4,4'-TRICHLOROCARBANILIDE (TCC) IN BAR SOAP. J. Demetrulics, H. North-Root. Armour-Dial, Inc., Scottsdale, AZ.

Studies performed by this laboratory have previously demonstrated the suitability of the hairless mouse as a model for predicting the human skin deposition of TCC. In this study, the skin deposition of 14C-TCC contained at various levels in bar soap was determined in the hairless mouse and the human. The bars used in these experiments differed slightly in composition and were processed by 2 different methods.

The results in hairless mice showed two-fold greater TCC skin residue following washing with 1.5% 14C-TCC soap made by method I versus method II. Further, TCC skin residues on mice washed with 0.4%, 0.9%, or 1.5% 14C-TCC incorporated into soap by method I were directly proportional to the concentration of TCC in the soap.

The flexor surface of human subjects' forearms was washed with 0.8% 14C-TCC soap processed by method I or 1.5% 14C-TCC soap processed by method II. The skin residue showed approximately the same TCC deposition from both soap preparations.

These results suggest that a change in formulation and/or manufacturing process may affect the skin deposition of ingredients, such as TCC, in rinse-off products.
The fate and distribution of $[^3]$H]T-2 toxin were examined in guinea pigs (GP). T-2, in methanol (MeOH) or DMSO, was painted on the shaved backs of GP; a screen barrier was applied; and GP were killed at 1, 2, 3, 7, 14, and 28 days. In the DMSO and MeOH groups, 82% and 8% of the radiolabel was absorbed from skin by day 28. The highest concentration of radiolabel was found in the GI tract in the DMSO group and in the carcass in the MeOH group. Radioactivity in bile and blood peaked by day 1 in the DMSO group and day 3 in the MeOH group. In a second experiment, urine and feces were collected daily for 28 days. In the DMSO group, 41% of the absorbed radiolabel was excreted equally in urine and feces by day 8. In the MeOH group, only 4% of the radioactivity was excreted in the urine (3%) and feces (1%). Urinary metabolites of T-2 were identified as HT-2, 2′hydroxy HT-2, T-2 tetraol, glucuronide-conjugates of T-2, HT-2, and T-2 tetraol, and several more-polar metabolites. In contrast to the MeOH group, application of T-2 toxin in DMSO resulted in the absorption and metabolism of a greater amount of toxin.

We reported that acute skin irritation responses to methyl salicylate, croton oil, and ethyl phenyl propionate (EPP) are produced by different mechanisms. To determine if repeated application of these irritants would result in different outcomes, i.e., cumulative irritation or exacerbations, and if responses which did develop were similar to the acute response, solutions containing the irritants were applied to one ear of laboratory mice daily for four days or at selected times during this period. Intensity of the inflammatory response was measured as ear thickness. Ears treated with methyl salicylate became unresponsive to methyl salicylate and to EPP; the response to croton oil was normal. Repeated applications of croton oil resulted in responses of increasing intensity. Croton oil inflammatory responses were maximum at 6 hours following both single and repeated exposures, however with each additional exposure the residual ear thickness at 24 hours increased. Following a single application of EPP, responses to EPP and to methyl salicylate were diminished, the response to croton oil was normal. After four daily applications of EPP, ear tissue was inflamed; however the inflammation was not similar to the acute response. These studies show that repeated application of chemicals producing irritation by different mechanisms result in different outcomes. Identification of acute mechanistic pathways may allow one to predict the effect of repeated exposure to chemicals.
The bioactivation of codeine to a toxic metabolite was investigated using freshly isolated hepatocytes as an in vitro model. Several pathways of metabolism were investigated which lead to the conclusion that a cytochrome P-450 metabolite of codeine was responsible for cell toxicity. Studies of potential codeine metabolites showed codeine to be the most toxic. The threshold concentration of codeine to cause a decrease in cell viability was 0.25 mM while that of codeine metabolite 0.025 mM. Both codeine and codeine metabolite caused dose related decreases in reduced glutathione (GSH) concentrations in hepatocytes. Codeine was shown to cause a non-enzymatic depletion of GSH through the formation of a conjugate, which was subsequently isolated by HPLC and identified by proton NMR. The GSH conjugate was formed by both liver microsomal preparations and hepatocytes and formation was inhibited by metyrapone. Data suggest that codeine metabolism to codeine by cytochrome P-450 may be responsible for codeine toxicity in isolated hepatocytes.

BHA pretreatment inhibits the covalent binding of AFB to rat hepatic DNA in vitro, but enhances binding of AFB to DNA in mouse liver slices in vitro. To determine the effects of BHA pretreatment on the binding of AFB to mouse hepatic DNA in vivo, female CD-1 mice were fed 0.75% BHA for 10 days. On the 11th day, half the mice were administered 3H-ABF (0.25 mg/kg, ip). After 2 hrs, binding of AFB to total hepatic macromolecules and purified DNA was determined. Remaining mice were used to determine in vitro metabolism of AFB via HPLC analysis for specific AFB metabolites. BHA decreased in vivo binding of AFB to hepatic DNA to 73% of control, but had no effect on binding to total macromolecules. In vitro, BHA increased the microsomal activation of AFB to 243% of control and increased the production of AFB-GSH in microsome-cytosol combinations to 462% of control. Overall metabolism of AFB to AFBGSH, AFB-triols, AFQ1, AFPA, AFPM and covalently bound metabolites was increased to 289% of control by BHA. These data suggest that dietary BHA protects against binding of AFB to mouse hepatic DNA via the induction of AFB-GSH transferases resulting in more efficient inactivation of the AFB-8,9-epoxide. (Supported by NIH Grants ES-03719 and T32 ES-07832).


Previous studies have found that the major AFB metabolite in bile is the glutathione conjugate (AFB-GSH), which accounts for 30-50% of total AFB-derived products in bile. A reversed phase HPLC system was developed which separates at least 17 individual AFB-related metabolites in bile. AFB-related peaks were isolated by preparative HPLC from bile collected from rats given 5 mg/kg AFB and further purified by HPLC. Three of 5 peaks more polar than AFB-GSH were hydrolyzed by B-glucuronidase, but sulfatase had no effect. Mass spectra were obtained by direct probe insertion, negative ion mass spectrometry. The aglycones of the two most polar peaks (peaks 1 and 2) had mass spectra consistent with alcoholic hydroxylation products of AFB. Peak 3 had a mass spectrum consistent with the mercapturic acid of AFB, and is greatly reduced, as is AFB-GSH, by depletion of GSH with buthionine sulfoximine and diethylmalate prior to AFB administration. Peak 5, the second most prevalent peak after AFB-GSH, was previously identified as AFQ1-glucuronide. AFQ1, AFPI and AFQI were detected in bile, but constituted less than 1% of the total AFB-derived metabolites. AFQ1 was not detected in bile. (Supported by NIH Grant ES-03415).


2,2,2-Trifluoroethanol (TFE) is the toxic metabolite of the anesthetic agent fluoxetine. TFE treatment (0.21 g/kg ip) of male Wistar rats significantly reduced peripheral WBC count, bone marrow nucleated cellularity, and dry weight of the small intestine. These effects were first observed at 8 to 16 hr after treatment, persisted for 96 hr, and were accompanied by severe diarrhea and edema of the small intestine. TFE increased the sensitivity of rats to bacterial endotoxin lethality by 1000-fold. Antibiotic and antiendotoxin pretreatment decreased the lethality of TFE but did not prevent the other toxic effects of TFE. Serum from TFE-pretreated rats (0.13 g/kg) stimulated the growth of an average of 65% fewer cultured bone marrow cell colonies compared to control serum. This suggests that TFE-induced bone marrow depression may be related to a decrease in colony stimulating factor activity as a result of TFE metabolism. The results indicate that TFE-mediated damage to the small intestine combined with prolonged leukopenia decreases the resistance of the rat to endogenous pathogens and leads to the rapid development of systemic bacterial infections. The increased sensitivity to endotoxin induced by TFE adds to the lethality. (Funded by NIH grant GM 23029)
Drug formulations frequently contain sorbitol and glycerin which have been reported to increase drug absorption. The purpose of this study was to determine the effects of sorbitol and glycerin on sucralfate absorption in dogs. 14C sucralfate was suspended in a preparation containing sorbitol and glycerin. The animals were given a single dose of 140 mg/kg sucralfate (U-14C) in suspension or dispersed in water. The pharmacokinetic parameters were obtained by using standard curve stripping techniques. Curve fitting was then performed by least-squares regression analysis using the NONLIN program. The time course after oral administration of sucralfate (U-14C) suspension and dispersion can be best described by a one-compartment pharmacokinetic model with the following major parameters: t1/2 = 6.002 hr (susp), 5.4608 hr (disp); Cmax = 0.4326 µg equiv/ml (susp), 0.5700 µg equiv/ml (disp); tmax = 1.808 hr (susp), 0.900 hr (disp); AUCmax = 6.5905 µg equiv/ml/hr (susp), 7.7113 µg equiv/ml/hr (disp); MRT = 22.80 hr (susp), 20.97 hr (disp). No significant differences between the two treatments were observed. It was concluded that the sucralfate suspension containing sorbitol and glycerin did not alter the sucralfate pharmacokinetic (absorption) or excretion pattern.


The identification of a polyamine uptake process in the lung which also accumulates the herbicide paraquat helps to explain the herbicide's selective toxicity to the lung. We have shown that this accumulation system is also present in the rat salivary gland and seminal vesicle although the apparent kinetic constants for the uptake differ in each tissue. The polyamine uptake system in the lung is identical or similar to that which has previously been described in human leukaemic cells. We have shown that murine leukaemic white cells (WEHI III) can selectively accumulate the polyamines and various drugs to a greater extent than non-transformed white cells (AD III cells). Similarly the anti-leukaemic drug methylglyoxalbis(guanhydrazone) is selectively accumulated into the lung. Furthermore paraquat selectively damages WEHI III cells in comparison with AD III cells. This polyamine accumulation system may well be relevant to the design of drugs targeted to specific cell types, and the knowledge of its existence may help to predict selective organ toxicity.

The interaction between phenycyclidine (PCP) and 1-phenylcyclohexene (PC). A.K. Chaturvedi and D.J. Kuntz. Dept. of Pharm. Sci./Tox., N.D. State Univ., Coll. of Pharmacy, Fargo, ND

PCP, an abused drug, is commonly administered by smoking, in which about 50% of PCP pyrolyzes into PC. The possibility for the interaction between PCP and PC exists in the PCP chronic smokers. It had been demonstrated that PC (1.1, 2.2 & 4.4 mmol/kg/day for 4 days, i.p., in corn oil) lowered the PCP-induced locomotion and pentobarbital (PET)-induced sleep in Swiss male mice (20-25 g). However, the mechanism by which PC exerts its effects has not been established. Therefore, the effect of the 4-day PC treatment on the hepatic mixed function oxidases (MFO) in mice was studied. At the 2.2 & 4.4 mmol/kg, PC increased (p<0.05; n=4) the in vitro rates of metabolism of phenacetin (42 & 103%) and benzopyrene (79 & 147%), while metabolic rates of amphetamine and amifostine were elevated only at the highest dose. The liver microsomal contents of P=450 were higher (p<0.5-0.1; 18-42%) than the control at 2.2 & 4.4 mmol/kg doses. PC also enhanced the in vitro metabolism of PET (212 in 120 min) and PCP (80 in 45 min). Therefore, it appears that the decrease in the effect of the 2.2 mmol/kg PCP is associated with their interaction at the metabolic level due to the induction of MFO. However, the possible interaction of these agents at other levels can not be completely ruled out.

The comparative cyanogenic potential of malononitrile (MN) and succinonitrile (SN). B. Bellantyne. Union Carbide Corporation, Danbury, CT.

The acute toxicity of MN and SN was investigated to assess their relative cyanogenic potential. Both were more acutely toxic in rabbits than rats. Thus, the peroral LD50 values (with 95% confidence limits) in mg/kg with female rabbits were 9.8(6.8-11.1) for MN and 28.2 (25.4-37.8) for SN, and with female rats 24.6(19.1-29.2) for MN and 458(394-527) for SN. After 2xLD50 of SN or MN, times to death (±SE) in min were longer in rabbits at 107±12 for MN and 24±18 for SN than for rats at 75±4 for MN and 14±5 for SN. Concentrations of cyanide and proportionate inhibition of cytochrome oxidase activity in brain and myocardium were respectively similar for rats and rabbits given MN or SN. However, whole blood cyanide concentrations were higher in rabbits than in rats receiving MN or SN. The times to death, tissue and blood cyanide concentrations, and cytochrome oxidase activities suggest that the lower toxicity of MN and SN to rats is due to a more efficient detoxification of cyanide in rats, which thus require larger doses than rabbits to produce a lethal tissue burden. This was confirmed by finding a significantly greater rate in increase of plasma thiocyanate in rats than in rabbits receiving SN. Additional studies showed higher 3-mercaptopyrurivate cyanide sulfur transferase activity in rats than rabbits. Mean values (±S.D.) in U/g, were for liver 186 (rat) and 56 (rabbit), for kidney 126 (rat) and 26 (rabbit), and for whole blood 61 (rat) and 3.3 (rabbit).
1001 STUDIES ON THE MECHANISM OF UROTEIC ACTIVITY OF N'-WITIENYL ACETYLNAPHTHOLINONE (IMAPN II): SPECIES DIFFERENCES IN TOXICITY. M.M. Matta & A.E. Ahmed, University of Texas Medical Branch, Galveston, TX 77550

IMAPN, a major component of NIKE catalyst EN8, causes urinary bladder dysfunction in exposed workers. In order to investigate the mechanism of action of IMAPN we carried out time course (0-72 hrs) and dose (200-700 mg/kg) response studies in rats and mice using various routes of administration. Bladder damage induced by IMAPN was characterized by distended bladders, punctate hemorrhagic spots on the posterior bladder wall, edema and ulceration of the epithelium. Differences in organ toxicity as well as lethality in both species as a function of route of administration was observed. Within 6 hours following oral IMAPN administration, urinary retention and bladder weight increased significantly compared to controls, remained high up to 36 hours and returned to normal levels between 90-72 hours. In rats there was a reasonable linear dose response relationship while in mice there was a maximum at 350 mg/kg IMAPN and at 700 mg/kg no IMAPN-induced hemorrhage was observed. The mice are more sensitive to IMAPN-induced hemorrhagic spot formation than rats. The excretion of IMAPN and/or its metabolites was studied in urine following extraction and gas chromatographic analyses. In the rat urine 40% of the administered dose was excreted unchanged while only about 5% was excreted in urine in mice. The extent of IMAPN metabolism to 8-a-monoxyoprotinol and cymene oxide, is higher in mice than rats. Thus species differences in metabolism play a significant role in the overall expression of IMAPN toxicity. (Supported by NIH Grant ES 01671).

1002 QUANTITATION OF POLAR URINARY METABOLITES OF NICOTINE ENANTIOMERS IN THE GUINEA PIG. C.G. Lozado, G.S. Godin, and P.A. Crooks, College of Pharmacy and Graduate Center for Toxicology, University of Kentucky, Lexington, KY. Sponsor: J.W. Flesher.

Previous in vivo studies have indicated that radiolabelled nicotine enantiomers are rapidly and extensively metabolized in the guinea pig to highly water soluble, polar urinary metabolites. This study was designed to identify and quantify these biotransformation products. Experiments were carried out on groups of male Hartley guinea pigs. Animal groups were administered either R- (+)- or S-(-)-nicotine (12 mg/day) over a period of 24 days via Alzet® 2014 osmotic minipumps inserted subcutaneously. A third group of animals were given saline. Animals were housed in glass metabolic cages and daily urine samples were analyzed by HPLC on a Partisil 10 SCX cation-exchange column using a NaOAc/MethOH/ErgN mobile phase. Calibration curves were prepared with authentic synthetic standards and these were used to quantify the urinary metabolites by integrating the area under the peak. Analyses revealed that 3-hydroxycotinine was the major urinary metabolite of both nicotine enantiomers; cotinine, nicotine and nor-nicotine were present in low amounts. S-(-)-nicotine formed only oxidativemetabolites, whereas R- (+)-nicotine underwent both oxidation and methylation. There was no evidence of enzyme induction in either the oxidative or the methylation pathway. (Aided by a grant from the Tobacco and Health Research Institute, Lexington, KY.)


Salicylamide (SAL) is an important compound used in drug disposition research. An HPLC method was developed to quantify SAL and its metabolites in mouse plasma and urine. Separation was achieved using a 5 µm Adsorbosphere C-18 reverse-phase column with subsequent detection at 240 nm. Mobile phase consisted of a 95:5 mixture of 1% acetic acid and tetrahydrofuran containing 1.5 mm tetraethylammonium hydroxide. SAL, gentisamide, 2,3-dihydroxybenzoic (2,3-DBA) and their glucuronic and sulfate conjugates were quantitated. The identity of 2,3-DBA was confirmed by proton NMR and mass spectrometry. Following administration of SAL (2 and 4 mmol/kg, i.p.), plasma and urine samples were collected. SAL and all metabolites except 2,3-DBA and 2,3-DBA sulfate were detected in plasma samples. All metabolites were detected in 24-hr urine samples. It should be noted that 2,3-DBA has not been previously reported as a significant product of SAL biotransformation. In this study, 11% of a 4 mmol/kg dosage of SAL appeared in urine as 2,3-DBA glucuronide. Thus, this method provides for the analysis of SAL and its metabolites from biological samples including one significant metabolite not previously reported. (Supported by USPHS grants ES-07079 and ES-07192 and a Stauffer Fellowship).

1004 IN VITRO METABOLISM OF SALICYLIC ACID BY ISOLATED KIDNEY AND LIVER MITOCHONDRIA. M.E. Kyle and J.J. Koessler, Dept. Pharmacology, Thomas Jefferson University, Philadelphia, PA

Mitochondria are known to contain a P-450-like system similar to that found in micosomes. Since previous studies from this laboratory have suggested that renal mitochondria may metabolize salicylate (SAL) to a reactive intermediate capable of protein binding, the ability of isolated kidney and liver mitochondria to activate SAL was investigated. Incubation of [14C] SAL with kidney but not liver mitochondria resulted in an NAPDH and oxygen-dependent increase in covalently bound radioactivity with time. Approximately 1% of the SAL present in the incubate was converted to 2,3-dihydroxybenzoic acid, the catechol analogue of SAL. The formation of 2,3-dihydroxybenzoate and the amount of radiolabel bound to mitochondrial protein was decreased in the presence of SKF 525-A; however, excess cold substrate had no effect on binding. These data indicate that kidney mitochondria activate SAL via a P-450-like system, but the binding species is not 2,3-dihydroxybenzoate itself. Oxidation of SAL at the ortho position and covalent binding of radiolabel, however, were also observed after the addition of ferrous iron and ascorbic acid to incubations containing [14C] SAL and bovine serum albumin. Mannitol decreased SAL oxidation and covalent binding, suggesting radical formation may also represent a mechanism for SAL activation by renal mitochondria. (Supported by BRSG Funds, Thomas Jefferson Univ.)
The mechanism whereby procainamide (PA) induces the formation of autoimmune antibodies in almost all patients receiving chronic therapy is unknown. It has been proposed that PA is metabolized to a reactive metabolite which is responsible for the immune aberrations. Recently indirect evidence for the in vitro formation of the hydroxylamine of PA has been published (Hetzrecht et al., Drug Metab. Dispos. 12:77, 1984). In the present study we have developed a method for the direct detection and quantitation of procainamide hydroxylamine (PHA) by HPLC with electrochemical detection. Using this method, the apparent rate of formation of PHA in hepatic microsomes from rat and man were determined. A V_{max} of 4.8 nmol/min/mg protein and apparent K_{m} of 86 uM were observed for PHA formation in rat liver microsomes. The V_{max} was 227 nmol/min/mg protein with an apparent K_{m} of 4.2 uM in human liver microsomes. The results of this study indicate that PHA is a probable metabolite of PA in man and the rat, with a formation rate much greater in the rat.

Vegetables of the Brassica genus contain an indolylacetyle-glcucosinolate, glucobrassacin (GB). GB autoxidation products include indole-3-carbinol (3C), indole-3-acetonitrile (13AN) and 3,3'-dindolemethane (dim3). All of which inhibit chemically-induced neoplasia in rodents. Methodology was developed to quantify this potentially important series of indolic metabolites in foods and to describe the thiolglutathione-mediated autoxidic process. Indole extraction is accomplished by successive homogenizations,filtrations with H2O, CH3OH and H2CO2L, followed by partitioning of the aqueous/organic phases. A portion of the organic phase is evaporated, redissolved in CH3OH:H2O (8:2) and eluted through 1.0 cm of C18 with 5.0 ml. An aliquot was analyzed by RP-HPLC using a CH3OH:H2O gradient of 5-80% over 60 min. Indoles were detected fluorometrically (exc 280nm, em 350nm) or via UV absorption (380nm). Recovery of all indoles is greater than 80% and the method can detect levels in the ng/mL range. Our results demonstrate the presence of a major, and as yet unidentified GB metabolite and indicate that the 3C is the major known product when plant tissue is disrupted, although its instability in the autoxidic milieu leads to a rapid decline (83%) in 24 hrs.

**PHARMACOKINETICS OF THE ANTI-INFLAMMATORY AGENT 5-(4-PHenyLYL)-6-(4-FLUOROPHENY)-2,3-DIMETHYLINDAZO(1,2-b)-THIAZOLE (SGAF 86002) IN SPRAGUE-DAWLEY RATS.**


SGAF 86002 represents a novel therapeutic agent for the treatment of inflammatory disease. Although the pharmacology of SGAF 86002 has been extensively described, essentially no information is available on its pharmacokinetics. Therefore, these studies were designed to: 1) Obtain preliminary information on the pharmacokinetics of SGAF 8602 and metabolites at doses used in animal pharmacology and toxicology experiments, 2) Define the dose range over which the pharmacokinetics of SGAF 8602 is linear in rats. Sprague-Dawley rats were administered a single dose of SGAF 8602 p.o. (10-80 mg/kg) or i.v. (5 mg/kg). After i.v. administration, the plasma half-life (TI/2) of SGAF 8602 was 21.04 and 28.06 hrs. TI/2 of sulfonamide was 4.9 ± 0.5 and 7.0 ± 1.4 hrs in male and female rats, respectively. TI/2 of sulfone was greater than 20 hours. After p.o. (20 mg/kg) administration, the TI/2 of parent was 1.63 ± 0.08 and 1.15 ± 0.28 hours in male and female rats, respectively. The apparent bioavailability of SGAF 8602 is greater than 80% in both sexes. These studies indicated that SGAF 8602 is extensively metabolized in the rat to its sulfonamide metabolite and ultimately to the sulfone. Furthermore, differences in i.v. and p.o. TI/2 of SGAF 8602 suggest that SGAF 8602 is absorbed slowly relative to its metabolism. The extended TI/2 of the oxidative metabolites of SGAF 8602 indicates that attention should be given to the potential for accumulation in toxicology studies.

**HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF ANTICARCINOGENIC INDOLES PRESENT IN BRASSICA OLERACEA.**

C.A. Bradford and L.F. Bjeel-Jansen. Dept. of Nutritional Sciences, Food Toxicology Group, University of California, Berkeley, CA.

Vegetables of the Brassica genus contain an indolylacetyle-glucosinolate, glucobrassacin (GB). GB autoxidation products include indole-3-carbinol (3C), indole-3-acetonitrile (13AN) and 3,3'-dindolemethane (dim3). All of which inhibit chemically-induced neoplasia in rodents. Methodology was developed to quantify this potentially important series of indolic metabolites in foods and to describe the thiolglutathione-mediated autoxidic process. Indole extraction is accomplished by successive homogenizations/filtrations with H2O, CH3OH and H2CO2L, followed by partitioning of the aqueous/organic phases. A portion of the organic phase is evaporated, redissolved in CH3OH:H2O (8:2) and eluted through 1.0 cm of C18 with 5.0 ml. An aliquot was analyzed by RP-HPLC using a CH3OH:H2O gradient of 5-80% over 60 min. Indoles were detected fluorometrically (exc 280nm, em 350nm) or via UV absorption (380nm). Recovery of all indoles is greater than 80% and the method can detect levels in the ng/mL range. Our results demonstrate the presence of a major, and as yet unidentified GB metabolite and indicate that the 3C is the major known product when plant tissue is disrupted, although its instability in the autoxidic milieu leads to a rapid decline (83%) in 24 hrs.

**METABOLISM OF THE ANTI-INFLAMMATORY AGENT 5-(4-PHENYLYL)-6-(4-FLUOROPHENY)-2,3-DIMETHYLINDAZO(1,2-b)-THIAZOLE (SGAF 86002) IN MALE SPRAGUE-DAWLEY RATS.**


SGAF 8602 represents a novel therapeutic agent for the treatment of inflammatory disease. Although the pharmacology of SGAF 8602 has been extensively described, essentially no information is available on its metabolism. Therefore, these studies were designed to: 1) Determine mass balance of [14-C]-SGAF 86002 in male rats, and 2) Isolate and identify metabolites found in rat plasma and urine. SGAF 86002 (20 mg/kg, p.o.) was metabolized to its sulfonamide and sulfone metabolites. Within 30 minutes after administration, plasma concentrations of sulfone were approximately twice those of parent drug. The sulfone was the major drug-related component in plasma within 8 hrs after administration. Mass balance studies indicated that 48% of the dose of [14-C]-SGAF 86002 was excreted in urine while 44% of the dose was excreted in feces, 3% of radioactivity still remained in the carcass 96 hrs after administration. HPLC analysis of the urine indicated that both SGAF 86002 and sulfonamide were present in urine. However, more than 50% of urinary radioactivity was accounted for as 5 distinct metabolites which were more polar than sulfonamide. In summary, these studies indicate that the metabolism of SGAF 86002 is extensive. While the primary metabolites found in plasma involve sulfur oxidation, most of the urinary metabolites are as yet unidentified.
DATA on the urinary excretion of DMPS and DMSA are fragmentary. Rabbits (N=4) were treated i.m. with either DMPS or DMSA at 0.2mmol/Kg. Urine collected at various times via a catheter was analyzed for DMPS or DMSA by bromobimane (BB) derivatization followed by HPLC separation and fluorescence detection. Only 5.0 ± 0.8 SE of the administered DMPS but 46.8 ± 3.5 SE of DMSA were excreted unchanged by 6hr after injection. A human male volunteer was given orally either DMPS (0.04mmol/Kg) or DMSA (0.2mmol/Kg) and urine was collected at various times. Only 2.1% and 5.1% of the dose of DMPS and DMSA, respectively, were excreted unchanged by 12 hr and only minute amounts 74 hr after ingestion. Treatment of urine with the reducing agent, NaBH₄, resulted in a 16-fold increase in detectable DMPS for both rabbit and man. When rabbits were given ¹⁴C-DMPS or ¹⁴C-DMSA, HPLC analysis of BB treated urine showed the presence of ¹⁴C-peaks other than unchanged ¹⁴C-DMPS or ¹⁴C-DMSA. By 6hr after injection, unchanged DMPS and DMSA represented 12% and 73%, respectively, of the urinary ¹⁴C. The differences in the excretion of the two dithiolels may be related to their different susceptibilities to oxidation. (NIHES RIIES03556).

DMPS has been used in the treatment of acute and chronic exposure to lead, mercury and arsenicals. Knowledge of the biotransformation of DMPS may aid in the better understanding of its pharmacokinetic and metal binding properties. Urine from rabbits (N=4), treated i.m. with 0.2mmol DMPS/Kg, was collected at various times via a catheter. Urine samples, with and without NaBH₄ treatment, were analyzed for DMPS by bromobimane derivatization, HPLC separation and fluorescence detection. Metabolites were isolated by HPLC and their structures were determined using fast atom bombardment-mass spectrometry. Six hr after injection, 5.0 ± 0.8 SE of the DMPS dose was excreted unchanged in the urine. The maximum amount was detected 0.5hr after injection. When urine was treated with the reducing agent, NaBH₄, the amount of DMPS detected increased to 81.0 ± 7.2 SE by 6hr after injection. The maximum amount was detected between 1 and 2hr after injection. The major metabolites found were DMPS tetrasulfide and intermolecular DMPS disulfide. The experimental results indicate that DMPS is rapidly biotransformed in the rabbit to the tetrasulfide most of which is excreted in urine within 6 hr. The disulfide form of DMPS appears to be an intermediate during the conversion of DMPS to the tetrasulfide. (NIHES RIIES03556).

In a recent NTP study, chronic gavage administration of DMNC to F344 rats and B6C3F1 mice caused malignant forestomach tumors in rats and mice and malignant tumors of the nasal and oral cavities of rats but not mice. We have investigated the metabolic basis of this difference in toxicity by studying the disposition of DMNC in both species. A single dose of 150mg/kg of 2-14C-DMVC was administered by gavage to male F344 rats and male B6C3F1 mice. Urine, feces and expired air were monitored for 24 hr after treatment. DMVC was rapidly metabolized and cleared by both species. Both rats and mice exhaled approximately 25% of the administered dose as 14CO₂ in 24 hr. The amount of unchanged DMVC expired in 24 hr is approximately 35% in rats and 5% in mice which may explain the occurrence of tumors in the nasal and oral cavities of rats but not mice as reported in the NTP study. The 24 hr urinary excretion of DMVC was 50% of the dose by mice and 35% by rats. Metabolism of DMVC appears similar in both species in that the HPLC profile of the urinary metabolites is qualitatively the same for both. The major metabolite in both species was identified as 2-amino-6-methyl-4-thia-5-heptan-1,7-diol acid.
URINARY METABOLITES OF 2,4- AND 2,6-DIMETHYLANILINE IN DOGS AND RATS. M.L. Hardy, C.R. Short, S.A. Barker. Dept. of Veterinary Physiology, Pharmacology, Toxicology, LSU School of Veterinary Medicine, Baton Rouge, LA.

The species selectivity, dose required to induce hepatotoxicity and histopathological effects produced by 2,4- or 2,6-dimethylaniline (DMA) are significantly different in the dog and rat. These divergent responses may be related to differences in metabolism. In this study male Beagles were orally dosed for 1 or 10 days with 2,6- or 2,4-DMA at 25 mg/kg. Male Fischer 344 rats were gavaged with 2,6- or 2,4-DMA at 25% of their respective LD50’s (202.5mg/kg; 117 mg/kg) for the same time period. 24 hr. urine samples were analyzed by GC and GC/MS/MS. The major urinary metabolite of 2,4-DMA in the dog was the hydroxylated parent compound. Minor metabolites included 4-amino-3-methyl-benzoic acid and N,N-dimethylaniline. The major urinary metabolites of 2,6-DMA were 2-amino-3-methyl-benzoic acid and the glucuronide conjugate of p-hydroxy-2,6-DMA. Minor metabolites included N,N-trimethylaniline, 2,6-dimethyl-nitroso-benzeno, and the glycine conjugate of the benzoic acid. Rats produced N-acetyl-2,4-dimethylaniline as the predominante metabolite of 2,4-DMA. Small amounts of N,N-trimethylaniline were also isolated. 2,6-DMA was excreted primarily as the sulfate conjugate of p-hydroxy-2,6-DMA in rats; a minor metabolite was the N-methyl compound.


Amiodarone (A) is an antiarhythmic drug known to undergo extensive distribution in human and animal tissues. A long half-life and a major metabolite, desethylamiodarone (DEA), have been well documented. However, no definitive data are available regarding the site or mechanism of metabolism of A. Pilot studies in our lab suggested the liver and/or intestine as possible sites of metabolism. In vitro metabolism experiments with hepatic and gut tissue fractions from rabbit and rat indicated significant A metabolism in the microsomal fractions. HPLC analysis indicated that biotransformation of A was greater for hepatic than for gut microsomes of both species. Three metabolites (I, II, III) were detected in the rabbit but not in the rat hepatic microsomal incubations. Hepatic P-450 involvement was confirmed for both species by significantly reduced metabolism in the presence of CO, 1 mM piperonyl butoxide, SKF 525-A, and n-octylamine. Rabbit gut microsomes produced DEA and II, while rat gut microsomes yielded only DEA. Cyt. P-450 involvement in the gut microsomal preparations was inconclusive. Metabolite I, also produced when only DEA was available as the substrate, is tentatively identified as deiodinated DEA. (Supported by HL-20622 and ES-07045-08.)


The metabolism of 1-nitro[14C]pyrene (NP) (8.1μM) and the binding of its reactive intermediates to DNA and protein of rabbit tracheal epithelial cells has been studied. Metabolites from the incubation medium and cell lysates were extracted, analyzed and quantitated by high pressure liquid chromatography. Metabolism of NP by tracheal cells occurred by both ring oxidation (1-NP-Diol and hydroxynitrophenols) and reduction of the nitro moiety to NAP and 1-AMP. Tracheal cells activated NP to reactive intermediates that were bound to DNA and protein. The highest rates of metabolism and binding occurred in cultures of tracheal cells treated immediately after isolation by a 1 hr protease digestion of the trachea at 37°C when compared to a 20 hr protease digestion at 4°C. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

METABOLISM AND PHARMACOKINETICS OF 4-NITRO-N-METHYLPHENYLAMIDE [CARBONYL-14C] (4-NPI) IN PREGNANT AND NULLIPAROUS RATS FOLLOWING ORAL ADMINISTRATION. R.C. Couch and L.M. Smith, Primate Research Institute, Holloman AFB, NM and General Electric Company, Pittsfield, MA.

The absorption, distribution, and excretion of 4-C-4-NPI equivalents in nulliparous and pregnant rats was studied after single oral dose administration of 1 or 100 mg/kg. Urine was the primary route of excretion regardless of reproductive condition or dose level. It accounted for 66 to 76% of the administered dose and the fecal route accounted for 12 to 20%. Less than 1% was eliminated via expired air as CO2. At the end of the 72-hour experimental period, all animals studied, only 2 to 4% of the radioactivity remained in the major organs and carcass. Transplacental studies over a 10-hour period indicated that the kinetics of 1-C-4-NPI equivalents in the fetus, amniotic fluid, and placenta parallel those observed in maternal whole blood. The levels of radioactivity in each of these reproductive components were generally less than the levels seen in maternal blood. These studies indicate that 4-NPI is readily metabolized and excreted in the urine as water soluble metabolites. Furthermore, there does not appear to be any significant bioaccumulation of 4-NPI in the body or reproductive unit.
Dinitrobenzenes, used as intermediates in the production of dyes and plastics, induce methemoglobinemia. The metabolism and excretion of C-labeled o-dinitrobenzene (o-DNB) was studied in male Fischer-344 rats after a single oral dose (0.15 mmol/kg). Urine and feces were collected over dry ice for 48 hours following the dose. Metabolites were separated by reverse phase HPLC and quantitated with an in-line radioactivity detector. Identification was made by enzyme hydrolysis, coelution with standards on HPLC, and mass spectrometry. Elimination of radiolabel was rapid; 85% of the dose was excreted within 24 hours. Urine was the primary route of elimination, accounting for 80-85% of the dose after 48 hours. S-(2-nitrophenyl)-N-acetylcysteine was the major urinary metabolite (42% of the total dose). The other metabolites were o-nitro-aniline-N-glucuronide (4%), an aminonitrophenylsulfate tentatively identified as 4-amino-3-nitrophenylsulfate (1%), o-(N-hydroxylamino)-nitrobenzene (excreted as a glucuronide) and an aminonitrophenol tentatively identified as 2-amino-3-nitrophenol. The hydroxylamine and 2-amino-3-nitrophenol coeluted and together accounted for 45% of the dose. Two unidentified metabolites totaling 42% were also found in urine. These results indicate that conjugation with glutathione is the major route of metabolism for o-DNB.

NPPD has allegedly been used as an agent for tracking U.S. diplomats by the KGB. In response to concern regarding human exposure and absorption of this compound, we have investigated its absorption and metabolism in male F344 rats. I.V. administration of NPPD in corn oil at dose levels of 0.8 and 8.0 mg/kg resulted in rapid excretion of urinary metabolites, two of which were identified as 4-nitrobenzoic acid (NBA) and 4-nitrohippuric acid (NHA). The highest concentration of metabolites was measured in urine 4 hrs after treatment. NPPD administered at 0.8 and 8.0 mg/kg by gavage in corn oil was rapidly absorbed and metabolized as indicated by high concentrations of NBA and NHA in urine within 4 hrs after dosing. Dermal administration to rats of NPPD (0.1, 1.0, or 10 mg/cm²) from an area resulted in low levels of NBA in urine over the 3 days that the animals were observed. The percent of dose absorbed decreased as the dose increased. Acetone extraction of the area of application after the 3 day observation period resulted in recovery of about 1/2 of the dose as parent compound. Thus, NPPD is readily absorbed through the G.I. tract and sparsely absorbed through the skin of male F344 rats. In either case most of the dose absorbed is rapidly metabolized and excreted.

DI-(2-ethylhexyl) terephthalate (DEHT) is used as a plasticizer for a variety of industrial and consumer products. Since little is known about the metabolism of DEHT, its fate and disposition were investigated in male Sprague-Dawley rats. DEHT and [14C]DEHT were mixed with corn oil and administered by gavage at 100 mg/kg body weight. Urine, feces, and expired air were collected up to 144 hrs at which time the rats were sacrificed. All tissues examined contained 14C; the highest concentrations were in liver and fat. Less than 2% of the dose remained in the carcass at the time of sacrifice. Radioactivity was eliminated in feces (56.5 +/- 12.1% of the dose) as DEHT (36.6%), mono-(2-ethylhexyl) terephthalate (MEHT, 2.5%), and polar metabolites. Radioactivity was expired as CO₂ (3.6 +/- 0.9%) and excreted in urine (31.9 +/- 10.9%) as metabolic products of MEHT and 2-ethylhexanol (2-EN). A major urinary metabolite was identified as terephthalic acid (TPA, equivalent to 51% of the dose on a molar basis). Our findings indicate that DEHT was metabolized differently than was its isomer, di-(2-ethylhexyl) phthalate (DEHP). DEHT was hydrolyzed predominantly to TPA. In contrast, results reported elsewhere indicate that DEHP is hydrolyzed largely to mono-(2-ethylhexyl) phthalate (MEHP).

PNPPD has allegedly been used as an agent for tracking U.S. diplomats by the KGB. In response to concern regarding human exposure and absorption of this compound, we have investigated its absorption and metabolism in male F344 rats. I.V. administration of PNPPD in corn oil at dose levels of 0.8 and 8.0 mg/kg resulted in rapid excretion of urinary metabolites, two of which were identified as 4-nitrobenzoic acid (NBA) and 4-nitrohippuric acid (NHA). The highest concentration of metabolites was measured in urine 4 hrs after treatment. PNPPD administered at 0.8 and 8.0 mg/kg by gavage in corn oil was rapidly absorbed and metabolized as indicated by high concentrations of NBA and NHA in urine within 4 hrs after dosing. Dermal administration to rats of PNPPD (0.1, 1.0, or 10 mg/cm²) from an area resulted in low levels of NBA in urine over the 3 days that the animals were observed. The percent of dose absorbed decreased as the dose increased. Acetone extraction of the area of application after the 3 day observation period resulted in recovery of about 1/2 of the dose as parent compound. Thus, PNPPD is readily absorbed through the G.I. tract and sparsely absorbed through the skin of male F344 rats. In either case most of the dose absorbed is rapidly metabolized and excreted.


Branched alkanes are known to produce nephrotoxicity in male rats. The purpose of this investigation was to compare the disposition of n-octane (non-nephrotoxic) and 2,2,4-trimethylpentane (TMP) (nephrotoxic). Male F-344 rats were given single oral doses (1 ml/kg, p.o.) of TMP [2,4-14C] or n-octane-[1(8)-14C] and placed in glass metabolism cages. Groups of 3 rats were killed at intervals of 1, 2, 4, 8, 12, 24, 48 and 72 hrs after dosing. Excretion of radioactivity (% of dose) 72 hrs after TMP dosing was: urine (42%), expired CO₂ (36%), feces (12%) and expired CO₂ (36%). In contrast, excretion of radioactivity 72 hrs after n-octane dosing was: urine (12%), expired CO₂ (10%), and expired CO₂ (36%). For both compounds, tissue radioactivity was present mainly in the liver and kidney. Radioactivity in the kidney of TMP-dosed rats was 6 times that of n-octane-dosed rats at 72 hrs. Also, at 72 hrs, the kidney-to-liver activity for TMP dosed rats was 17.6 compared to 0.35 for n-octane dosed rats. In conclusion, distinct differences in the disposition of n-octane and TMP were observed. The more titer of TMP metabolites in the kidney may help to explain why TMP is nephrotoxic to male rats and n-octane is not. Additional work is needed to assess the type of TMP metabolites (free or bound) present in the kidney.
DISPOSITION OF HEXACHLORO-1,3-BUTADIENE IN MALE FISCHER 344 RATS AND B6C3F1 MICE. R.S.N. Yang, K. Jefferio, and J.B. Matthews. National Toxicology Program, NIEHS, RTP, NC 27709 and Research Triangle Institute, RTP, NC 27709.

The disposition of 14C-hexachloro-1,3-butadiene (HCBD) was studied in rats and mice. Approximately one-half of single oral or IV doses of HCBD at 0.58-34 mg/kg (rat) or 2-24 mg/kg (mouse) was excreted in feces over 3 days. Lesser amounts of the dose (rat: 12-20%; mouse: 18-30%) were excreted in 3 day urine, almost entirely as metabolites. Volatile organics, presumably excreted via breath and consisting primarily of HCBD, accounted for another 3-6% of the dose to rats, 8-11% to mice. At higher dosage levels in both species, the rats and relative amounts excreted in urine and feces were altered. Thus, excretion of 14C was significantly depressed after oral doses of 166 mg/kg of HCBD to rats or 50 mg/kg to mice, indicating that metabolic or clearance mechanisms may have been saturated. Mice were more sensitive to intoxication by HCBD than rats. This greater sensitivity appears to be related to glutathione conjugation. Tissue distribution studies indicated that in the 166 mg/kg rat group, fat had the highest concentration of radioactivity; this was followed by the kidney. In all other treatment groups in both species, kidney had the highest concentration of radioactivity.


Picric acid (2,4,6-trinitrophenol) is widely used in industry, by the military, and as a research/clinical chemistry reagent. Characterization of the toxicity of this chemical has been limited. Thus the acute toxicity, distribution, and metabolism of picric acid were investigated using Fisher 344 rats. The LD50 for picric acid following oral dosing of male and female rats was established as 450 and 200mg/kg, respectively. Blood gas analysis indicated metabolic acidosis was the specific cause of death in acute intoxication. Metabolism of picric appeared limited with picramic acid being the only detectable metabolite. The th for elimination was 13.4h with an absorption coefficient (Ka) of 2.45/h. Two h following oral administration of 14C-picric acid (100mg/kg), the primary depot of radioactivity (per g tissue basis) were blood, kidney, liver, omentum, lung, and spleen. Restricted tissue ratios were 0.54, 0.31, 0.31, 0.25, and 0.22. All other tissues assayed had partition ratios<0.20 with brain and adiase tissue having the least amount of radioactivity. Tissue/blood ratios were essentially maintained over a 48h post-administration period. Binding (in vitro) of 14C-picric acid to plasma proteins (whole blood preparations) demonstrated both high and low affinity binding sites, with dissociation constants of 2.85×10^-6 and 3.18×10^-4M.

METABOLISM OF n-OCtANE IN FISCHER 344 RATS. C.T. Olson, K.O. Yu, M.P. Serve. Bussey G. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH. Sponsor: Dr. B.O. Stuart.

n-Hexane has been shown to produce neurotoxicity in man and experimental animals. Pentane is also believed to have neurotoxic characteristics similar to those of hexane. Isocyanates are nephrotoxic to male rats, and n-octane can cause cancard, cardiac sensitization, and respiratory paralysis, but neurotoxicity has not been demonstrated. Metabolism of hexane and heptane leads to the production of diketones which, although not the major metabolites, are thought to be the neurotoxic agents. This study was conducted to identify the major urinary metabolites of n-octane and determine if there were differences between male and female rats. Rats were dosed by gavage every other day for 14 days with 2 mL/kg n-octane and urine was collected for the first 48 hours. Monohydroxylation (3- and 2-octanol) and oxidation to keto-acids (5-oxo-hexanolic and 6-oxo-heptanolic acids) were the major metabolic processes and products were the same in both male and female rats. Of interest is the formation of keto-acids with reduced C chain length which were major metabolites. One kidney from each animal was used for histopathologic examination and the other for extraction of octane metabolites. No metabolites were identified in kidney extracts of either sex and no signs of neurotoxicity or nephropathy were observed.

DISPOSITION OF 2-HYDROXY-4-METHOXYBENZOPHENONE (HMB) IN RATS DOSED BY GAVAGE, INTRAVENOUS INJECTION, OR DERMAL APPLICATION. S.M. El Darree, R.F. Tillery, J.R. Kahn, and D.L. Hill. Southern Research Institute, Birmingham, AL. The disposition of HMB, which is used as a sunscreen compound and as an ultraviolet stabilizer in plastics, was evaluated in male Fischer 344 rats. Oral doses of [ring-14C]-HMB in the range of 2.01 to 2570 mg/kg were administered by gavage to rats. No dose-dependent processes were evident. Measured at 72 hr after dosing, urinary excretion of radioactivity ranged from 63.9 to 72.9% and fecal excretion from 19.3 to 41.7% of the dose. An intravenous dose (4.63 mg/kg) distributed rapidly throughout the body of rats and appeared in the urine in an amount (67.5%) similar to those for the oral doses. [14C]-HMB was administered to rats topically, either as an ethanolic solution (0.05, 0.2, or 0.8 mg/cm²) or in a lotion (0.05 mg/gm). At 72 hr, 60% of the low dose and 30% of the high dose was absorbed. The vehicle had little or no effect on the rate of absorption. Of the radioactivity absorbed, 49.8 to 62.0% was excreted in the urine. Gut tissue, liver, and kidneys contained the highest concentrations of radioactivity, regardless of the route of administration. For rats with biliary cannulas, 36.5% of an intravenous dose (4.46 mg/kg) was recovered in the bile in 4 hr; the initial half-life for elimination in the bile was 48 min. Four radioactive components, none of which was intact HMB, were noted in the bile. (Supported by Contract NO1-ES-1-5008 from NIEHS, NIH, DHHS).
The metabolic fate of hydroquinone was studied in Fischer 344 rats at 5, 25, and 50 mg [14C]hydroquinone/kg body weight. Dosed rats were placed in metabolism chambers to collect urine, feces, and expired air. Feces, tissues, and carcasses were extracted with acetone and the residues burned. Urines were analyzed by reverse phase hplc, and gc/ms following acid or enzyme hydrolysis. Test animals rapidly absorbed and excreted the 14C at all doses. Urinary conjugates were the major metabolites. By 8 hrs, male rats dosed by gavage excreted 61-64% of the dose as hydroquinone glucuronide. 19-26% as hydroquinone sulfate and about 12% as unchanged hydroquinone. Female rats showed a slightly smaller glucuronide to sulfate ratio. No significant differences were observed between male rats dosed by intratracheal instillation and those dosed by gavage. Metabolism of hydroquinone was independent of the route of administration. There were no dose related differences in the ratio of urinary metabolites formed over the dose range tested. The liver and kidneys showed the highest concentration of tissue radioactivity. The primary metabolic pathway for hydroquinone was conjugation with glucuronic acid, and to a lesser extent, with sulfonic acid. There was no evidence that hydroquinone was metabolized to any other substances.

Dermal absorption is a prevalent route of exposure for many industrial chemicals. To assist in determining exposure to MDA studies were performed in male F344 rats treated topically with [14C]-MDA at 2 or 20 mg/kg. Conditions of exposure including dosage, dose regimes and occlusion were assessed. During 96 hr after the low dose, 43% was recovered in urine, 10% in feces and 2% in tissues. Only 25% was excreted and the remainder (26%) was recovered by extraction and skin solubilization. After the high dose, only 4.8 and 1.3% was eliminated in the urine and feces respectively; 0.4% was recovered in tissues. 62% in skin wash and 24% from the application area. Although the percentage absorbed decreased by increasing the dose, the total amounts absorbed (0.2-0.25 mg/rat) remained similar after both doses. Occlusion appeared to enhance absorption. Skin washing did not remove all applied 14C even when it was performed 5 min after application. Washing with acetone/water was less effective than soap/water and, in addition, acetone facilitated absorption. Significant amounts of dose remained associated with the skin. If MDA associated with the skin is available for systemic circulation, the extent of dermal absorption would be considerably higher (80%) than calculated from excretory and tissue distribution data only (54%).

To assist in determining workplace exposure to MDA, studies were performed in rats, guinea pigs, and monkeys treated topically with [14C]-MDA. Conditions of treatment included dosage, dose regimens and occlusion. The studies in rats (accompanied by abstract) and g. pigs were performed at 2 dose levels (2 and 20 mg/kg). The monkeys were treated with the low dose only. In g. pigs, 10% of the low dose was excreted in urine and 18% in feces; 1% was recovered in tissues, 41% in the skin wash and 29% from the application area (extraction and skin solubilization). After the high dose, recoveries in urine, feces and tissues averaged 3, 4 and 1%; 70% was recovered in dose wash and 14% from the application area. In monkeys, 19% and 2% were eliminated in urine and feces during 168 hr. Washing efficiency studies showed higher and more consistent recoveries with soap/water compared to acetone/water. However, both washing methods were incapable of removing all the applied material from the skin. Significant amounts of doses remained associated with the skin. Under similar conditions of treatment (application of low dose for 24 hr and extraction collection for 96 hr) comparable absorption was demonstrated in g. pigs and monkeys (18%) and higher absorption in rats (43%). If MDA associated with the skin is considered available for systemic circulation, absorption would be considerably higher for rats, g. pigs and monkeys.

As a part of an evaluation of the disposition behavior of MDA after different routes of exposure, the elimination and tissue distribution of [14C]-MDA were determined after i.v. dosing to Fischer 344 rats, Hartley guinea pigs and rhesus monkeys. Excreta from rats and guinea pigs were collected for 96 hr then the animals sacrificed for tissue sampling. The studies in monkeys were extended to 168 hr and only excreta were sampled. In rats, most of the dose was eliminated by 24 hr. Recovery at 96 hr averaged 57% in urine and 31% in feces. Most of the doses were eliminated by 48 hr in guinea pigs and monkeys. In guinea pigs, 35% of dose was excreted in urine and 57% in feces during a 96 hr period. Monkeys excreted 84% of dose in urine and 10% in feces during a 168 hr period. In rats, 14C in blood and tissues averaged 19% of dose at 6 hr which declined to 2% by 96 hr. The highest levels were demonstrated in liver which was 5-9 times higher than blood. In guinea pigs, 3% of the dose was recovered in blood and tissue at 96 hr. The highest content was demonstrated in the spleen (5 times higher than blood) followed by liver. The data suggest considerable elimination through the biliary route especially for guinea pigs.
Detailed characterization of alterations in caffeine elimination during pregnancy are essential in assessing the potential exposure of the fetus to caffeine and its metabolites. Forty female monkeys (Oecaxa fascicularis) were exposed to caffeine in their drinking water seven days per week before, during, and after pregnancy. The low dose corresponded to levels sometimes consumed by pregnancy women (30-15 mg/kg/d) while the high dose was above normal human consumption (25-30 mg/kg/d). Blood samples and 24-hour urine samples were collected every two weeks and analyzed for caffeine and metabolites by HPLC. Prior to pregnancy, mean serum caffeine concentrations were approximately 2.5 and 8.1 µg/ml and serum theophylline concentrations were 6.6 and 13.2 µg/ml for the low and high dose groups respectively. During pregnancy, serum caffeine concentrations increased by 75% for both dose groups while theophylline concentrations did not change. Caffeine concentrations returned to pre-pregnancy levels after parturition. The amount of caffeine and theophylline excreted in the urine over 24 hours increased during pregnancy and then returned to pre-pregnancy levels following parturition. The changes in caffeine elimination occurred at week seven of pregnancy and may be related to hormone changes that occur at approximately the same time.

DISPOSITION AND METABOLISM OF A SINGLE ORAL DOSE OF [14C]16I-Q-CRESYL PHOSPHATE (TCP) IN HENS. E.Suwita*, A.A. Nomeir* and M.B. Abou-Donia*. Duke University Medical Center, Durham and NIHS, Research Triangle, NC.

Hens were given an oral dose of 50 mg/kg (4.6 µJ/kg) of [14C]TCP. Four groups of three hens were killed after 0.5, 1, 2 and 5 days. 47% of the dose was eliminated in the excreta during the first 12 hr. Radioactivity level in the tissues reached a peak of 4.2% of the dose at 1 day. After 5 days, 99% of the dose was eliminated as excreta. The highest concentration of 14C outside the GI system was detected in the bile followed by kidney, liver and lungs while sciatic nerve contained the highest concentration among the nervous tissues. The half-lives of 14C in the nervous tissues ranged from 2.6-3.2 days while plasma had a t1/2 of 2 days. TCP reached its highest concentration in plasma at 0.5 day. The predominant metabolites in the plasma at 1 day were salicylic acid and di-o-cresyl hydrogen phosphate while TCP and o-cresol were present in trace amounts; these concentrations dropped significantly as time passed. Appreciable concentrations of saligenin, cyano-o-tolyl phosphate, which is believed to be the active neurotoxic metabolite, was detected in the plasma as well as in the kidney and lungs at all time points. Supported by NIOSH Grant No. OH82003 and NIEHS Grant No. ES03411.


The objective of this study was to determine the kinetics of absorption, distribution, and elimination of DBCP after intravenous (iv) administration in plasma, and after oral administration in water or corn oil, to conscious fed male Fischer 344 rats. Rats were prepared with an external jugular vein canula and were dosed with 0.1, 1 or 10 mg/kg DBCP into the penile sinus, or orally as a solution in water or corn oil. Blood was sampled at various times up to 12 hr, concentrations of DBCP were determined, and data were evaluated by classical pharmacokinetic techniques. Absorption was more rapid after oral administration in water than in corn oil, with peak blood levels being reached after about 12 min and 1.6 hr, respectively, but the extent of absorption (AUC) was similar for both vehicles. The distribution and elimination phase of DBCP was bi-exponential for all experimental groups, with terminal half-lives ranging from 2.02 to 3.55 hr. These results suggest that, at these doses, systemic toxicity of DBCP may be independent of the vehicle and route of administration, but that effects at the site of presentation, i.e. the stomach, may be dependent on the vehicle.

THE EFFECTS OF ETHANOL CONSUMPTION ON THE MICROBIAL METABOLISM OF PESTICIDES BY INBRED STRAINS OF MICE. W. Baensel and E. Hodgson. North Carolina State University, Toxicology Program, Raleigh, NC.

The effects of ethanol consumption on xenobiotic metabolism were investigated in three inbred mouse strains: C57BL/6J, C3H/He and DBA/2J. Ethanol was administered as a liquid diet by isocarotic substitution of ethanol for dextrose from a nutritionally balanced diet. To examine the effects of carbohydrate reduction, the same diet was administered with corn oil substituted for dextrose. The mice received up to 30% of their calories from ethanol in the liquid diet. With this concentration of ethanol in the diet, cytochrome P-450 levels per unit protein in hepatic microsomes increased until they had doubled in approximately 10 days. The effects of ethanol consumption on several monooxygenase pathways were investigated using pesticides as substrates. The epoxidation of aldrin to dieldrin was significantly increased. The metabolism of parathion, however, was not affected to the same degree. The type III spectra produced upon the metabolism of piperonyl butoxide were similar for both induced and uninduced microsomes. Piperonyl butoxide was also used as a synergist to examine its ability to inhibit the reactions studied. Studies involving other pathways and lung microsomes are in progress.
Numerous epidemiologic and anecdotal reports have suggested an association between sudden death in narcotic addicts and the concomitant intake of ethanol. This study was undertaken to determine whether this phenomenon might be associated with an alteration in the kinetics of opiates in the presence of ethanol. Male Sprague-Dawley rats (190-240 g) were administered a single intravenous dose of morphine sulfate (2.5 mg/kg) one hour following an oral gavage of either 50% ethanol (3 g/kg) or an equal volume of saline. Blood samples, obtained over the next four hours by orbital sinus puncture, were assayed for both morphine and ethanol. Kinetic parameters of morphine were calculated for each individual rat. Elimination of morphine was biphasic in both ethanol-treated and control animals. The presence of ethanol, however, resulted in a significantly different pattern of morphine elimination with steeper slopes (shorter half-lives) for both the alpha and beta phases of the disappearance curve. At the same time, there was no significant change in the overall rate of morphine elimination indicated by area-under-the-curve (AUC) and clearance. The observed changes suggest that ethanol may enhance morphine toxicity by facilitating morphine’s penetration into the CNS.


Excess urinary folate excretion is one mechanism by which chronic ethanol ingestion could lead to folate deficiency. Acute ethanol administration to rats produces a marked increase in urinary folate excretion, the mechanism of which is unknown. One possibility is an increased delivery of filterable folate to the nephron, resulting from an increased amount of free plasma folate. Ethanol was administered orally in a four doses of 1 g/kg each at 0, 1, 2, and 3 hr to male Sprague-Dawley rats; isocaloric glucose was given similarly to controls. Blood samples were collected at timed intervals and centrifuged to obtain plasma. Plasma samples were treated with acid and then coated charcoal to remove endogenous folate from the binder. Folate binding capacity (FBC) in stripped (Total FBC) and untreated (unsaturated FBC) plasma was assayed using a radiobinding assay. TFBC and UFBC were both decreased about 40% from control values at 4 hr after ethanol treatment, with a return towards normal after 6 hr. No marked changes were observed in total folate levels in the plasma. A decrease in folate binding in the plasma suggests a greater amount of free folate available for filtration by the glomerulus, which might be one mechanism for the increased urinary folate excretion due to ethanol. (Supported in part by NIAAA grant AA05308.)

Enhanced Hepatotoxicity of Allyl Alcohol in Female Rats. R.M. Panchar, W.C. Kershaw and G.L. Lage, Dept. of Pharmacology & Toxicology, Philadelphia College of Pharmacy & Science, Philadelphia, PA, 19104

The sex dependent nature of allyl alcohol (AA) induced hepatotoxicity and in vitro metabolism of AA has been evaluated. AA (5, 25, 32, 42, or 52 mg/kg) was administered ip to male and female SD rats which were killed 24 hr later. Additional animals were killed 6, 12, 24, 36, or 48 hr after AA (42 mg/kg) administration. Indices of hepatotoxicity employed were: liver morphology, liver/body weight ratio, and serum activities of alanine aminotransferase and sorbital dehydrogenase. AA is activated by alcohol dehydrogenase (ADH) to acrolein (ACR), the ultimate reactive metabolite. ADH activity and rate of ACR formation in liver cytosol were determined in control male and female rats. Hepatotoxicity was greater in female rats 24 hr after AA (32, 42 & 52 mg/kg) and 6 through 48 hr after AA (42 mg/kg). At the 52 mg/kg dose, females showed 80% mortality, whereas all the males survived. Female liver cytosol had significantly more ADH activity (35%) and produced more ACR (50%) than male liver cytosol. These results indicate a greater hepatotoxic effect of AA in female rats, due to increased ADH activity paralleled by toxic activation of AA to ACR.
Benzyl alcohol (BA), a bacteriostat in parenteral preparations, was implicated in 1962 as the agent responsible for precipitating "The Gaspig Syndrome" in premature neonates. To further evaluate its toxicity, BA was given i.p. to adult (23-26 g) and neonatal (2-7 g) CD-1 male mice. The LD50 at the end of 4 hr was 1000 mg/kg for both groups. Rapid absorption and conversion of BA to its primary metabolite, benzaldehyde (BZ), was demonstrated by gas chromatographic analysis of plasma from both experimental groups with levels being comparable in both groups. In an attempt to alter the toxicity of BA, pyrazole and disulfiram were used to inhibit the activities of alcohol dehydrogenase and aldehyde dehydrogenase, respectively. Pretreatment with pyrazole before BA exposure resulted in BA levels increasing to 25% of control, BZ concentrations decreasing to 40% of control, and toxicity being markedly increased. Although pretreatment with disulfiram led to BZ levels which were significantly increased to 250% of control, toxicity was unchanged. These data imply that the acute toxicity of BA, which includes sedation, dyspnea and loss of motor function, is due to BA itself and not to its metabolite, BZ.

Alpha-beta unsaturated aldehydes have been implicated as highly reactive cytotoxic agents which are potential substrates for detoxification by hepatic aldehyde dehydrogenase (ALDH). Our study has examined the oxidation by rat liver ALDHs by 4-hydroxynonenal (4-HN), hexenal (HE), and crotonaldehyde (CA). Livers were obtained from male Sprague-Dawley rats for preparation of semi-purified high and low affinity ALDH isozymes from the cytosolic (C), mitochondrial (M), microsomal (S) fractions. 4-HN is oxidized by the high affinity M and C ALDHs, and by the low affinity M ALDH. The respective Km (μM) and Vmax (nmol/min/mg protein) values are 6.5, 505.1, 9.0, and 1.01, 57.6, and 0.91. Hexenal is oxidized by the S, M, low affinity, and C low affinity ALDHs with Km and Vmax values of 84.0, 0.83, 16.7, and 5.09, 0.47, 1.25 respectively. Oxidation of CA occurred only by the S ALDH with a Km of 207.6 μM and a Vmax of 0.46. (Supported by NIH Grant 53527)

Crude preparations of liver mitochondrial ALDH, obtained from male Sprague-Dawley rats, were used to study the oxidation of t-4HN, t-4HH and t-2H. Concentrations of t-4HN ranging from 1.0 mM to 20 μM resulted in linear Lineweaver-Burk plots. Values of 70.6 μM and 16.2 mmoles NADH/mg protein were obtained for the apparent Km and Vmax, respectively. Cytosolic preparations of ALDH were unable to metabolize t-4HN to any significant extent. The use of t-4HH resulted in first order kinetics from concentrations ranging from 1 to 12.0 mM. However, when t-2H was substituted as a substrate for mitochondrial ALDH, Michaelis-Menten kinetics were observed for concentrations ranging from 2 mM to 50 μM. The apparent Km value for this substrate was 0.4 mM with an apparent Vmax of 38.7 mmole NADH/min/mg protein. These results are in support of the possibility that ALDH may play an important role in the detoxification of t-4HN arising through the process of mesosomal lipid peroxidation. The results of the ALDH oxidation of t-4HH by the rat differ from what we have previously reported for mouse mitochondria (Toxicologist 5(1): 975, 1985). These latter findings suggest differences between rat and mouse isoforms of mitochondrial ALDH. (Supported by NIH, ES03343)

Ethylene glycol is biotransformed to glycolaldehyde (GA) which, in subsequent reactions, is converted to highly acidic metabolites that cause proximal tubular necrosis. The present study has examined and compared the oxidation of GA by aldehyde dehydrogenases (ALDH) in rat and dog liver and kidney. Rat ALDH activity with GA was highest in liver (1.5±0.1, 1.2±0.1 and 1.0±0.05 μmoles NADH/min/mg tissue in liver, cortex and medulla, respectively). In contrast, dog ALDH activity with GA was highest in the cortex followed by liver and medulla (2.5±0.4, 1.7±0.2 and 1.6±0.3, respectively). A similar ranking for both species was observed for propionaldehyde an analog of GA. The greatest activity with GA in rat cortex and medulla was observed in the cytosolic fraction (70% total) whereas dog kidneys possessed similar activities in mitochondrial and cytosolic fractions (45% total each). Minimal activity was found in the microosomal fraction of samples for either species. Studies with disulfiram revealed species differences in the extent of inhibition of ALDH activity toward GA (liver cytosolic ALDH, dog; rat cortex mitochondrial ALDH, rat; dog). These findings demonstrate that dog and rat cortex exhibit a high capacity to oxidize GA.

The effects of ethanol and 4-methylpyrazole (4-MP) on the toxicity and pharmacokinetics of ethylene glycol (EG) in the dog were compared. Dogs were randomly assigned to 3 groups: EG-treated only, (173 mmol/kg EG, po.), EG and ethanol (15.3 mmol/kg, iv 3, 37, 14 and 24 hrs after EG) and EG and 4-MP (0.24 mmol/kg, iv 3 hrs after EG. 0.16 mmol/kg at 24 hrs and 0.06 mmol/kg at 36 hrs). EG produced a rapid onset metabolic acidosis within 3 hrs and acute oliguric renal failure (48 hrs) and ethanol or 4-MP greatly attenuated these effects. EG half life in serum was 10.8±0.7 hrs in the EG only treatment group, 6.8±0.7 (p<0.05) in the EG and ethanol group and 9.8±0.9 hrs in the EG and 4-MP group. Approximately 10% and 48% of the dose of EG was excreted unchanged in the urine at the 0-3 and 3-72 hr periods, respectively. 4-MP increased the amount of EG excreted in the urine (71% of the dose, 3-72 hrs) whereas ethanol did not (51%). However, both ethanol and 4-MP increased the rate constant of EG excretion into urine approximately 70%. Prevention of EG-induced toxicity by inhibition of alcohol dehydrogenase with ethanol or 4-MP was associated with an increase in the rate constant of EG excretion in the urine and not with a prolongation in EG half-life.

1043 METABOLISM AND BINDING OF 1,2-DICHLOROETHANE (EDC) IN RATS TREATED CHRONICALLY WITH 4-METHYL-PHYRAZOLE (4-MP) AND ETHANOL. M. El-hawari1, M. Stoltz1, F. Pallas, K. Christianson2, K. Cheever3 and E. Meisburger3, MRL, Kansas City, MO, NIDDK, Cincinnati, OH, and NCI, Bethesda, MD.

DS potentiation of 1,2-dibromoethane carcinogenicity prompted studies of such interactions with other halo-carbons. S.D. rats were exposed to EDC (50 ppm by inhalation), EDC plus OS (0.05% in diet), or EDC plus ET (5% in water). Control rats were exposed to filtered air, DS or ET. At termination (24 mo), levels of EDC were determined in blood. Rats were then treated orally with 14C-EDC (150 mg/kg) and 14C elimination in expired air, urine and feces was measured, and urinary metabolites were analyzed by HPLC. Rats were also treated with 14C-EDC and were sacrificed at 6 hr for assessment of DNA binding. In control rats, 14C was eliminated in expired air (28-30%), primarily as EDC, urine (47-55%) and feces (1-2%). In DS-treated animals, excretion in expired air increased to 41-45% and in urine decreased to 35-36%. The major (thioglycolic acid and its sulfoxide) and monochloroacetic acid) urinary metabolites were not affected by DS or ET. Hepatic DNA binding was low (36-44 µmol/mol DNA) and was not altered by DS or ET. A major effect of treatment was the increased levels of EDC in blood of DS-treated rats which, with modified EDC elimination, may have contributed to the higher incidence of hepatic, testicular and mammary tumors demonstrated in rats exposed to EDC and DS.


Glycol ethers (GEs) are known to be toxic to the liver, central nervous system, kidneys, and hematopoietic and reproductive systems. Their mechanisms of toxicity and biotransformation are not well understood. Aldehyde dehydrogenase (ALDH) may function in the biotransformation of ethylene glycol and monomethyl ether (EGME) but not of propylene glycol monomethyl ether (PGME). To test this hypothesis, LD50s of EGME and PGME were determined in the presence and absence of disulfiram (DSF), an inhibitor of ALDH, using male white Swiss-Webster mice. DSF was given one hour before exposure to the GEs. The LD50 of EGME, but not PGME, was significantly lower in the group pre-treated with DSF. These results suggest that the alcohol dehydrogenase/ALDH pathway is important in the biotransformation of EGME but not PGME. Furthermore, it may be concluded that the accumulation of a metabolic intermediate, i.e. an aldehyde derivative, is at least partly responsible for the acute toxic effects of EGME.

1044 KINETICS OF INHALED METHANOL IN MALE FISCHER-344 RATS. V.L. Horton, M.A. Higuchi, K.L. Wong, and D.E. Ricker. CITI, Research Triangle Park, NC, and Curiculum in Toxicology, University of North Carolina, Chapel Hill, NC.

Exposure of humans to methanol is primarily by inhalation at low concentrations. To determine the kinetics of inhaled methanol and its metabolites, four rats were exposed in a head-only chamber for six hours to an atmosphere of methanol or air. Gas chromatography showed that the peak blood methanol concentrations were 3.3±0.7 (mean±SE), 32.2±1.5, and 90.6±10.0 µg/ml in rats exposed to 200 (6 hr-end of exposure), 1200 (6 hr), and 2000 (1 hr postexposure) ppm methanol, respectively. These results indicate a nonproportional increase in blood methanol concentration with the atmospheric exposure level. Steady-state methanol concentrations were only attained during the 200 ppm exposure. Semilogarithmic plots showed monoeponential decay of blood methanol concentrations; half-lives of 0.8±0.6 hr (200 ppm), 3.3±1.1 hr (1200 ppm), and 2.4±0.1 hr (2000 ppm) were calculated. The mean peak blood formate concentration ranged from 9.6 to 12.2 µg/ml in all four groups, and while about two-times the average pre-exposure concentration, the elevation was not due to the methanol exposure. The results indicate that formate does not accumulate in F-344 rats and that methanol elimination was not linear over the range of exposure concentrations studied.

Computer simulations generated by a physiological pharmacokinetic model of dichloromethane (DCM) disposition in rats were used to establish a dose-equivalence relationship between 6-hour inhalation exposures up to 1500 ppm and oral doses up to 1000 mg/kg. The equivalency was determined by comparing relevant measures of internal exposures that resulted from the two routes. These measures included the area under the concentration-time curve (AUC) for blood and target tissues (liver, lung) and the cumulative metabolite production by each of two biotransformation pathways that convert DCM to CO and CO₂. The results showed that as dose level increased, inhalation exposures led to disproportionately lower tissue levels and to more rapid saturation of DCM metabolism than when compared to oral administrations over the examined dose ranges. It was concluded that the pulmonary first-pass effect and the driving forces which controlled the absorption of DCM into the body were responsible for the route-dependent differences.

COMPARATIVE PHARMACOKINETICS OF INHALED DICHLOROMETHANE IN RATS AND MICE. T. Green, J.A. Nash, and W.M. Proven, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TA, U.K. Sponsor: E.A. Loeck.

The marked species differences seen in the carcinogenicity of inhaled dichloromethane (DCM) in rats and mice (NTP 1985) suggests that the fate of DCM may be significantly different in the two species. In this study, the pharmacokinetics of C-14 DCM have been determined following exposure of F344 rats and B6C3F1 mice to 500, 1000, 2000, and 4000 ppm for 6 hr. Urine, feces, expired DCM, CO, and CO₂ were collected at short time intervals for 48 hr after exposure. At the top dose level the body burden of CO was similar in both species. Significantly more radioactivity appeared in urine and as CO₂ in the mouse than in the rat. The half-life of elimination of unchanged DCM was 18 min in the mouse and 86 min in the rat. This resulted in markedly different time courses for the elimination of both CO and CO₂ in the two species. The data suggests that there are significant differences between the two species both in the extent of DCM metabolism and in the distribution of metabolites between the two pathways.

MODELING BLOOD AND TISSUE SOLUBILITIES OF HALOGENATED METHANES, ETHERS, AND ETHYLENES. P. G. Seybold, M. A. May, *M. L. Gargas, and *E. E. Andersen, Wright State University, Dayton, Ohio, and *AMRL/THB, Wright-Patterson AFB, Ohio.

Solvent:a:air and tissue:a:air partition coefficients have been determined for 25 halogenated methanes, ethers, and ethylenes in saline solution, olive oil, and in blood, liver and fat tissues from rats by vial equilibration methods. Correlation equations were developed using theoretical molecular modeling techniques. These equations relate solubilities to various properties of the chemicals. Two graphic theoretical approaches (the distance method of Wiener and the connectivity index method of Randic, Kier, and Halli) and an approach utilizing ad hoc molecular descriptors were employed. Satisfactory regression models were obtained with both the Randic-Kier-Halli approach and the ad hoc descriptor approach but not with the Weiner approach. The method of ad hoc descriptors revealed that fluorine substituents decrease tissue solubilities, whereas both chlorine and bromine substituents increase tissue solubilities, with the relative influence being 8Cl > 7Cl. Tissue solubilities could also be conveniently represented in terms of contributions from oil and saline solubilities.

IN VIVO METABOLISM OF C-METHYLENE CHLORIDE (MEC). R. H. Reitz, F. A. Smith, and *M. E. Andersen, Dow Chemical USA, Midland, Michigan, and *AMRL/THB, Wright-Patterson AFB, Ohio.

Male B6C3F1 mice exposed to 1500 or 50 ppm C-MEC for 6 hr metabolized 2.92 and 0.28 mmol/kg, respectively. In the 6 hr exposure period, 61 and 70% of the total metabolites were collected and 39 and 30% in the 6-24 hr post-exposure period. At 1500 ppm, 1.62 mmol CO/kg and 0.78 mmol CO₂/kg were recovered and at 50 ppm, 0.13 mmol CO₂/kg and 0.12 mmol CO/kg. Kinetic constants of MEC metabolism can only be determined when these volatile metabolites are collected both during and after the exposures. MEC is metabolized by microsomal oxidation and glutathione conjugation. Satura-tion of oxidation is evident by the nonproportionality of CO production. With mice treated with pyrazole (320 mg/kg) to inhibit oxidation, there was, respectively, 60 and 83% inhibition of CO and CO₂ production at 1500 ppm; at 50 ppm there was 62 and 69% inhibition. These data are consistent with the suggestion (Gargas et al., 1985) that oxidation yields both CO and CO₂. At low MEC concentrations oxidation appears to give equal amounts of CO and CO₂, and is predominant in MEC metabolism. Oxidation and conjugation may play different roles in MEC toxicity and their relative activity will vary depending on exposure conditions.
EFFECT OF A SINGLE DOSE VS 7 DAILY DOSES OF CCl₄ ON TOXICANT METABOLISM AS DETERMINED BY IN VITRO ENZYME ACTIVITY AND A MODEL SUBSTRATE ASSAY.


The effect of a single i.p. injection of 0, 20, 200 or 1000 µl/kg CCl₄ on the metabolism of the model substrate lindane and on the activity of a variety of in vitro hepatic enzymes was compared to the effect of daily i.p. injections of 0, 20, 200 or 1000 µl/kg CCl₄ for seven days in weanling, female Fischer 344 rats. A single injection of CCl₄ produced significant dose-related inhibition of phase I and phase II reactions both in vivo and in vitro. On the other hand, repeated pretreatment with CCl₄ increased lindane metabolism in a dose-related manner. Metabolites excreted as glucuronides, sulfates and mercapturic acids were significantly increased by repeated treatment. Glutathione-S-transferase employing 1,2-dichloro-4-nitrobenzene but not 1,2-epoxy-3-(p-nitrophenox) propane was induced in vitro. Moreover UDP-glucuronyl transferase activity was stimulated when naphthol but not chloramphenicol was used as the substrate. Aldrin epoxidase was induced in one experiment but not in another. The low and middle doses of CCl₄ stimulated ethylmorphine demethylase while the highest dose inhibited activity. Results from this study suggest that the rat may exhibit metabolic adaptation to repeated low level CCl₄ exposure.

COMPARISON OF THE UPTAKE, DISTRIBUTION AND MACROMOLECULAR BINDING IN MICE OF 1,2-DIBROMOMETHANE AND CHLOROFORM ADMINISTERED IN CORN OIL OR WATER.


Mice were administered orally 1,2-dibromomethane -¹⁴C (DBE) or chloroform-¹⁴C in either corn oil or water and the uptake and macromolecular binding in blood and organs determined. The peak blood level of CHCl₃ and DBE were obtained in 3 min when administered in water and in 10 to 20 min when administered in corn oil. The uptake and macromolecular binding of CHCl₃ in the kidney and of DBE in the blood, kidney, liver, stomach and testis were greater when administered in water than when administered in corn oil throughout the four hour study. Water vehicle caused a greater uptake of chloroform in blood and liver and greater macromolecular binding in the liver only during the first 20 min. The uptake and macromolecular binding of CHCl₃ and DBE in either vehicle were related linearly to the administered dose. Water vehicle at all dose levels tested resulted in a greater uptake and macromolecular binding of DBE in the blood and organs. Administration of CHCl₃ in water resulted in a greater uptake and macromolecular binding in the blood and kidney at all doses and in the liver only at the highest dose. Thus, the type of vehicle, i.e., water or corn oil, can alter the blood and organs in terms of uptake and macromolecular binding of CHCl₃ and DBE. This abstract does not necessarily reflect EPA policy.

URINARY Porphyrins AS A MEASURE OF ARSENIC EXPOSURE IN THE RAT. W.T. Bellamy and D.E. Carter, Dept. of Pharmacology and Toxicology, Coll. of Pharmacy, University of Arizona, Tucson, AZ.

Previous studies on porphyrin excretion by Wood and Fowler (1978) and Martinez (1983) have shown that both As V and As III enhanced the urinary excretion of uroporphyrin and coproporphyrin when given in the drinking water to rats. Gallium arsenide administered intratracheally to rats also showed this increase (Webb 1984). The purpose of this study was to determine if there was a dose related increase in urinary porphyrins after acute As (III) administration. Male Sprague Dawley rats (200-230g) were placed on a special arsenic-free diet for one week and then dosed i.p. with sodium arsenite at 0.1, 1.0, or 10.0 mg As/kg. Control animals were dosed with 0.9% saline. The animals were placed in individual metabolic cages and urine samples collected at 12 and 24 hours. Blood samples were taken at 24 hours and analyzed for arsenic. Urinary porphyrins were separated by hplc and measured with a fluorescence detector. The high dose caused acute lethality. Uroporphyrin was significantly increased over control values (p<0.05) at the 0.1 and 1.0 mg As doses. Coproporphyrin III was significantly elevated in the 1.0 mg As group (p<0.05) but not in the 0.1 mg As group. The increases did not however follow a dose related pattern when compared to the blood arsenic levels. (Supported by NIH GM-07076 and T32 ES 07091.)
INDICATORS OF SYSTEMIC TOXICITY IN THREE ANIMAL SPECIES FOLLOWING ARSENIC III EXPOSURE. R.D. Mitchell, B.J. Snider and D.E. Carter. Dept. of Pharmacology and Toxicology, Col. of Pharmacy, University of Arizona, Tucson, AZ.

In order to assess the comparative toxicity of Arsenic (III), seven species of rodents (Sprague-Dawley rats, B6CF1 mice and Golden Syrian hamsters) were dosed intraperitoneally with sodium arsenite at 0.1 and 1.0 mg/kg. Tissues were collected at time points ranging from 15 minutes to 24 hours and assayed for the following indices: 1) blood: total As, glucose, creatinine and urea nitrogen; 2) kidney: pyruvate dehydrogenase (PDH) activity; and 3) urine: porphyrin excretion.

Though sporadic changes were seen in some of the indicators of toxicity, the only consistent apparently significant differences between saline controls and experimental animals occurred in all three species in blood As levels and kidney PDH activity. PDH activity appears to be significantly depressed at early time points in mice and hamsters and at later time points in rats. In mice only, coproporphyrin and uroporphyrin appeared to be significantly elevated in experimental animals at time points greater than 2 hours. (Supported by OH 02076 and T32 ES-07091.)

INTRANUCLEAR ARSENIC INCLUSIONS: STRUCTURE AND COMPOSITION. H.B. Sorensen. Department of Pharmacology and Toxicology, The University of Texas, Austin, TX.

The arsenic inclusion was first reported to occur in freshwater fish in the nuclei of parenchymal hepatocytes. Inclusions are composed of amorphous subunits which are arranged linearly to form a complex structure which twists and curls throughout the volume of the nucleus. Three-dimensional evaluation using stereomicroscopy revealed subunits of variable size arranged in what appears to be a helical pattern. The inclusion lacks a limiting membrane and is morphologically distinct from the nucleus. Ultrastructural cytochemical studies were conducted using a variety of enzymatic digestion procedures on thin sections. Trypsin, amylase, pepsin, or protease, as well as periodic acid, hydroquinone, hydrochloric acid, and/or ethylenediamine-tetraacetic acid (EDTA) were used with control sections (exposed concurrently to digest solutions lacking the enzymes or other agents). Loss of electron density from the inclusion following digestion was used as an indication of the composition of the inclusion. Exposure to EDTA caused the most pronounced loss of electron density in the inclusion—indicative of metal/metalloid composition of the inclusion. This study corroborates previous X-ray microprobe analysis data which indicates that the inclusion does contain arsenic in a six to one ratio with sulfur. In addition, the preservation and staining of specimens without the use of osmium, lead, or uranium resulted in less dense inclusions. Therefore, the marked electron density characteristic of the intranuclear arsenic inclusion is thought due to the deposition of arsenic, osmium, lead, and/or uranium at the site of the biochemical lesion originally induced by arsenic.


Toxicity of inorganic arsenicals is influenced by methylation, primarily to dimethylarsonic and methylenarsenic acids (DNA and MAA). Uptake and metabolism of arsenic were compared in rat hepatoma-derived (H4A-RH 7777; H4T-EC-3) and kidney epithelium (NRK-52E) cell lines and freshly isolated normal rat hepatocytes. Uptake kinetics were similar for both As (III) and (V) in hepatoma-derived and kidney epithelial cell lines, with greater uptake of As (III). After 8 hr exposure to 1.3 uM As (III) or (V), uptake was 15 X greater for As (III). Similar uptake of As (III) was seen for the NRK-52E cells, but uptake of As (V) was 3 X higher. Hepatocytes and MAA-RH 7777 cells incubated with 0.25 uM As (III) methylated 70% to DMA, while NRK-52E cells methylated less than 20%. No DMA was detected after incubation of hepatocytes with As (V), but up to 10% was methylated to DMA by NRK-52E cells. Such data indicate arsenite can be methylated by hepatoma-derived and kidney epithelial cell lines, as well as by hepatocytes; kidney-derived cells had the least activity. With arsenate, only kidney-derived cells had an even lower rate of methylating activity. DMA was always the major metabolite. (Supported by The University of Alabama, Research Stimulation Award)

DEPENDENCY ON BOTH THE ORGANIC CONSTITUENT AND METAL GROUP FOR DEALKYLATION OF ORGANOMETALTS BY RAT HEPATOCECTY IN PRIMARY CULTURE. D.L. Novick, P. Mushak, W.R. Krigman University of North Carolina, Chapel Hill, NC 27514

Dealkylation of a coherent series of tetraalkyl- and trialkyl- tin and lead compounds were investigated in primary hepatocyte cultures. The hepatocytes were obtained from young adult (200-250gm) male Long Evans rats and the cultures were prepared using a standard collagenase perfusion method. The cultures were allowed to equilibrate overnight (19 h.). Toxicants (Et4Pb, Et3Pb, Me4Pb, Me3Pb, Et4Sn, Et3Sn, Me4Sn, Me3Sn) were added after media replacement. The dealkylation was followed by determining the hydrocarbons present in the atmosphere of cells cultured in stopped tissue culture flasks. Hydrocarbons were identified and quantitated by gas chromatography. The tetraalkyl compounds, irrespective of the metal group, are dealkylated more rapidly than the corresponding trialkyl congener. The lead compounds were dealkylated more rapidly than the corresponding tin compounds. The ethyl compounds are more rapidly dealkylated than methylcompounds. In a mixed diethyl- and dimethyldil, the deethylation reactions are favored over demethylation reactions. Inhibitors of microsomal xenobiotic metabolism also seem to differ in their effects on deethylation and demethylation reactions. (P01 ES01104 and T32 ES07017)
EFFECTS OF TRIMETHYLIN (TMT) ON CELL AND SYNAPTIC FUNCTION IN THE RAT DENTATE GYRUS. D.A. Janiero, P.A. Schwatzkroin and L.G. Costa, Depts. of Neurological Surgery and Environmental Health, Univ. of Washington, Seattle, WA.

The effects of TMT on dentate granule cell (GC) passive properties and synaptic activity have been investigated in hippocampal slices in vitro. Intracellular recordings from GC were obtained before and after 15 min. exposure to TMT (1-10 μM), which increased cell input resistance (R_i) from 34,1+3.6 to 45.6±4.1 and 64.7±14.7 megohms, respectively. This was accompanied by 8-10 mV depolarization and occurred 5-30 min. after TMT exposure. In about half the cells, TMT caused an increased EPSP amplitude; decreased IPSP amplitude was also observed, but developed with longer delays following TMT exposure. Extracellular recordings from the GC layer in response to paired pulse stimulation of the perforant path showed a decrease of the ratio between the amplitudes of the first and the second population spikes (at inter-pulse intervals of 5-9 msec) from 1.8±0.14 to 0.8±0.08, while the amplitude of the test pulse remained unchanged, suggesting that TMT was causing a specific decrease of inhibitory neurotransmission. The field potential effects appeared 2-3 hours after TMT perfusion. We hypothesize that TMT affects cell excitability by increasing R_i and later, by decreasing inhibition. TMT effects in vitro seem to be much less dramatic than those seen in vivo, possibly due to briefer TMT exposure times.


TMT is a potent neurotoxicant but there is little evidence of pronounced systemic effects. In this study female BALB/c mice were administered either 0.9% saline or 2.75 mg TMT/kg ip. The mice were then housed in either room air (RA) or in glass chambers flushed with either 10%, 40% or 100% oxygen and sacrificed at 4, 8, 24 or 48 hr. The adrenals were analyzed for various biogenic amines and S-adenosylmethionine (SAM) by ion-pairing HPLC, and blood ketones determined by UV spectrometry. Tissue sections were evaluated for histopathological changes by EM. With exposure to TMT in RA, adrenal epinephrine (EPI) and norepinephrine (NE) levels were significantly depressed after 8 hr. Supplementation with 40% oxygen did not attenuate this effect but in the case of mice treated with TMT and housed in 100% oxygen for 48 hr actually exacerbated the adrenal NE and EPI depletion. None of the conditions used in this study caused a decrease in adrenal dopamine, 5-HIAA, 5-HT or SAM levels. TMT treatment significantly increased blood ketene bodies indicating additional metabolic dysfunction. These effects of TMT preceded the appearance of both clinical and histopathological changes in the hippocampus by 12-24 hours.


The immunotoxicity of nickel varies with the compound, animal, route and exposure time. NiSO4 was administered until equilibrium occurred to determine threshold responses and to identify the reticuloendothelial cells involved. Female B6C3F1 mice were given NiSO4 in water at 0, 1, 5, or 10 g/L for 180 d. There was no mortality. Decreases in body and organ weights were proportional to dose. Blood nickel was measured at 4-23 wk exposure; equilibrium was reached in 8 wk at 5 g/L, but levels continued to increase for 23 wk at 1 and 10 g/L. Change in immune functions were restricted to a reduction in the in vitro lymphocyte response to LPS, indicating a low degree of immunosuppression. The primary toxic effect of NiSO4 appeared to be in bone marrow stem cells. There were dose-related decreases in bone marrow cellularity and proliferation of CFU-GM and CFU-GM stem cells. Bone marrow cells were harvested; cellularity decreased proportional to dose. Cells were separated on Percoll and frozen.


The effects of subcutaneous magnesium acetate (MgAcet) treatment on the acute toxicity of intraperitoneally injected nickelous acetate (NiAcet) in rats were studied. Male Fischer F344 rats, 150-200 g, received either NiAcet alone, MgAcet alone, NiAcet + MgAcet, or saline. The dose of NiAcet was 115 μmol/kg for the lethality and 95 μmol/kg for all other tests. MgAcet was given in 400 μmol/kg daily doses at -24, 0, and +24 hr relative to NiAcet for the lethality study, or at -24 and 0 hr for all other tests. Treatment with MgAcet increased 14-day survival (p<0.02) of the NiAcet-injected rats and diminished Ni concentrations (24 hr) in kidneys, lung, and liver, but not in blood, spleen, heart, or brain. MgAcet also slightly increased (10% in 0-24 hr) urinary excretion of Ni. MgAcet had no effect on NiAcet-induced hyperglycemia (0-5 hr), or lipid peroxidation in liver and kidneys (24 hr). NiAcet-induced decrease in renal cytochrome P450 content (24 hr) was also unaffected by MgAcet. Neither NiAcet nor MgAcet had any effect on the ATPase activity in heart and brain at 2.5 hr after injection. These results suggest that MgAcet inhibits the lethality of NiAcet by alterations in the pharmacokinetics of nickel(II) and not by direct protection against the adverse biochemical effects of NiAcet described above.
Both nickel and chromium compounds are thought to be human carcinogens. While both agents damage DNA, Ni(II) has been shown to exhibit little or no mutagenic activity in bacterial or mammalian cells in contrast to chromium which is potentially mutagenic. To examine the metal induced aberrations and Sister Chromatid Exchanges (SCE), 10y phase Chinese hamster ovary (CHO) cells were treated with NiCl₂, NIS, and CaCrO₄ and chromosomal aberrations and SCE were examined. NiCl₂ induced a 4 fold increase in aberrations, a large proportion of which were found in the centromeric heterochromatic regions. In addition to inducing the same type of changes, NIS induced excessive aberrations in the heterochromatic arm of the X-chromosome. CaCrO₄ increased aberrations six fold. They were localized in the euchromatic regions of the chromosomes. Two fold increase in SCE level was observed after Ni(III) treatment and 8% of the cells showed multiple exchanges in the heterochromatin of the X-chromosome. In contrast, a 5 fold increase in SCE level was observed in CaCrO₄ treated cells and they were randomly distributed on all the chromosomes. These results suggest that the differences in metal interaction with chromatin may explain their mutagenicity.

CaCrO₄ induces damage and cytotoxicity in mammary link cells. M. Sugiyama and M. Costa. Department of Pharmacology, University of Texas Medical School, Houston, TX and Department of Environmental Medicine, New York Medical School, New York, N.Y.

Compounds containing chromium are potent toxic and carcinogenic agents. However, the role of DNA damage in the cytotoxicity of chromium is not well understood. To examine the relationship between induction of DNA damage and cytotoxicity, asynchronous and synchronous cultured Chinese hamster ovary cells were treated with CaCrO₄ and DNA damage was analyzed by the alkaline elution technique. Colony formation was utilized to assess cytotoxicity in these studies. CaCrO₄ induced DNA-protein crosslinks (DPC) and single strand breaks (SSB). DPC induced by non-toxic dose were rapidly repaired whereas DPC induced by toxic doses remained at the same level or increased between 4 to 24 hr. following the removal of CaCrO₄. Only at very low concentration was repair of DPC. In contrast SSB were rapidly repaired even at highly cytotoxic doses. Cells in early G phase of cell cycle exhibited the greatest sensitivity towards TEM and cytotoxicity compared with cells in other phases of the cell cycle (i.e. G₀, M, and G₂). These results suggest that DPC play a role in the cytotoxicity of CaCrO₄. The slow accumulation and persistence of DPC suggests that this lesion may play an important role in the carcinogenic effects of CaCrO₄.

GOLD INDUCED CHANGES IN COPPER, ZINC AND METALLOTHIONEIN IN RAT TISSUES. K.J. McVety and Z.A. Shaikh. University of Rhode Island, Kingston, R.I.

Nephrotoxicity is an important side effect of antiarthritic gold drugs. The present studies were designed to evaluate the metabolism of gold and its interaction with tissue zinc, copper and metallothionein. Female SD rats were injected with gold sodium thiosulfate (10 mg/kg/day, s.c.) for eight weeks. Hepatic gold levels increased linearly with time to 88 μg/g tissue by week eight whereas renal gold rose to near 300 μg/g by week two and remained at that level throughout the study. Plasma gold increased linearly to 2.5 μg/ml by week eight. No change in hepatic MT was noted but renal MT increased 28-fold over controls by the second week of treatment. No change in plasma MT was seen but urinary MT levels were significantly higher in treated animals after week two. No changes in hepatic zinc or copper were noted. Renal zinc and copper increased 13- and 28-fold respectively by week two. No change in plasma zinc or copper was noted. Since increases in renal copper and zinc levels accompany the accumulation of gold and an increase in renal MT levels, then a possible role of copper or zinc in the mediation of MT synthesis by gold is indicated. (Supported by USPHS Grant No. ES 03187.)
EXCESS COPPER DEPOSITION IN THE LIVER OF ADJUVANT ARTHRITIC RATS FOLLOWING CHRONIC TREATMENT WITH COPPER ASPIRINATE. V. Kishore, and R.F. Ochillo. College of Pharmacy, Xavier University of Louisiana, New Orleans, Louisiana.

While some copper complexes have already been used experimentally in the treatment of arthritic diseases in humans, some others, such as copper-aspirin complex (CA), have been proposed for such use. However, since long-term treatment with copper complexes may result in the accumulation of toxic levels of copper in the body, a study was done to explore such a possibility. Forty male Sprague-Dawley rats (4 groups of 10 each) were induced with adjuvant arthritis (day 1) and treated daily with CA (0, 100, 200, and 400 mg/kg, p.o.) for up to day 22. Animals were sacrificed on day 23 and samples of plasma, liver, heart, brain, bone, kidney, pancreas, stomach, and skeletal muscle were analyzed for total Cu concentration by atomic absorption spectrometry. Of all the tissues analyzed, only liver showed a significant accumulation of copper. Liver Cu concentrations (mean±SE) for 0, 100, 200, and 400 mg/kg groups respectively were found to be 43±6, 112±22, 42±57, and 64±34 mg of Cu/g of dry liver. Results clearly demonstrated that while most tissues were not affected, liver Cu concentration increased by as much as 10 times the normal concentration following 22 days of treatment with CA. Financial support from NIH Grant No. 3506 RR00008-16-91 is gratefully acknowledged.

GOLD SODIUM THIOLOLATE INHIBITS THE RAT KIDNEY ENDOPLASMATIC RETICULUM CALCIUM PUMP. L. Moore. Department of Pharmacology, Uniformed Services University, Bethesda, MD. Sponsor: J.H. Wills

Disruption of cellular calcium regulation by a toxic agent is thought to be an important link between the toxic insult and cell death. Gold sodium thiolulate (GTM) is an antirheumatic agent that produces proximal tubular damage in 5 to 8% of patients that receive it. Administration of GTM (7.5-75 mg/kg ip) to male Sprague-Dawley rats (200-300 g) causes dose-dependent inhibition of the kidney endoplasmic reticulum (ER) calcium pump within 2 hrs (19-84% inhibition). After 24 hrs inhibition does not increase. Another ER enzyme, glucose-6-phosphatase, is not inhibited by this dose range at 2 hrs. Kidney plasma membrane and liver ER calcium pumps are not inhibited by GTM in vitro. However both kidney and liver ER calcium pumps are inhibited by GTM (>10^-7 M) in vitro. Inhibition of the kidney ER calcium pump can be partially reversed in vitro by glutathione, dithiothreitol or mercaptoethanol. These data suggest that GTM may increase cytoplasmic calcium levels in the kidney by inhibiting the renal ER calcium pump. Inhibition may be due in part to oxidation of sulfhydryl group(s) in the pump protein. Because disruption of calcium sequestration by GTM in vivo is selective for renal ER, inhibition of the kidney ER pump may play a role in the organ-specific damage produced by GTM. (NHES Grant E50 3437.)

GOLD-INDUCED POTENTIATION OF ACETAMINOPHEN (AAP) HEPATOTOXICITY IN RATS. B.P. White, A.M. Blacker, G.F. Casale, and R.C. Schnell, Dept Pharmacodynamics-Toxicology, Univ Neb Med Ctr, Omaha, NE 68105.

Experiments were undertaken to examine the effect of gold (thiomolate) on AAP hepatotoxicity in male rats. Pretreatment with gold (100 mg Au/kg, ip) 24 hr prior to AAP (800 mg/kg, ip) potentiated the AAP induced an increase in serum ALT and SDH activities, a decrease in hepatic GSH levels, and an increase in vivo covalent binding of AAP to hepatic macromolecules. Gold treatment did not alter hepatic cytochrome P-450 levels. However, the urinary excretion of AAP and metabolites were decreased over 72 hr in gold treated rats. Excretions of AAP-glucuronide and AAP-mercapturate were decreased over 12 hr and AAP and AAP-sulfate over 48 hr. In the livers of rats receiving gold treatment, the distribution of AAP was significantly increased with the increase occurring in all subcellular fractions. These data support the view that gold potentiates AAP hepatotoxicity probably by decreasing the available GSH levels and increasing the distribution of AAP to the liver. Supported by Burroughs-Wellcome Toxicology Scholar Award (RCS) and Research Starter Grant from Pharmaceutical Manufacturers Association (AMO).

CLEARANCE OF SODIUM ORTHOVANADATE AND VANADIUM PENTOXIDE FROM RAT LUNG AFTER INTRATRACHEAL DEPOSITION. S.J.S. Flora, R.P. Sharma, D.B. Brown and S.G. Oberg. Center for Environmental Toxicology, Utah State University, Logan, UT

Occupational exposure to vanadium pentoxide dust is a known cause of pulmonary problems in industrial workers. However, previous studies have reported a rapid translocation of vanadium pentoxide after direct deposition in lungs. Clearance and tissue distribution of sodium orthovanadate (a soluble form) and vanadium pentoxide (a relatively insoluble form) was investigated in male Sprague-Dawley rats which were injected intratracheally with 500 μg of sodium orthovanadate in 2% dextrose solution and 10 mg of vanadium pentoxide in 0.5% carboxymethylcellulose. The insoluble form of vanadium (vanadium pentoxide) was eliminated from lungs at a slow, but exponential rate, whereas the soluble form was translocated rapidly from this organ and exhibited complex kinetics. Significantly smaller amounts of soluble vanadium were retained in lungs at 7 and 28 days after intratracheal instillation than the insoluble form. In the case of sodium orthovanadate, significantly higher residues were observed in major organs 1 day after the treatment; liver, kidney, spleen and bone exhibiting nearly 4 times the concentration as compared to animals treated with vanadium pentoxide. The results suggest a long-term retention of insoluble forms of vanadium and possible health effects after repeated occupational exposures.
The nonenzymatic oxidation of NADH was studied spectrophotometrically in the presence of three vanadium compounds, sodium orthovanadate, sodium metavanadate, and vanadyl sulphate. At physiological pH (7.4) and in 25 mM sodium phosphate buffer, very small differences were found in the ability to stimulate NADH oxidation by the various forms of vanadium. Addition of the synthetic thiol, dihydroethriitol (DTE, 100 μM) resulted in a marked increase of NADH oxidation in the presence of vanadyl sulphate, and to a lesser degree, in the presence of ortho and meta vanadates. All oxidations were dose dependent with respect to the vanadium compounds. Cysteine and cysteamine substituted for DTE, whereas glutathione did not. In all reactions both the chelating agent EDTA and the superoxide anion scavenger, superoxide dismutase, completely inhibited the vanadium stimulated NADH oxidation. Catalase had an inhibitory effect only on the vanadyl sulphate reaction, and the hydroxyl scavengers mannitol and thiourea inhibited the vanadyl sulphate reaction to a greater extent than that of the vanadates. These results provide a partial explanation for the ability of vanadium compounds to both decrease cellular reducing equivalents and to promote lipid peroxidation.


Two macaques inhale trace levels of 54MnCl2 for 30 min and subsequently, the chest, head, and fecal radioactivity were monitored for over a year after exposure. The chest disappearance curve required three exponentials to attain a satisfactory fit (mean half-time of 0.3, 19, and 140 days). Head levels peaked 40 days after acute inhalation (mean half-time of 9.9 days) and remained elevated for over a year (mean half-times of 245 days). The elimination of manganese after acute inhalation through the feces could be described by a function with two exponentials. One had a half-time of less than a day and the second had a half-time of 55 days. A third macaque received a six week continuous exposure through a subcutaneous osmotic pump containing higher levels of 54MnCl2.

After the pumps were removed, manganese cleared the head at a faster rate than after acute inhalation (half-times of 4 and 53 days). Fecal elimination following continuous exposure resembled that following acute inhalation. The long half-times of the manganese in the head probably reflected both slow disappearance from the head and replenishment from other depots, such as the lung. (Supported by ES-01247, ES-01248, AA05188-02.)

HOMEOSTATIC CONTROL OF MANGANESE EXCRETION IN THE NEONATAL RAT. N. Ballatori and J.E. Clarkson. Liver Center, Yale Univ. School of Medicine, New Haven, CT, and Division of Toxicology, Univ. of Rochester School of Medicine, Rochester, NY.

Previous studies have shown that neonatal animals have a greatly diminished capacity to excrete Mn, and were therefore considered to be unable to regulate tissue Mn concentrations. In contrast, the present studies indicate that suckling rats have the capacity to excrete excess Mn at rates comparable to those of adults. Eight to ten day old rats given a tracer dose of 54MnCl2 (carrier free) either via gavage or by ip injection showed little elimination of the 54Mn until the 18-19th day of life, when there was an abrupt increase in the rate of the metal's excretion. However, when Mn was given in doses of 1 and 10 mg/kg, the young animals excreted from 20-70 % of the dose in only 4 days. This enhanced rate of excretion was further accelerated at the 18-19th day of life. Biliary excretion of Mn, the primary route for the elimination of the metal, was only slightly lower in 14-day-old rats when compared to adults, at doses ranging from tracer to 10 mg Mn/kg. For both the 14-day-old and adult rats, an apparent biliary transport maximum was reached at a dose of 10 mg Mn/kg. These studies indicate that the Mn excretory pathways are well developed in the neonatal rat: the avid retention of tracer quantities of Mn by the neonate may be a consequence of the scarcity of this essential trace metal in its diet.

EFFECT OF PLETTRAN ON CALCIUM PUMP ACTIVITY IN RAT HEART SARCOPLASMIC RETICULUM. Kabeer I. Ahmadd Sabih and Duriska defisiah. Dept. of Neurology, Univ. Miss. Med. Ctr., Jackson, MS. 39216

The effects of Plettran (P), ap protein inhibitor, on Ca2+ uptake and Ca2+ATPase were studied in vitro and in vivo in rat heart sarcoplasmic reticulum (SR). Heart SR was isolated by differential centrifugation as described by Landemman et al. (1983). Uptake of Ca2+ by SR in P administered rats decreased up to 32 and 37% in 30 and 60 mg/kg doses respectively. The Ca2+ATPase also showed a significant decrease in all the dose groups. P inhibition of both these processes was found to be dose dependent. When Ca2+ uptake and Ca2+ATPase were assayed in vitro in the presence of different concentrations of P, a concentration dependent inhibition was observed. Isoproterenol stimulated uptake (14-30%) and Ca2+ATPase (15-35%) of SR and the stimulation was not concentration dependent. In addition to its effects on basal enzyme, P also inhibited isoproterenol stimulated uptake (53-90%) and Ca2+ATPase (35-65%) at all the concentrations tested. Since cardiac relaxation mediated by β-adrenergic stimulation is regulated by Ca2+ uptake by SR, the inhibition of Ca2+pump by P may result in alterations in cardiac Ca2+ fluxes leading to cardiac dysfunction.
INHIBITION OF METABOLIC COOPERATION BY METALS.
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Several metals were tested for their ability to inhibit metabolic cooperation in cell cultures. Metabolic cooperation is the sharing of low molecular weight compounds among cells joined by directly communicating junctions. To monitor inhibition of metabolic cooperation, we cocultured wild-type Chinese hamster V79 cells that were proficient in metabolism of 6-thioguanine (6TG) with mutant V79 cells that were unable to metabolize 6TG to its toxic metabolite. In the presence of 6TG, inhibition of metabolic cooperation was observed as increased recovery of mutant cells compared to background levels in untreated cocultures. The toxic metal compounds arsenic acid, mercuric chloride, lead acetate and nickelous chloride significantly (p<0.05) increased the recovery of mutant cells at concentrations that did not alter cell viability. Cadmium chloride increased mutant cell recovery only at toxic concentrations. Aluminum sulfate and zinc sulfate did not significantly increase mutant cell recovery. These data demonstrate that some metal compounds inhibit metabolic cooperation at otherwise nontoxic concentrations, and suggest that inhibition of junctional communication may be a mechanism underlying the toxic effects of some metals. Supported by NIOSH grant #1 K01 OH00024-02 and NCI grant CA 21104.

DISTRIBUTION AND BIOCHEMICAL EFFECTS OF ALUMINUM CHLORIDE FOLLOWING IP ADMINISTRATION IN RATS.
K.H. Jeffery, H.T. Jansen, and J.A. Bellinger.
University of Illinois, Department of Veterinary Biosciences, Urbana, IL

The effects of aluminum chloride (AlCl₃) on hepatic and renal subcellular distribution, hepatic microsomal lipid peroxidation and cytochrome P450 dependent metabolism of ethylmorphine and p-nitrophenol were examined. Six male rats weighing between 225 and 250 g were injected once daily with 0 or 10 mg/kg in saline. Animals were killed, livers and kidneys were removed and homogenized to 5% in cold isotonic KCl for centrifugal separation of cellular fractions. Tissue samples of the treated animals contained approximately five times greater aluminum than controls. Aluminum was concentrated in the cytosolic fractions of both liver and kidney. The kidney contained approximately ten times more aluminum (per mg protein) than the liver in both control and treated animals. Cytosol was fractionated on a Sephadex G-75 column and aluminum eluted at about twice the void volume. These results indicate that aluminum may bind to a metallothionein-like protein. Levels of P450 and ethylmorphine N-demethylase were depressed in treated animals by 24 and 30% respectively. p-Nitrophenol O-deethylase was not affected. Lipid peroxidation was reduced by 43% in microsomes from aluminum treated rats.

DIFFERENTIAL REACTIVITIES OF Pd(II), Pt(II) AND Rh(III) WITH LIVER ELECTRON TRANSPORT SYSTEMS.
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Institute for Environmental Studies and Department of Biochemistry, Louisiana State University, Baton Rouge, LA. Sponsor: C.R. Short

Platinum group metals, used widely as industrial catalysts are known nephro- and hepatotoxins. Their unique redox chemistry prompted our studies of the relative reactivities of Pd(II), Pt(II) and Rh(III) with redox dependent liver microsomal and mitochondrial electron transfer systems (etc). In microsomal etc, Pd(II) directly oxidized NAD(P)H and mediated the reduction of cytochrome c in a reaction that was superoxide dependent. Pd(II) mediated NAD(P)H-dependent reduction of cytochrome c even in the absence of microsomes. Kinetic studies suggest that Pd(II) is reduced preferentially by NADPH over cytochrome P-450 reductase in microsomal etc. Thus, Pd(II) could serve to shunt electrons from cytochrome P-450 in microsomal etc, Pt(II), which did not mediate NADPH-dependent cytochrome c reduction, was a potent inhibitor of microsomal etc. Pt(II) and Pd(II) were both potent inhibitors of heavy beef heart mitochondrial etc. Rh(III) in contrast to Pt(II) and Pd(II) did not alter microsomal or mitochondrial etc. The differential redox effects of these metals may be important with respect to their relative toxicity mechanisms. Supported by LSU Center for Energy Studies.
Selenium has been shown to be essential to health in warm-blooded animals, and to cause deleterious effects in excessive doses. An early report showed that hepatic glycogen levels in the fed rat were reduced following the administration of multiple doses of less than 0.5 mg selenium over 3-7 days. The purpose of this investigation was to determine the effect of selenium on total hepatic glycogen content in fasted animals. Male albino Sprague-Dawley derived rats (175-200 g) received sodium selenite (Na2SeO3) in doses of 0.1, 0.15, and 0.2 mg Se/kg via the intraperitoneal route. Controls received normal saline. Animals were fasted for 24 hrs prior to experimentation. Forty-eight hours following treatment animals were sacrificed, livers rapidly excised and frozen in a tissue clamp which had been previously cooled in liquid nitrogen. The glycogen contained in the resulting powder was converted by enzymatic hydrolysis to glucose which was then estimated spectrophotometrically. Results indicated that selenium in doses of 0.1, 0.15, and 0.2 mg/kg increased total hepatic glycogen content to 176, 293, and 247%, respectively of control values. Thus, selenium appears to promote enhanced glycogen storage in fasted animals. (Supported by NIH grant RR 08111)

The testes are a target of cadmium carcinogenesis despite very low levels of cadmium localized in this organ. An absence of metallothionein (MT), a protein normally associated with cadmium tolerance, may explain this susceptibility. This study was performed to clarify the nature of the low-Mt-metal-binding proteins in rat testes. Control testes were compared to livers of zinc-treated (1.0 mmol/kg, sc, 24 hr prior to isolation) rats, the latter which is known to have high MT levels. Gel-filtration of testicular and hepatic cytosol revealed major zinc-binding proteins of about 10,000 M, that could also bind cadmium and could be partially purified from either source by heat-treatment and sequential acetone precipitation. Such extracts were further separated by reverse phase HPLC, and two major forms with similar retention times were seen in each organ. Amino acid analysis of proteins derived by HPLC confirmed the presence of Mt in the liver on the basis of Cys content (> 27%). However, the two testicular forms were notably different from MT, being distinctly lower in Cys content (< 2.0%). Admixture of testicular and hepatic preparations prior to elution by HPLC resulted in separation of the second forms. These results further suggest that the low-Mt, cadmium-, zinc-binding protein in the rat testes is not MT.

Persistent granulomatous masses were seen at the site of the ear tag in 39 of 250 male Wistar rats 100 weeks after tagging. This was an unexpected finding in an on-going bioassay. Microscopically, these lesions varied from initial thickening and mineralization of cartilage adjacent to the tag with mild to severe dermatitis and epidermal hyperplasia to areas of metaplastic lamellar ossification with inflammation and ulceration. Finally, invasive osteosarcomas developed consisting of scattered areas of osteoid ground substance and bone, multinucleated giant cells and pleomorphic mononuclear round to spindle-shaped cells. The first such tumor was seen 79 weeks into the study and no lesions have yet become evident in the contralateral pinna. Metal analysis of tags from tumor-bearing rats averaged 59.6% Ni, 39.4% Cu, 0.5% Mn, 0.4% Fe and 0.1% Cr with traces of Zn and Cd but no detectable Pb or As. Internal prostheses made of nickel alloys have been reported to induce local malignancies both in humans and animals (J. Bone Joint Surg. 1984) 66B, 625) and the present observations provide further evidence pertaining to this carcinogenic hazard posed by implantation of nickel-containing alloys.

In isolated hepatocytes cell toxicity, depletion of glutathione and formation of malondialdehyde by several metals were shown (J. Toxicol. Environ. Hlth. 9, 277, 1982). We therefore studied in vivo the acute hepatotoxic effects by measuring serum SH-activities and lipid peroxidation by monitoring ethane exhalation after single sublethal iv doses of CdCl2 (3 mg/kg), NaVO3 (6 mg/kg) and HgCl2 (2 mg/kg) 3 h as well as 24 h after treatment in normal and GSH-depleted mice. GSH-depletion was accomplished by pretreatment with phe- rone (250 mg/kg ip) 2 h before treatment with metals. Both CdCl2 and HgCl2 but not NaVO3 significantly enhanced the SH-activity 24 h after treatment. These increments were further aggravated in GSH-depleted mice. Exhalation of ethane, indicating lipid peroxidation were not at all enhanced after treatment with the 3 metals. We conclude that hepatotoxicity following treatment with CdCl2 and HgCl2 is enhanced by GSH-depletion but seems not to be associated with lipid peroxi- dation; with NaVO3 even no hepatotoxic effects were observed. Our results therefore indicate discrepancies with previous studies using isolated hepatocytes.
Most methods used to quantify metallothionein lack specificity to distinguish isoforms of this protein. As a result, little is known about the induction of MT-I and MT-II. In this study, induction of MT-I and MT-II by Cd and Zn was determined with an HPLC method. Rats were injected with Cd (1-100 umol/kg, sc) or Zn (100-10,000 umol/kg) and 24 hours later, hepatic concentrations of MT-I and MT-II were measured. In controls, only MT-I was detected in liver. Cd increased MTs at all dosages such that concentrations of MT-I and MT-II were approximately equal. Lower dosages of Zn (100-1000 umol/kg) increased concentrations of MT-II about 2.5-3 times higher than MT-I, but higher dosages increased both isoforms to similar levels. Time-course experiments indicated that at all times after Cd administration (30 umol/kg), concentrations of MT-I and MT-II were similar. However, with Zn (1000 umol/kg), MT-I and MT-II were similar at 6 hours, but thereafter MT-I was 3 times higher than MT-II. Thus, in liver, Cd induces MT-I and MT-II similarly over a wide range of dosages. However, Zn preferentially increases MT-II at low dosages but increases MT-I and MT-II to similar levels at high dosages. (Supported by USPHS grants ES-01142 and ES-07079 and a Procter and Gamble Fellowship).

CHILDOOD LEAD POISONING AND IMPAIRED RELEASE OF PITUITARY TSH. C.R. Angle, C.A. Husezen, C.M. Moriarity, and M.S. McIntire, Deps. of Pediatrics and of Physiology and Biophysics, Univ of Neb Med Center, Omaha, NE.
The role of the neuroendocrine toxicity of Pb to impaired growth was supported by studies of 2 girls, 7 y.o., pre and post chelation for blood lead (PbHg) 40-70 ug/dl. The following was normal: T3; T4; somatostatin; C; hCG response to L-dopa and insulin; prolactin response to TRH; LH and FSH response to LRF. The TSH response to TRH was below age normal on 6 challenges. This prompted in vitro studies of TSH release by rat pituitary cells, incubated for 2 hours with Pb at 0.1, 1.0, and 100 umol followed by one hour with TRH. In the absence of Pb, TSH release was dose related to TRH. Pb does not interfere with the radioimmuno assay of TSH. There was no dose related inhibition of basal and stimulated TSH beginning at 0.1意味 Pb (0.2意味/dl). Pb inhibition of TRH-stimulated release of TSH related to the kinetics of 45Ca in pituitary slices. Incubation x 1h with 1意味 Pb impaired the mass (45 to 180 min) efflux of 45Ca. The decrease in the size and exchangeability of the most tightly bound pool of intracellular Ca was similar to the effect of lead on hepatocytes. The pituitary cell culture system provides a model for the decreased TSH release of lead poisoning and supports the biologic plausibility of a neuroendocrine effect on growth, and indicates Pb/Ca interaction as basic to the inhibitory mechanism.

EFFECT OF ZINC ON ACETAMINOPHEN BIOTRANSFORMATION IN MALE RATS. A.M. Pour, M.M. Davies, A. Blecker, S.A. Weir, and R.C. Schnell, Dept. Pharmacodynamics-Toxicology, Univ Neb Med Ctr, Omaha, NE 68105.

Previous studies have shown that acute pretreatment with zinc (acetate) can ameliorate acetaminophen (AAP) hepatotoxicity in rats. These studies were undertaken to examine the effect of zinc on AAP biotransformation as a possible mechanism for its protective effect. In animals pretreated with zinc (6 mg Zn/kg, ip) at 48 and 24 hr prior, hepatic cytochrome P-450 content was reduced by 28%. Urinary excretion of AAP metabolities over 72 hr was increased by 25% in Zn treated rats, with the excretions of AAP, AAP-glucuronide, and AAP-sulfate being significantly increased, with that of AAP-glutathione being decreased, and that of AAP-mercaptopurate being unchanged in Zn treated animals. Bilary excretion of AAP metabolities over 4 hr was decreased in Zn-treated rats (18.6% vs 25.8%) while the pattern of metabolites was unchanged. Urinary excretion quantity and pattern were the same as before. Plasma decline of AAP in Zn-treated rats was increased with half life in Zn-treated rats 120 min vs 154 min in controls. These studies provide evidence that Zn may protect against AAP hepatotoxicity by altering both Phase I and II biotransformation of AAP. Supported by Burroughs-Wellcome Toxicology Scholar Award.


Since oxygen radicals appear to be important in ischemic, toxic and immunologic models of acute renal failure, we sought to characterize the response of isolated renal proximal tubule segments (PTS) to reoxygenation following a 30 minute hypoxic period or to graded oxidative stress with tert-butyl hydroperoxide (TBH, 1.2 or 4.8 mM). Hypoxia or TBH treatment evoked different injury patterns measured as lipid peroxidation [malondialdehyde formation], total glutathione, respiratory rates and tubule potassium, calcium and ATP levels. Exposure to TBH caused an immediate increase in lipid peroxidation which was coincidental with a loss of cellular glutathione and an imbalance in cellular electrolytes. After a delay of 60-120 minutes there was a significant supression of mitochondrial respiration and a decrease in energy production (ATP). During reoxygenation following hypoxia, there was only a small increase in lipid peroxidation and a decrease in cellular glutathione and potassium. During hypoxia the most dramatic changes were the immediate increase in intracellular calcium and the disruption of mitochondrial respiration and energy production (ATP). Thus the mechanisms of cellular injury following hypoxia/ reoxygenation or TBH appear to be different.
Endogenous protective mechanisms against free radical-induced injury include the enzymes superoxide dismutase, catalase and glutathione peroxidase and endogenous antioxidants such as glutathione. Using TBH to mediate free radical injury in Pts we have shown that this chemical induces lipid peroxidation (LP), depletes cellular glutathione and disrupts cellular respiration and electrolyte balance. In an attempt to identify the biochemical mechanism of TBH-induced injury we have pretreated our Pts suspension with antioxidants and sulfhydryl protective agents. The antioxidants, diphenyl phenylene diamine (20 μM) and butylated hydroxytoluene (200 μM) did not prevent the initial burst of TBH-induced LP (moleons malondialdehyde/mg protein) but did reduce the level of LP 30% and 120% after addition of TBH. TBH induced injury was only partially prevented by these antioxidants. Pretreatment of Pts with the sulfhydryl protective agent thioerythritol (2 mM) resulted in only a small reduction in TBH-induced lipid peroxidation and a greater improvement in the tubule viability parameters measured. These studies demonstrate that the mechanism of TBH-induced Pts injury involves both lipid peroxidation and inactivation of proteins with essential sulfhydryl groups.


A rat monoclonal antibody, 411-52B, was developed that binds specifically to fully mature, terminally differentiated murine type I pneumocytes. The 411-52B antigen is membrane associated, cryoprotein sensitive, and is not expressed in the lungs of newborn mice until 21 days after birth. MoAb 411-52B was used to monitor changes in the respiratory epithelium of BALB/c mice in a chemically induced model of diffuse interstitial pulmonary fibrosis. Immunoperoxidase staining with 411-52B indicates that a single 400 mg/kg intraperitoneal injection of butylated hydroxytoluene (BHT) destroys essentially 100% of type I cells throughout the lung. Mice receiving 70% oxygen for 6 days following BHT develop a diffuse interstitial pulmonary fibrosis whereas mice placed in air following BHT repair the initial lesion within 3 to 4 weeks. Staining of type I cells with 411-52B returns in lungs of mice receiving air beginning 21 days following BHT Injection while fibrotic lungs fail to stain with the MoAb. This supports the hypothesis that diffuse interstitial fibrosis stems from an inability to repair the respiratory epithelium following primary lung damage. (Research sponsored jointly by NII Grant No. CA 7433 and the ONER, USDOE, under contract DE-AC05- 840R14400 with the Martin Marietta Energy Systems, Inc.)


Previously, we have observed that the interaction of particulate with alveolar macrophages (AMs) stimulates these cells to produce and release a factor which is chemotactic for both AMs and polymorphonuclear leukocytes (PMNs). The purpose of this study was to evaluate the interaction mechanism by which diesel particulate (DP) interacts with AMs to produce this response. In particular, we used the effect of artificial surfactant on the phagocytosis of DP as a tool to study what effect changes in phagocytosis in vitro. The effect of surfactant on phagocytosis was assessed by measuring the uptake of labeled particulate and was found to increase 4-5 fold in the presence of artificial surfactant. Chemotaxis was assessed using a modification of the Boyden method with and without surfactant. Surfactant had no effect on AM chemotaxis either alone or in combination with DP. However, surfactant did increase AM chemotaxis in response to DP incubated with AMs. These results suggest that surfactant enhances the phagocytosis of DP and it is this increase in phagocytosis that stimulates the production and release of chemotactic factors by AMs. Therefore, phagocytosis may be part of the mechanism by which inhaled particulate interacts with AMs to recruit additional AMs and PMNs into the lung.
PNEUMOTOXIC EFFECTS OF METHYLCYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT). D. N. Cox and G. J. Treiger. Dept. of Pharm. and Tox., Univ. of Kansas, Lawrence, KS 66045

Removal of experimental animals with MMT suggest that lung injury is mediated in part by the sympathetic nervous system (Toxicological 5, 135, 1985). The present studies were done to determine if the sympathetic nervous system plays a role in the pneumotoxicity and mortality of MMT when dosed by ip injection. Systemic arterial pressure was measured in unanesthetized unrestrained rats catheterized 24 hrs prior to dosing. We report here that systemic arterial pressure was unchanged during the 10 hr period following a lethal dose of MMT (63.1 mg/kg). Adrenalectomy or phenoxbenzamine pretreatment failed to protect against MMT-induced lethality. Spontaneously hypertensive rats were not more susceptible to MMT than normotensive controls as judged by their respective LD50 values. Experiments designed to investigate changes in pulmonary capillary endothelial cell integrity demonstrated a significant decrease in the pulmonary content of angiotensin converting enzyme. Our data suggests that MMT mortality resulting from ip dosing is not associated with a marked increase in systemic arterial pressure, but may instead be due to changes in capillary integrity and permeability of the lung vasculature. (Supp. by Ethyl Corp).

DNA STRAND BREAKS IN CULTURED PULMONARY ENDOTHELIAL CELLS INDUCED BY REACTIVE OXYGEN SPECIES. R. L. Thies and A. F. A. Duxbury. UBC Pulmonary Res. Lab., Dept. of Pathology, University of British Columbia, Vancouver, B.C., V6T 1W6

Confluent endothelial cell monolayers (EC) derived from bovine pulmonary arterioles, were exposed to reactive oxygen species generated from oxidizing dithionite (DHF). DNA strand breaks were assessed by a DNA unwinding technique in which increased strand breakage is represented by a decrease in percentage of double stranded DNA (% DS-DNA). EC's incubated 30 min with DHF showed a concentration-dependent change in % DS-DNA; 400 µg/ml DHF resulted in 46% DS-DNA, compared with 96% DS-DNA for untreated control EC's. EC's incubated with 400 µg/ml DHF, plus either 38 U/ml superoxide dismutase (SOD), 600 U/ml SOD, 100 U/ml catalase (CAT), 1.5 mM deferoxamine (DFX) or 5 µM 3-aminobenzamide (3AB) resulted in 94, 92, 94, 87 and 46% DS-DNA respectively. These data suggest that O2-, H2O2 and H2O2 generated from DHF induced intracellular DNA strand breaks which can be prevented by SOD, CAT or the iron chelator DFX, but not by the poly(ADP-ribose) synthetase inhibitor 3AB.

BCNU-INDUCED TOXICITY IN ISOLATED PULMONARY TYPE II CELLS. A.C. Smith, Y. Nakagawa, D.J. Reed and J.B. McMahon. Lab. Exp. Ther. & Metab. NCI, Bethesda, MD.

BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) is a potent anticancer drug and pulmonary toxin. BCNU given at pulmonary toxic doses inhibits lung GSSG reductase and alters lung GSH redox status. Histopathological studies indicate that BCNU is toxic to pulmonary Type II cells in vivo. Isolated Type II cells were used to study the toxicity of BCNU in vitro and to determine the effects of BCNU on Type II cell antioxidant defense mechanisms. The purity of lung Type II cell cultures, isolated from F344 rats, was assessed by rabbit anti-rat surfactant apoprotein antibodies and confirmed by electron microscopy. BCNU (50 µM) inhibits Type II cell GSSG reductase by 90% one hour after BCNU addition. The inhibition of GSSG reductase results in a 46% decrease in cellular GSH levels four hours after BCNU. The alteration of GSSG reductase activity and cellular GSH levels is accompanied by a decrease in fatty acid synthesis, a marker for BCNU-induced lung toxicity, by 54% and an increase in cellular LDH leakage by 100%. These data demonstrate that BCNU is toxic to isolated lung Type II cells. The changes caused by BCNU in isolated Type II cells mirrored the changes seen in the lung in vivo after BCNU administration.

DEVELOPMENT OF INTERSTITIAL FIBROSIS IN PERIPHERAL LUNG ORGAN CULTURES. A COMPARISON BETWEEN MULTIPLE TEST ARTICLES AND TISSUE FROM DIFFERENT SPECIES. M. E. Placke and G. L. Fisher. Battelle, Columbus, OH

Peripheral transverse lung explants derived from mature F344 rats, exposed in vitro, via the airways to respirable fractions of asbestos, silica, quartz, glassbeads, bleomycin, paracetamol or media alone were cultured for up to four weeks. In addition, distal lung tissue of hamster, bovine, or human origin was exposed to asbestos and cultured. Tissue samples were removed at weekly intervals and examined microscopically or total collagen was estimated by determination of hydroxyproline content. Asbestos, silica, bleomycin and paracetamol each caused a sequela of parenchymal lesions, which varied in severity and distribution according to the test substance and species of organ, but each culminated in the development of pulmonary interstitial fibrosis. Tissue damage typically began with early degeneration of pneumocytes, and septal thickening followed by fibroplasia, focal pneumocyte hyperplasia and progressive deposition of histologically reactive collagen. These changes were both compound and dose related. Collagen content was elevated in tissue exposed to higher concentrations of each fibrotic substance, but there was no dose relationship. Tissues exposed to glassbeads or media alone were histologically and biochemically normal. Supported by Corporate Technical Development, Battelle Memorial Inst.
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Rabbit antibodies to the phenobarbital (PB) inducible rat liver cytochrome P450 isozymes (P450b & c) and to the 3-methylcholanthrene (MC) inducing isozyme (P450c) were utilized to examine these forms in rat lungs. Western blots of lung microsomes demonstrated that 100 pmol P450b/mg protein (and no detectable P450c) was present in untreated lungs and that pretreatment with either PB or MC caused no significant changes in expression of these isozymes. Microsomes from control and PB treated lungs contained no detectable P450c (<1 pmol/mg), but MC induced 450c to 120 pmol/mg. Immunocytochemistry (peroxidase-antiperoxidase staining) was used to demonstrate immunoreactivity (IR) to these isozymes in specific cell types. Neither isozyme was detectable in endothelial cells from control or PB treated lungs, but MC induced P450c in pulmonary endothelial cells. Mast-like cells were identified by metachromatic staining. In control, MC, or PB treated rats, type I alveolar cells showed distinct IR to P450c, but not to P450c. In control, MC, and PB treated lungs individual Clara cells stained for either P450c or P450c and double staining of MC treated lungs demonstrated co-localization. Unexpectedly, a consistent increase in Clara cell IR to P450c was not observed after MC induction. In summary, P450c is highly inducible by MC in rat lung, as detected by microscopy (Western blot) and specifically in endothelial cells (by immunocytochemistry). P450c is present in mast-like cells from control, MC, or PB treated lungs. 2) P450c is detectable in rat lung microsomes, and IR to P450c is localized in alveolar type II, Clara, and mast-like cells. 3) This is the first report of P450c in pulmonary mast cells.

**1093**

**SPEZIES DIFFERENCES IN RELEASE OF MEDIATORS OF INFLAMMATION IN RESPONSE TO INHALED DIESEL EXHAUST.** R.F. Henderson, M.W. Leung, A.G. Harns, and R.O. McClean. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

In lifespan studies in CO-1 mice and F344/Crl rats, inhaled diluted diesel exhaust was highly fibrogenic in rats but not in mice. This was despite the higher lung burden in mg. soot/g lung achieved in mice compared to rats. We tested the hypothesis that the greater fibrogenicity of the soot in rats was due to greater release of mediators of inflammation from alveolar phagocytic cells in rats compared to mice. Female F344/N rats and B6C3F1 mice were exposed for up to 17 days to diluted diesel exhaust containing 3.5 mg/m3 of soot. Bronchoalveolar lavage fluid was analyzed for LTb2, LTC4, PGE2, PGF2α, and TxB2 after 2, 12, and 17 days of exposure. Control rats had - the same amounts of PGE2, PGF2α, and LTC4/g lung in airway washings as control mice, but higher levels of TxB2 (16-fold), and LTb2 (6-fold) than control mice. Exposed rats had fivefold increases in PGF2α and twofold increases in LTb2. These effects were not observed in mice exposed to diesel exhaust.

**1094**

**EFFECTS OF OZONE ON THE BIOSYNTHESIS OF ANTI-OXIDANT ENZYMES IN ESCHERICHIA COLI.** C. Whitaker and H. M. Hassan. North Carolina State University, Raleigh, NC. Sponsor: E. Hodgson.

Oxidative damage has been implicated in ozone (O3) toxicity and in other oxidant stress. In this study, we investigated the effects of O3 on the biosynthesis of the antioxidant enzymes, catalase and superoxide dismutase (SOD), in Escherichia coli to determine their role in the defense against ozone toxicity. Inhibition of growth was observed in cultures exposed directly to ozone as well as in cultures inoculated into media which had been pre-exposed to ozone. Results also showed an induction of CAT and SOD in cultures exposed to ozone. Cessation of O3 exposure resulted in one hour of continual high rate of catalase synthesis followed by a gradual decrease in the level of the enzyme approaching that of the unexposed cultures. This phenomenon may be explained by the fact that upon removal from ozone exposure the cells, no longer stressed, decreased their accelerated rate of biosynthesis of these enzymes, while continuation of growth resulted in the induction of pre-existing levels of the enzyme. The increased enzyme levels in response to O3 exposure were shown to be true induction rather than activation of the apo-enzyme. Exposure of pure CAT and copper-zinc SOD to O3 resulted in nearly complete inactivation of both enzymes.

**1095**

**BINDING OF 1,2-DIBROMOETHANE TO PLASMA MEMBRANE LIPIDS OF RAT LIVERS.** L.W. Chang, A.B. DeAngelo and M.A. Pereira. U.S. Environmental Protection Agency, Health Effects Research Laboratory, Cincinnati, OH.

1,2-Dibromoethane (DBE), a known liver mitogen, is toxic to the kidney, liver and testes in mice and rats. In this study, we investigated the effect of 1,2-DBE on the plasma membranes of rat liver cells. 1,2-DBE-14C was incubated at 0° and 37°C in the absence of additional cofactors with either isolated rat hepatocytes or liver plasma membranes prepared by the two-phase polymer system of Bunsen and Tull (J. Membr. Biol., 5:215-224, 1971). Results demonstrated the binding of 1,2-DBE-14C to diacylglycerol (DG) and triacylglycerol (TG) but not to phospholipids (PL) at both temperatures. When mixed with individual lipids, 1,2-DBE-14C bound to DG and TG containing only unsaturated fatty acids (FA) such as oleic and linoleic acids but not to DG and TG containing saturated FA such as palmitic and stearic acid; nor did DBE bind to PL such as phosphatidylcholine and phosphatidylethanolamine. Tiacylglycerol has been implicated as a signal in the stimulation of mitogenesis in liver. These results suggested that 1,2-DBE might exhibit a direct effect by binding to DG and TG in liver plasma membranes resulting in either a toxic and/or a mitogenic response. The abstract does not necessarily reflect EPA policy.
A variety of the title compounds were synthesized by the reaction of thiols with 3-bromo-1,1,1-trifluoropropan-2-one. The resulting compounds were potent inhibitors of mammalian microsomal esterases hydrolyzing a variety of substrates including diethyl succinate, clofibric acid, 4-nitrophenyl acetate, and malathion. Differences in inhibitory potency were observed depending upon the tissue, species, and substrate used. 3-Hominythylidene-1,1,1-trifluoropropan-2-one acted as a synergist for malathion when both compounds were administered IP. When attached to a solid matrix, the compounds served as effective affinity ligands allowing one-step purification to apparent homogeneity of the insect enzymes metabolizing juvenile hormones. In some cases quantitative yields were obtained with purification factors of over 1000 X. This class of compounds promises to be valuable for the characterization and affinity purification of a variety of enzymes of toxicological significance. This work was supported in part by NIH grant GM-EE002710-05.


We have previously reported that chloroform is a potent stimulator of rat hepatic ornithine decarboxylase activity. To examine more thoroughly this effect we have investigated a number of biochemical and pharmacological manipulations on this stimulatory phenomenon. Attempts to duplicate this activity in vitro using primary hepatocyte cultures and partially purified enzyme preparations have been largely unsuccessful. The following studies have been conducted: In Vivo, the following treatments resulted in ODC activity (pmole 14C/mg protein/30 min): Control (1.09 + 0.32); Reserpine (2.53 + 0.92); Reserpine + Chloroform (7.817 + 14.46); partial Hepatectomy (PH) (15.02 + 1.42); PH + Chloroform (52.11 + 5.86); Propranolol + PH (19.70 + 4.82); Propranolol + PH + Chloroform (27.66 + 14.17); Diethylmaleate (DEM) (1.55 + 0.28); DEM + Chloroform (130.87 + 15.02); DEM (Females) (1.35 + 0.17); DEM + Chloroform (Females) (160.70 + 13.51); and Chloroform (Males) (63.25 + 20.63). Studies are currently in progress employing additional manipulations to better understand this effect. This abstract does not necessarily reflect EPA policy.

A NEW PROCEDURE FOR THE AFFINITY PURIFICATION OF CYTOSOLIC EPOXIDE HYDROLASE FROM MOUSE LIVER. R.M. Wixtron, M.N. Silva and R.D. Hannoch. Depts. of Entomology & Environmental Toxicology, University of California, Davis, CA.

Epoxy hydrolase constitutes approximately one percent of total cytosolic protein in mouse liver. It appears to play an important role in the metabolism of lipid epoxides. We have developed an improved affinity column technique for purifying cytosolic epoxy hydrolase (EHy) from normal and clotfibrate-induced mouse liver. This one-step procedure uses benzyl mercaptan coupled to epoxy-activated Sepharose CL-6P for the selective adsorption of the enzyme and 4-fluorocatechol oxide (a EHy inhibitor) for its selective elution. The EHy from clotfibrate-induced mouse liver was purified 110-fold, appeared to be >99% pure by laser densitometry scanning of SDS polyacrylamide gels, exhibited a specific activity of 1400 pmol/min/mg protein, and was obtained with 75-100% recovery of enzyme activity. As compared with a previously published affinity procedure ([1980], the present method offers the advantages of less variability, a more pure enzyme preparation, and a commercially available column ligand. This simple, rapid purification technique will facilitate the isolation of EHy in large quantities for further studies of the enzyme's role and mechanism of action.
Affinity purification of cytosolic epoxide hydrolase from human, nonhuman primate, rabbit, and roent liver. M.H. Silva and P.D. Hershock. Dept. of Environ. & Toxicology, University of California, Davis, CA.

Microsomal (mEH) and cytosolic (cEH) epoxide hydrolase from livers of rhesus monkey, baboon, human, rabbit, control and clofibrate induced rats and Balb/c mice showed significant hydrolysis rates in unpurified, subcellular fractions that tested with cis- and trans-stilbene oxide (TSO) as well as benzo[e]pyrene-4,5-oxide (BPO). Hydrolysis rates of TSO, C50, and BPA in cytosol and microsomes of Balb/c mammary gland were also studied. Substrate selectivity was demonstrated by Balb/c mammary gland and liver epoxide hydrolases, but was not observed in other samples.

Cytosol EH, purified by a single step affinity elution from methacrylomethyl 4-thioli-Sepharose with 4-azidocholesterol oxide as eluting compound yielded one main band by SDS-PAGE from liver cytosol of all samples examined or gland of Balb/c mice. The data from this work indicate that specific activities among and within species vary widely when selected substrates for unpurified mEH and cEH were used. The affinity purification procedure, originally developed for mice, can be applied to a wide range of species.

Effect of age, sex, and PCN treatment on digoxin metabolism and toxicity in rats. M. P. Arlotta, A. J. Sonderfan, M. M. McKinney and A. Parkinson. Department of Pharmacology, Toxicology & Therapeutics, Kansas University Medical Center, Kansas City, KS 66103.

The aim of the present study was to investigate whether the mechanism by which pregnenolone-16a-carbonitrile (PCN) protects rats from digoxin toxicity was dependent on the induction of liver microsomal cytochrome P-450 and/or the UDP-glucuronosyl transferase activity toward digoxigenin monodigloxidoxide (UDP-dtG). The age-dependent decline in constitutive cytochrome P-450 levels in female but not male rats resulted in a marked sex difference in the rate of digoxin toxicity. In female rats, the activity of UDP-glucuronosyl transferase toward digoxigenin monodigloxidoxide (UDP-dtG) increased dramatically at puberty in both male and female rats. However, no sex difference in digoxin toxicity was observed in either immature or mature rats. In contrast, cytochrome P-450 activity increased dramatically at puberty in both male and female rats. However, no age differences in digoxin toxicity were observed in rats of either sex. The results indicate that cytochrome P-450 and UDP-glucuronosyl transferase activity toward digoxigenin monodigloxidoxide (UDP-dtG) can be independently regulated in rat liver and that large changes in these microsomal enzymes have no effect on digoxin toxicity. This brings into question the postulated role of these enzymes in the toxicity of digoxin. Supported by NIH grant ES 03765 and the PMA Foundation.

In vitro effects of helenanin and alantolactone on mixed function oxidase activities. D.E. Chapman, D.J. Holbrook, I.M. Hall and S.G. Cheney. Toxicology, Biochemistry, Medicinal Chem. Univ. of North Carolina, Chapel Hill, NC.

The in vitro effects of the sesquiterpene lactones (STL) helenanin (a potential antitumor drug) and alantolactone on mouse hepatic microsomal mixed function oxidase activities were determined. Microsomes, from male BDFJ mice, were prepared from perfused livers by differential centrifugation. Helenanin (1.0 mM) and alantolactone (0.6 mM) inhibited aminopyrine demethylase activities by 73% and 78%, respectively. Aniline hydroxylase activities were inhibited 30% and 32% by helenanin (1.0 mM) and alantolactone (0.6 mM), respectively. Inhibition of enzyme activity was maximal within 10-15 min of STL addition. The STL inhibited aminopyrine demethylase activity noncompetitively and exhibited a mixed inhibition of aniline hydroxylase activity. The STL, at the concentrations used above, did not inhibit MDPH cytochrome P-450 reductase activity and did not convert cytochrome P-450 to cytochrome P-420. Both helenanin and alantolactone exhibit a characteristic type I binding spectra with oxidized hepatic microsomes. These results suggest that inhibition of substrate binding to cytochrome P-450 is a possible basis for the STL inhibition of mixed function oxidase activity in vitro.

Testosterone hydroxylation by the PCN-inducible form of rat liver microsomal cytochrome P-450. A. J. Sonderfan, M. P. Arlotta, D. R. Dutton and A. Parkinson. Department of Pharmacology, Toxicology & Therapeutics, Kansas University Medical Center, Kansas City, KS 66103.

Treatment of adult female rats with pregnenolone-16a-carbonitrile (PCN) caused a 40-fold increase in liver microsomal testosterone 26- and 15s-hydroxylase activity, as determined by HPLC. Liver microsomes from untreated adult female rats catalyzed these pathways at 5-10% of the rate catalyzed by microsomes from immature female, immature male or mature male rats. Treatment of these latter 3 groups of rats with PCN resulted in a 5-fold increase in testosterone 26- and 15s-hydroxylase activity. These data corroborate our recent report (Carcinogenesis 6, 1985, 612-614) that the major PCN-inducible form of cytochrome P-450, P-450s, is constitutively expressed at a much higher level in adult male rats compared to adult females. However, in contrast to our initial proposal (that cytochrome P-450 is selectively expressed in adult male rats), the present results indicate that this sex difference results from the selective repression of cytochrome P-450 in adult female rats, as recently reported by Waxman et al. (Biochemistry 33, 1985, 4404-4417). Whether cytochrome P-450 is solely responsible for catalyzing the 26- and 15s-hydroxylation of testosterone remains to be established. Supported by NIH grant ES 03765 and the PMA Foundation.
INDUCTION OF RAT LIVER CYTOCHROME P-450 ISOZYMES BY SUBSTITUTED POLYCHLORINATED BENZENES AND BIPHENYLS. D. Duffett, S. Li, M. Denommee, B. Leece, S. Safe, and A. Parkinson. Department of Pharmacology, Toxicology and Therapeutics, Kansas University Medical Center, Kansas City, KS 66103; University of Guelph, Ontario, Canada, and Texas A & M University, College Station, TX

A series of substituted pentachlorobenzenes and 4'-substituted 2,3,4,5-tetrachlorobiphenyls were synthesized and examined for their ability to induce various rat liver microsomal cytochrome P-450 isozymes and epoxide hydrolase. The substituent groups were H, Pd, Cl, Br, CH₃O-, and CN-, NO₂-, CF₃-, OH-, CH₃, (CH₃)₃CH₂ and (CH₃)₂C=. In both the polychlorinated benzene and biphenyl series, those analogs substituted with a H-, OH-, CH₃- or CH₃O- group were relatively ineffective inducers. In contrast, those analogs substituted with certain electronegative groups (F-, Cl, Br- I-, NO₂-, CN-, CF₃) were potent inducers of rat liver microsomal cytochromes P-450α, P-450c (particularly in the biphenyl series). The Cl-, Br, I- and CF₃- derivatives in the biphenyl series were the most potent inducers (10 to 11 fold) of cytochrome P-450α identified to date. The effect of substituent structure on the ability of the same polychlorinated benzene and biphenyl analogs to displace TCDU from a high affinity receptor in rat liver cytosol was also determined. Supported by NIH grant ES 03765 and the PMA Foundation.


The yeast, Saccharomyces cerevisiae (S.c.) has cytochrome P-450 (P-450) system(s), and produces 17β-estradiol. This suggests parallel pathways in yeast and mammals for steroid biosynthesis.

A S.c. gene library was screened for plasmids conferring resistance to the P-450 inhibitor ketoconazole, and a structural P-450 gene was isolated. DNA fragments of this gene used as probes of S.c. genomic DNA indicated additional P-450 genes. These genetic bands have been further characterized by their relatedness to the bovine steroidogenic P-450’s G21 and 17α-hydroxylase(s) and the side-chain cleavage enzyme.

The S.c. library was screened using specific DNA probes for the S.c. and bovine genes, and multiple genomic clones were isolated. These clones are now being tested to determine the number of genes isolated. This research demonstrates that multiple P-450 genes exist in yeast and that these genes are related to those of mammals at the level of DNA sequence. Yeast will provide an important model for understanding mammalian P-450 mediated metabolism. We thank Dr. M. Waterman for access to the bovine gene probes. This research was supported in part by a CEEFA grant to JCL and by a Sigma Xi award to TRS.

EVIDENCE THAT HEXACHLOROBENZENE (HCB) INDUCES P-450d (RAT3) AND P-450c (C57BL/6 MICE) BY A MECHANISM NOT INVOLVING THE AH RECEPTOR. P. Linko, M. Dasiwicz, H.N. Yeowell and J.A. Goldstein, NIH, Research Triangle Park, NC and *University of Rochester, Rochester, NY.

The P-450 isozymes induced by HCB in rats and mice were characterized using radiolmmunoassay and immunoblotting. HCB was found to be a mixed-type inducer in female rats. The predominant isozymes in HCB-induced livers were P-450dp450d = P450c. Lower chlorinated benzenes tested induced only P-450b. HCB had little effect on specific binding of 2-TCDD to the cytosolic Ah receptor in vitro by the hydroxyapatite assay (less than decrease at 10⁻⁶ M) indicating weak or negligible binding to the Ah receptor. Using antibodies to rat liver P-450s on Western blots, we also examined the effects of HCB on P-450 isozymes in C57BL/6 mice congeneric except at the Ah allele: homozygous non-responsive (aa), heterozygous responsive (Aa), and homozygous responsive (AA). Induction of a protein recognized by anti-P-450c (P-450) by 3-methylcholanthrene (MC) segregates with the Ah responsive allele. However, induction of the protein recognized by anti-P-450d (P-450) by both HCB and MC was comparable in aa, Aa and AA mice, indicating induction of P-450 on P-450c is mediated by the Ah locus.

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1108 CYTOSKELETAL PERTURBATION INDUCED BY HERBICIDES
2,4-DICHLOROPHENOXACETIC ACID (2,4-D) AND
2,4,5-TRICHLOROPHENOXACETIC ACID (2,4,5-T).
Wande Li, Yinzi Zhao, and I.N. Chou. Department of Microbiology, Boston University School of Medicine, Boston, MA. Sponsor: A.C. Rogers.

Because of their extensive use, 2,4-D and 2,4,5-T are of considerable concern in environmental health. To understand the mechanism of toxicity of these two related agents, we have studied the effects of 2,4-D and 2,4,5-T on cytoskeletal organization, particularly, microtubules (MT) and microfilaments (MF), and the synthesis of cytoskeletal proteins and DNA in mouse 3T3 cells. Exposure of cells to 2,4,5-T (1.25 mM, 20 hr) resulted in the appearance of rope-like MT bundles, onionskin-like MT organization and MF degradation as revealed by fluorescence microscopy. 2,4-D is less effective under the same conditions. Similarly, 2,4,5-T is more potent than 2,4-D in inhibiting DNA synthesis (10% at 0.90 and 2.21 mM respectively). However, the synthesis of cytoskeletal proteins as analyzed by stepwise fractionation, is essentially unaffected by 2,4-D and actually increased by 2,4,5-T (1.25 to 1.86 mm for 20 hr). These results suggest that dramatic alterations in cytoskeletal morphology induced by 2,4-D and 2,4,5-T are probably due to a structural reorganization. Supported by NIH grant ES 03574.

1109 INHIBITION OF GLYCOSAMINOGLYCAN SULFATION AND INDUCTION OF DIFFERENTIATION IN HUMAN HL60 CELLS.
D. Carson, D. DeBord, and C.S. Baxter. Univ. of Cincinnati Coll. of Med., Cincinnati, OH.

Glycosaminoglycans (GAGs) have been suggested to play a role in regulation of cell growth, differentiation and transformation. We therefore chose to investigate GAGs in HL60 human promyelocytic leukemia cells, which have been shown to differentiate into monocytes after treatment with phorbol diesters such as 12-O-tetradecanoylphorbol-13-acetate (TPA). Differentiation was accompanied by morphological changes, non-specific esterase activity, and adherence, assessed 24 hours after treatment. We have recently discovered, however, that TPA caused the production in HL60 cells of undersulfated chondroitin sulfate within only 4 hours. This increased sulfation was both dose and time dependent and was also seen with phorbol-12,13-dibutyrate (PDBu) and dioctanoylglucol (D86), a diacylglycerol of the type proposed to be an endogenous ligand for the high-affinity phorbol diester receptor. The ELD50 of the PDBu dose-effect curve was, furthermore, similar to the K of PDBu binding to the HL60 phorbol diester receptor. These findings suggest that a decrease in chondroitin sulfation may be an initial event in monocytic differentiation in HL60 cells, and is mediated through the phospholipid-dependent protein kinase, which is activated by diacylglycerols, and which has been proposed to partially constitute the phorbol diester receptor. Supported by Grant ES 07073.

1110 PROTECTIVE ROLE OF CELLULAR NON-PROTEIN SULFHYDRLS AGAINST HELENALIN CYTOTOXICITY IN P-388 LYMPHOCYTIC LEUKEMIA CELLS.
G.B. Roberts, D.E. Chapman, I.H. Hall, S.G. Chaney, D.J. Holbrook. Toxicology, Biochemistry, Medicinal Chemistry, Univ. of North Carolina, Chapel Hill, NC.

Studies suggest that the \( \alpha \)-methylene-\( \gamma \)-lactone and the \( \alpha \),\( \beta \)-unsaturated cyclopentenone moieties of helenalin (HL), a potential antitumor drug, react with reduced glutathione and selectively alkylate thiol-containing enzymes and thereby suppress DNA and protein synthesis. In vitro effects of HL on endogenous non-protein sulfhydryl (NPS) levels and on viability (determined by lactate dehydrogenase leakage) of P-388 lymphocytic leukemia cells were examined. One hour of incubation with 640 \( \mu \)M HL reduced P-388 NPS to 60% of control and viability to 53% of control. P-388 cells were treated with either diethylmaleate (DEM) or \( N \)-acetyl-L-cysteine (NAC) to modify intracellular NPS content. One hour of incubation with 500 \( \mu \)M DEM reduced NPS to 62% of control while 2 hours of incubation with 400 \( \mu \)M NAC increased NPS to 214% of control. Treatment with 400 \( \mu \)M NAC prior to HL exposure reduced HL cytotoxicity, while pretreatment with 500 \( \mu \)M DEM enhanced the cytotoxic effects. Thus manipulation of the sulfhydryl content prior to HL treatment demonstrated the protective role of cellular NPS against HL cytotoxicity. (ES 07126, CA 26466).

1111 ENHANCED CYTOTOXICITY OF MISONIDAZOLE IN HYPOXIC TUMOR CELLS.

Misonidazole, a nitroheterocyclic drug, is selectively toxic under hypoxic conditions. Colony-forming assays were used to determine survival of cells treated with Misonidazole, and alkaline elution techniques were used to assess DNA damage. Survival of EMT6 mouse mammary tumor cells was decreased 80% by 2-hr exposure to misonidazole at 5 mM in hypoxia, whereas survival ofoxic cells decreased 30%. Glutathione was depressed more than 60% in cells made hypoxic for 24 hr; furthermore, these cells showed an impaired ability to replenish glutathione. Pretreatment with diethylmaleate (DEM) at 0.1 mM enhanced the cell-killing effects of misonidazole under hypoxic conditions. DEM depleted glutathione to 10% in oxic and hypoxic cells. By 22 hr, the concentration of glutathione had returned to 90% of control levels in DEM-treated oxic cells but remained depressed at 11% in DEM-treated hypoxic cells. Misonidazole produced dose-dependent single-strand breaks in DNA in hypoxic cells but not in oxic cells. Moreover, pretreatment with DEM potentiated this damage. These data indicate that cells in hypoxic environments may be less able to maintain their glutathione levels and therefore be more susceptible to mutagenic agents. (Supported in part by grants CA-274 from the American Cancer Society and CA-36946 from the National Cancer Institute)

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A method for developing a stratified, squamous epithelium in vitro upon cultivating rat keratinocytes on nylon membranes has been described (Vaughan, Gray & Bernstein, 1965, In Vitro: IN PRESS). This epidermal-like culture is being used to obtain a better understanding of mechanism of skin vesication after topical exposure to the sulfur mustard, bis-(beta-chloroethyl) sulfide (BCES). Radiolabeled macromolecular precursors, thymidine, uridine and leucine, were used to study the effect of BCES on the synthesis of DNA, RNA and proteins, respectively, after controlled exposure to concentrations of 50-500 mmoles/cm². From these and subsequent studies it was determined that even at the lowest concentration (50mM/cm²), significant inhibition in the uptake of all three macromolecular precursors persisted 24 h after the initial 30 min exposure and that no recovery of the inhibition was evident. This indicates a very early lesion following exposure to BCES.

EVIDENCE FOR LOW-LEVEL DNA DAMAGE BY ACRYLONITRILE IN VIVO. L. L. Ugoz AND F. P. Guengerich, Departments of Pharmacology and Biochemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, TN 37232

The question of in vivo DNA adduct formation from acrylonitrile has not been resolved yet. We find that administration of acrylonitrile to rats (50 mg/kg ip) causes unscheduled DNA synthesis (UDS) in liver but not brain. Acrylonitrile did not cause increased DNA synthesis in rat liver or brain. When acrylonitrile was infused into perfused rat liver, 2-cyanoethylene oxide was recovered. However, administration of 2-cyano- [2,3-3H]-ethylene oxide to rats (ip, 0.6 mg/kg) did not yield detectable hepatic or brain DNA adducts (>1/10⁷ bases). DNA and RNA from rats treated with acrylonitrile or 2-cyanoethylene oxide were hydrolyzed and analyzed by HPLC; < 1 nM-ethenoadenine derivative was present per 2 x 10⁷ bases in liver. 2-Cyanoethylene oxide also reacts with DNA in vitro to form N²-(2-cyanoethyl)guanine derivatives. We used NaH²H₄, postlabeling method and detected one N²-(2-cyanoethyl)guanine adduct per 3 x 10⁷ bases in hepatic DNA following a single ip administration of acrylonitrile (50 mg/kg) or 2-cyanoethylene oxide (6 mg/kg) to rats; the levels in brain DNA were about 1 adduct/2 x 10⁷ bases after such treatments. The UDS and N²-(2-cyanoethyl) guanine identification results indicate that acrylonitrile has a small but finite ability to form DNA adducts in vivo after metabolic activation.


Alpha-ketoglutaric acid (a-KGA) has been shown to be an effective antagonist against cyanide-induced lethality. The mechanism of this antagonism is hypothesized to be due to binding of a-KGA with cyanide. Various molar ratios of a-KGA:cytadine were injected into a high pressure liquid chromatograph. Cyanide reduced the peak area of a-KGA at a molar ratio of greater than 0.5:1:0. Blood from naive male ICR mice was spiked with a-KGA and cyanide. Headspace above these blood samples were injected into a gas chromatograph and analyzed for the released hydrogen cyanide. a-KGA reduced the quantity of hydrogen cyanide released into the headspace at molar ratios of greater than 0.4:1:0. Inhibition of cytochrome oxidase is an accepted target enzyme for cyanide-induced lethality. The ability of a-KGA to prevent cyanide-induced inhibition of brain cytochrome oxidase (BCYTOX) activity was investigated. The effect of a-KGA on BRDCTOX activity was determined and found to be greater than 0.06M a-KGA resulted in inhibition. It was observed that 10⁻⁵M cyanide completely inhibited BCYTOX activity and that this inhibition was prevented at 0.04M a-KGA. Thus, these data suggest that a-KGA does bind with cyanide and this binding can possibly account for the antagonism by a-KGA of cyanide-induced lethality. (Supported by USAMRC Contract DAMD17-85-L-5766.)


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COMPARISON OF ULTRAVIOLET INDUCED DNA DAMAGE AND REPAIR BY SUNLITET, SUNLIGHT, BOOTH, AND GERMICIDAL LAMP. J. R. Hincks and P. A. Fuellenhe, Jr., Center for Environmental Toxicology, Utah State University, Logan, UT

The use of sunlamp booths by the general public for either health or cosmetic reasons has increased in popularity. Research investigating the possible harmful effects of tanning booths is limited. In this study, DNA single-strand breakage (SSB) and subsequent repair induced by ultraviolet (UV) from sunlight, sunlamp booth, and 254 nm light in the MRCK epithelial cell line were monitored by gravity-flow alkaline elution. The effect of time exposure on DNA SSB was dose-dependent for all 3 systems. The tanning booth required half the time to produce the equivalent DNA SSB compared to sunlight. The proper dose to induce equivalent amount of DNA damage was determined for the 3 light sources and used in the repair study. For sunlight, repair (50%) was rapid over the first 15 min, then slowed until full repair was attained at 3 hr. The tanning rays induced rapid repair (70%) in the first 1 hr to produce complete repair by 2 hr. The repair of DNA SSB due to 254 nm UV light was considerably slower showing slight repair even at 4 hr. Results indicate that DNA damage and repair mechanisms induced by sunlight or tanning rays are similar while the mechanisms underlying 254 nm UV-induced DNA damage and repair are different. Supported in part by USPHS grants ES03591 and ES07097.
1116 DELTA-S-4-TETRAHYDROCANNABINOL ENHANCES HERPES SIMPLEX VIRUS TYPE 2 (HSV2) RELEASE IN VITRO. E.M. Nishkin, P.J. McNerney, and G.A. Cabral. Dept. of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA. Sponsor: A.E. Munson.

This study was undertaken to determine whether Delta-9-tetrahydrocannabinol (Delta-9-THC) modulates HSV2 replication in vitro. Virus-infected Vero cells pre-treated for 24 hr with 10-8 M to 10-6 M Delta-9-THC yielded amounts of total infectious virus similar to vehicle controls. However, 100-fold increases in infectivity extracellular virus occurred with 10-8 M or 10-6 M of drug. Autoradiograms of SDS-PAGE profiles of radiolabelled infected cell homogenates revealed a drug dose-dependent decrease in ICSP9, the virus capsid protein, and ICSP16, the virus major envelope glycoprotein complex. Immunofluorescence with antisera to ICSP16 showed a decrease in cell surface glycoproteins. Scanning and transmission electron microscopy demonstrated cell surface cavitons, plasma membrane dissolution, and pronounced intracytoplasmic membrane distortion and vacuolization in drug-treated cells. These results suggest that Delta-9-THC does not stimulate HSV2 replication. Rather, the drug perturbs cytoplasmic and cell surface membranes, with the consequence of enhanced release of residual intracellular HSV2. This release is accompanied by reduction in virus-specific cell-surface glycoproteins, suggesting that infected cells could be "masked" in the infected host.

1118 INCORPORATION OF ISOTOPES BY STRESS PROTEINS (Sps) AFTER DL-ISOPROTERENOL (IPR) AND SODIUM ARSENITE (NaAsO2) ADMINISTRATION IN SORTED NUCLEI. J.F. Anson, J.L. Pipkin, W.G. Hinson, E.R. Burns* and D.A. Cassiano. National Center for Toxicological Research, Jefferson, AR and *University of Arkansas for Medical Sciences, Little Rock, AR. Sponsor: G.L. Wolff

The incorporation of radioactive leucine and/or phosphate by several polypeptides extracted with imidazole and/or 0.35 M sodium chloride from nuclei sorted physically from the G0+G1 phase of the rat submaxillary gland cell cycle was observed by two-dimensional microscale polyacrylamide gel electrophoresis. IPR, a human therapeutic drug, induced cell cycling and the labeling of several Sps. NaAsO2, an environmental pollutant, had no effect on the cell cycle but did stimulate incorporation by the same Sps in these intact glands. After dosing with IPR, newly labeled Sps ranging in molecular weight from 20 to 130 KDa were observed during the G0+G1 phase as compared with controls. Treatment of animals with NaAsO2 prompted phosphate labeling of two Sps (25 KDa and 130 KDa) in the non-dividing G0 phase of the submaxillary gland. Enhanced incorporation of polypeptides was exhibited after combined dosing of IPR and NaAsO2, but the newly incorporated polypeptides were seen in G0+G1. The identical mobilities of five Sps, as determined by co-electrophoresis of stress marker proteins, suggested that those polypeptides were homologous.

1117 LIPOPROTEIN-MEDIATED UPTAKE AND TRANSPORT OF LIPOPHILIC XENOBIOTICS. D. Busbee, R. Zipirin, J. Norman, R. Wilson, and S. Ragsdale. Division of Toxicology, College of Veterinary Medicine, Texas A&M University, and Veterinary Toxicology and Entomology Research Laboratory, USDA, College Station, TX. Sponsor: T.D. Phillips

The lipoprotein-associated uptake and transport of ingested xenobiotics was investigated using gastrically instilled aflatoxin B1 (AFB1) and benz(a)pyrene (BaP). Cannulae surgically implanted in the thoracic duct were used to collect lymph. AFB1 and BaP concentrations were determined in thoracic duct lymph. Lymph was separated into major components by size exclusion HPLC, showing BaP, but not AFB1, uptake and transport with chylomicron fractions. BaP plasma clearance was coincident with clearance of circulating chylomicrons. Partitioning studies showed that BaP, but not AFB1, was taken up by lymph and plasma lipoproteins (LP), and that BaP uptake correlated with the triglyceride and total lipid concentration of the respective LP. Exteriorization of the thoracic duct lymphatic flow preceded ingested BaP, but not ingested AFB1, entry into the peripheral circulation. BaP internalization into fibroblasts or hepatocytes in vitro was lipoprotein-mediated, while AFB1 was internalized as a function of concentration regardless of the LP content. Supported in part by NIH grant HL31973, Council For Tobacco Research Grant 1449, The Texas Agriculture Experiment Station, and the U.S. Department of Agriculture.


Nuclei were isolated from labeled mouse lymphoma cells, rat submaxillary glands and livers and stained with propidium iodide (PI). They were sorted with a fluorescence activated cell sorter from partitions of the cell cycle, and the nuclear proteins were analyzed by two-dimensional autoradiography using O'Farrell polyacrylamide gels. The incorporation of leucine and/or phosphate of five stress proteins (Sps), ranging in molecular weights from 20 to 130 KDa, from each of the three tissue sources was observed after isoproterenol and/or sodium arsenite administration. An altered light cycle schedule also prompted labeling of several of these Sps. The Sps (mostly labeled in G1) were isolated from gel autoradiographs and their homology was compared with one another. Coelectrophoresis, immunohistochemical blotting and protease V8 peptide mapping confirmed the identical nature of these five Sps. These data imply that there are relatively few Sps, they are ubiquitous in all organisms, they are conserved during evolution and they possess some elemental and essential function. They possibly can serve as universal indicators (bio-markers) for toxic stress.
The phenobarbital (PB) inducible rat hepatic proteins, cytochromes P-450b and P-450e, are extremely homologous members of a complex gene family that play important roles in a variety of bioactivation and detoxication reactions. Employing highly discriminatory synthetic 18-mer DNA hybridization probes, the tissue specific mRNA expression and responses to inducing substances were assessed. The mRNAs for P-450b and P-450e were coordinately inducible by PB in the rat liver, but not in the lung, kidney, or testis. P-450b mRNAs were however expressed constitutively in the lung and testes at approximately 10% and 8%, respectively, of the levels observed in the PB pretreated liver, but were not detected in the kidney. P-450e mRNAs were not detected in the extrahepatic tissues studied, but, unlike P-450b mRNAs, were detected in trace amounts in the uninduced liver. 3-methylcholanthrene did not appear to alter the constitutive expression of any of these mRNAs in any tissue examined. The available data indicate that even closely related P-450 genes can be differentially regulated in a highly tissue specific manner. Supported by NIH grant GM-22281.

Under an interagency agreement between the Agency for Toxic Substances and Disease Registry and the National Toxicology Program (NTP), the NTP is participating in Public Health Service activity related to the Comprehensive Environmental Responses, Compensation and Liability Act (Superfund Act) by conducting toxicity testing on chemicals that were identified in priority hazardous waste sites or otherwise released into the environment for which adequate toxicology data may not be available. As part of this endeavor, a project was initiated on toxicological studies of chemical mixtures of environmental concern, specifically ground water contaminants derived from hazardous waste disposal. Following internal deliberations, a research proposal from the NTP was recently reviewed by an ad hoc committee consisting of 11 scientists representing academic, industrial, governmental and international organizations. As an initial attempt, three areas of research will be pursued: (1) Health effects on drinking water contaminants; (2) Investigation of frequency of occurrence of toxicological interactions; (3) Mechanistic approach to the study of toxicological interactions.

The lack of a consistent and rational public behavior with respect to environmental chemicals is a major problem in our society and has led to inappropriate use of both economic and scientific resources. A significant contributor to this problem is the lack of public understanding of the relative risks that various chemicals pose. Although part of this is due to subjective public estimates of risk acceptability, there are two other important factors. One is the limited knowledge of the basic principles of toxicology. The second is a poor understanding of the risk assessment process and, as a result, of the risk assessment information that is imparted to the public. This paper will illustrate some of the ways that risk assessment information is commonly transmitted and some alternatives that are available. In addition, it will present educational materials which were developed to provide both general toxicology information and specific knowledge about risk assessment. Copies of some of the materials will be available for distribution. Others interested in this topic are encouraged to bring their own ideas and materials to share. It is hoped that this interaction will lead to better methods of communicating risk assessment information to the public and, ultimately, to better public policy.

This study investigates the relationship between the probability of detecting a statistically significant linear trend in the proportion of animals observed to have tumors for the modified pathology protocol currently being employed by The National Toxicology Program (Buff, et. al., 1985). The results indicate a small loss in the probability of detecting a statistically significant result will occur when the modified protocol is used instead of the previous pathology protocol. This loss varies as a function of the design of the study in terms of dose and animal allocation and as a function of the steepness of the dose-response relationship. The absolute difference between the probability of a statistically significant result for the original pathology protocol and that for the modified pathology protocol never exceeded 0.03. This difference was always less than 8% of the probability of a statistically significant result for the original pathology protocol.
ONCODYNAMICS AND RISK ASSESSMENT OF ETHYLENE OXIDE

Two rat inhalation bioassays are integrated into the risk assessment on the carcinogenicity of Ethylene Oxide (EO). The biological basis for the selection of gliomas as the endpoint used as the incidence data is reviewed. The duration of exposure in the rat bioassays is compared to the maximum working lifetime occupational exposure on the basis of percent life span. Three methods for calculating effective dose are used (exposure concentration x time, surface area correction for metabolic rate, and area under the plasma concentration-time curve). Since evidence indicates that EO is a primary carcinogen/mutagen and is rapidly metabolized into nonreactive metabolites, the area under the plasma concentration curve is further explored using allometric calculations for plasma half-time in man based on both rat and dog pharmacokinetic studies. The risk, based on glioma incidence, for working lifetime occupational exposure were estimated for daily exposures of 1.8 µg/l using the three different methods for calculating dose and two mathematical extrapolation models.


The United States Environmental Protection Agency is currently testing a series of chemicals to aid in the development of regulations under the Resource Conservation and Recovery Act of 1976, as authorized by the Hazardous and Solid Waste Amendments of 1984. Chemicals being tested are isobutanol, n-butanol, methanol, methyl isobutyl ketone, o-, m-, and p- cresols, crotonaldehyde, ethyl ether, ethyl acetate, acetone, and 2,3,4,6-tetrachlorophenol. Each chemical is being tested using a standard protocol for 13-week subchronic toxicity studies in rats. When indicated, additional teratology and neurotoxicology studies are being conducted. Preliminary results are presented and discussed. General patterns of toxicity and trends in the data are analyzed and their applicability to the risk assessment process are evaluated.

EPA'S DRINKING WATER HEALTH ADVISORY PROGRAM.

The Office of Drinking Water’s non-regulatory Health Advisory Program provides technical guidance on health effects, analytical methodology and treatment technology that would be useful in dealing with contamination of drinking water. Health Advisories also describe concentrations of contaminants in drinking water at which adverse effects would not be anticipated to occur. A margin of safety is included to protect sensitive members of the population.

The Health Advisories are developed from data describing non-carcinogenic end-points of toxicity. For those chemicals which are known or probable human carcinogens according to the proposed Agency classification scheme, non-zero one-day, ten-day, longer-term Advisories may be developed, with attendant caveats. Advisories for lifetime exposure may not be recommended. Projected excess lifetime cancer risks are provided to give an estimate of the concentrations of the contaminant which may pose a carcinogenic risk to humans.

The risk assessment methodologies used in the development of Health Advisories will be described. Examples of the appropriate application of the Advisories also will be given.


Toxicology in drug development has three basic roles - to ascertain which drugs at which dose levels in which animal species cause toxicity to which organs or systems - to investigate the mechanism of that toxicity in order to allow the risk associated with administration of the drug to man - to design models which assist the selection and design of follow-up compounds. The purpose of such studies is not to avoid toxicity but to define it in terms which allow lower risk dosage and meaningful clinical monitoring in the exposure of patients to the drug. Such information is also essential in the design of follow-up compounds to prevent uneconomic development programmes. Whilst regulatory authorities and the state of scientific knowledge dictate that long term exposure of animals is ultimately necessary for the protection of human subjects, development programmes and early clinical trials necessitate the use of shorter term investigative studies in appropriate models. Examples will show how investigations into species specificity, short-term target organ effects and mechanistic models in vivo and in vitro can not only provide vital information in relation to safety of drug usage in patients but also play a crucial role in an economic drug discovery and development programme.
CHEMICAL SCORING SYSTEM FOR HAZARD ASSESSMENT
T. O'Bryan, R. H. Ross, and P. Y. Lu
Existing Chemicals Assessment Division, U.S.
Environmental Protection Agency and Information
Research and Analysis Section, Oak Ridge National
Laboratory.

To assist in the preliminary evaluation of com-
ounds of toxicological and environmental inter-
est to the U.S. Environmental Protection Agency
(USEPA), a scoring system was devised as a colla-
borative effort between the USEPA and the Oak
Ridge National Laboratory. The scoring system
combines objective guidelines with professional
judgment to evaluate chemicals and consists of
seventeen separate scoring parameters, one of
which pertain directly to toxicology, e.g., oncogen-
icty and genotoxicity. The remaining parameters
are related to environmental fate and occupa-
tional and consumer exposure. This scoring sys-
tem is designed to rapidly score chemicals in a
minimum amount of time with readily available in-
formation. It is used by the Office of Toxic
Substances of the USEPA as a tool to help set
priorities in conjunction with other criteria.
It is particularly useful in performing prelimi-
nary evaluations involving large chemical class-
es, such as petroleum distillates.

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A EUROPEAN TOXICOLOGY DATABASE - THE
ESTABLISHMENT AND POTENTIAL. C E Lumley,
S R Walker, Centre for Medicines Research,
Carshalton, Surrey, ENGLAND. Sponsor: D Conning

Increasing reliance on animal studies for pre-
dicting the safety of medicines in man demands a
reappraisal of conventional animal testing
procedures. A unique database has been estab-
lished containing toxicological information on 122
pharmaceutical compounds studied in one or
more species - rat (102), dog (72), primate (33)
and mouse (5). The data were provided by 21
companies in Europe and include study details,
salient drug-related findings and the time and
dose levels at which each effect occurred.
Major therapeutic classes include cardiovascular
system, central nervous system and anti-infective
agents; 54 of the compounds are marketed, 20 are
under development and research has been
terminated for 48. An analysis of the data from
both short- and long-term studies provided no
evidence to support the need for animal tox-
ico1ogy studies of longer than 6 months apart from
those to detect carcinogenicity. The information
in the database enables the identification of
target organs and the species which is best for
predicting effects in man. Any prospect of
rationalising the number and design of animal
studies must stem from better use and judicious
analyses of the available data on the toxicity
of compounds previously investigated. One
approach must be through the use of such a
toxico1ogy database.

A COMPARATIVE ANALYSIS OF SHORT- AND LONG-TERM
STUDIES OF PHARMACEUTICAL COMPOUNDS. S R Walker,
C E Lumley, Centre for Medicines Research,
Carshalton, Surrey, England. Sponsor: D Conning

Recommendations for the minimum duration of
chronic toxicology studies in animals to support
marketing authorisation of pharmaceuticals vary
from 6 to 18 months, although the value of those
longer than 6 months has not been established. A
comparative analysis of data from short and long-
term toxicity tests has been carried out to de-
determine whether any significant new information
is obtained from studies longer than 6 months.
In 34/38 studies with comparable data at 6 and
12/18 months and in 33/46 studies with data at
only 1/3 months and 12/18 months, all significant
effects were identified within 6 months. In the
remaining 17 studies, any new findings after 6
months did not influence the progression of the
compound to market or termination of research.
In 13/17 of these studies, as 6 month data are
not available it is not possible to conclude
whether the new findings would have been observed
at 6 months. These data do not support the need
for animal toxicity studies of longer than 6
months duration, apart from those to detect car-
cinogenicity. Erroneous conclusions may be drawn
regarding the value of long-term studies if
careful attention is not given to study design.
Rather than prolonging animal studies to deter-
mine toxicity, the emphasis should be on post-
marketing surveillance to determine safety in man.

SELECTION CRITERIA AND PROCEDURES USED TO IDENTI-
FY CHEMICALS AND INFORMATIONAL SOURCES FOR THE
HAZARDOUS SUBSTANCES DATA BANK (HSDB). A.A. Wykes
and V.W. Hudson. Toxicology Information Program,
National Library of Medicine, Bethesda, MD

The Hazardous Substances Data Bank (HSDB) is a
computerized toxicology information resource of
the National Library of Medicine (NLM) designed
to support a variety of scientific communities.
The data bank consists of more than 4,100 records
that offer toxicological abstracts, with 138 data
fields, of technically reviewed scientific infor-
mation relevant to chemicals with hazardous phys-
ical/chemical properties or that possess a poten-
tial or proven toxicity for humans, animals and/
or the environment, with special emphasis upon
chemicals with high production volume, exposure
potential or usage. The identification and selec-
tion of chemicals and literature sources for the
HSDB is a dynamic process. Often newly-identified
chemicals require additional literature/data
sources. Some sources must be continually as-
sessed for their timeliness and utility. To en-
sure maximum usefulness of the HSDB to both public
and private sector users, NLM scientists have
established selection criteria and review pro-
cedures for the identification and prioritization
of chemicals for the HSDB, and for the high qual-
ity tertiary, secondary, and primary literature/data
sources required for each HSDB chemical.
Current attention is focused upon chemicals for
the HSDB regulated under 1980 Comprehensive En-
vironmental Response Compensation and Liability Act.

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PB-PK models offer significant advantages over conventional empirical models for interpreting and extrapolating data, but their increased power is obtained at the cost of an increased number of parameters. Although most parameters are based on physiology or derived from in vitro studies, there are often several which must be estimated by matching model predictions to kinetic data. We have compared numerical techniques for performing this process with a manual method in which an investigator interactively varies model parameters to obtain a visual match between model output and data. Both gradient and direct search techniques were used to minimize weighted least squares objective function. The number of estimated parameters ranged from one to ten, and the PB-PK models and data sets represented a range of complexity. The performance of the numerical methods was often disappointing. When initial parameter estimates based on a manual fit were used, there was little improvement. When other initial estimates were used, they were generally unable to match important features of the data. In this paper we describe our attempts to improve parameter estimation by using objective functions incorporating analysis of data curvature and use of Bayesian priors.

ANTHEMATICAL APPROACHES TO MATHEMATICALLY ANALYZING THE BIODETACHMENT DATA FOR 2,3,7,8-TCDD. T.L. Sielken, R.W. Carling, D.J. Paustenbach, M.P. Shy, and F.J. Murray, Professor of Statistics, Texas A&M University, College Station, TX. 2Statistical Consultant, St. Charles, IL. 3Syntex (U.S.A.), Inc., Palo Alto, CA.

This paper will critically evaluate the approaches currently used by regulatory agencies in assessing the cancer risk of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). These agencies use mathematical models when estimating cancer risk by extrapolating from high doses, where animal tumor data exist, to very low doses, where human exposure is likely to occur. These models are based on the hypothesis that cancer is an expression of a permanent, replicable change in cellular genetics, i.e., an initiator. These models typically assume a linear, non-threshold, irreversible response at very low doses for all chemical carcinogens. Due to the assumptions inherent in the various models, the virtually safe dose (VSD) estimated by these models may vary by 3-4 orders of magnitude. There is no convincing evidence that TCDD possesses initiating activity. The data from mutagenesis assays, DNA binding studies and tumor promotion studies strongly support a non-genotoxic mechanism for TCDD carcinogenesis. Specifically, this paper will discuss the VSDs predicted by various linear, non-threshold models and present arguments that the resulting VSDs are likely to be overly conservative, given what we know about the mechanism of action of TCDD. We will discuss alternative and more justifiable methods for analyzing the TCDD cancer data. These alternative methods will incorporate such data as time-to-tumor development, correction for early death, a threshold or a non-linear dose-response relationship, receptor-mediated mechanism, and limited low-dose linearity. We will demonstrate that as the histopathology of the lesion progresses to that of a tumor, the dose-response curve becomes more and more non-linear. This phenomenon has also been seen with other non-initiating carcinogens, including saccharin.

AN EXAMINATION OF CRITICAL ASSUMPTIONS IN HEALTH RISK ASSESSMENTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN CONTAMINATED SOIL. D.J. Paustenbach, M.P. Shy, and F.J. Murray, Syntex (U.S.A.) Inc., Palo Alto, CA.

Environmental limits for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are currently being considered by regulatory agencies worldwide. Among these are limits for tap water, soil at industrial sites, residential soil, fish, ambient air and fly ash. Thus far, in the United States, no standards have been promulgated but a few have been suggested. This presentation will review several critical assumptions used in proposed approaches to setting limits for TCDD in contaminated soil within industrial sites. For example, it will be shown how the results of the risk assessment and, subsequently, the magnitude of the recommended limits can be profoundly affected by more justifiable assumptions regarding the quantities of soil typically consumed by children and adults, TCDD's nongenotoxicity, its degradation at the soil surface, and its bioavailability after oral or inhalation exposure. These assumptions will be compared and contrasted with the approach used in the United States.


Lifetime studies of mice and rats showing evidence of tetrachloroethylene (perc) cancer-causing activity serve as the basis for estimation of carcinogenic risk. Experimental data on metabolism, pharmacokinetics, and covalent binding properties were evaluated for incorporation into risk assessment. Evidence indicates liver and kidney toxicity and carcinogenicity potential of perc are dependent on its metabolic conversion to biologically reactive intermediates. Reactive perc metabolites bind irreversibly to cellular macromolecules. In vivo binding is independent of route, but proportional to amount metabolized. Indices of hepatocellular toxicity and cellular damage have been shown to correlate linearly with metabolism. No comparative experimental evidence shows the metabolic pathways for perc qualitatively differ for rats, mice, and humans. Data for these species suggest metabolism of perc after oral or inhalation exposure is rate-limited and proceeds according to Michaelis-Menten kinetics. Methods for establishing metabolized dose-tumor incidence relationships for the bioassays and for their extrapolation to man are outlined. When the animal/human pharmacokinetic data are incorporated into the quantitative assessment, various methods result in similar cancer risks.
Historically, most health hazard determinations were done by informal means. Several years ago, we developed a new Material Introduction process to improve the production use of additives which have passed performance testing in Research and Development trials. However, the OSHA Hazard Communication Standard requires that employers evaluate the physical and chemical hazards of the chemicals they use through a documented procedure. We currently use a two-part process. First, a Toxicity Profile is prepared from available literature, computer database and manufacturer information. Chemical identification and physical/chemical information are included for descriptive purposes. Exposure limits, basic safety precautions and handling are given for industrial hygiene considerations. Each major toxicity category is summarized: acute, chronic, carcinogenicity, mutagenicity, reproductive, neurologic and immunologic. Synonyms are included for cross-reference. Second, a Health Hazard Determination is made in each of OSHA’s designated categories. The outcome is an overall health hazard assessment which can be used for employee training, product Material Safety Data Sheets and customer information.

Neurotoxicity is much easier to prevent than it is to cure. Prevention can occur in a work environment where the early signs and symptoms of neurototoxicity are monitored. The nervous system is highly sensitive to the effects of many toxic chemicals. This sensitivity allows the implementation of a prevention program so that serious neurologic and other disease can be reduced or avoided.

A three step approach is used: 1) workers are monitored for early symptoms that may be caused by neurotoxicity; 2) specialized tests of neuropsychologic function are administered when employment begins, and repeated every 6-12 months; and 3) neurophysiologic tests are administered, using simple instrumentation that can be assembled and protocol followed in remote laboratories. Workers who show signs of neurotoxicity are selected for special education and/or treatment.

This program is designed to reduce 1) occupational disease; 2) accidents from subtle and unmonitored neural dysfunction; 3) medical and disability costs; and 4) lawsuits and even criminal charges of negligence against corporations and corporate managers.

The safety of biomaterials currently is assessed using the USP mouse safety test. This test, although widely used, requires 48 hr to complete and is relatively expensive in terms of the number of animals required to conduct the test. The purpose of this investigation was to evaluate the effectiveness of a bacterial luminescence inhibition assay in assessing the toxicity of compounds which are released from biomaterials. Luminescence from a strain of bacteria most closely resembling Photobacterium phosphoreum was measured using a Microtox (Model 2055) Toxicity Analyzer. The concentration that inhibited luminescence by 50% (EC50) was determined for each plasticizer, monomer and additive. The intravenous (IV-ALD) and intraperitoneal (IP-ALD) approximate lethal doses were determined using mice. By ranking the reference compounds toxic/ non-toxic a comparison was obtained for the IV-ALD and IP-ALD toxicity data. While there was only a 13% agreement for the IP-ALD and EC50 values, there was a 75% agreement between the IV-ALD and EC50 values. Although additional validation is required, these results demonstrate that the bacterial luminescence inhibition assay is a promising alternative method to evaluate the intravenous toxicity associated with the use of biomaterials.

Neurotoxicity is much easier to prevent than it is to cure. Prevention can occur in a work environment where the early signs and symptoms of neurototoxicity are monitored. The nervous system is highly sensitive to the effects of many toxic chemicals. This sensitivity allows the implementation of a prevention program so that serious neurologic and other disease can be reduced or avoided.

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This program is designed to reduce 1) occupational disease; 2) accidents from subtle and unmonitored neural dysfunction; 3) medical and disability costs; and 4) lawsuits and even criminal charges of negligence against corporations and corporate managers.

The dog is widely used as an animal model to study a variety of thrombotic diseases in man. Because it is also a conventional model for toxicity studies, it is important that appropriate and sensitive laboratory assays for evaluating the canine fibrinolytic system be properly validated and agreed upon to conduct safety and efficacy trials for new fibrinolytic agents. Recent advances in the understanding of the molecular structure of serine proteases, their substrates and activators, have led to the development of practical techniques for assessing fibrinolysis in man. We adapted to the dog, and validated, assays for plasminogen and alpha-2-antiplasmin that employ a chromogenic substrate (S-2251) selective and specific for plasmin activity. The optimal conditions for each assay were established. Critical among these were the plasma dilutions employed and the substitution of streptokinase (used for the assay in man) with urokinase as the activator of plasminogen. The assays were highly reproducible and linear from approximately 12% to 120% of normal activity. The sensitivity of these tests was established over the course of a study with urokinase, a well-established fibrinolytic agent. We conclude that these modified assays are useful as tests for fibrinolysis in the dog.
THE LABORATORY ASSESSMENT OF STREPTOKINASE- AND UROKINASE-INDUCED FIBRINOLYSIS IN THE DOG.

The dog has been used extensively to study the efficacy and safety of both new and conventional fibrinolytic agents. However, tests for fibrinolysis in the dog have yet to be critically examined and correlated with other hemostatic parameters over the course of drug-induced systemic fibrinolysis under controlled conditions. Assays for plasminogen and alpha-antiplasmin were recently adapted to the dog in our laboratory. We studied in dogs the effect of a single I.V. bolus injection or a 3-hour I.V. infusion of two known fibrinolytic agents (streptokinase and urokinase) on these assays and the following parameters: platelet count, PT, APTT, thrombin time, fibrinogen and fibrin degradation products. Treatment-related changes in fibrinogen concentration, thrombin time, plasminogen, alpha-antiplasmin, and fibrin degradation products (FDP) were observed with both agents. The onset and duration of these effects varied with the agent administered and the rate of infusion employed. We conclude that the plasminogen and alpha-antiplasmin assays, measurement of FDP's and the conventional tests for hemostasis are useful indicators of systemic fibrinolytic activity in the dog and will be important tools in evaluating the toxic potential of novel thrombolytic agents.


The use of 3H-DFFP to measure erythrocyte survival is well documented, however, the procedure has not been widely utilized in animal research. A red cell life span study using 3H-DFFP was conducted in the F-344 rats as well as in rats of the Wistar and Sprague-Dawley strains. The latter two strains served to validate the procedure as significant data exist for the erythrocyte life spans of these rats. Eight-week old rats of each strain received 20 µCi 3H-DFFP i.v. via the jugular vein. Blood samples were collected from a tail vein beginning 8 days post-labeling and roughly 3x/week until day 33. Blood samples were individually prepared for scintillation counting and blood radioactivity, corrected for sampling loss, was expressed as a percent of the total activity at the time of the initial sampling. Data were analyzed by the least-squares method of linear regression. Results, expressed as a mean erythrocyte life span (days) were 86.5±6.3 for F-344, 59.8±6.18 for Wistar and 61.0±7.70 for Sprague-Dawley rats. These results are in agreement with published data for Wistar and Sprague-Dawley rats and thus validate the 3H-DFFP method for erythrocyte life span determination and demonstrate an erythrocyte life span for the F-344 rat comparable with other rat strains.


Groups of F344 rats and B6C3F1 mice of both sexes were administered 4-chloro-2-nitroaniline by gavage in corn oil for 13 weeks at dose levels of 0, 50, 100, 200, 400, and 800 mg/kg for rats, and 0, 75, 150, 300, 600, and 1200 mg/kg for mice. Reduced survival was observed among chemically exposed rats of both sexes but was not clearly related to dose level; mean body weights of male rats given 800 mg/kg and male mice given 1200 mg/kg were reduced relative to controls from week 4 to the end of the study. Mean absolute and relative liver weights were increased in both sexes of rats and mice at the top 3 dose levels. Microscopic pathological changes associated with compound administration were observed in the livers of male mice (multinucleate hepatocytes and hepatocellular hypertrophy), in the kidneys of male and female rats (brown pigmentation of proximal convoluted tubules; hyaline droplets in epithelial cells of proximal convoluted tubules in males only) and in the spleens of all chemically exposed rats and mice (hematosiderin deposition). Thus liver, kidney, and spleen were identified as major target organs of 4-chloro-2-nitroaniline.

EFFECT OF HIGH DOSE, SHORT TERM ETHYLENE OXIDE (EO) EXPOSURE IN MICE. D. Popp, S. Lock and R.A. Popp. Oak Ridge Natl. Lab., Oak Ridge, TN 37831

C57BL mice survive 50 exposures to 255 ppm EO over a period of 10 weeks but experience perturbations in hematopoietic populations. SEC mice similarly exposed for 4 weeks. Because humans are more likely to be exposed to short bursts of high levels we have examined the effect of 1500 ppm EO for 10 minutes 2 times/day in both strains. Groups of 6-7 mice were exposed to a monitored concentration of EO in a bubble chamber. Blood was obtained from the orbital sinus 2 hours after removal from the chamber and again 6 days later. Hematological parameters were quantitated on an Ortho ELT-8/ws Hematology Analyzer calibrated for mouse blood. The effects of 2, 4, 6 and 8 exposures on hematological parameters and the ability of bone marrow to replace the cellular populations depleted by bleeding were measured. Erythroid parameters were not affected following EO exposure and were depressed equally 6 days after bleeding in control and exposed mice of both strains. Platelet levels were unchanged in exposed groups and slightly elevated following blood loss in control and experimental groups. Significant depression in lymphocytes and granulocytes occurred in both strains after 2 and 4 exposures. EO exposed SEC mice recover from blood loss better than exposed C57BL mice. (Research sponsored by OER, DOE, under contract DE-AC05-840K21400 with the Martin Marietta Energy Systems, Inc.)

BD, a copolymer in the production of synthetic rubber, has recently been demonstrated to be a potent leukemogen in B6C3F1 mice (Huff et al., 1985). The effects of BD on hematopoietic stem cell development were examined in male B6C3F1 mice following inhalation of 1250 ppm BD (6 hr/day, 5 days/ wk). Exposure for 6 weeks resulted in no alteration in the frequency of CFU-S (colony forming unit-spleen); however, CFU-S colonies from treated animals were smaller than controls. Following exposure for 31 weeks, a significant decrease in the number of CFU-S was observed. Similarly, no difference in the number of CFU-GM (colony forming unit-granulocyte/macrophage) was observed after 6 weeks, however, a significant decrease occurred after 30 weeks exposure. Bone marrow cultures were derived from control and treated mice after 6 weeks of exposure. CFU-GM were reduced after 14 days in cultures derived from BD-treated mice; however, after 28 days they were increased relative to controls. These results suggest that BD results in delay in stem cell maturation, and that alterations in stem cell development may play an important role in the pathogenesis of BD-induced thymic lymphoma.


Spleenectomized and sham operated mice were exposed to 0.10, or 100 ppm benzene for 6 hr/day. Both erythroid (CFU-e) and granulocytic (GM-CFU-e) progenitor cells were assayed after 5, 10, 15 and 30 exposures. In the 10 ppm groups CFU-e growth was depressed in both spleenectomized and sham operated mice on day 5; after which time the CFU-e levels in the sham operated mice returned to control levels. The spleenectomized mice, however, exhibited a loss of CFU-e hemostasis from day 5 through day 30. Exposure to 100 ppm benzene resulted in a loss of CFU-e hemostasis in both spleenectomized and sham operated mice from day 5 to day 30. GM-CFU-e growth was initially increased at day 5 among spleenectomized, 10 ppm benzene exposed groups. By day 10 and thereafter, however, GM-CFU-e growth was normal in these animals. In the 100 ppm-exposed groups GM-CFU-e growth was markedly elevated at day 5 in both spleenectomized and sham operated mice, however, thereafter GM-CFU-e growth was depressed in both spleenectomized and sham operated mice. It appears that spleenectomy in combination with benzene exposure affects the hemostasis of the erythroid precursors at low and moderate exposure levels. Granulocytic precursors, however, show greater resistance to the combined treatments at low benzene exposure levels.


Thymic lymphoma is known to be the primary cause of death in B6C3F1 mice following chronic exposure to BD (Huff et al., 1985). In view of the established role of bone marrow damage in radiation-induced murine lymphocytic leukemia, the effects of BD on hematolgy in B6C3F1 mice were examined. Exposure to BD resulted in a macrocytic anemia in male B6C3F1 mice following inhalation of 1250 ppm for 6-24 weeks. Changes evident after 6 weeks included decreases in circulating erythrocytes, total hemoglobin and hematocrit and an increase in mean corpuscular volume. Leukopenia, and a 5-6 fold increase in circulating microcytes were also observed. However, no significant increases were observed in circulating reticulocytes or nucleated erythrocytes. No consistent alterations in bone marrow cellularity were found; however, analysis of bone marrow cell cycle kinetics revealed a 44% increase in proliferative index, due to an increase in the proportion of cells in S phase. These findings are consistent with a treatment-related macrocytic-megaloblastic anemia and indicate the bone marrow to be an important target organ for BD toxicity.


Mice inhaled 10 ppm benzene (current TLV) for 50 days (6 hr/day x 5 days/wk). This was followed by a hematopoietic dose of phenylmethazine (PHZ). Both erythroid (CFU-E) and granulocytic (GM-CFU-C) progenitor cells were then assayed. Although the benzene exposures alone produced minor changes, benzene exposures followed by PHZ treatment resulted in marked depressions in CFU-E and GM-CFU-C growth. In contrast, 5 days exposure to 10 ppm benzene followed by PHZ treatment induced increases in CFU-E and GM-CFU-C growth. In a similar study, mice inhaled 10 ppm benzene for 5 days and then received hydroxyurea (HU) which kills cells in the S phase of the cell cycle. Benzene-exposed mice exhibited increased CFU-E growth at 48 and 72 hr and GM-CFU-C growth was elevated at 24 hr post HU treatment. The effect of 10 ppm benzene exposure on the production of lung conditioned media (LCM) was also assessed. LCM promotes colony formation by GM-CFU-C. LCM from benzene exposed mice stimulated greater GM-CFU-C colony formation than LCM from air-exposed mice suggesting either an increased production of colony stimulating factor (CSF) or production of a more potent CSF. These results indicate that exposures to low levels of benzene cause subtle but observable perturbations in the hemopoietic system.
1147 BENZENE METABOLITES INHIBITORY TO DNA SYNTHESIS IN MOUSE BONE MARROW CELLS IN VITRO. E. W. Lee, C. D. Garner, J. V. Johnson, Biomedical Science Dept., General Motors Research Labs, Warren, MI

Formerly, we showed that DNA synthesis of mouse bone marrow cells (MBC cells) was inhibited by benzene (B) metabolites (s) formed during incubation of the cells with B (Toxicologist, 5:146, 1985). Here we report the effects of added B metabolites on the DNA synthetic activity of MBC cells in vitro. Effects of metabolites were evaluated by a 30-minute incorporation of [H]TdR into DNA following 30-minute interaction with the cells in McCoy's 5a medium. Phenol and muconic acid did not inhibit DNA synthesis. However, catechol(3), 1,2,4-benzene-trotiol (BT), hydronquinone (HQ), and benzoquinone (BQ) were able to inhibit 52, 64, 79 and 98% of the DNA synthetic activity, respectively, at 24 μM. The inhibitory effect of CA was reversible while that of BQ was not. The HQ and BQ effects were partially reversible. In a cell-free DNA synthetic system, CA and HQ did not inhibit incorporation of [H]JTP into DNA up to 24 μM, but BT and BQ did. The effect of BQ was completely blocked in the presence of 1,4-dithiothreitol (1 mM). Furthermore, when DNA polymerase was replaced by DNA polymerase 1 in the cell-free assay, CA and HQ were able to inhibit DNA synthesis. These data suggest that the B metabolites are capable of inhibiting DNA synthetic activity of MBC cells through step(s) not involving gene damage.

1149 HYDROQUINONE SUPPRESSION OF BONE MARROW STROMAL CELL SUPPORTED HEMOPOIESIS IN VITRO IS ASSOCIATED WITH PROSTAGLANDIN E2 (PGE2) PRODUCTION. K. Gaido, D. Wierda, West Virginia Univ. Med. Ctr. Dept. Pharmacology/Toxicology, Morgantown, WV.

Hydroquinone, a metabolite of benzene, will inhibit bone marrow stromal cell supported hemopoiesis in coculture. Stromal cells are known to produce both an inducer (CSF) and an inhibitor (PGE2) of hemopoiesis. This research was conducted to determine if PGE2 was involved in the suppression of stromal cell function by hydroquinone. Stromal cell cultures from male C57BL/6J mice were treated with doses of hydroquinone (10⁻⁷ to 10⁻⁴ M). Some cultures were treated with indomethacin (10⁻⁵ M) 1 hour prior to treatment with hydroquinone. The cultures were incubated for 3 days and then used in coculture with non exposed bone marrow cells overlaid in agar. Stromal cell function was measured by the number of granulocyte/monocyte (G/M) colonies which developed in agar after incubation. Hydroquinone, at doses which did not significantly alter stromal cell number, increased PGE2 levels and significantly inhibited stromal cell supported G/M colony formation. Indomethacin, decreased PGE2 levels in culture and protected against hydroquinone toxicity. These results demonstrate that the suppression of bone marrow stromal cell supported hemopoiesis by hydroquinone is related to PGE2 production and suggests that PGE2 production may be involved in myelosuppression associated with benzene exposure.


Factor IX Complex, including Factors II, VIII & X is a human blood fraction indicated for procoagulant factor deficiencies. Five rats/sex/dose received iv 150 or 317 u/kg of Factor IX Complex (approximately 3 & 6 times the projected human dose). Control animals received 0.9% saline solution. Animals were observed for 7 days post treatment. Within 4 hours after the infusion a number of rats that received the high dose developed swollen, black tongue which protruded from the mouth. This swelling of the tongue started to subside the following day. On the second day a number of animals died. No signs of toxicity were observed in rats treated with low dose of Factor IX Complex. Animals that died showed intravascular thrombosis, hemorrhage & severe parenchymal damage of liver & kidneys & congestion of lungs. Animals sacrificed after 7 days showed granulation tissue traversing the liver parenchyma with areas of cell necrosis at the high dose level. In comparative experiments, iv administration of Factor IX Complex to rabbits at a dosage level of 83 u/kg produced no adverse effects. In conclusion, Factor IX Complex was thrombogenic to rats when administered at 317 u/kg whereas the lower dosage level was well tolerated without any adverse effects.

1150 INDOMETHACIN PROTECTS AGAINST IN VIVO BENZENE INHIBITION OF STROMAL CELL FUNCTION. D. Wierda AND K. Gaido West Virginia Univ. Med. Ctr. Dept. of Pharmacology/Toxicology, Morgantown, WV.

Benzene exposure in vivo can inhibit bone marrow stromal cell supported hemopoiesis. Two factors which are involved in the regulation of hemopoiesis are colony stimulating factor, an inducer of hemopoiesis, and PGE2, an inhibitor of hemopoiesis. The goal of this study was to demonstrate a role for prostaglandin biosynthesis in the expression of benzene toxicity. Male C57BL/6J mice were given benzene (100 mg/kg), indomethacin (2 mg/kg), or a combination of benzene plus indomethacin 1 p., twice a day for 4 consecutive days. Indomethacin was administered 1 hour prior to benzene administration. On Day 5 marrow cell suspensions were removed from the mice and assayed for (1) the number of granulocyte/monocyte (G/M) precursors present, (2) the number of stromal cell colonies which developed, and (3) the number of G/M precursors from untreated mice which could be supported by stromal cells from treated mice in an agar coculture system. Benzene had no effect on bone marrow cell number, G/M precursor number, or stromal cell colony number, but stromal cell function in vitro was significantly decreased by 27%. Mice treated with indomethacin prior to benzene had values comparable with control levels. These results demonstrate that prostaglandin biosynthesis is involved in benzene myelosuppression.

The toxicity of mouse interferon-beta to suckling mice (Crl/lbN) was investigated using MuIFN preparations (2 x 10⁶ IU/mg protein) derived from L-cells with NDV virus. MuIFN inhibited the growth of mice, caused hepatic necrosis with degeneration and induced glomerulonephritis when it was injected daily at levels of 10⁷ or 10⁸ IU/kg from 1 to 8 days after birth.

In addition, there was severe anemia and decreased hematopoiesis, with RBC, Hb, and Ht levels only 50% of the controls in suckling mice receiving MuIFN. The influence of this mouse interferon-beta on erythropoiesis was antiviral titer dependent and species specific, since human interferon-beta does not cause such abnormalities. Furthermore, suckling mice receiving MuIFN from days 8–15 after birth showed less hematological changes and no hepatic lesions. It is likely that MuIFN initially depresses erythropoiesis with subsequent development of the liver lesion.


Although alterations of the endocrine (E) events occurring within the testes can result in decreased (i) spermatogenesis, these events are poorly understood. The purpose of these studies is to investigate E events within the testes which occur in response to treatment with known reproductive toxins. This includes measurement of serum and interstitial fluid (IF) testosterone (T) and in vitro T release in response to hCG stimulation. Androgen binding protein (ABP) levels in serum, IF, and epididymis (EPI) are also measured, providing a means by which Leydig cell and Sertoli cell function can be assessed independently. Studies with dibutyl phthalate (DBP) and diethylstilbestrol (DES) demonstrate that different E profiles are associated with altered spermatogenesis. For example, DBP has no effect on serum T, while increases (i) in IF and hCG stimulated T in EPI of untreated rats, and decreased (i) in serum ABP in untreated rats caused a + in all three measures. ABP concentrations were + in serum, IF and EPI of DBP treated rats, while DES caused + in all three measures. Serum FSH was + by DBP but unaffected by DES. Serum PRL was + by DES and unchanged by DBP. Serum LH was not altered by DES, but + by DBP. These results suggest that DES and DBP-induced alterations in spermatogenesis are associated with strikingly different E profiles.

AEROBIC AND ANAEROBIC OXIDATION OF HUMAN BLOOD HEMOGLOBIN BY SODIUM NITRITE. H. Chiidi and J.C. Mohler. Los Angeles County-University of Southern California, School of Medicine, Los Angeles, California. Sponsor: A. Sevianian

In the presence of oxygen, human hemoglobin (Hb) in a buffered solution is oxidized to methemoglobin (MetHb) by nitrites via an autocatalytic reaction. Under anaerobic conditions the oxidative autocatalytic reaction is replaced by a much slower oxidative reaction. Whether the autocatalytic reaction also occurs in human whole blood is still unclear. It was the aim of this study to measure MetHb formation as a function of time in human whole blood exposed to sodium nitrite (NaNO₂). A flowthrough tonometer at 37°C was used to expose the blood to NaNO₂. Blood pH ranged from 7.20 to 7.40. MetHb was measured in a calibrated IL 282 CO-Oximeter immediately after the sample was drawn from the tonometer. Hb monomer to NaNO₂ ratio was 1:1 mol. After one minute exposure to NaNO₂ there was 15.7 ± 0.43% of MetHb formed anaerobically and 23.9 ± 2.53% under aerobic conditions. After 3 minutes 33.3 ± 0.5% and 43.1 ± 2.6%, after 10 minutes 38.0 ± 0.5% and 37.3 ± 1.9% MetHb, respectively were formed. Exposure of nitrite treated blood to 100% CO for 15 minutes produced 9% of carboxyhemoglobin (COHb) under anaerobic conditions and 40% COHb aerobically. Our unpublished electron spin resonance data on nitrosylhemoglobin (NOHb) shows that the smaller percentage of COHb in the anaerobic experiment is due to the presence of NOHb which is not present in the aerobic experiment. In whole blood the Hb oxidation by nitrite, whether aerobic or anaerobic, does not show any catalytic effect. The difference in the amount of MetHb formed seems due to the oxygen presence in aerobiosis which oxidizes the NOHb to MetHb as soon as it is formed.

INFERTILITY AND PARTIAL RECOVERY AFTER A SINGLE EXPOSURE TO 1,3-DINITROBENZENE (DNB) IN THE MALE RAT. S.D. Perregault1, R.E. Linder1, R.A. Hess2, and L.E. Strader3. USEPA, HERL, DBB, Reproductive Toxicology Branch, and Northrop Services Inc.4, Research Triangle Park, NC. Sponsor: Neil Charnoff.

Adult male SD rats were given a single dose of DNB (48 mg/kg) or vehicle (corn oil) by gavage on d 0. Sperm fertilizing ability (FA) was evaluated by breeding 1 group of males (8 treated, 8 control) at intervals over 175 d, and examining eggs flushed from oviducts the afternoon of estrus for evidence of fertilization. FA remained high in controls (80–100%) but declined and recovered in treated males as follows: FA = 96% (d 16); 65% (d 24); 0% (d 32); 7% (d 62); 64% (d 96); 70% (d 162). Two males never recovered (0% eggs fertilized) although they did breed. Terminal studies on other groups (8 treated, 8 control) of unbred males revealed significant decreases in testis/epididymis weights, testicular sperm head counts and cauda epididymal sperm reserves, morphology and motility by d 16. These effects were maximal by d 24 and 32. Substantial recovery of these parameters occurred between d 72 and 175 in some, but not all, males. At d 175 histopathology showed either recovery of spermatogenesis or severe seminiferous tubule atrophy with epididymal occlusion. Thus a single dose of DNB caused irreversible infertility in some animals.

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The reproductive capacity of most mammalian males remains intact into late life, although there are age-related changes in the neuroendocrine processes regulating fertility. At present, the relative susceptibility of reproductive functions to environmental toxins is unclear. The aim of this study was to evaluate reproductive function in 4, 12, and 24-month-old male Long-Evans rats following a 2-wk exposure to diethylstilbestrol (DES). Rats received DES (2.2 or 10 μg/kg/d) via Alzet minipumps implanted sc. At sacrifice, trunk blood was collected for serum hormone analyses. Pituitaries (pits) were weighed and hemisected with one-half used for hormone conc and the other half for hormone release in vitro using a perfusion procedure. Hypothalamic GnRH conc was also determined. An age x dose interaction was observed in pit wt, serum prolactin (prl), pit LH, FSH and prl conc and hypothalamic GnRH conc. Serum testosterone decreased while testis wt increased with age. Significant age, dose and age x dose interactions were noted in pit LH, FSH and prl release in vitro. In summary, the significant age x dose interactions indicate that the effect of DES on the hypothalamic-pituitary-testicular axis is greater in the aged male and points to the need to consider advanced age as a significant variable when evaluating the effects of toxins upon the reproductive system.

It is unclear whether the testicular toxicity of DBCP is due to the parent compound or to metabolites that may be produced by oxidative dehalogenation. Therefore, the cytotoxicity of DBCP, epichlorohydrin (ECH), alpha-chlorohydrin (ACH), beta-chloralactate (BCL), and 1,2-dibromoethane (EDB), a structural analog of DBCP, was compared at equimolar doses using spermatogonocytes isolated from 30-day-old S-D rats. 2X10^6 cells in 1 ml of MEM were incubated (32°C) with 1, 3, and 9 μmol of test compound, or with ethanol. LDH release and trypan blue exclusion (TBE) were monitored as parameters of cytotoxicity. TBE for control cultures averaged 90% after 80 min of incubation. LDH release from cells treated with 1 μmol DBCP was increased after 20 min (p<0.05); at 80 min LDH release was more than twice control and TBE was 0%. Cytotoxic doses for ECH and EDB were respectively 3 and 1 μmol, while ACH and BCL were without cytotoxic effects at the doses examined. These data indicate that DBCP is a more potent cytotoxicant to spermatogonocytes than the other compounds tested. Therefore it is unlikely that DBCP cytotoxicity is mediated by its metabolism to these compounds. (Supported by a gift from General Motors Corp. and NIEHS ES03451)

The structural and functional integrity of the plasma membrane is essential for differentiation of male germ cells (MGC). Perturbation of membranes is a basis for toxicological phenomena including chemical promotion of cancer. The purpose of this study was to confirm biochemically and measure the activity of membrane bound adenylyl cyclase (AC) in rat MGC. In addition, the effects of promotor agents on AC alone and in the presence of follicle stimulating hormone (FSH) were evaluated. Radioimmunoassay was used to quantitate cAMP formation as an index of AC activity. Male germ cells were isolated using a two step enzymatic digest and washed exposed to 4,0-TPA and TPA (1.7 x 10^-19M) for 1 h at 35°C. Purified plasma membranes were exposed to ATP (0.5 M) and FSH (1.39 x 10^-7 M) for 10 min at 37°C. Preliminary data indicate stimulation of AC activity by both ATP and FSH in all pretreatments. Both phorbol esters attenuated the FSH induced stimulation of AC observed in vehicle controls. These data indicate that rat MGC contain measurable AC activity and that this activity can be altered by physiological and promotorial agents.

Spermatogenesis takes place in the lumen of the seminiferous tubules. Sertoli cell (SC) provide structural and functional support of the developing germ cells. In rat testes, SC convert 95% of their glucose to lactate; this is thought to be an adaptation to provide lactate to the GC which preferentially utilize exogenous lactate as a substrate. Since this metabolic dependence of GC on SC exists, the effect of lead acetate (PbAc2) on glucose utilization by SC, and lactate utilization by GC was studied. SC cultures were exposed to 0.01, 0.05, 0.10 mM PbAc2. Glucose utilization was measured by the liberation of 14CO2 from [14C]-glucose was significantly depressed (43.3% of control) by 0.10 mM PbAc2. Glucose transport (into SC) as measured by the use of 3-O-methyl-[U-14C]-glucose was not affected. Lactate utilization by GC was measured by the liberation of 14CO2 from [14C]-lactate was significantly depressed (46.4% of control) by 0.10 mM PbAc2. Viability of SC and GC as measured by Trypan Blue exclusion and LDH release into the culture medium was not affected under the experimental conditions. These results suggested that PbAc2 may inhibit spermatogenesis by the disturbance of either GC or SC metabolism or both. Supported by NIEHS ES03451 and the GM Corp.
The effects of 2,5-hexanediol (2,5-HD), acrylamide (ACR), and mono-(2-ethylhexyl)phthalate (MEHP) on Sertoli cell-enriched cultures. R.E. Chaplin, T.J.B. Gray, S.L. Dutton, and J.C. Lamb, IV National Toxicology Program, NIEHS, Box 12233, RTP, NC 27709 and #BIBRA, Woodmansterne Rd, Carshalton, Surrey, SM5 and 4DS, England

Primary cultures of testicular cells are being used increasingly to characterize and define the effects of toxicants on these cells. We used Sertoli-enriched cultures from 18-d Fischer 344 rats to compare the effects of MEHP with those of ACR and 2,5-HD on secretory and synthetic endpoints in the media and cellular monolayer. The cultures were 80-90% Sertoli cells, and were responsive to FSH, as measured by changes in media lactate and cell shape. MEHP produced a dose-dependent increase in lactate secretion, a decrease in media pyruvate, and a decrease in cellular ATP levels. 2,5-HD also stimulated media lactate levels in a dose- and time-dependent manner, with no change in media pyruvate or intracellular ATP. While MEHP had no effect on [3H]-leucine incorporation into secreted and cellular proteins, 2,5-HD produced a dose-dependent increase in cellular protein synthesis. For both compounds, these changes occurred in the absence of effects on cellular morphology. For ACR, in contrast, only those doses which altered cellular morphology also changed media lactate or protein synthesis. The presence or absence of FSH in the media had no effect on the response of the cells to these toxicants.

Biochemical response of primary rat Sertoli cell cultures to cadmium. S.R. Clough, M.J. Welsh, and M.J. Brabeck, Program in Toxicology, The University of Michigan, Ann Arbor, MI 48109.

The "vascular necrosis" seen in acute testicular insult by cadmium makes it difficult to discern the potential cellular target(s). In vitro observations made on different testicular cells in our lab suggest that the Sertoli cell may be particularly sensitive to cadmium. We therefore exposed primary Sertoli cell cultures to relatively low concentrations of CdCl₂ (0.25 - 2.25 μM) and measured production of lactate, conversion of C₆-glucose to CO₂, incorporation of [3H]-leucine, and cadmium binding capacity. After two hours exposure, there was a marked decrease in production of CO₂ at all dose levels. A significant decrease in [3H]-leucine incorporation could be observed at 1.0 and 2.0 μM. Induction of cadmium binding activity could not be seen until 12 hours of exposure, and only at 0.5 and 1.0 μM. Lactate production was dose related and linear over time. At 12 hours, 0.75 and 2.25 μM caused an increase of 2 and 4.5 times that of control, respectively. It is concluded that low levels of Cd (less than the 72 hr LD50) can rapidly affect biochemical parameters of Sertoli cell function. Since Sertoli cells delineate the blood-testis barrier and support spermatogenesis, a differential sensitivity to Cd might explain the selective toxicity of this element in the testis. Supported by a gift from General Motors Corp. and NIEHS grant #ES03431.
Cephalosporin antibiotics containing a N-methylthioetetrazole (MTT) side chain, as well as the MTT side chain itself, are known to cause testicular atrophy in juvenile rats. The purpose of the present study was to test cefonicid, which has a modified MTT side chain, for possible effects on male sexual maturation in rats. The drug was subcutaneously administered to male rat offspring from days 6-36 postpartum. Moxalactam (-MTT) and cephalexin (-MTT) were used as positive and negative controls, respectively. Moxalactam caused reductions in reproductive organ weights and histopathological lesions in the testes in juvenile (day 37) and adult (day 102) rats at all dose levels. Treated males were subfertile, and control females inseminated by moxalactam-treated males had smaller litter sizes. When autopsied at 102 days of age, moxalactam-treated rats had significantly reduced testicular sperm production rates and cauda epididymal sperm numbers. Cephalothin and cefonicid had no biologically and/or statistically significant adverse effects on male sexual maturation.

Previous reports of various neurotoxic agents having reproductive tract toxicity prompted us to investigate the effect of tri-o-cresyl phosphate (TOCP) in the male rat. Animals were dosed continuously with 10-150mg TOCP/kg/d, po and sacrificed between 3 and 63 days. There was an inhibition of sperm motility and number/mg cauda in a dose-(50-100%) and time-(50% at d 5, 100% by d 10) dependent fashion. An inhibition of testicular non-specific esterase (NSE) and neurotoxic esterase activities was observed. Histochemical localization studies indicated the majority of NSE activity and TOCP induced inhibition of NSE was located in the testicular interstitium. Pairfed and parathion treated rats served as controls for effects due to weight loss and acetylcholinesterase inhibition. None of these animals showed any signs of testicular toxicity. Plasma and testis interstitial fluid testosterone concentrations as well as plasma luteinizing hormone levels were normal. These data support a non-hormonally mediated mechanism for TOCP's action on the testis. Supported by NIOSH grant no. OH02003.


The onset and development of the testicular lesion after TOCP dosing have been determined. Male rats (200g) were administered 150mg TOCP/kg/d in corn oil (po, 16/group) for 3, 5, 7, 10, 14 and 21 days. A vehicle treated group (n=5) served as control. Sections from formalin fixed, methacrylate embedded testes showed by day 5, numerous detached spermatid heads at the basement membrane of the seminiferous tubules. Columnar vacuolations of the epithelium, radiating from the basement membrane to the lumen of the tubule were also observed. Electron micrographs (EM) revealed these to be localized in Sertoli cells (SC). Widespread dilation of SC smooth endoplasmic reticulum was also present. By day 7, large numbers of pink staining cytoplasmic bodies (CB) were present in the lumen of stage VIII tubules along with giant cells (GC). The lesion progressed with increasing vacuolations of the epithelium and numbers of CB and GC (10, 14, 21d). The epididymis has CB and GC by day 10. There was also a decrease in sperm density/tubule (0 by d 14). These morphological results indicate a possible selective effect on SC. Spermatogenesis is affected as seen by the decrease in sperm density and increase in necrotic spermatids. Supported by NIOSH grant no. OH002003.


Selective lowering of GSH in the epididymis has been associated with enhanced ethyl methanesulfonate-induced mutagenicity. The purpose of this study was to extend these observations to determine the effects of other known hepatic GSH depletors on epididymal and testicular GSH. Rats were administered a single i.p. dosage of tri-methyl phosphate (TRM) (600 mg/kg), 2,3-dichloropropylphenol (50 mg/kg), pentachlorophenol (25 mg/kg), methyl mercury (1 mg/kg), naphthalene (500 mg/kg), isophorone (500 mg/kg), methyl iodide (100 mg/kg), acetaminophen (1,500 mg/kg) or diethylmaleate (DEM) (500 mg/kg). TMP, DEM and isophorone significantly reduced GSH in the testes and epididymis. DEM decreased epididymal and testicular GSH to 43 and 82% of control, respectively. The other compounds reduced hepatic GSH but failed to perturb testicular or epididymal GSH. GSH in the male reproductive tract was resistant to some chemical-induced alterations, while other chemicals were able to reduce GSH. The functional significance of chemical-induced lowering of epididymal GSH is possibly a potentiation of chemical-induced germ cell mutations. Chemical groups capable of altering GSH in the male reproductive tract appear to be the alkylating agents. (USPHS grant ES02824).
Progressive atrophy of seminiferous tubules is characteristic of the injury caused by the tricho-thecene mycotoxin angudine (diacetoxycirpenol) in mice. The effects of angudine on testicular function and structure were evaluated in 15-week-old male Lewis rats given 1.7 mg angudine/kg body weight (75% of the LD50) ip in 10% dimethyl-sulfoxide and studied at 1, 3, 7, 30, 60 and 90 days. Angudine caused a progressive loss of testicular weight beginning at 60 days. The number of sperm/testis, sperm/g testis and the sperm production rate, were decreased by 30 days and continued to decrease through 90 days. The sperm production rate of angudine-treated rats was 40% of control by 90 days. Epididymal sperm reserves were decreased by 3 days and remained low through 90 days. Angudine had no effect on sperm transit time in the epididymus. Focal degeneration of seminiferous epithelium was seen at 1 day. There was a progressive increase in the number of atrophic tubules thereafter. The progressive tubular atrophy and decrease in sperm production rate 30 to 60 days after angudine exposure are consistent with injury to proliferating cells early in the maturation sequence. (Supported by contract DAMD 17-82-C-2235 from the U.S. Army Medical Research and Development Command).


DBCP produces a rapid decrease in fertility in laboratory animals after acute exposure. Previous studies linked the sterilizing effect of DBCP to a post-glycolytic inhibition of sperm carbohydrate metabolism. The present studies were aimed at identifying the specific site of inhibition. 14CO2 generation from epididymal sperm of F344 rats was measured using 14C-labeled acetyl-CoA, citrate, α-ketoglutarate, or succinate as substrates. There was 10-30% inhibition of CO2 generation at 0.5mM DBCP and 80-95% inhibition at 3mM DBCP with all four substrates. The similarity in response for all substrates suggests that there may be a common site of inhibition. We explored the possibility that the inhibition occurs in the mitochondrial electron transport chain. DBCP (3mM) inhibited endogenous oxygen consumption rates, but had no effect on succinate oxidation. Oxygen consumption rates in the presence of α-ketoglutarate or malate were inhibited about 80% by 3mM DBCP, however, the activity of α-ketoglutarate dehydrogenase was not affected by DBCP. These results indicate that DBCP inhibits sperm carbohydrate metabolism at the NADH dehydrogenase step in the mitochondrial electron transport chain.

**Hepatic Peroxisome Proliferation and Testicular Toxicity of Di(2-Ethylhexyl)Phthalate (DEHP) in Neonatal Rats After Direct Dosing and Exposure to DEHP Via Breast Milk.** L.A. Dostal, W.L. Jenkins, M.D. Ross, S.A. Stefanek, and B.A. Schuetz. National Toxicology Program, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709

To determine the relative sensitivity of neonatal vs. adult rats to the toxicity of the plasticizer DEHP, 5 daily oral doses of DEHP (0.01, 0.1, 1.0, 2.0 g/kg) in corn oil were given to 1, 2, 3, and 6-wk-old rats. Two doses of 2 g/kg caused death in 1, 2 and 3-wk-old rats while 6-wk-old rats showed no lethality after 5 doses. Similar increases in liver weight (% of BW) and in the activities of hepatic peroxisomal enzymes palmitoyl Co A oxidase (PCO) and carnitine acetyl transferase (CAT) (9-17-fold) were observed at all ages after 1 g/kg DEHP for 5 days. Plasma cholesterol and triglycerides were decreased in 3 and 6-wk-old rats. Testis weight (% of BW) was decreased at all ages after 1 g/kg but histological changes (eg. loss of spermatids and/or spermatocytes) were more dramatic in 3- and 6-wk-old rat testes than in the younger rat testes. Therefore, neonatal rats show similar increases in liver weight and peroxisomal enzymes to those observed in weaned rats, but the type of testicular damage varied with age. Five daily oral doses of DEHP (2 g/kg) to lactating rats caused 2-fold increases in PCD and CAT activities in 1-wk-old suckling pups suggesting transfer of DEHP through breast milk.


The present study was designed to determine if the reduction of epididymal reserves through frequent copulation would enhance the detection of ethoxy-ethanol (EE)-induced spermatotoxicity. Male Long-Evans rats were assigned to one of two mating schedules: mated every other day (bidally) or sexually-inactive. The rats were then intubated with 0.150, or 300 mg/kg of EE for 6 weeks. At sacrifice cauda epididymal sperm count (ESC), testicular spermatid count (TSC), and sperm morphology (SM) were assessed. Bidally mating per se did not differentially influence TSC which was significantly reduced by EE treatment. At 300 mg/kg EE, there was a significant decline in ESC for both mating conditions. However, these effects were significantly greater in the males mated bidally. Of greater interest was the fact that at 150 mg/kg EE, only the mated animals showed a significant decline in ESC. SM showed identical effects to those seen for ESC. These data suggest that this approach may provide a powerful tool for more accurately estimating the risk for a given reproductive hazard. (March of Dimes 59-104; NIEHS ES-07073).
Recent studies from this laboratory implicated ACR as a male reproductive toxicant in rats at doses that failed to produce peripheral neuropathies (Zenick et al., 1985; Smith et al., 1986). The current research was designed to define the stage of spermatogenesis most adversely affected by ACR treatment. Adult, male Long-Evans rats received either 0, 30, 45, or 60 mg/kg of ACR (p.o.) for five consecutive days (N=15/group). Males were serially mated beginning two days after the last dose and then on weeks 2, 3, 4, 7, and 10 post-exposure. Females were sacrificed on day 15 of gestation and examined for evidence of pre- and post-implantation loss. ACR produced significant pre- and post-implantation losses at all doses employed with effects primarily seen in the first three weeks of mating. No treatment-related effects were observed at weeks 7 and 10. These data suggest that spermatozoa and spermatids represent the most sensitive germ cell stages. This abstract does not necessarily reflect policy or opinion of the U.S. EPA.

Male F344 rats and B6C3F1 mice were treated with 0, 50, 100, or 200 mg/kg (mouse), 0, 50, 100, 200, 400, or 600 mg/kg (rats) tricresyl phosphate (TCP) containing <0.1% TCP by gavage in corn oil, or 0, 1700, 3300, or 6600 ppm (rats) or 0, 50, 1000, or 2100 ppm (mouse) TCP in feed for 13 weeks. Sperm concentration, motility, and morphology were evaluated. The reproductive tract was examined for histopathologic lesions. Mice exposed by gavage exhibited a decrease in sperm concentration and an increase in abnormal sperm morphology at 200 mg/kg; a decrease in sperm motility was found in all TCP dose groups. Tricresyl phosphate treatment significantly affected (p<0.01) all male reproductive parameters in a dose-dependent manner in rats exposed to TCP by gavage. In animals exposed to TCP in the feed, sperm parameters were adversely affected in 2100 ppm group mice and 6600 ppm group rats. Preliminary histopathologic findings indicate multifocal testicular degeneration in rats but not mice by both dosing routes. At comparable dose levels (mg/kg) corn oil gavage administration of TCP results in greater male reproductive toxicity in rats than mice. (Supported by Contract Nos. N01-ES-75653-03 and N01-ES-45050 from NTP).

Tricresyl phosphate (TCP) is used commercially as a plasticizer and a flame retardant. The reproductive effects of TCP (<9.0% TCPP) were examined. Male Long-Evans rats received 0, 100, or 200 mg/kg and females received 0, 200, or 400 mg/kg TCP in corn oil by gavage. 100 mg/kg TCP males mated with 200 mg/kg TCP females, and 200 mg/kg TCP males bred with 400 mg/kg TCP females. Males were dosed for 56 days and females for 14 days prior to breeding and throughout the 10-day breeding period. Following breeding the males were necropsied and evaluated for sperm parameters and reproductive tract histopathology. Females were dosed throughout gestation and lactation. Pups and adult females were necropsied on postnatal day 21. Sperm concentration, motility, and progressive movement were decreased for 200 mg/kg dose group males. A dose-dependent increase in abnormal sperm morphology was observed for males in both TCP dose groups. The percent of sperm positive females per group was unchanged, but the number of females delivering live young was severely decreased by TCP exposure. Litter size and pup viability was decreased in the 400 mg/kg dose group. Pup body weight and developmental landmarks were unaffected by TCP exposure. Supported by EPA project no. CR810862. This abstract does not represent the policy or opinion of the USEPA.

Male rats were exposed to target concentrations of 250, 1000 and 2500 ppm MeBE for 5 D/Wk, 6 Hr/D for 12 weeks and were mated to female rats exposed to the same concentrations for a 3 week period. Exposure continued through the mating period, gestation and lactation. A second litter (P1,) was produced under the same mating and post-mating exposure regimen. No adverse effect of treatment was observed with the adult animals (Po) throughout the one-life portion of the study. No adverse findings were observed for pregnancy rates, male fertility and mating indices of both mating intervals (P1 and P2). Pup viability indices at birth were comparable for control and treated groups for the P1 litters, but the P2 mid- and high-dose groups displayed a very slight, but significant (p<0.05) decrease. In the absence of a consistent trend between litter intervals, no treatment related effect was indicated in the incidence of litters with pup mortality. Litter survival indices were comparable between control and treated groups for both litter intervals. Pups of mid- and high-dose females had slightly lower (not significant) mean weights at days 14 and 21 of lactation. MeBE possesses little adverse reproductive toxicity potential as measured in a two litter, single generation reproduction assay.
1176 REPRODUCTIVE TOXICITY OF METHYLOXACETIC ACID (MAA) IN THE DRINKING WATER TO MOUSE BREEDING PAIRS. J.D. George, J.R. Reel, C.B. Myers, A.D. Lawton, and J.C. Lamb, IV, Research Triangle Institute and National Toxicology Program, NIHES, Research Triangle Institute, NC, 27709.

The effect of MAA on fertility and reproductive performance of CD-1 mice was evaluated using a continuous breeding protocol. After an 18-week exposure to MAA-dosed drinking water, fertility was completely suppressed in 0.4% MAA-treated pairs; reduced fertility and prenatal viability was observed in 0.1% and 0.2% MAA-treated pairs, relative to controls. A crossover mating trial immediately following the 18-week exposure revealed that fertility was profoundly reduced in pairs with either a Δ or γ 0.4% MAA-treated partner. No live pups were born to control Δ x 0.4% MAA γ pairs; only one live litter was born to 0.4% MAA Δ x control γ pairs. Sperm concentration and % motility were decreased in 0.4% MAA vs. control males; these effects were accompanied by atrophic seminiferous tubules and reduced testicular, epididymal, prostate, and seminal vesicle weights. No treatment-related histopathology was observed in female 0.4% MAA mice. A mating trial of control vs. 0.1% MAA F₁ pairs revealed complete infertility in MAA treated mice, accompanied by testicular toxicity that was similar to but less severe than that observed in F₀ mice. Thus, MAA was a reproductive toxicant in Δ and γ F₀ and F₁ mice. (Supp. by NIH/NTF Contract No. NIH-ES-25014).

1177 REPRODUCTIVE EFFECTS OF CHLORAMINE ADMINISTERED BY GAVAGE TO LONG-EVANS RATS. R.D. Carlton, F.B. Barlett, H.K. Smith, Battelle Columbus Laboratories, Columbus, OH and USEPA, HERL, Cincinnati, OH.

Chloramine is one water disinfectant under consideration by the USEPA as an alternative to chlorination. The reproductive effects of mono-chloramine (NH₂Cl) administered by gavage were studied using Long-Evans rats. Animals received 0, 2.5, 5.0, or 10.0 mg/kg NH₂Cl for a total of 66-73 days. Males were dosed for 56 days and females for 14 days prior to breeding and throughout the 10-day breeding period. Three to 7 days after completion of the breeding period, males were necropsied and evaluated for sperm parameters and reproductive tract histopathology. Females were dosed throughout gestation and lactation until necropsy on lactation day 21. Pups were necropsied at weaning on postnatal day 21. No differences were observed when fertility, viability, litter size, or the day of pup eye opening or female pup vaginal patency were evaluated. Sperm concentration, motility, morphology, and direct progressive movement (μm/sec) were unaltered by chloramine treatment. No hematologic abnormalities or histopathologic lesions were observed in any chloramine treatment group. Supported by EPA Project No. CR810862. This abstract does not represent the policy or opinion of the USEPA.


Buprenex (B) is a potent agonist-antagonist analgesic when administered either orally or parenterally and was non-mutagenic as shown in 4 in vitro assays. B administered subcutaneously (sc) to male rats at 0, 0.1, 1.0 or 5.0 mg/kg/day for 60 days prior to mating caused no effect on fertility, however, all drug treated males gained less weight than the controls. B administered sc to female rats at the same dosage levels for 14 days prior to mating, during mating, and throughout the gestation period caused no effect on fertility or gestation indices. All drug treated females gained less weight than controls and the viability and lactation indices were less than controls. Among females necropsied on day 13 of pregnancy, no significant effect was observed on the number of viable young or resorption sites. In females rearing young, no significant effect was observed on litter size at birth. Pup weights for all drug treated groups were significantly less than controls from birth to day 21. Pup mortality was increased in all drug treated groups. No drug induced external or internal anomalies were observed grossly at necropsy of the pups at weaning.
The reversibility of reproductive toxicity induced by THC was studied. Groups of 10 male and 10 female (6 weeks old) F344/N rats and B6C3F1 mice were administered THC in corn oil by gavage at 0, 5, 15, 50, 150 or 500 mg/kg, 5 times a week for 13 weeks followed by a 60-day recovery period. After 13 weeks dosed male and female rats showed dose-related reductions in body weights. No histopathologic changes were found in rats dosed at 500 mg/kg. THC at 150 or 300 mg/kg lowered testicular and epididymis weights, induced focal testicular atrophy and increased abnormal sperm numbers in male rats. These doses caused uterine and ovarian hypoplasia and prolonged estrous cycles in female rats. After a 60-day recovery period body weights of all dosed male and female rats were comparable to those of controls; uterine weights, ovarian morphology and estrous cycles were normal but testicular weight and focal testicular atrophy persisted in the 500 mg/kg males. After 13 weeks body weights and reproductive systems of dosed male and female mice were normal except minimal uterine and ovarian hypoplasia were observed in 500 mg/kg females. These changes persisted for 60 days after dosing. Atrophy of testes in male rats and hypoplasia of reproductive organs in female mice after 13-week administration of high doses of THC were not reversed in a 60-day recovery period.

The reproductive toxicity of 7 glycol ethers and oxalic acid was assessed according to a new protocol designated "Pertinuous Fertility Assessment by Continuous Breeding" (PFA/B). PFA/B shares the same fundamental endpoints as conventional reproductive toxicity test systems but is designed to improve the reliability and sensitivity of fertility testing. PFA/B consists of four tasks: dose finding, continuous breeding, identification of the affected sex, and offspring assessment. At the conclusion, animals are necropsied and selected organs are weighed and fixed for histopathological examination. CD-1 mice were used and all test articles were administered in drinking water. Ethylene glycol monomethyl ether was the most potent reproductive toxicant among the 7 glycol ethers. Ethylene glycol monooctyl ether, ethylene glycol monoethyl ether acetate, ethylene glycol monobutyl ether (ECBME), and 2-ethoxy acetic acid (2-EAA) also adversely affected both fertility and reproduction. ECBME was effective mostly at toxic levels and the reproductive toxicity of 2-EAA occurred at levels where daily water consumption was below normal. Ethylene glycol only marginally affected fertility and reproduction. Propylene glycol and oxalic acid were designated negative.
Inhibition of the Decidual Cell Response of the Female Rat by Methoxychlor. A. M. Cummings, USEPA, HERL, DBB, Reproductive Toxicology Branch, Res. Tri. Park, NC. Sponsor: N. Chernoff.

Chemically-induced peri-implantation embryonic loss can result from direct fetal effects or changes in maternal reproductive physiology. This study was designed to develop a protocol to identify toxicant-induced alterations in the potential capability of the uterus to implant a fertilized egg. The DCR was used because this method can separate maternal and fetal effects. Pseudopregnancy was induced by cervical stimulation on d 6 of the estrous cycle. A decidual induction was performed by surgical trauma of the endometrium on d 4, thus mimicking the effect of the blastocyst. Each animal was necropsied on d 9, and the uterus was weighed. The normal response is a 10-fold increase in tissue mass known as a deciduoma which is histologically and biochemically similar to decidua of early pregnancy. Estrone, a positive control, produced a full inhibition of the DCR at a dose of 5 μg/ret when injected sc d 1 - d 8 of pseudopregnancy. The estrogenic pesticide methoxychlor (M) also inhibited the DCR in a dose-dependent manner. M was gavaged d 1 - d 6. A dose of 100 mg/kg M was without effect. A dose of 200 mg/kg produced a partial (44%) but significant inhibition of the DCR. Other doses used (300, 400, 500, and 600 mg/kg/day) produced significant inhibition of the DCR of 83-96%. These data show that M exerts direct maternal effects.


The pesticide Mxc has been shown to possess a weak estrogenic action and has been found to have a number of toxic effects upon the rodent reproductive system, primarily at the gonadal level. The purpose of this study was to extend the influence of chronic, low doses of Mxc upon pituitary (pit) and hypothalamic components of the male reproductive system. At 21 d, male Long-Evans rats were gavaged daily with 25 or 50 mg/kg Mxc (in corn oil) for 8 wks. Controls received vehicle only. At sacrifice, trunk blood was collected for serum hormone analyses. Anterior pit and mediobasal hypothalami (MHB) were rapidly removed for measures of tissue hormone conc (pit LH, FSH, prolactin (PRL) and MHB gonadotropin-releasing hormone (GnRH)) and assessment of hormonal release in vitro. No significant changes were seen in serum hormones, nor in pit LH or FSH. Pit PRL was elevated for both doses, and peripit fragments released more PRL. MHB GnRH was higher, but only for the 50 mg/kg dose. At this dose, there was also an increase in the KCl-stimulated release of GnRH. The data suggest that previously reported reproductive effects of Mxc may be mediated, at least in part, through an elevation in PRL conc and release, which in turn is able to influence GnRH secretion from the MHB.


Ethylene oxide (EtO) at airborne concentrations of 255 ppm, 6 hr/day, 5 days/week kills SEC males before 20 exposures whereas C57Bl males survive more than 50 exposures. Dominant lethal studies showed that the germinal tissues of these two strains also responded differently to EtO. Males were mated to females on weekends after 5 daily exposures to 150 ppm of EtO for 4 weeks. Exposed C57Bl males remained fertile during the period of 20 exposures and a significant number of dead implants was observed in pregnant females through 2 weeks after the last exposure. In contrast, SEC males were sterile during the period of 20 exposures and through 4 additional weeks. Histological examinations showed that EtO caused extensive damage in the testis of SEC males. Pachytene and later stage spermatocytes contained necrotic nuclei; undifferentiated spermatocytes and Sertoli cells were observed in the lumen of many seminiferous tubules; and many tubules contained very few primary spermatogonias and spermatocytes. In C57Bl males, EtO induced dominant lethal mutations by affecting mostly mid and later stage spermatids; and in SEC males, EtO induced sterility by affecting primary spermatocytes as well. (Research sponsored by OHER, DOE under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.)

This study investigated the effects of high LET radiation on male reproductive competence using an in vitro embryo system for determining dominant lethal mutations and by assessing sperm parameters at sacrifice. Additionally, the presence of reciprocal translocations were assayed in spermatocytes. Male mice (20 pups) received a dose of 0, 0.5, 1.0, 1.5, 2.0, or 2.5 Gy 95 keV/μm accelerated Argon ions, and were monitored over a 6 month period. Two-cell embryos, recovered from females mated with treated males on a biweekly schedule, were cultured until blastocyst formation. The blastocysts were transferred to conditions where they formed a trophoderm outgrowth and an inner cell mass. Losses at the blastocyst stage were scored as preimplantation losses, and decreases in the inner cell mass formation reflected postimplantation losses. There were dose related increases in pre- and postimplantation losses from sperm treated as spermatogonia, spermatids, and spermatocytes. Treated spermatogonia displayed postimplantation losses that were still present six months post exposure. There were dose-related decreases in body weights and testis weights. Research supported by Dept. of Defense Contract DNA 001-84-C-0170, and NIHs grant CA 09215.


Acrylamide (ACR), a well known neurotoxin, is inactive as a mutagen in bacterial test systems. Zenick et al. (1985) reported reproductive failure but normal sperm parameters in male rats exposed for 70 days to 100 ppm of ACR. The present study was designed to examine the dominant lethal effects of ACR in subchronically exposed male Long-Evans Rats. Males were given 0, 15, 30 or 60 ppm of ACR ad libitum in drinking water for 11 weeks. At week 10 each male was mated to two unexposed females. Pre- and post-implantation losses were evaluated at mid gestation. Half of the males in each group were sacrificed immediately after fertility testing. Fixed preparations were made of branches of the sciatic nerve and a testis was removed for cytogenetic analysis. ACR caused a dose-related increase in post-implantation loss. Pre-implantation loss was increased only at the high dose. No changes were seen by light microscopy in 1-2 sections of the branches of the sciatic nerve. No chromosome aberrations were found in spermatogonia or spermatocytes. This abstract does not reflect the policy or opinion of the U.S. EPA.


This study evaluated reproductive effects following the dietary administration of LONTREL herbicide (3,6-dichloropicolinic acid) to Fischer-344 rats. Male and female animals were fed 0, 150, 500 or 1500 mg/kg/day through 2 generations with 2 litters/generation. Parental effects were generally limited to rats fed 1500 mg/kg/day and included decreases in body weight, increases in liver weight, and observations of focal hyperkeratotic changes in the gastric mucosa of 1 of 30 F0 and 2 of 30 F1 males. Microscopic examination of parental reproductive organs (0 and 1500 mg/kg/day dose levels) revealed no treatment-related effects. No adverse effects on reproductive parameters or neonatal survival were noted in animals from F1a, F1b, F2a or F2b litters. Gross examination of all weanlings revealed no treatment-related lesions and no histopathologic changes were noted in tissues examined from F2b weanlings (0 and 1500 mg/kg/day dose levels). Based upon these results, the dietary administration of LONTREL herbicide to rats at dose levels up to 1500 mg/kg/day for 2 successive generations resulted in no adverse effects on reproductive performance.

*Trademark of The Dow Chemical Company.

1190 TRIDIPHANE: TWO-GENERATION DIETARY REPRODUCTION STUDY IN FISCHER 344 RATS. K.S. Rao, J.A. John, T.K. Jeffries, B.H. Scotttichini and J.T. Young. Mammalian and Environmental Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, MI 48674

A two-generation reproduction study was conducted to determine the effects of chronic ingestion of tridiphane [2-3(3,5-dichlorophenyl)-2-(2,2-trichloroethyl)oxirane], an herbicide, on the reproduction of rats. Male and female Fischer 344 rats, approximately 6 weeks of age (f generation), were given diets providing 0, 10, 30, or 30.0 mg tridiphane/kg/day through 2 generations with 2 litters/generation. Treatment-related effects in the f, and f1, adults at 30.0 mg/kg/day consisted of increased relative liver weights and decreased body weights. No adverse effects on fertility, reproduction or neonatal survival were observed among animals in the f or f1 litter. An apparently lower fertility index was observed at the two higher dose levels in the f2 generation. However, as these fertility indices were within the historical control range in this strain of rat in our laboratory, this observation was not considered to be treatment-related. Based on these results, it is concluded that dietary administration of tridiphane to rats at levels up to 30 mg/kg/day for two successive generations did not adversely affect the reproductive parameters evaluated.
A study was conducted to assess the potential adverse effects of Filarbits® Plus chewable tablets on the reproductive capabilities of the F0 and F1 generations and the development of the F2 generation through weaning. Filarbits® Plus was administered as a daily dose to one group (7 males, 14 females) of pedigreed AKC registered outbred Beagles. The F0 animals were exposed for a minimum of 75 days prior to breeding through termination. A dose of twice the intended use level was selected. A control group received placebo tablets at a rate equal to the treated group. All females were allowed to whelp. At approximately 49 days of age, the F1 pups were exposed for a minimum of 288 days prior to breeding through termination. Ten F2 offspring selected at random from each dose group and all F1 adult animals received a complete pathological evaluation. Clinical laboratory parameters were measured in the F1 and F2 generations. Administration of Filarbits® Plus chewable tablets to the F0 and F1 parental generation did not adversely affect survival, general physical condition or behavior, body weight gain or food intake. No pathological systemic effects were observed in the F1 and F2 generation dogs; clinical laboratory parameters were unaffected. Filarbits® Plus had no adverse effect on the reproductive capability of the F0 or F1 generations or the survival, growth and development of the F2 generation to weaning.

We have employed in vitro rodent embryo culture to assess the direct embryotoxicity of narrowleaf sunweed (Iva angustifolia), an abortive plant when consumed by cattle during the second to third trimesters of gestation. Whole Iva plants were air dried, ground, and extracted with ethanol for 24 hours; the extracted residue was dissolved in DMSO at a concentration of 1.3 mg of sunweed per ul, while Sprague-Dawley embryos, explanted at 10.75 days of gestation, were cultured with 50% Weymouth's media 75/25, 50% fresh rat serum, and 15 ul/ml of S9 fraction obtained from Amcol-Induced rats. Twenty four hours after culture initiation and treatment over a 10-fold dose range of Iva extract, embryotoxic effects derived from Iva-associated components included: decreased crown-rump length, deformities in the telenephalon, deformed tails, and absence of red blood cell circulation through the yolk sac. These findings support observations on Iva embryotoxicity in the field and present a useful system with which to more precisely identify those toxic Iva constituents present.

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ME given by gavage on gestation day (gd) 11 specifically induces paw malformations in CD-1 mice. This effect is significantly attenuated when ethanol (EtOH) is administered with or after ME. EtOH presumably reduces teratogenicity by competing with ME for oxidation by alcohol dehydrogenase and thus inhibits its conversion to methoxyacetic acid (MAA), the putative proximate teratogen. MAA has been identified as accumulating in rat embryos at concentrations twice those of maternal blood after ME exposure. In CD-1 mice dosed with labeled ME (350 mg/kg), gd 11 embryos accumulated 14C above that of maternal blood. The relevance of embryonal accumulation of radioactivity to ME teratogenicity was further examined by monitoring 14C (250 mg ME/kg) in maternal blood for up to 6 hr after gavage with or without EtOH (2 g/kg). The EtOH treatment, which attenuates ME teratogenicity, neither influenced 14C kinetics in maternal blood nor altered embryonal accumulation of 14C (embryo/whole blood 14C ratio = 1.7). Our observations suggest that either the chemical form of the accumulated 14C is not the teratogenic factor or that MAA is not the proximate teratogen in vivo.

The fetal tissue of pregnant C57BL mice exposed to 255 ppm of EtO for 6 hours/day is a sensitive indicator of the cytostatic effects of EtO (Toxicologist 5:89, 1985). In the present study, pregnant BALB/c mice were exposed twice/day for 10 minutes to 1500 ppm EtO for either 2 or 3 successive days between days 10 and 16 of pregnancy. Control and treated animals were sacrificed on days 13 through 17 of pregnancy about 18 hours after the last exposure. The weight of the uterus with contents and each embryo in its amniotic sac were obtained. Fetal viability was determined before the weight of each fetus was acquired. The highest fetal mortality (51%) was found in mice exposed on days 12-14 of pregnancy and sacrificed on day 15. Three days of exposure with a sacrifice on day 14 resulted in 41% fetal mortality and 3 days of exposure with sacrifice on day 16 resulted in 19% mortality. All other exposure/sacrifice regimens resulted in less than 5% mortality. Body weight of living fetuses was not grossly affected by the EtO exposures. It is concluded that there is a potential risk to the fetuses associated with the exposure of pregnant mice to short term high concentrations of EtO. (Research sponsored by OHER, DOE, under contract DE-AC05-84021400 with the Martin Marietta Energy Systems, Inc.)
Pregnant rats (N=7) were dosed o.p. with 005-TMP (LD50=87 mg/kg) at 20 mg/kg on days 6, 8, and 10. For reference of effect of body weight loss on fetal resorption, animals (N=7) were treated with corn oil and paired fed. Five naive animals were used for control. The abdomen was palpated on day 15 and if no mass was detected, a cesarean section was done on the same day. Otherwise rats were maintained until day 21. In four animals out of the seven treated with 005-TMP, complete resorption was observed on day 6, 15. All pair-fed and naive animals maintained pregnancy until day 21. Total number of resorption/number of implants (%) was 60/95 (63.2%) in 005-TMP animals, 21/82 (25.6%) in pair-fed animals and 4/65 (6.5%) in naive animals. Both the incidence of complete resorption and % resorption were significantly higher (P<0.03 and P<0.01) in comparison with those in pair-fed animals. By external, soft tissue and skeletal examinations, no malformation was detected in any fetuses. However, in 005-TMP animals, slight growth retardation was detected. Fetal resorption associated with 005-TMP treatment seemed to result from maternal toxicity.

Butyraldehyde (BA) and crotonaldehyde (CA) were selected for study as a result of an aldehyde class study. The chemicals were administered orally (gavage, corn oil) to male and female rats and mice for a total of 12 dose days. Feed and water were provided ad libitum. CA, but not BA showed dose-related mortality in rats and mice. Mean body weight gains were variably depressed in BA treated rats and mice, but a dose-related depression was observed in CA treated rats and mice. In BA, there were increased (P<0.05) liver body weight ratios in 2.5 and 1.25 g/kg treated male and female mice and 1.25 g/kg female rats. CA treatment resulted in increased (P<0.05) liver-BW ratio in the surviving highest dose (75 mg/kg) female rats. Lung-BW ratio in 37 and 75 mg/kg female rats was decreased. Both BA and CA induced stomach lesions in surviving animals. Gastric lesions (erosion, ulceration, inflammation, and hyperplasia) were dose-related. The no-effect level for oral administration was determined: BA (rats and mice) - 625 mg/kg, CA (rats and mice) - 18.75 mg/kg. These results were used to determine appropriate dose-levels for NTP 13-wk studies for comparing BA and CA toxicity by oral and inhalation routes of exposure to F344 rats and B6C3F1 mice.

This study was performed to investigate the delayed implantation induced by butyrophenone neuroleptics, haloperidol and timiperone, in rats. When female rats were intravenously administered either drug from day 0 to day 4 of gestation, the implantation of ova was delayed in a dose-dependent manner. The experiment was performed at a dose level of 6.4 mg/kg where the delayed implantation was observed in 83% of the treated rats. The ovum entry into the uterus, the development of ova, and the decidual reaction of the uterine endometrium were also delayed in the treated rats. Moreover, serum levels of LH and FSH, and ovarian venous blood level of estradiol were significantly lower in the treated rats than in the control rats. All of the delays in ovum development and transport, zona pellucida shedding, decidual reaction, and ovum implantation induced by the drugs were not observed by a single injection of 0.1 μg of estradiol or 20 IU of HCG or 20 IU of PMSC on day 3 of gestation. These results suggest that a decrease of estrogen secretion, which is due to a decrease of LH and FSH secretion, is attributed to the delayed implantation of ova by the neuroleptic drugs.
1199 TOXNET—THE NATIONAL LIBRARY OF MEDICINE’S NEW TOXICOLOGY DATA NETWORK. B.M. Vasta, H.M. Kissmeyer. National Library of Medicine, Toxicology Information Program, Bethesda, MD

The Toxicology Data Network (TOXNET) is a new data retrieval system, developed by the National Library of Medicine (NLM), that became publicly available on July 1, 1985. The initial TOXNET component files consist of an expanded Toxicology Data Bank (TDB) and a larger, more comprehensive file, called Hazardous Substances Data Bank (HSDB). TOXNET is expected to expand to include a number of factual databases related to toxicology, pharmacology, environmental sciences and occupational health. Soon to be added to TOXNET are the National Cancer Institute’s Chemical Carcinogenesis Research Information System (COCIRS) and, later, the National Institute for Occupational Safety and Health’s Registry of Toxic Effects of Chemical Substances (NTECS).

As a fully integrated software system, TOXNET is composed of several modules that provide for: (1) record building and editing; (2) online, interactive technical review of data records; (3) electronic mail and messaging; (4) in-process control and tracking of records in various stages of preparation and review; and (5) enhanced online search and retrieval. This presentation will highlight technical developments, scientific review of data content, Express Updaing of data records, and future plans for TOXNET and its files.


Calcitriol (1,25-dihydroxycholecalciferol) is under development as a parenteral supplement for the naturally occurring hormone which helps regulate calcium homeostasis. A six-month toxicity study was performed in which calcitriol was administered subcutaneously to rats at dosage levels of 0.006, 0.023 and 0.092 mcg/kg/day. Apparent signs of toxicity observed at 0.092 mcg/kg/day included emaciation, dehydration, weakness, corneal opacities and significantly decreased body weight gain and food consumption. Significant increases in urine output, urinary calcium and phosphorus values, relative organ weights (liver, adrenals, kidneys, heart, brain and testes) were also evident in rats given 0.092 mcg/kg/day. Microscopic lesions found at 0.092 mcg/kg/day included renal tubular nephrosis, thickening of trabecular bone and deposits of mineral in the various organs. Renal tubular nephrosis along with renal and enhanced osseous mineralization were also observed in some rats given 0.023 mcg/kg/day. The maximum tolerated dosage in rats for six months was considered to be 0.006 mcg/kg/day.


The acute toxicity of helenalin (HL), a potential antitumor drug, was examined in male BDF1 mice. The LD50 for a single i.p. dose of HL was 42 mg/kg. Mice receiving i.p. doses of 0, 3, 8, 15 and 25 mg HL/kg/day for three days exhibited significant reductions in liver, spleen and thymus weights, increased serum alanine aminotransferase (ALT) and urea nitrogen (BUN) levels, elevated hematocrits and polymorphonuclear neutrophil counts and decreased lymphocyte counts at the 15 and 25 mg HL/kg doses. Histologically, a substantial loss of cortical lymphocytes in the thymus was observed. Pretreatment of mice with diethylmaleate (3.7 mmol/kg 0.5 hr before the i.p. injection of 8 or 15 mg HL/kg) enhanced the lethality of HL and the ability of HL to increase serum BUN and sorbitol dehydrogenase (SoDH) levels. These results suggest that HL toxicity is dependent on the glutathione status of the animal. A single i.p. injection of 25 mg HL/kg increased serum ALT, SoDH and BUN levels within 6-12 hr. HL (25 mg/kg for three days) reduced liver microsomal cytochrome P450 and 65 contents by 74% and 37%, respectively. Aminopyrine demethylase and aniline hydroxylase activities were decreased by 70% and 75%, respectively. (ES 07126, CA 26666)


When the survival distributions are heterogeneous among the treated groups in an oncogenicity study, due to differential toxicity or uneven sacrifice schedule, unadjusted analysis of tumor incidences produce misleading conclusions. The standard practice among many investigators is to use a modified Hoel and Wasberg method as proposed by Peto et al in the famous IARC Monograph. However, because of intercurrent mortality differences, interval selection by the above method is difficult and will often produce incorrect conclusions regarding treatment effects. Alternative methods which are free of subjective interval selection and which can incorporate concomitant variables as well, are investigated with examples and the most efficient algorithm currently available in statistical literature is proposed for analysis of "incidental" tumor data.
ACUTE INTRAVENOUS TOXICITY OF CI-937, AN ANTHRACYCLINE ANTI-CANCER DRUG CANDIDATE. D. G. Pegg, S. N. Kim, Warner-Lambert/Parke-Davis Pharm. Res., Ann Arbor, MI

CI-937 is an intercalating agent which selectively inhibits DNA synthesis. In beagle dogs, single doses of 0.09, 0.18 and 1.8 mg/kg were administered to groups of two animals/sex. One animal/sex was sacrificed after eight days and the remaining animal after 63 days. After 1.8 mg/kg one male died and the remaining animals in the dose group were sacrificed in a moribund state on Day 8. These animals exhibited emesis and anorexia which progressed to include dehydration, diarrhea and decreased body weight. There were no treatment related clinical signs in the lower dose groups and no consistent electrocardiographic or clinical biochemical changes among groups. Dogs primarily in the 1.8 mg/kg group exhibited hypoplasia of intestinal mucosa, bacterial colonies in lymph nodes, tonsil, lung and intestine, and congestion and/or hemorrhage in heart, lungs, gastrointestinal tract and lymphoid organs. The most pronounced effect of CI-937 treatment was a severe reduction in total white blood cell count and marked depletion of hemopoietic cells in bone marrow, primarily in animals administered 1.8 mg/kg. The bone marrow of these animals was devoid of all immature cells of myeloid and erythroid series. There were no alterations in hematologic parameters of animals from the 0.09 mg/kg dose group.

PRECHRONIC DOSE FEED TOXICITY STUDIES OF 4,4'-THIOBIS-(6-T-BUTYL-m-CRESOL) IN RATS AND MICE. C.A. Mansur, I.A. Muni, American Biogenics Corporation (ABC), Woburn, MA, 01801, and L.S. Bierbaum, NIH, Research Triangle Park, NC 27709

4,4'-Thiobis-(6-t-butyl-m-cresol)(TBBC) is used as an antioxidant in the rubber industry. Prechronic studies by the dosed feed route were conducted in F344 rats and B6C3Fl mice of both sexes. The 14 day study animals were given TBBC in the diet at 0.00, 0.10, 0.25, 0.50, 1.0 and 2.5% (w/w). At these doses levels the target organs included the gastrointestinal tract, thymus and adrenal glands. After evaluating the 14 day study data, the subchronic dosed feed levels were set at 0.0, 0.025, 0.050, 0.100, 0.250 and 0.500% for rats and 0.0, 0.01, 0.025, 0.05, 0.10, and 0.25% for mice. In the subchronic study, leukocytosis with neutrophilia was noted in high level rats and an increase in immature neutrophils was noted in high level mice. Macrophage enlargement was noted in the liver and mesenteric lymph nodes of high dose level rats and mice. Cortical tubular necrosis of the kidney was also noted in high dose level rats. In rats, treatment with TBBC caused liver toxicity as evidenced by increased levels of alkaline phosphatase, GOT and GPT activities. Based on these data, it is concluded that TBBC toxicity targets the reticuloendothelial system in rats and mice and the liver and kidney in rats. (Supported by NIEHS Contract No. NO1-ES-28004-02).

IMPARED CLEARANCE, ELIMINATION AND METABOLISM OF PLASMA CHOLESTEROL ESTER ASSOCIATED WITH HYPERCHOLESTEREMIA IN MICE FED CYCLOPROPOEN FATTY ACIDS. J. F. Mallock, Exxon Corp. and J. E. Nixon, Oregon State University Sponsor: G. F. Egan

Swiss-Webster mice were fed corn oil control diet or 0.7% cyclopropeen fatty acid (CPFA) diet for eight weeks and dosed iv with an equimolar suspension of [3H]-cholesterol palmitate and [14C]-cholesterol palmitate. Blood decline of labeled sterol was biphasic. Within treatments, there were no differences in the in vivo plasma cholesterol ester metabolism, elimination kinetics or focal elimination rate for labeled sterol from [3H]-cholesterol palmitate or [14C]-cholesterol palmitate. However, compared to controls, CPFA animals diverted significantly more labeled sterol into saturated and diunsaturated cholesterol ester, less into mono- and tetra-unsaturated esters and showed decreased blood clearance and fecal elimination of labeled sterol. Biliary elimination was not impaired by depressed hepatic cholesterol ester synthesis activity in CPFA-fed mice. The fundamental effect of CPFA on serum cholesterol concentration appears to reside in a severely imbalanced cholesterol ester profile. Results indicate that CPFA alters normal fatty acid profile of serum cholesterol esters by proportionally altering the C-2 fatty acyl composition of serum phospholipid, which is the substrate for lathosterol acyltransferase, the major source of plasma cholesterol esters.

ULTRASTRUCTURAL CHANGES IN RATS TREATED NEUROTRANSLY 6-MERCAPTOPURINE. A. A. Abraham, B. Veronesi, E. R. Allegra and T. Balazs. NIH, Washington, DC. 2EPA, Chapel Hill, NC. 3FDA, Washington, DC.

We characterized the ultrastructural changes in hind-limb muscles of 2- and 6-month-old rats (2 control, 2 treated at each age) that were injected with 6-mercaptopurine monohydrate (6-MP) at 2 mg base/kg sc daily from 2 to 22 days of age. Light microscopically, focal Zenker-type degenerative changes were seen in treated rats at 2 months of age without atrophy of the involved muscles. The earliest ultrastructural alterations were disorganization of fibrils at the Z-bands and loss of myofibrils. In the most extensive lesions, the entire fiber was involved with vacuoles within the disorganized fibers. The sarcolemmal nuclei were large and showed a prominent nucleolus and folding of the nuclear membrane. In the six-month-old rats, similar lesions were seen with atrophy, fatty infiltration and replacement of the degenerated muscle fibers. Controls showed no changes. The peripheral nerves and vessels showed no alterations. Light microscopic examination of plastic-embedded thin sections of the brain, spinal cord at several levels, dorsal root ganglia and peripheral nerves revealed no remarkable. The latter negative findings, coincident with muscular pathology, argue against a primary neural involvement in this myopathy.

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Numerous chromatographic techniques (based on TLC and/or HPLC) have been reported for the resolution and detection of mixtures of mycotoxins. A major disadvantage of such techniques is the lack of chemical identification based on structural information. Our recent findings indicate that capillary GC/quadrupole mass spectrometry can be effectively employed as a one step analytical/diagnostic tool for the detection, quantification and structural confirmation of mixtures of mycotoxins from a variety of agricultural commodities and biological samples. Our basic procedure for sample cleanup and extraction involved partitioning homogenized samples by polarity with aqueous acetone and hexane, followed by extraction with chloroform. The chloroform extracts were combined, concentrated under nitrogen and redissolved in a known volume of solvent prior to GC/MS analysis (without derivatization). Extraction procedures and GC/MS conditions were modified according to chemical classes of mycotoxins in the mixture and source. Using this approach, we successfully resolved and detected mixtures of trichotheccenes (T-2 toxin, HT-2 toxin, T-2 tetraol, DON, and DAS), zearalenone, aflatoxins (B1, B2, G1, G2, M1 and aflatoxicol), ochratoxins (A, alpha, and 4-hydroxy A), and patulin and penicillic acid in samples of grain, mixed feeds, blood, urine and tissues. This procedure provides rapid and reliable multi-mycotoxin analysis. (Supported by U.S. Department of Agriculture Project 84-CRSR-2-2434 and USDA CRSP Project 02-53095-2).

These studies represent a continuation of our investigations of the application of drug carrier mechanisms as an approach in the antagonism of toxicants. In a prior report bovine rhodanese was encapsulated into murine erythrocytes by hypotonic dialysis to detoxify cyanide. This research is on the in vivo viability of these carrier erythrocytes in the rat and in one instance the chicken. Preliminary studies with $^{14}C$-sucrose as the biological marker indicate that after a single dose of $^{14}C$-sucrose loaded carrier erythrocytes, radioactivity-time data monitoring in blood indicated a biexponential decay. Initially, a rapid decline in $^{14}C$-sucrose radioactivity in whole blood ($t_{1/2}$ = 23 minutes) is followed by a slower decline ($t_{1/2}$ of 11 days). With the unencapsulated $^{14}C$-sucrose, greater than 90% was eliminated from circulation within 10 minutes. Any $^{14}C$-sucrose leaking out of the carrier erythrocytes is minimal since less than 2% of the initial dose of encapsulated $^{14}C$-sucrose appear in plasma. In view of these encouraging results with radioactive sucrose, survival studies on the circulatory life of rhodanese encapsulated murine rbc has been initiated. These preliminary results suggest that carrier erythrocytes represent viable approaches to the detoxification of cyanide. (Supported by funds from NIEHS, USAMRC, and Texas A & M Univ.)

The use of powdered compound/diet mixtures in oral rodent toxicity studies poses a risk of contamination of the animal room, personnel and control animals. We have demonstrated that this can be significantly reduced by pelleting the mixture.

The levels of contamination which occurred in the facility and on personnel with the pelleted and gavage regimes were generally five fold less than with the powdered regiments. Fluorescein contamination of the hair and plasma of the pelleted and gavage animals occurred in all 3 regiments demonstrating contamination of control animals with the test compound.

Whilst pelleting is indeed an effective way of reducing contamination, a background level appears inevitable. Fluorescein contamination of bedding is likely to have been a major source of this generalised contamination.
DETERMINATION OF 4-BROMO-METHYL-BENZOATE FOLLOWING CHROMIC ACID OXIDATION OF LIVER EXTRACTS FROM RATS TREATED WITH BROMIFACOUM OR BROMADIOLONE. A.C. Ray, M.J. Murphy, M.D. DuVal, J.C. Reagor. Texas Veterinary Medical Diagnostic Laboratory, College Station, TX

An assay to determine residue concentrations of the anticoagulant rodenticides bromifacoum (BF) and bromadiolone (BD) in liver was developed. Adult rats were fed baits containing either 50 ppm BF or 50 ppe BD in measured amounts (50-90 g, 3-4.5 mg BF or BD). After death occurred (5-7 days), the livers were ground with Na_2SO_4 and extracted with CHCl_3:CH_3OH (9:1). The residues were oxidized by heating with chromic acid solution after cleanup with silica cartridges. The primary oxidation product of BF and BD is 4-bromo-benzoic acid, which was extracted with CHCl_3 and methylated with trimethylsilyl hydroxide to form 4-bromo-methylbenzoate (BB). BB was analyzed using gas chromatography/mass spectrometry by monitoring total ion or single ion (183 or 185 m/ε) abundances. Concentrations of from 0.9 to 21 ppm BF or BD were detected in rat livers. BF or BD toxicosis in dogs was diagnosed by using this method for field cases.

DETERMINATION OF CYCLOPIAZONIC ACID IN SKELETAL MUSCLE BY HIGH PRESSURE LIQUID CHROMATOGRAPHY. W. P. Norred, R. J. Cole and J. W. Deer. Toxicology Unit, Russell Research Center, Athens, GA and National Peanut Lab, Dawson, GA, ARS/USDA

The toxic fungal metabolite, cyclopiazonic acid (CPA), is produced by Penicillium and Aspergillus fungi, and occurs as a contaminant of many foods and feeds. We previously reported that orally-administered CPA distributes largely to muscle of rats. Distribution in the meat of domestic animals could be a cause of concern for human exposure. Therefore a method for determining presence of CPA in muscle is needed. Varying amounts of CPA was added to ground chicken meat and chloroform/methanol (4:1) extracts were partitioned against 0.1N NaOH. The aqueous layer was acidified and CPA extracted with dichloromethane (DCM). Interfering components were removed from the DCM extract by minicolumn chromatography with petroleum ether and chloroform. The CPA-containing fraction was eluted with methanol:acetic acid (99:1), and subjected to ligand-exchange HPLC with a F-phosphonyl reversed-phase column and a mobile phase containing 4-dodecyltriethylamine, zinc acetate, ammonium acetate, 2-propanol, and acetonitrile. Recovery from spiked samples was 70.7% and 14.3% (n=38), and the analysis was linear from 0.016- to 16-mg CPA/kg muscle. Chickens were administered an oral dose of 0, 0.5, 5 or 10 CPA/kg body weight. After 3 hr, meat of CPA-dosed birds was analyzed by the method and contained ca. 20% of the CPA dose.

A THIRTEEN WEEK FEEDING STUDY ON DIISONONYL PHTHALATE (DEP) IN RATS. M. O. Bird, R. W. Kape, C. A. Keller*, A. W. Linton. Exxon Corporation, Research and Environmental Health Division, Linden, NJ. *Exxon Chemical Company, Linden, NJ.

Groups of 15 F-344 rats per sex were fed diets containing 0, 1,000, 3,000, 6,000, 10,000, and 20,000 ppm of DEP for a period of 13 weeks. There were no deaths prior to study termination. A significant depression in food consumption and body weight gain was noted in the high dose group. No significant hematologic changes were observed. Clinical chemistry values for triglycerides showed a dose-related decrease while albumin and globulin ratios showed a dose-related elevation. Absolute and relative kidney and liver weights of various treated groups (≥ 3,000 ppm) were increased as compared to controls. Kidney lesions (nephrotic and regenerative changes) were found only in males treated with ≥ 3,000 ppm DEP. A dose-related diffuse cytoplasmic eosinophilic hyper trophy of liver hepatocytes was observed in all treatment groups. Electron microscopy examination of the liver, showed a dose-related proliferation of peroxisomes and smooth endoplasmic reticulum of groups exposed to ≥ 6,000 ppm. In summary, high doses of DEP produce liver and kidney effects in rats with a no observed effect level at 1,000 ppm.

GLYCOLIC ACID DETERMINATION BY HIGH PRESSURE LIQUID CHROMATOGRAPHY FOR DIAGNOSIS AND MANAGEMENT OF ETHYLENE GLYCOL INTOXICATION IN HUMANS. T.P. Hewlett, A.J. Lauro, and K.E. McMartin. Louisiana Regional Poison Control Center and Department of Pharmacology. Section of Toxicology. Louisiana State University Medical Center, Shreveport, LA and Emergency Department, Charity Hospital, New Orleans, LA.

Glycolic acid is the metabolite of ethylene glycol (EG) that accumulates in the highest concentration and may be the major contributing factor to the acute toxicity of EG. Serum and urine levels of glycolic acid have been found to correlate directly to clinical symptoms and mortality in poisoning cases, making it a valuable diagnostic tool. A high pressure liquid chromatographic method (HPLC) for quantitation of glycolic acid in serum was used in three cases of ethylene glycol ingestion presented to the Louisiana Regional Poison Control Center. Serum levels of EG were determined by gas chromatography and levels of each case upon admission were as follows: 9 mg%, 15 mg% and 142 mg%. In the latter case, the glycolate levels were 12.2 mmol/L and 14.7 mmol/L within the first two hours after ingestion. These levels quickly dropped to zero when ethanol therapy was initiated even when EG levels were still over 30 mg%. The data collected in this study supports the use of glycolate determination by HPLC to monitor the status of patients poisoned by EG.

When an insect is intoxicated by pyrethroids, it quickly develops hyperexcitation and tremors, which are followed by paralysis. We have done acute toxicity studies with the extracts in mice and rats and observed initial sedation, excitement, tremor, abnormal locomotor activity, prostration, paralysis and death. The LD50 (i.p.) in mice and rats were 2.20 mg/kg and 1.80 mg/kg respectively. We hypothesized that the extracts have direct inhibitory effects on acetylcholinesterase leading to accumulation of acetylcholine which in turn cause some of the observed toxic symptoms. The effects of the extracts on acetylcholinesterase was determined by spectrophotometric method using purified erythrocyte acetylcholinesterase. A 3.3x10^-6 mg/ml and 3.3x10^-5 mg/ml inhibited the enzyme by 11% and 2% of control respectively. However, higher doses, 3.3x10^-5 mg/ml, 3.3x10^-4 mg/ml and 3.3 mg/ml stimulated the activities of acetylcholinesterase (% of control) by 8, 15 and 48 respectively. Although the results of the effects of the extracts are at variance with our working hypothesis, the positive modulation of the enzyme by pyrethroid has not been reported before and therefore provide an interesting research frontier.

This investigation was supported by Grant 3506 RR00808 from NIH.

SODIUM PENTOBARBITAL ADSORPTION BY SUPERCHAR, DARCO G-60, AND USP ACTIVATED CARBON. C.D. Caud, and J.J. Stewart, Dept. of Pharmacol. and Ther., Louisiana State University School of Medicine, Shreveport, LA. Sponsor: J.E. Mann.

A study was designed to compare the relative adsorption of sodium pentobarbital (SP) by SuperChar, Darco G-60 and USP activated carbons. Solutions of SP ranging in concentration from 1.25 to 160 mg were prepared in distilled water with (14C) added to serve as a concentration marker. Two ml of each solution was added to test tubes containing from 10 to 320 mg of the activated carbons. The pentobarbital-charcoal slurries were mixed thoroughly and incubated at 37°C for 10 mins. The concentration of drug remaining in the supernatant was determined and the amount of drug bound by the activated charcoal was then calculated. The data were analyzed by two methods. Langmuir isotherms were used to calculate the maximum weight of solute adsorbed per unit weight of charcoal (Qm, mg/g). Qm (mean ± SEM) was 102.7 ± 6.7, 211.7 ± 21.1, and 424.1 ± 24.3 for Darco G-60, USP, and SuperChar respectively. Plots of percent drug bound vs. quantity of charcoal were used to calculate the amount of charcoal required to bind 50 percent of the drug (B=50). B=50 values were largest for Darco G-60, followed by USP and then SuperChar. Both methods establish that relative adsorption of SP is greatest for SuperChar, followed by USP and Darco G-60, respectively. (Supported by NIH grant RR-03922-06).

AN IMPROVED "FLOATING SHEET" METHOD OF ULTRA-STRUCTURAL PREPARATION OF CULTURED CELLS FOR TOXIC STUDIES. J.R. Arnold, R.L. Harrison, R.M. Hymaith, P.J. Box, Department of Pathology, University of Texas Medical Branch, Galveston, TX. Sponsor: A.E. Ahmed

Cultured cells provide isolated systems for both biochemical and morphological examination of toxic effects. Previous methods of processing cell culture specimens for electron microscopy (SEM) have been limited to sectioning either a monolayer or centrifuged cell suspensions which are not morphologically intact. In our improved method, N-butyl glycycyl ether is added to cell cultures (2-5 min with agitation) following in situ fixation (3.0% glutaraldehyde in 0.1M Pipes pH 7.2 for 20 min, osmium tetroxide 4% for 20 min). A thin pliable "sheet" of cells floats free from the plastic culture device and can be manipulated (centrifuged or folded) to obtain a vast number of morphologically intact cells for examination. We have examined several cell types (vascular smooth muscle, lung, liver, and endothelial cells) grown in two types of plastic culture flasks (Nunc and Falcon). This new method provides excellent EM morphology, maximizes number of cells examined and allows determination of cell orientation since a remnant of the dissolved flask remains loosely bound to the bottom of the cells. Supported by NIH Grants HL 26189 and HE 00929.

CHARACTERIZATION OF POTENTIALLY NEUROTOXIC EFFECTS USING AN IN VITRO HIPPOCAMPAL SLICE TEST BATTERY. S.B. Foutain and T.J. Teyle. Department of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272. Sponsor: Z. Annau.

Studies were conducted to determine the feasibility of using the in vitro hippocampal brain slice preparation for characterizing potentially neurotoxic effects of chemical agents during the initial stage of neurotoxicologic assessment. The brain slice technique involves maintaining living slices of rat hippocampus in vitro at the interface of a medium pool containing artificial cerebrospinal fluid (c.s.f.) and an atmosphere providing high oxygen content. Agents of interest were introduced either as vapor via the atmosphere of the slice chamber or in solution via the artificial c.s.f. medium pool. Established electrophysiological methods were used for differentiating the effects of agents on excitatory systems, inhibitory systems, and properties of neuronal plasticity. Ethanol and aspiramine exposure potentiated the response of hippocampal CA1 pyramidal cells, whereas amnion exposure suppressed the excitability of CA1 cells. In contrast, acetone exposure compromised recurrent inhibitory systems. Thus the hippocampal slice technique can begin to differentiate possible mechanisms underlying potentially toxic effects during the initial stages of assessment. (Supported by NIDA 03755).
The Draize eye irritation evaluation is the standard test for determining the eye irritation classification of industrial and agricultural chemicals. With many chemicals and formulations, a period of up to 21 days of observation may be required to assign an eye irritation classification. Since eye irritation classification is primarily based upon the duration of corneal involvement, it was reasonable to assume that early changes in the cornea may reliably predict the eye irritation classification. In order to test this hypothesis, a variety of test materials including aqueous solutions of sodium hydroxide, alcohols, cyclohexanone, hexane, and a shampoo were tested for eye irritation in rabbits. Eight rabbits were used for each test material. A 0.1 milliliter aliquot of each test material was placed in the conjunctival sac of the left eye in each rabbit. The eyes were evaluated for irritation and corneal thickness for up to 21 days using a slit lamp with a pachymeter attachment. The ratio of the corneal thicknesses of the treated eye to the control eye for the first 3 days had a high correlation (0.86) with the duration of corneal cloudiness. This could shorten the observation period and reduce the need for the use of laboratory rabbits.

As a means of exploring the biochemical mechanism underlying surfactant production in the lung, the incorporation of [1-14C]palmitate or [Methyl-1-14C]choline or [2-14C]acetate into lipid in freshly isolated type II cells was compared with that in alveolar macrophages. Cells isolated from rat lungs and suspended in a phosphate-saline buffer were incubated with 14C-labeled precursors. Among all the substrates used in this study, palmitate was the most actively utilized by type II cells. In freshly isolated type II cells, the incorporation of [1-14C]palmitate into phosphatidylcholine (PC) and phosphatidylglycerol (PG) were one to two- and five to six-fold higher, respectively, than in the macrophages. However, the incorporation of [1-14C]palmitate into tripalmitin was three to four-fold higher in the macrophages than in the type II cells. Type II cells and macrophages synthesized similar quantities of total lipid from [Methyl-14C]choline with PG accounting for more than 80% of the total lipid. The incorporation of [2-14C]acetate into total lipid was at least 10-fold higher in the type II cells than in macrophages. The results suggest that the response of type II cells to toxic substances that may compromise the synthesis or secretion of surfactant could be studied in freshly harvested type II cells in vitro.

Acute oral toxicity tests require a single dose to young rats, traditionally delivered by gavage. Certain plastic resin powders are not miscible with aqueous or oil based liquids; gavage dosing can lead to particulate separation and inaccurate dosing. Thus, a system of oral dose self-administration was developed. For 1-2 weeks prior to study initiation, young rats (95-130 g) are trained to eat a mash diet as one meal per day. Initially, exposure to the meal is 8 hours/day. Later, it is reduced to 4 and then to 2 hours/day for 3 days prior to testing. On the day of the test, test article is mixed with 3 g of mash diet and offered to the rats. After the mixture is consumed (within 1-2 hours), the rats are offered mash diet ad libitum for the 14 day test period. Meal-fed rats essentially maintain body weight prior to study initiation and show a compensatory increase in weight gain after ad libitum access to diet. A meal-feeding regime may compromise the normal response to the additional stress of test article administration. However, since the plastic resin powders have acute oral toxicity of greater than 5 g/kg in meal-fed rats, this method is useful as an initial screen.

Many commercial microencapsulation processes are inappropriate for encapsulating chemicals for toxicology studies. An evaluation of these processes has shown some to be of limited utility due to unacceptable residues of solvents, toxic shell reactants, indigestible coatings and adverse reaction with the test chemical. Other factors considered in process selection were adequate chemical load and shell permeability. The effect of the encapsulation process on the test chemical also was studied. Methods for evaluating the integrity of encapsulated chemicals by an impurity profiling method will be described.
It is common clinical practice to adjust drug dosage given to a patient with altered pharmacokinetics so that the steady-state serum concentration (Cp) is the same as in normal patients. This should also be done in experimental studies of the interaction of drug toxicity and a disease state. Dosage adjustment reduces average Cp but the resulting concentration-time profiles of the normal and diseased groups will be of different shapes. Model-independent deconvolution analysis was utilized to derive the infusion schedule needed to achieve a constant Cp followed by a predetermined nonexponential decline. The resulting exponential infusion was applied using a microcomputer driven pump to attain identical serum gentamicin profiles in pairs of a subtotally nephrectomized (NX) and normal dog during a 12 hour infusion. Preinfusion terminal elimination slopes (h⁻¹) were 0.85 ± 0.09 in normal and 0.37 ± 0.03 in NX (mean ± SEM) compared to 0.18 ± 0.02 and 0.21 ± 0.01 from the controlled decline, supporting the feasibility of this approach. [Supported by NIH NO1-CM-AM 31062 and NIHES - DE 67046.]

1225 A CORRELATE IN VITRO AND IN VIVO STUDY OF SKELETAL MUSCLE IRRITANCY

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The experimental evaluation of skeletal muscle injury is usually based upon various morphological and biochemical parameters measured in the intact animal. The purpose of this study was to evaluate injury produced in rat skeletal muscle following a single IM injection of a number of therapeutic agents, and to correlate various morphological and biochemical alterations with the results obtained from testing the same agents in vitro. Local skeletal muscle injury was evaluated in vivo by observing and grading the lesions based upon the degree of hemorrhage, necrosis, and measurement of enzyme activities (CK, LD, AST). Irritancy was evaluated in vitro using cultures of transformed rat skeletal muscle myoblasts (L-6) and cells derived from trypsin disaggregated newborn rat skeletal muscle. Gross morphology of the cultures, cell viability, and the measurement of enzyme activity following a 24 hour treatment with log dose concentrations of the test agents was used to examine cell injury. A direct correlation was made between the injury observed in vivo and the extent of injury in vitro. This model system may represent an alternative to in vivo testing of skeletal muscle irritancy.

1226 DETERMINATION OF QUATERNARY AMMONIUM METABOLITES BY MASS SPECTROMETRIC ANALYSIS


Quaternary ammonium metabolites are often difficult biotransformation products to identify and quantify in biological fluids, due to their water solubility, high polarity and non-volatility. This study utilizes HPLC-mass spectrometry in the structure elucidation of the quaternary N-methylated urinary metabolites of R-(+)-nicotine in the guinea pig. The relative usefulness of electron impact (EI), chemical ionization (CI), thermal spray and fast atom bombardment (FAB) techniques for the identification of quaternary nicotine metabolites after prior separation by cation-exchange HPLC, are compared. A procedure, involving initial reduction of quaternary nicotine metabolites with sodium borohydride, followed by GLC-electron impact mass spectrometric analysis is presented as an alternative method to direct analysis. Analysis in the EI and CI mode does not afford a molecular ion, but gives spectra of the corresponding parent N-demethylated compound. Thermal spray and FAB both give a good molecular ion but no fragmentation was observed. Reduction and GLC/MS gives a volatile derivative that afforded good diagnostic spectra of the quaternary N-methylated nicotine metabolites. [Aided by a grant from the Tobacco and Health Research Institute, Lexington, KY.]

1227 A NOVEL IN VITRO CULTURE SYSTEM FOR CARDIOTOXIC (CDT) DRUG SCREENING


Animal models of CDTX are expensive, time-consuming and can depend on semi-quantitative histopathological assessments of CDTX. Similarly, many in vitro methods rely on non-quantitative assessments of beating rates and enzyme leakage as indicators of cell viability. Because of these limitations, we developed a novel culture system for plating viable rat neonatal myocytes in a glucose-free medium. Optimal cultures were obtained using hearts from 3-4 day old rats digested with 0.2% crude trypsin. A "pour off" technique reduced fibroblasts to 5%. Intracellular ATP content (by luciferase bioluminescence) was selected as a novel index of viability based on ATP's essential and quantitative role in maintaining metabolically active tissues. ATP values were normalized to total cellular protein. Developmental studies revealed cultures at Day 4 to be optimal for ATP/protein ratios (15.4 ng/mg), beating rates (120 beats/minute), DNA synthesis indices (11.1%) and low % fibroblasts (8.1%). Day 4 myocyte cultures were then used to evaluate CDTX of a model antitumor agent, doxorubicin. Preliminary results showed a time- and dose-dependent decrease in both the ATP/protein ratio and in beating rates following DOX exposure. ATP/myocyte cultures represent a novel system for rapid and sensitive CDTX drug screening. [Supported by NCI-CA-17094.]
1228 SELECTED ORGAN PATHOLOGY FOLLOWING ACUTE STREPTOZOTOCIN IN GUINEA PIGS: MODIFICATION BY INSULIN THERAPY. M.J. Schlosser, A.W. Bannister and A.J. Verlander. Atherosclerosis Research Laboratories, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS. Sponsor: W.L. Guess.

Streptozotocin (STZ) administered acutely to rats and mice provides researchers with a model of insulin-dependent diabetes. We have previously shown specific organ weight and histopathological changes following STZ in the guinea pig, a species rarely used in diabetes research. The present study will assess whether these changes are due directly to STZ or to the diabetic condition STZ produces. Male Dunkin-Hartley guinea pigs received STZ (150 mg/kg, intracardiac) or vehicle (control). After 5 days, STZ animals were given daily s.c. injections of either insulin (STZ-INS) or saline (STZ-SAL) for 25 days. Controls were treated with saline. Insulin improved the initial body weight loss of the STZ-INS group. Relative organ weights (g/kg) of the adrenal, kidney and spleen were significantly elevated in the STZ-SAL group compared to controls, while similar increases were not seen in STZ-INS animals. Both STZ groups had a higher relative heart weight than control animals. Histopathological changes occurred in the kidney and spleen of STZ-SAL animals; these changes were not as evident in the STZ-INS group. Insulin therapy, therefore, appears to prevent or reverse organ pathology associated with acute STZ administration in guinea pigs. (Supported by AHAFA, Washington, D.C.).


A comparison of the response observed in the rabbit vaginal model with that of the ovariectomized rat and the sheep was conducted using superabsorbents. The rabbits and rats which do not readily retain solid materials intravaginally were dosed daily for 10 days with 24 hour saline extracts of four superabsorbent laminate materials. The laminates were placed in the sheep vaginas and replaced each day for ten consecutive days. Observations of each species were made each day for external vaginal irritation. On the tenth day, the animals were sacrificed. A gross necropsy was performed on all animals and the following tissues were taken and fixed for histopathological evaluation: urinary bladder, vagina, cervix and uterus. There was no mortality during the study and no significant external irritation or unusual observations attributed to test materials. Gross necropsy revealed minor irritation to the sheep vaginal epithelium consistent with and attributed to the dosing procedure. Histopathology revealed no unusual observations. Thus, one can conclude that the four superabsorbent laminates studied produce no significant vaginal irritation or toxicity to the observed organs.


The oxidative derivatives of uroporphyrinogen and coproporphyrinogen are excreted in increased amounts in inherited and chemically induced porphyric diseases. An alteration in the normal isomeric profile of uro or coproporphyrin would be expected to be of value in detecting an impairment of heme biosynthesis in various tissues. Previously published porphyrin extraction methods were not found to be applicable to tissue samples since the porphyrin extraction procedures were concerned primarily with blood and urine and require a long series of differential extractions. A simple rapid procedure has been developed, utilizing disposable C18 mini-columns, for extraction of uro and coproporphyrin isomers from biological tissues. The relative ratios of known standards of uro I and III and copro I and III were obtained from liver, kidney, testes and bone marrow of the rat. The extracted samples were analyzed by HPLC. The recoveries from liver, were 90, 99, 89, and 96 percent for copro I, copro III, Uro I and Uro III respectively. Similar recoveries were obtained with kidneys, testes and bone marrow. This method is suitable for the study of chemically induced porphyria characterized by alterations of the ratios of the I and III isomers of uro and coproporphyrin. (NIH grant #ES-02524).

1231 COMPARATIVE STUDY ON EMBRYOTOXICITY OF NICOTINE ADMINISTERED BY INJECTION AND INFUSION. M. Prevo and R. Ballard, San Jose State University, San Jose, CA. Sponsor: D. Halley.

This study evaluated the effects on embryotoxicity of nicotine administered via an ALzet mini-osmetic pump compared to twice daily injections. Timed pregnant rats were treated with 0-13 mg/kg/day nicotine. On Day 8 of pregnancy, a pump was implanted s.c. or twice daily s.c. injections were begun. Dams were killed on Day 16. Traumatic maternal responses, ranging from tremors to severe convulsions and coma, were seen after s.c. injections of nicotine and were dose-dependent. This was not observed in rats implanted with the pumps. Both dosing methods resulted in a dose-dependent decrease in fetal weight, length, and placental weights. Continuous infusion of nicotine resulted in a slight shift to the left in the dose response curve, indicating increased embryotoxicity curve compared to injection. Plasma nicotine levels for the highest dose showed that continuous infusion produced more stable levels than the nicotine injections. This research indicates that the continuous administration of nicotine was a viable substitute for the intermittent injections attributed to test materials. Evaluation of a compound by a regimen and at doses relevant to the use situation should be used in the study of the pharmacological and toxicological properties, and direct comparisons of pulsed and continuous administration are relevant.

Animal models were compared to evaluate effects of drug formulations on gastrointestinal mucosa. Cross-model assessment was done on cat esophagus (Carlberg & Densert, Eur. Surg. Res., 12:270, 1980), dog intestine and colon (Casper), and rabbit colon. Under anesthesia, a 15-20 cm bowel segment is opened along its antimesenteric border and, with blood supply intact, is clamped in a multichambered cell to form the floor of each chamber. Each chamber is perfused with 37°C Ringer's at 2 ml/min; test agents are placed directly on mucosal tissue for up to 8 hr. More than one substance can be evaluated at the same time on adjacent areas and exposure time varied to allow macroscopic observation of events as they progress and histologic study later. Tissues are scored and an index (0-27) used to reflect severity and area of irritation. Using Slow-K9 as a positive control because of its documented human g.i. toxicity, 3-h exposure on rabbit colon yielded the following irritation indices for commercial products: doxycycline, 27; propranolol, 17; Slow-K9, 14; aspirin, 5; and for drug formulations: Na indomethacin, 4; Na naproxen, 2; ibuprofen, 0.1; indomethacin, 0; placebo, 0. We found the rabbit colon model to be sensitive, reliable, and representative of all models evaluated to assess topical g.i. irritation.


Met-Enk is a naturally occurring opioid pentapeptide (Try-Gly-Gly-Phe-Met) that may be involved with intercellular communication in the nervous, endocrine, and immune systems. Because of potential therapeutic utility of this peptide, subchronic intravenous toxicity studies were performed in rats (28 days) and dogs (27 days). Met-Enk was administered intravenously at dosages of 1.25, 6.25, and 25 mg/kg at rates of 2 ml/min (rat) and 2-4 ml/min (dog). Treatment groups were compared on the basis of survival, overt signs of toxicity, body weight gain, changes in organ weights, ophthalmological assessment, urinalysis, hematological and clinical chemistry profiles, and gross and microscopic tissue changes. No lethality occurred in either species. Clinical signs observed only in dogs treated with Met-Enk included emesis, excessive salivation, diarrhea, and apparent hyperphagia in all dosage groups. Relaxation of the nictitating membrane also occurred, preponderantly in the higher dosage groups. No toxicologically significant findings were evident in any of the clinical pathology parameters evaluated or in the gross or microscopic tissue assessments of either species.


In order to conduct intravenous (i.v.) preclinical safety studies for a new drug with minimal aqueous solubility, a series of eleven vehicles were developed that contained non-aqueous solvents, complexing agents, or surfactants to enhance the drug's solubility. The vehicles had in common the capability of solubilizing the drug to approx. the same extent. The vehicles (and saline, as control) were given i.v. to rats as a single bolus dose of 2.5 ml/kg once daily for two weeks. The parameters evaluated included general clinical condition (including the tail vein injection area), body weights, hematologic, clinical chemistry, and urinalysis parameters. Clear differences in acceptability were discerned among the vehicles. In general, vehicles containing ethanol, propylene glycol, and/or polyethylene glycol and the vehicle with Emulphor® EL-719 had fewer adverse effects than other vehicles. The types of adverse effects seen included changes at the tail vein injection area, changes in general clinical condition, decreased body weights, and hemolysis as evidenced by red-brown urine and/or urine with occult blood. This in vivo hemolysis data corresponded with in vitro hemolysis data. From these data, several of the vehicles were judged to be acceptable for use in i.v. preclinical safety studies.

1235 EFFECT OF HEXACHLOROBENZENE ON THE SPECIFIC BINDING OF 2,3,7,8-TCDD TO THE HEPATIC AHH RECEPTOR. M.L. Hahn, T.A. Gasiewicz, J.A. Goldstein*, and P. Link*. Div. of Toxicology, University of Rochester, Rochester, NY 14642; and *NIHES, Research Triangle Park, NC 27709.

In the rat, both hexachlorobenzene (HCB) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induce the hepatic cytochrome P-450 isozymes P-450c and P-450d and produce a similar hepatic porphyrin. To investigate the possibility that these effects of HCB are mediated by the Ah receptor, as proposed for TCDD, we examined the ability of HCB to interact with this receptor in vitro and in vivo. HCB, up to 1.0 μM, was not potent in vitro compared to the specific binding (SB) of [3H]-TCDD (0.3 nM) to rat hepatic cytosol. In a separate experiment, the SB of [3H]-TCDD was measured in hepatic cytosol of rats fed a diet containing 3000 ppm HCB for various times (4 to 7 d). The SB of [3H]-TCDD was decreased after 4 h on the HCB diet; maximal reduction (to approx. 65% of control) occurred after 1 to 7 d of HCB treatment. The decrease in binding appeared to be a reduction in the total number of binding sites, although an effect of HCB on the affinity of the receptor for TCDD cannot be ruled out. These results suggest that HCB may be able to interact with the Ah receptor in vivo. The role, if any, of this interaction in the P-450 induction and porphyrin caused by HCB remains to be determined. (Supported in part by NIH Grant ES02515 and EHS Center Grant ES01247.)
1236  EFFECT OF THYROID STATUS ON AH RECEPTOR AND
ENZYME INDUCIBILITY BY 2,3,7,8-TCDD IN RAT LIVER.
E.C. Henry and T.A. Gasiewicz, Div. of Toxicology, University of Rochester, Rochester NY 14642

In rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), serum thyroxine is decreased and TSH may be elevated. Furthermore, hypothyroidism has been shown to offer some protection from TCDD-induced weight loss, lethality, and immunotoxicity (Rozman et al, Tox Appl Pharm 72, 372, 1984). We therefore compared (1) Ah receptor properties and (2) TCDD-inducibility of enzymes (a receptor-mediated response) in livers of thyroidectomized (TXH) and euthyroid rats. Post-mortem, however, the protection afforded by hypothyroidism. In untreated THX animals, there was no consistent or signif.
ificant quantitative or qualitative change in Ah receptor compared to pair-fed or ad libitum con.
trols. Total cytochrome P-450 level and activity of NADPH-menadione oxidoreductase (NMOR) were not altered by thyroid status, while 7-ethoxycoumarin O-deethylation activity (ECOD) was approximately 50% lower in TXH animals. At 3 and 10 days after TCDD treatment (10 μg/kg, i.p.), P-450 concentrations, and NMOR and ECOD were induced by approximately the same proportion in both TXH and pair-fed intact rats. However, the absolute levels of induced enzyme activities in THX groups were lower than in pair-fed controls. It was concluded that hypothyroidism does not modulate TCDD toxicity via changes in the hepatic cytosolic Ah receptor.
(Supported by NIH Grants ES02315, ES02859 and Center Grant ES01247)

1238  UPTAKE OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
(TCDD) FROM PLASMA LIPOPROTEINS BY CULTURED HUMAN

The effects of human plasma lipoproteins on cellular uptake of TCDD were studied using normal human skin fibroblasts and familial hypercholesterolemia (FH) mutant fibroblasts which lack the normal cell membrane receptor for low density lipoprotein (LDL). Confluent monolayers of cultures were incubated in medium with serum- or lipoprotein-associated [3H]TCDD to study time- (10-240 min.), concentration- (5-50 μg LDL/ml) or temperature- (4 or 37°C)-related uptake of the toxin. Cellular uptake of [3H]TCDD was greatest from LDL, intermediate from high density lipoprotein (HDL) and least from serum. A significantly greater uptake from LDL by the normal cells compared to the mutant cells indicated the involvement of the LDL receptor-mediated pathway. Cellular uptake at 37°C of [3H]TCDD varied linearly with the toxin conc. at constant LDL conc. Since [3H]TCDD could be removed from cells efficiently by incubation with 20% serum > HDL > LDL, it is suggested that the physiologic vehicles (either serum- or LDL-associated TCDD), rather than organic solvents, should be used in experiments with cultured cells or perfused organs for metabolism studies.

1239  PATTERNS OF HEPATIC ENZYME ACTIVITIES FOLLOWING
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) TREAT-
MENT IN THE RAT. C.K. Kelling, L.A. Menahan, and
R.E. Peterson, University of Wisconsin, Madison, WI.

To assess functional significance of changes in serum thyroid hormone levels following TCDD treatment, activities of several hepatic enzymes were examined in adult male rats pretreated with TCDD (6.25, 25 or 100 μg/kg) for one week. Serum concentrations of L-thyroxine at all doses tested. Triiodothyronine was unchanged at 6.25 and 25 μg/kg and elevated by 20% at 100 μg/kg TCDD. Total liver mass increased proportional to dose, but total hepatic DNA content remained unchanged. Total liver protein content and that per mg DNA were increased in TCDD-treated rats when compared to pair-fed rats. Specific activity (μg/mg protein) of succinic dehydrogenase and a-glycerol phosphate dehydrogenase (a-GPDH) was unchanged or slightly decreased, respectively, in livers of TCDD-treated rats. Specific activity of the cytosolic marker enzyme lactate dehydroge-

nase was increased in all TCDD-treated groups to a similar extent. However, the specific activity of three NAD-dependent cytosolic enzymes ("ma-
ic" enzyme (ME), glucose-6-phosphate dehydro-
egase and 6-phosphogluconate dehydrogenase) were increased following administration of TCDD in a dose-dependent manner. Activities of two thy-
roid-responsive hepatic enzymes, a-GPDH and ME in TCDD-treated rats would suggest that thyroid status was not altered by TCDD (NIH Grant ES01332).
BIOCHEMICAL AND TOXICOLOGICAL EFFECTS OF TCDD CONGENERS IN FEMALE RATS. M.A. Shara and S.J. Strehl. University of Nebraska Medical Center, Omaha, NE

The capacity of six TCDD congeners to induce toxicity and biochemical changes was investigated relative to TCDD. The following were administered at a dose of 40 or 400 µg/kg/day for 3 days P.O.: 2,3,7,8-tetrachlorodibenzop- dioxin (TCDD); 1,2,4,6-tetrachlorobenzop-p-dioxin; 2,3,7,8-tetrachlorodibenzo-p-dioxin (Eq. 1); 3,3',4,4',5-pentachlorobiphenyl (PCB); 2,2',4,4',5,5'-hexachlorobiphenyl. Six days after treatment the animals were killed. Lipid peroxidation and glutathione peroxidase (GSH-Px) activity were determined in liver and kidney. Hepatic arylhydrocarbon hydroxylase (AHH) activity was determined 48 hrs following the administration of 400 µg/kg of each congener or 40 µg/kg of TCDD. With the exception of PCB and TCDD, the other congeners showed no toxicity at the doses given as determined by the above parameters. GSH-Px (400 µg/kg) resulted in a 4-5 fold increase in lipid peroxidation and a 65% decrease in GSH-Px activity. These results were comparable to the effects of a 40 µg/kg dose of TCDD. PCB treatment resulted in a 67% decrease in AHH activity, and a 5-6 fold increase in AHH activity which was comparable to the effects of TCDD. A correlation appears to exist between the ability to induce AHH activity and lipid peroxidation.
The toxicity of TCDD has been examined in C57BL/6J (B6) mice, which have a high affinity cytotoxic Ah receptor for TCDD, and DBA/2J mice, which lack this receptor. The Ah receptor has been correlated with the toxic effects of TCDD, with B6 mice being more sensitive to TCDD than DBA/2J mice. The metabolism of TCDD was examined by incubating hepatocytes isolated from control and TCDD pretreated B6 and DBA/2J mice with purified 14C-TCDD (2.2 μM) for 8 hr. Mice were pretreated with TCDD at doses that maximally induced ethoxyresorufin-O-deethylase (EROD) activity, a measure of Ah locus responsiveness to TCDD (B6, 3 μg/kg, ip; D2, 30 μg/kg, ip). Control B6 and DBA/2J hepatocytes were no different in cytochrome P-450 content, while TCDD pretreatment increased P-450 content 4-fold in B6 and 3-fold in DBA/2J mice. Hepatic EROD activity was similar in control B6 and DBA/2J mice (81.7 and 101.7 pmol/min/mg P-450, respectively), and was increased 18-fold in B6 and 10-fold in DBA/2J mice after TCDD administration. The rate of hepatic TCDD metabolism was similar in control B6 and DBA/2J mice, although some qualitative differences in the metabolites were detected by HPLC. TCDD pretreatment produced no quantitative or qualitative changes in TCDD metabolism. These results suggest that the rate of hepatic TCDD metabolism does not correlate with genetic differences at the Ah locus. (Supported by NIEHS Grant ES-02693).

The acute toxicity of TCDD exhibits marked interspecies variability, with the guinea pig being the most sensitive species. The metabolism and disposition of TCDD was investigated in guinea pigs for 45 days following a single exposure to purified [3H]-TCDD (0.36 μg/kg, ip). Urinary and fecal excretion of TCDD-derived [3H] followed apparent first-order kinetics, with an elimination half-life of 93.7 ± 15.5 days (mean ± SD). HPLC analysis of urine and bile indicated that all of the [3H] represented metabolites of TCDD, demonstrating that these routes of elimination are dependent on prior metabolism of TCDD. However, 70 to 90% of the [3H] in fecal samples was found to represent unmetabolized TCDD, suggesting that excretion resulted from the direct intestinal elimination of the toxin. Furthermore, the cumulative excretion of TCDD-derived [3H] over 45 days indicated that 74.3% of the [3H] was excreted as unchanged TCDD in feces, while 25.3% of the [3H] was excreted in urine and feces as TCDD metabolites. The metabolism of TCDD does not play a major role in the ultimate elimination of the toxin from the guinea pig. This may in part explain the relatively long excretion half-life for TCDD in the guinea pig and may contribute to the remarkable sensitivity of the guinea pig to the acute toxicity of TCDD. (Supported by NIEHS Grant ES-02693).

3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is one of the most toxic compounds known to man. Environmental contamination is widespread, and exposure generally occurs in populations which are heterogeneous with respect to age. The effects of age on TCDD intestinal absorption were studied using adult male Fischer-344 rats of 3 ages: 13 weeks (young), 13 months (middle) and 26 months (senescent). Absorption was measured using an in situ intestinal perfusion procedure. 3H-TCDD (20 ng/ml) was perfused through a 30cm intestinal segment at 1 ml/min for 60 minutes. Absorption was measured as the disappearance of label from the perfusate as well as the appearance in the liver. Absorption expressed as ng TCDD absorbed/g intestinal dry wt/hr decreased slightly from 402 ng/g/hr (young) to 332 ng/g/hr (senescent). Absorption was also calculated in terms of ng TCDD/g mucosal dry wt/hr since the mucosa represents the actual absorptive surface of the intestine. Absorption expressed in this manner increased significantly (P<0.05) from 1422 ng/g/hr (young) to 816 ng/g/hr (senescent). TCDD is often found in combination with polychlorinated biphenyls. It was demonstrated that TCDD absorption was unaffected by 2,4,5,5',6,6'-hexachlorobiphenyl (HCB) in the perfusate, but that HCB absorption was (P<0.01) enhanced by the presence of TCDD. The biological significance of these effects remains to be evaluated (NIR Grant ES-07126).

The acute toxicity of TCDD exhibits marked interspecies variability, with the guinea pig being the most sensitive species. The metabolism and disposition of TCDD was investigated in guinea pigs for 45 days following a single exposure to purified [3H]-TCDD (0.36 μg/kg, ip). Urinary and fecal excretion of TCDD-derived [3H] followed apparent first-order kinetics, with an elimination half-life of 93.7 ± 15.5 days (mean ± SD). HPLC analysis of urine and bile indicated that all of the [3H] represented metabolites of TCDD, demonstrating that these routes of elimination are dependent on prior metabolism of TCDD. However, 70 to 90% of the [3H] in fecal samples was found to represent unmetabolized TCDD, suggesting that excretion resulted from the direct intestinal elimination of the toxin. Furthermore, the cumulative excretion of TCDD-derived [3H] over 45 days indicated that 74.3% of the [3H] was excreted as unchanged TCDD in feces, while 25.3% of the [3H] was excreted in urine and feces as TCDD metabolites. The metabolism of TCDD does not play a major role in the ultimate elimination of the toxin from the guinea pig. This may in part explain the relatively long excretion half-life for TCDD in the guinea pig and may contribute to the remarkable sensitivity of the guinea pig to the acute toxicity of TCDD. (Supported by NIEHS Grant ES-02693).

OCDD is a widespread environmental contaminant which is relatively non-toxic after acute exposure. In order to better assess the significance of chronic exposure to OCDD, the disposition of 14C-OCDD was studied in male F344 rats. Animals were treated with 50 μg OCDD/kg iv, and 50, 500, and 5000 μg OCDD/kg po, and held in individual metabolism cages for 3 days. Feces was the major route of elimination with less than 1% of the dose appearing in the urine. No 14CO2 was detected. Absorption was readily saturated, less than 10% of the oral low dose being absorbed. The same body burden was achieved at 500 and 5000 μg/kg. Liver was the major tissue depot, containing over ten times the level in adipose tissue. No metabolites of OCDD could be detected in tissues or excreta. Likewise, the OCDD-derived radioactivity in bile was all parent compound. To determine the persistence of OCDD in tissues, the rats were treated iv at 50 μg 14C-OCDD/kg, and held metabolism cages for up to 56 days. The half-lives in liver and adipose tissue were three and one month, respectively. Thus, OCDD is poorly absorbed after oral exposure, concentrated in the liver, and excreted unchanged in the feces. The slow elimination would allow for accumulation during chronic exposure.
Hepatic plasma membrane epidermal growth factor (EGF) receptor alteration in inbred strains of mice in response to a single ip dose (115 µg/kg) of TCDD was compared. 125I-labeled EGF binding to its receptors on the C57BL/6J, was markedly inhibited (90-97%) in strains (A/J, SEC/ReJ, and CBA/J) which have a high affinity tyrosine kinase TCDD-receptor. In strains (DBA/2J, RF1/J, AKR/J, SWR/J and 129/J), which have a low affinity TCDD-receptor, the reduction in EGF binding was only 20-45%. TCDD also enhanced the phosphorylation status of several membrane proteins among which are EGF-receptor (170 kd) and its cleavage product (150 Kd). Exposure of new born mice to TCDD through mother's milk (10 ug/kg) caused early eyelid opening and tooth eruption in the high affinity strain (C57BL) similar to those caused by exogenously administered EGF. The study concludes that in vivo administration of TCDD modulates EGF receptor in inbred strains of mice. Such a modulation of expression of the receptors is a physiologically important biochemical event and suggests that some of the toxic expressions evoked by TCDD (i.e., cleft palate, differentiation, etc.) could be due to its functional alteration. (Supported by NIH Grant No. ES-03575).


Our studies focus on evaluation of epidermal growth factor (EGF) and Ah receptors, 6-glutamyltranspeptidase (GTP) TCC, and DNA synthesis occurring during the course of TCDD promotion of rat hepatocarcinogenesis. Initiation doses of diethylnitrosamine (DEN) (200 mg/kg, ip) or saline (S) were given to N-10 adult Sprague-Dawley rats followed by repetitive treatments (every 2 weeks) with corn oil (CO) or TCDD in a dose equivalent to 100 ng/kg/day. After 22 weeks of promotion, greater numbers of GTP-positive foci were found in DEN-TCDD rats than in the other groups. The rate of hepatic DNA synthesis was greatest in the DEN-TCDD animals. TCDD induced microsomal BPH activities 5 to 6-fold above saline and DEN controls, respectively. DEN treatment alone also resulted in a persistent elevation of BPH activity. Concentrations of liver cytosolic TCDD-Ah receptors were significantly increased in TCDD-treated rats. Initiation influenced the effect of TCDD on the EGF receptor. A decrease in EGF receptor number and phosphorylation occurred in S-TCDD rats; while in DEN-TCDD rats an increase or no effect was observed. Receptor mediated events such as those controlled by the EGF and Ah receptors may play a critical role in TCDD hepatocarcinogenesis by influencing the growth and metabolic capacity of preneoplastic cells.


Twelve male Sprague-Dawley rats (233 ± 1 g) were housed in cages equipped for measurement of O2 and CO2 concentration. Four rats were dosed ip with 925 µg/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in corn oil, whereas 8 rats received vehicle alone. Four of these 8 rats were pair-fed with the TCDD-treated rats and 4 rats had free access to feed. Oxygen and CO2 consumption were determined daily. TCDD rats were significantly (p < 0.05) lower than that of pair-fed controls. CO2 production was not different between TCDD-treated and pair-fed controls, when corrected for metabolic body size. TCDD rats showed significantly (p < 0.20) lower CO2 production than either pair-fed or ad libitum fed controls. It is concluded that TCDD-treated rats do not adapt their energy metabolism to starvation as do pair-fed controls.
PROTECTIVE EFFECT OF ALPHA-NAPHTHAFLAVONE ON TCDD-INDUCED IMMUNOSUPPRESSION. J. Blank, A. Tucker, J. S. Sweatlock, and T. Gasiewicz. NIH, RTP, N.C. 27709 and University of Rochester, Rochester, NY 14642

Differentiation of B-lymphocytes into antibody-producing cells can be inhibited by TCDD in vitro, and this assay has been proposed as a system in which TCDD toxicity can be examined at the molecular level. The immunosuppressive effect of TCDD has been correlated with its ability to bind to the Ah receptor. Alpha-naphthaflavone (ANF) antagonizes the inhibition of B-cell by TCDD when added to lymphocyte cultures at a molar concentration 100 fold in excess of TCDD. In contrast, B-naphthaflavone (BNF) appears to enhance but does not antagonize the effect of TCDD on lymphocyte maturation. Using ethoxyresoruifin-O-deethylase (EROD) activity as an indicator of cytochrome P-448 mixed function oxidase activity, we demonstrated that BNF will induce lymphocyte EROD activity and that ANF, which does not induce EROD activity, blocks the TCDD-induced increase. Furthermore, receptor binding studies using rat liver cytosol preparations show that ANF competes with TCDD in a competitive manner for binding to the receptor having a Kd value of 14nM. These data suggest that ANF blocks TCDD suppression of B-lymphocyte differentiation by antagonizing TCDD binding to its receptor and further supports the concept that the inhibition of B-cell differentiation is related to the induction of protein(s) encoded by the Ah locus.

Influence of Isoproteorenol on Tension Development and Rate in Atria Isolated from Rats Treated with 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). C.K. Kelling, L.A. Menahan, and R.E. Peterson. University of Wisconsin, Madison, WI

The functional significance of changes in serum thyroid hormone levels following TCDD treatment was assessed by examining mechanical responses of isolated atria to isoproteorenol and enzyme activities of energy metabolism in ventricular muscle from adult male rats pretreated with TCDD (6.25, 25 or 100 µg/kg) for one week. Basal tension development of electrically-stimulated left atria was increased similarly at all doses of TCDD tested. Basal rate of spontaneously-beating right atria was depressed in rats receiving 100 µg/kg TCDD. In left atria from rats treated with 100 µg/kg TCDD, basal tension development and maximal inotropic responses to isoproteorenol and 1-methyl-3-isobutylxanthine were enhanced to the same degree. Right and left atria from rats receiving 100 µg/kg TCDD had an increased sensitivity to the chronotropic and inotropic effects of isoproteorenol, respectively, when compared to pair-fed rats. The ratio of ventricular mass to body weight and activities of pyruvate kinase and citrate synthase in ventricular homogenates were not influenced by TCDD treatment at the doses tested. Findings with isolated atria and ventricular muscle suggest that functional thyroid status of the heart was not influenced by TCDD treatment (NIH Grant ES01332).

TCDD, DIETARY IRON AND IRON DISTRIBUTION IN FEMALE RATS. Z.A. Al-Bayati, B.J. Stohs and W.A. Al-Turk. College of Pharmacy, University of Nebraska Medical Center, Omaha, NE

Previous studies have shown that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces lipid peroxidation. The involvement of iron in lipid peroxidation is well known. We have therefore examined the effect of TCDD administration on subcellular distribution of Fe in livers of female rats fed defined diets containing deficient, normal (control) and excessive (supplemented) levels of Fe for 17, 24 and 31 days. Half the animals on each diet received 40 µg TCDD/kg on days 6-9 prior to sacrifice. Fe content was determined by atomic absorption spectroscopy. On the deficient diet the greatest decrease in Fe occurred in microsomes (-42.5%). On the Fe supplemented diet, 11.4 and 41.0% increases in Fe occurred in whole homogenate and microsomes, respectively. TCDD treatment caused decreases of 44.0, 31.1 and 41.8% in Fe content in microsomes from animals on control, Fe deficient and Fe supplemented diets, respectively, while producing 46.2, 28.1 and 71.4% increases, respectively, in Fe content of mitochondria. Thus, TCDD treatment produces significant alterations in Fe content of microsomes and mitochondria. The increase in microsomal lipid peroxidation induced by TCDD is not due to an increase in microsomal Fe content.

The Effects of Altered Rat Hepatic Receptor Levels on the Enzyme Induction Activities of Toxic Halogen BPhenols. B. Leese, M.A. Domonné, M.A. Li, R. Towner, C. Kamps, G. Mason and S. Safe. Department of Chemistry, University of Guelph, GuelphOntario, Canada and Veterinary Physiology and Pharmacology, Texas A&M College Station, TX 77843

Treatment of immature male rats with 2,2',4,4',5,5'-hexachlorobiphenyl (HCB, 300 µmol/kg) resulted in 2- to 3-fold increases in the rat hepatic 2,3,7,8-TCDD cytosolic receptor protein. The effects of increased receptor levels on the aryl hydrocarbon hydroxylase (AHH) inducing activities of 3,3',4,4',5,5'-pentachlorobiphenyl (PeCB) 2,3,7,8-TCDD and 3-methylcholanthrene (MC) were determined using dose levels which elicited submaximal and maximal induction responses. Sequential treatment of rats with 2,2',4,4',5,5'-HCB and the AHH inducers resulted in non-additive increases in AHH induction by 2,3,7,8-TCDD and 3,3',4,4',5,5'-PeCB but not MC when administered at submaximal induction doses. In contrast, administration of 2,2',4,4',5,5'-HCB and dose levels of the inducers which elicited a maximum induction response did not significantly change the activities of the halohydrocarbons but resulted in increased (non-additive) induction by MC. (Supported by NSERC).

The percutaneous absorption of HCB through the skin of rats was investigated following the dermal application of a 10 mg/kg dose of HCB in a tetrachloroethylene vehicle. Greater than 85% of the administered dose was recovered from the skin at the dosed site, or the occlusion bandage covering the dosed site at all sacrifice times post-dosing. Approximately 10% of the dermally applied dose of HCB was absorbed through the skin of rats by 72 hr post-dosing, but only about 1% of the applied dose was absorbed by 6 hr post-dosing. Percutaneous absorption occurred by an apparent first-order process with an absorption half-life of about 22 days. The concentration of HCB in the blood, liver, carcass and fat increased as a function of the length of exposure. Total recovery was approximately 95% at all sacrifice times. Repeated soap and water washing of the dosed skin at 6 hours after dosing removed approximately 35% of the applied dose and decreased the amount absorbed over 72 hours by more than one-half. These data suggest that some of the HCB was absorbed dermally by the rat, distributed to other tissues within the body, and then slowly eliminated.

ACUTE, SUBACUTE AND SUBCHRONIC ORAL TOXICITY STUDIES OF 1,1-DICHLOROETHANE (DCE) IN RATS. S. Muralidhara, R. Ramachandran, C. E. Dallas and J. V. Brunner, Department of Pharmacology and Toxicology, College of Pharmacy, University of Georgia, Athens, GA.

The objective of these studies was to assess the acute, subacute and subchronic toxicity of oral DCE. In the acute study, adult male 5-0 rats were given a single oral dose of 0, 0.5, 1.0, 2.0, 4.0 or 8.0 g DCE/kg. Urine and organ samples were taken at 24 hr. No treatment-related effects were seen on serum and urinary enzyme levels, organ weights or tissue morphology, though there was significant mortality in the 8.0 g/kg group. In the subacute study, rats were given a single oral dose of 0.5, 1.0, 2.0 or 4.0 g DCE/kg each day for 5 or 10 consecutive days. There was a dose-dependent increase in glutathione (GSH) in the kidney after both 5 and 10 days of exposure, though liver GSH levels and most other indices were not significantly different from controls. In the subchronic study, rats received single oral doses of 0, 0.5, 1.0, 2.0 or 4.0 g DCE/kg five days/week for up to 12 weeks. Marked CNS depression and high mortality were manifest in the 4.0 g/kg group. Little evidence of toxicity, other than transient CNS depression, was seen with consistency at the lower dosage levels. DCE thus appears to have very limited potential to cause toxic injury in rats upon either short- or long-term ingestion. (Supported by U.S. EPA Coop. Agreement CR 811215).

EFFECTS OF D-BENZYL-P-CHLOROPHENOL (BCP) ON PORPHYRIA EXCRETION IN F344 RATS. H. S. Chang, M. R. Heitmancik, A. C. Peters, and L. S. Birnbaum*. Battelle, Columbus, OH, and *National Toxicology Program, RTP, N.C.

BCP, an aryl halide biocide, produced renal lesions in rats and mice in a subchronic study. A significant increase in absolute kidney weight and a reddish-yellow stain around the urogenital area were observed in BCP treated rats. Rats were treated p.o. with 0, 60, 120, or 240 mg/kg BCP in corn oil in females and 0, 30, 60, or 120 mg/kg for males, 50/wk for 13 wks. Liver porphyrins, urinary coproporphyrins and uroporphyrins as well as urinary N-acetyl-β-D-glucosaminidase (NAG), galactosidase, lactate dehydrogenase, and alkaline phosphatase (AP) were evaluated. The excretion of coproporphyrins increased in a dose dependent manner in the treated rats. Negligible uroporphyrins were detected. There was no dose dependent effect of BCP on liver porphyrins. These results suggest that BCP may induce a porphyrinuria rather than porphyria in rats. Both urinary NAG and galactosidase decreased in female rats. All treated rats exhibited an increase in urinary AP. These observations are consistent with the renal tubular abnormalities produced in the subchronic study. (Supported by NTP Contract No. N01ES4502)


The capacity for induction of microsomal metabolic enzymes by Tetrathal® (tetrachlorophthalic anhydride, TCFA) was evaluated in Sprague-Dawley rats and CD-1 mice. The rats were orally dosed for 7 days with TCFA suspended in corn oil at 25, 100, 250 and 500 mg/kg. Following this treatment a dose dependent reduction in the xoxozoneal paralysis time occurred over the dose range 100-500 mg/kg in the rat. No effect on the hexobarbital sleep time was observed at any test level. TCFA was found to produce a statistically significant increase in hepatic aminopyrine-N-demethylation, aniline hydroxylase and cytochrome P-450 in the rat at 500 mg/kg. In addition statistically significant increases were seen in aniline hydroxylase and cytochrome P-450 at 25 mg/kg. Mice were orally dosed with TCFA for 7 days at 250, 500 and 1000 mg/kg. There was no effect in the oxazolome paralysis time or hexobarbital sleep time in this species. Hepatic microsomal enzyme levels were not measured in the mouse. These results suggest that following oral dosage TCFA is a weak inducer of microsomal enzymes in the rat. A similar effect was not observed in the mouse for the parameters tested.
Toxicity induced by trans-1,2-dichloroethylene (DCE) administered for 14 and 30 days was evaluated in male rats. At 30 days, final body weight was not different from controls, while only kidney weight was significantly higher for the treated group. Creatinine and BUN values were both significantly depressed for the treated group. There were no differences found with electrolytes, CBC, differentiation of white cells, or parameters of the drug metabolizing enzyme system between the two groups. At 30 days, final body weight was also not different from controls, while liver weight was higher and lung weight lower for the treated group. AST and creatinine values were depressed for the treated group which also showed significantly elevated calcium values. Electrolytes, red cell count, hemoglobin and hematocrit were significantly altered for the treated group. Ethylmorphine N-demethylase and aniline hydroxylase activity and cytochrome P-450 content were depressed in the 30 day treated group. Histopathological examination of various tissues showed no changes after 14 or 30 days of DCE treatment. (Supported by Burroughs-Wellcome Toxicology Scholar Award.)

The effects of dichloromethane exposure on the lungs and livers of rats and mice
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Sponsor: E.A. Lock

Dichloromethane (DCM) has been shown to cause a significant increase in lung and liver tumours in B6C3F1 mice, but not F344 rats, exposed to 2000 and 4000 ppb by inhalation (NTI 1985). The effects of exposure to DCM at these levels for either one or 10 days have been assessed to determine the role of cytotoxicity in tumour development in B6C3F1 mice. Significant increases in liver/body weight ratios (120% of control) were seen in mice, but not rats, after exposure at either dose level for 10 days. A highly selective vacuolation and pyknosis of the mouse lung Clara cell was seen after one day of exposure at either dose level. Clara cells also contained pale enlarged mitochondria, necrotic cell debris and myelin whorls. At 10 days the vacuolation of the Clara cells had disappeared, but enlarged mitochondria and myelin whorls were still evident. No treatment related effects were found in the lungs of rats or in the livers of rats or mice other than an accumulation of lipid and glycogen in the livers of both species. The sustained cytotoxicity seen in a specific cell type in the mouse lung may be significant to the development of tumours in that organ.

Cytogenetic effects of dietary tetrachloroazoxybenzene exposure to mice
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Cytogenetic evaluations were made of weanling mice consuming control, 40 ppm 3,3',4,4'-tetrachloroazoxybenzene (TCAB), or 100 ppm cyclophosphamide (Cy; positive control) for 28 days. TCAB was tested because it is an approximate stereoisomer of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), binds with high affinity to the Ah receptor, and results in the same clinical signs of toxicity; but is several orders of magnitude less toxic. The TCAB-treated mice exhibited an increased number of chromatid breaks over control values. This increase occurred without a corresponding change in isochromatid breaks, and suggests damage after DNA replication. These lesions may be caused by an alteration in the synthesis or attachment of the chromosomal proteins involved in structural integrity. However, chromosome rearrangements (triradials, quadriradials) were seen in the mice consuming TCAB, indicating direct damage to DNA is also occurring. TCAB did not result in an increase in sister chromatid exchanges (SCE). Alkylating agents (Cy) are known to damage during the S phase of cell growth and are potent inducers of SCE. S independent mutagens (radiation, bleomycin) are poor SCE inducers. Therefore, TCAB may be acting similar to the S independent compounds.
Although other tissues are also affected, the liver is the main target organ of PFDA toxicity. In this study, the distribution of PFDA in liver, kidney, testes, fat, and blood was measured 2 hrs, 24 hrs, 4, 8, 12, 16, and 30 days after an ip injection of 50 mg PFDA/kg. The major portion of the PFDA was in the blood, liver, and kidney with the peak concentrations between 4 and 8 days. No PFDA was found in fat or urine in the first 10 days after injection. While PFDA levels in the other tissues decreased over the 30 day period, levels in liver remained constant on a μg/g basis. Since the liver to body weight ratio in PFDA rats is almost twice that of control rats, the absolute amount in liver was actually highest at 30 days. Liver lipids were separated by thin layer chromatography and PFDA eluted with the most polar fraction containing mainly phospholipids. This fraction was further separated using another solvent system and PFDA eluted after most of the phospholipids indicating conversion of parent PFDA to a more polar metabolite which we believe is a toxic metabolite of PFDA.

Rats treated with PFDA exhibit toxic signs which are similar to those observed in a vitamin A deficient state. Male Sprague-Dawley rats were given a single ip injection of 75 mg/kg and killed 2,4,8,15,22, and 36 days later. Serum, kidney, and hepatic concentrations of vitamin A were determined with HPLC. PFDA caused a dramatic depression in serum retinol concentration as early as 2 days post-exposure (X = 0.064 ± 0.019 μg/ml) which was significantly different (P<0.01) from control or pair-fed control (pfc) animals (X = 0.324 ± 0.055 and 0.299 ± 0.055 μg/ml, respectively). A gradual recovery to control serum vitamin A values at day 36 was observed. Similar to a vitamin A deficiency, PFDA caused renal accumulation of retinol relative to pfc rats (X = 1.49 ± 0.17 and 0.90 ± 0.01 μg/g, respectively). PFDA also caused renal accumulation of retinyl palmitate relative to pfc rats (X = 2.28 ± 0.48 and 0.358 ± 0.122 μg/g, respectively). Hepatic vitamin A content was not significantly affected throughout the course of the study. The data suggest that PFDA induced changes in serum vitamin A levels may be partially responsible for the signs of hypovitaminosis A. (NHI ES05347).

The effects on physiological and hormonal parameters were investigated in male rats (300-350 g), 7 days after dosing with PFDA (20, 40 or 80 mg/kg, ip). PFDA caused dose-dependent reductions in: body weight, feed intake, resting and total O2 consumption, resting and total CO2 production, and basal metabolic rate. These changes were also observed in pair-fed control rats. Effects of PFDA, not mimicked by pair-feeding, included: thyroid gland weight, plasma T4, free thyroxine index (FTI), plasma T3, respiratory quotient (RQ). Body temperature and spontaneous motor activity (SMA). Plasma T4 and FTI were affected to the same extent by pair-feeding to the high dose group but were unchanged in low dose pair-fed controls. Reduction in thyroid weight and body temperature observed in pair-fed controls of the high dose group were less extensive than in the respective PFDA group. SMA was unchanged in all pair-fed control groups, whereas RQ was increased in controls pair-fed to the high dose group. Plasma T3, unaffected in the high dose group, was markedly reduced in its pair-fed controls. Metabolic changes appear to result from PFDA-induced hypo-phyagia rather than a direct effect of PFDA on thyroid function. (Supported by AFOSR).

TCDD, 2,3,4,5,7,8-hexachlorodibenzofuran (HCB), and perfluorochemicals similar to a vitamin A deficiency, TCDD and HCB increased while PFDA decreased serum retinol. These chemicals plus 2,4,5,2',4',5'-hexachlorobiphenyl, a non-toxic PCB oenogen, and vitamin E, a non-competitive inhibitor, were tested for the in vitro inhibition of hepatic retinyl palmitate hydrolyase (RPH) activity. Only PFDA inhibited at concentrations which seemed physiologic. Male Sprague-Dawley rats given a single ip dose of 50 or 100 mg/kg PFDA and sacrificed at 2,8 or 11 days exhibited symptoms characteristic of TCDD toxicity. Hepatic RPH activities were depressed in both treatment groups (p<0.025) and correlated with serum retinol. Extraction of hepatic homogenates with acetone removed PFDA and increased RPH activities 2- and 3-fold for the low and high dose groups, respectively. Analysis of partially purified RPH showed PFDA to be a non-competitive inhibitor; KI=340 μM. We conclude that, in contrast to TCDD, PFDA causes a decrease in the mobilization of vitamin A from the liver by non-competitive inhibition of RPH. (NHI ES08385).
1268 IMPAIRMENT OF CALCIUM HOMEOSTASIS BY HEXACHLOROBENZENE EXPOSURE IN FISCHER 344 RATS.
J.E. Andrews, K.D. Donaldson, J.E. Andrews, K.D. Donaldson.* U.S.E.P.A., MERL, RTP, NC; *NCSU, Toxicology Program, Raleigh, NC.

Human exposure to HCB has resulted in the conditions of osteoporosis and resorption of distal phalanges. These conditions were observed in 35-60% of examined individuals and found to persist for at least 20-30 years. Fischer 344 male rats were dosed with 0, 0.1, 1.0, 10.0, or 25.0 mg HCB/kg body weight for 5 weeks while being fed normal rat chow or vitamin D3 deficient chow. Rats receiving normal chow had a dose related decrease in body weight gain, increased liver weight and increased liver to body weight ratio when compared to their controls. Serum triglycerides, cholesterol and ALT were significantly elevated. Serum 1,25-dihydroxy vitamin D3 and PTH concentrations were significantly elevated when compared to the control values. In the vitamin D3 deficient diet group, there was a dose related increase in liver weight, liver to body weight ratio and kidney to body weight ratio. Serum triglycerides and cholesterol were significantly elevated along with 1,25-dihydroxy vitamin D3 concentrations. Urinary calcium decreased significantly with increased HCB dose level indicating conservation of calcium. The data from this study indicate that HCB does affect calcium homeostasis and that more detailed studies are needed. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.


Meat-type chickens were fed diets with 0 or 10 ppm PCBs for 14 days. The diets with PCBs were withdrawn and replaced by diets containing 5% Vasoline®, 5% mineral oil, 5% colestipol, or 5% propylene glycol, and each of these was fed at 10% with diets restricted to 50% of control intake. At twenty-one days of treatment, all of the chickens (9 per treatment) were euthanized bloodlessly with CO2 and the carcasses ground for PCB analyses. Chickens previously fed PCBs but not subsequently treated (negative controls) had body burdens of 5.95 mg/bird. Those that received 10% control intake averaged 108% of that value. Vasoline reduced body burdens to 69%, Vasoline plus restricted feeding to 47%; propylene glycol reduced burdens to 90%, propylene glycol plus restriction to 57%; colestipol reduced body burdens to 67%, and colestipol plus restriction to 77% (no additional benefit). The major 1 reduced burdens to 73%, but when combined with restricted feeding burdens were only 32% of the positive control. Thus of the treatments applied 10% mineral oil in the diet with 50% dietary restriction was the most effective for hastening removal of PCBs.

1270 PURIFICATION AND CHARACTERIZATION OF THE DOG HEPATIC CYTOCHROME P-450 ISOZYME(S) RESPONSIBLE FOR THE METABOLISM OF 2,2',4,4',5,5'-HEXACHLOROBENZENE. D.B. Duignan, J.R. Harbert, T.B. Leonard, and I.G. Sipes, Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ, and Smith, Kline & French Labs., Philadelphia, PA.

Previous in vitro studies have shown that among the dog, monkey, rat and human, only the dog metabolizes 2,2',4,4',5,5'-hexachlorobenzene (265-HCB) to a significant degree. We have shown that pretreatment of dogs and rats with phenobarbital (PB) enhances the in vitro metabolism of 265-HCB. In addition, at least two PB-induced isozymes of cytochrome P-450 have been detected in dog hepatic microsomes as evidenced by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and column chromatography. In a reconstituted system, PB-B, the major PB-induced isozyme in the rat, can metabolize 265-HCB, and antibodies raised against PB-B show cross-reactivity towards the two PB-induced isozymes in the dog. By use of Ocatylamino-Sepharose, Hydroxylapatite and DEAE-Sepacel column chromatography, one of the isozymes (which we call PB-D) has been purified (>95%) as determined by SDS-PAGE, and shows activity towards 265-HCB. These results, along with the spectral characteristics of PB-B and PB-D, suggest a similarity between these two isozymes. By use of anti-PB-D antibodies, we hope to better elucidate the role of this isozyme in the metabolism of 265-HCB, and thus better understand why the dog is so efficient in its metabolism of this compound. (Supported by NIH Grant #T32 ES 07091.)

1271 COMPARISON OF IN VIVO AND IN VITRO METABOLISM OF 1,2,4,5 TETRACHLOROBENZENE IN THE DOG AND RAT. E.E. Sikorski, R.G. Schnellmann, and I.G. Sipes, Dept. of Pharmacology and Toxicology, Col. of Pharmacy, University of Arizona, Tucson, AZ.

Previous studies in our laboratory have shown that among the species tested, the dog is unique in that it readily metabolizes and eliminates 2,2',4,4',5,5'- hexachlorobenzene (265-HCB). Because 1,2,4,5-tetrachlorobenzene (TCB) is structurally related to 265-HCB (no unsubstituted, adjacent carbon atoms). We compared the in vitro metabolism and in vivo elimination of TCB in the male mongrel dogs and Fisher 344 rats. By 7 days after administration of 14C-TCB (10 mg/kg, iv) rats excreted 22% and 8% of the dose in the urine and feces, respectively. In this same time, dogs excreted only 1.2% and 1.1% in the urine and feces, respectively. Incubation of 14C-TCB by dog or rat hepatic microsomes produced phenolic metabolites at comparable rates, 14.9 and 10.9 pmoles/nmole P-450/min, respectively. The major metabolite produced by rat microsomes was 2,3,5,6-tetrachlorophenol, while dog microsomes produced a mixture of 2,3,5,6- and 2,3,4,6-tetrachlorophenols. Because of the previous results with 265-HCB, the slower elimination of TCB was unanticipated. The reason for the species difference is not known, but does not appear to relate solely to differences in phase I metabolism. (Supported by NTP-N01-ES-3031.)
1272 EFFECT OF NEONATALLY-ADMINISTERED AROCLOR 1254 ON HEPATIC MICROSONAL TESTOSTERONE HYDROXYLASE ACTIVITY IN MALE ADULT RATS: J.M. Haake, M. Romkes, M. Kelley, and S. Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, Texas 77845.

The effects of neonatal exposure to the PCB mixture Aroclor 1254 on adult hepatic testosterone hydroxylase enzyme activities were examined in Long Evans rats. Aroclor 1254 was administered IP at a dose of 500 μg/kg to male rat pups on day 4, and the hepatic microsomal testosterone hydroxylase activities were determined in 60-day old rats by HPLC analysis of the metabolites. Significant increases in the basal activities of 2α, 6α, 6α, 15α, 16α, 16β, and 17α-testosterone hydroxylases and decreases in 7α-testosterone hydroxylase were observed in the 60 day old rats. Moreover, decreased 6α, 15α, and 17α-testosterone hydroxylase and increased 7α-testosterone hydroxylase inducibilities by Aroclor 1254 were observed in neonatally exposed male rats demonstrating that neonatal exposure of male rat pups to Aroclor 1254 can cause permanent changes in the adult hepatic drug metabolizing enzyme activities. (Supported by the Texas Agricultural Experiment Station).

1273 ARYL HYDROCARBON HYDROXYLASE INDUCTION BY TCDD CONTAMINATED SOILS FROM TIMES BEACH, MISSOURI, AND NEWARK, NEW JERSEY.

Thomas H. Umbreit, E.J. Hesse, and M.A. Gallo. University of Medicine and Dentistry of New Jersey, Rutgers Medical School, Busch Campus, Piscataway, New Jersey 08854.

TCDD is a potent, but not exclusive, inducer of aryl hydrocarbon hydroxylase (AHH). Four rats per sex per group were gavaged either once or on four consecutive days, and sacrificed 24 hours after the final dose. Groups were soil from Times Beach, and Newark, and TCDD-positive control soil, at 10 μg TCDD/kg. Cytochrome P-450 and AHH were measured in liver microsomes. Polycrylamide slab gel electrophoresis was performed on liver microsomes to distinguish the P-450 isoenzymes induced. AHH was induced 3.6 to 10x background with recontaminated soil, while Newark soil induced 2 to 5.4x while Times Beach induced 2 to 4.1x. Gels showed induction of P-450 and b by positive control soil and both site soils. (Supported by EPA contract #CR812114-01-1).

1274 COMPARATIVE TOXICITY OF TCDD CONTAMINATED SOILS FROM TIMES BEACH, MISSOURI, AND NEWARK, NEW JERSEY.

Thomas H. Umbreit, E.J. Hesse, and M.A. Gallo. Dept. of Environmental and Community Medicine, University of Medicine and Dentistry of New Jersey, Rutgers Medical School, Busch Campus, Piscataway, NJ 08854.

Toxicity of TCDD contaminated soil has been reported to be very high for Times Beach soil, but very low for Newark soil. This study compares the toxicity of the two soils in guinea pigs. Ten animals per sex were gavaged with Times Beach or Newark soils (1 to 10 μg/kg). After the first death that could be attributed to TCDD, two animals per group were sacrificed. Remaining guinea pigs were sacrificed after 60 days. Cause of death was confirmed by autopsy. The Newark soil was less toxic (LD₅₀ > 10 μg/kg) than Times Beach soil (LD₅₀ < 10 μg/kg). Severe hepatic necrosis was seen in guinea pigs treated with TCDD controls and moderate liver pathology was seen in the Times Beach group. Little or no pathology was seen in the Newark soil group. (Supported by EPA contract #CR812114-01-1).


The effects of dioxin on adult mink was assessed through three studies in which TCDD was administered as a single oral dose (from 2.5 to 7.5 μg TCDD/kg b.w.) via the diet (0.001 to 100 ppb TCDD). In all studies, there was a dose dependent decrease in feed consumption with corresponding weight loss (up to 50% by time of death on the higher TCDD levels). Gross necropsy revealed motting and discoloration of the liver, spleen, and kidneys in all groups exposed to TCDD. Red and white blood cell counts, hematocrits, and hemoglobin concentrations in surviving mink were not significantly altered. Response of peripheral blood lymphocytes to mitogenic stimulation by concanavalin A was significantly altered by single oral doses of 7.5 μg TCDD/kg b.w. Twenty-eight-day LD₅₀, 28-day and 125-day dietary LC₅₀ values of 4.2, 4.3 ppb TCDD, and 0.85 ppb TCDD, respectively, were calculated for the mink in these studies. These results indicate that mink are among the most sensitive species to 2,3,7,8-TCDD and that they can serve as a valuable model to study the impact of environmental dioxins on mammalian species.
CONCENTRATIONS OF POLYCHLORINATED BIPHENYS IN LACTATING COWS AND THEIR CALVES. L. B. Willett, N. I. Durst, and Z-T. Y. Liu. Ohio State University/Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

Polychlorinated biphenyls (PCB) occasionally enter the feed supplies of lactating dairy cattle. It is important to understand the distribution of residues in animals that produce and are used for human food. Four Holstein cows were orally dosed with 10, 100, and 1,000 mg/day of Aroclor 1254 in sequential 60-day periods. Average concentrations of PCB (µg/g) in blood plasma (BP), milk fat (MF), and adipose tissue (A), at the end of the dosing periods were: 10 mg/day BP=0.005, MF=1.9, and A=1.4; 100 mg/day BP=0.02, MF=10, and A=6.9; and 1,000 mg/day BP=0.14, MF=91, and A=70. Cows were maintained through 40 days of the subsequent lactation which was between 250 and 300 days after the last PCB dose. The cows, plus their calves which were conceived during dosing and fed milk from their dams, were necropsied after 40 days post-partum. Concentrations of PCB (µg/g) in cows averaged BP=0.05, A=17.7, liver (L)=0.30, kidney (K)=0.11, and brain (B)=0.18; and in calves BP=0.08, A=26.3, L=1.03, K=0.33, and B=0.41. High concentrations of PCB had no apparent adverse affect on health or productivity. (Supported in part by Monsanto Contract No. RF715688.)


N balance (B) and NH₃ metabolism were studied in ARO (300 mg/kg, 4d) or vehicle-treated, pair (PF) and ad lib (AF)-fed, male Harlan-Sprague-Dawley rats (175-200 g). Treated rats had decreased body weights (BW) (ARO 10%, PF 11%) and control rats had increased BW (AF 19%). NB (N balance) and N retention (N-N₆ feces + urine)/N-N₆ (feces) were less in ARO vs. AFs (p<0.05). Urinary N and urea were similar for ARO vs. AF and lower for PF vs. AF. Fecal N and NH₃ were similar for ARO and PF which were less than AF. Blood urea was increased in ARO 12 min after NH₃ acetate (2.5 mmol/kg, i.p.) and was greater than AF at this time. ARO and PF were hypoglycemic vs. AF after treatment. Blood glucose of AF was increased 12 min after NH₃ acetate and was greater than ARO. Differences in blood glucose and urea appearance in blood between ARO and AF were also observed when expressed in µmol/100 g BW. The data correlate ARO-induced BW loss with N deficits and altered glucose and urea metabolism. Results for ARO were indistinguishable from PFs and could be attributed to the same caloric intake. Differences between ARO vs. AF, therefore, were attributed to ARO treatment. Since hepatic mitochondria initiate glucose and urea synthesis from NH₃, the data indicate that ARO treatment altered mitochondrial NH₃ metabolism and regulation. (Supported by USPHS grant E00267.)
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583 BENZO(a)PYRENE(BP) AND ITS METABOLIT/F DNA ADDUCTS WITH
669 BENZO(A)PYRENE-INDUCED DOWN-REGULATION OF INTERLEUKIN-2
655 BENZO(A)PYRENE/UBCHRONIC PULMONARY EXPOSURE OF MICE TO
667 BENZO-(A) PYRENE IMMUNOTOXICITY./HE CELLULAR TARGETS OF
668 BENZO-(A)PYRENE INDUCED MODULATION OF INTERLEUKIN 1,2
1211 BENZOATE FOLLOWING CHRONIC ACID/TION OF 4-BROMO-METHYL-
95 BENOZIDAZEPINE RECEPTOR/IONSHIP TO THE PERIPHERAL-TYPE
339 BENZOYL-1,3-INANDIONE ANTIAGGULANT RODENTI/QSR OF 2-
340 BENZOYL-1,3-INANDIONE ANTIAGGULANT RODENTI/QSR OF 2-
1037 BENZYL ALCOHOL./TOXICITY AND METABOLISM OF
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639 BILARY ELIMINATION OF METHYLXYR IN THE MARINE/SLOW
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605 BILARY EXCRETION OF APLATOXIN /ETHYL MALEATE (DEM) ON
54 BILARY EXCRETION OF CHOLEPHIL/POTZOTOCIN (STZ) ON THE
444 BILARY EXCRETION OF GLUTATHIONE/-SPECIES VARIATIONS IN
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BLOOD SAMPLING METHODS FOR RAT TOXICIT/AN EVALUATION OF
BLOOD TOLUENE LEVELS AFTER ORAL AND INHAL/COMPARISON OF
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BROMADIOLONE./CTS FROM RATS TREATED WITH BRODIFACOUM OR
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BROWN ADIPOSE TISSUE (IBAT) IN/ LIVER AND INTESTSCULAR
BSO) AND DIETHYL MALEATE (DEM/ BUTHIONINE SULFOXIMINE (BSO)
BUCHE) OF MUSCULARIS MUSCLE OF/D BUTYRCHOLINESTERASE (BUCHE)
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BUTHIONINE SULFOXIMINE (BSO) TREATMENT OR OF/EFFECTS OF
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C ACTIVITY IN EL4 CELLS./2,3,7,8-TCDD ON PROTEIN KINASE C REDUCTASE ACTIVITY AND BAP/OME P450, NADPH CYTOCHROME C3H 1OT1/2 CELL TRANSFORMATION/RISON OF THE BALB/3T3 AND C57BL/6 MICE./ AND LIVER NODULE FORMATION IN C3H/He MICE./ (PB)-INDUCED HEPATIC NODULES IN C57BL AND C57BL/6 MICE./ PHENOBARBITAL(PB)-INDUCED LIVER NODULES IN C5 IN 0,0,5-TRIMETHYL PHOSPH/P THE COMPLEMENT COMPONENT C57/B6 MOUSE./VIVO AND FETAL AND MATERNAL TISSUES OF THE C57BL AND C3H/He MICE./ (PB)-INDUCED HEPATIC NODULES IN C57BL AND STERILITY IN SEC MAL/UCES DOMINANT LETHALS IN C57BL MICE./YGIN (VAN)-INDUCED NEPHROTOXICITY IN FEMALE C57BL/6 MICE) BY A MECHANISM N/450d (RATS) AND P3-450 (C57BL/6 MICE./ AND LIVER NODULE FORMATION IN C3H/He AND C57BL/6 SPLENOCYTES./ON NATURAL KILLER CELL ACTIVITY IN C57BL/6J AND DBA/2J MICE./IN (TCDD) BY HEPATOCYTES FROM C57BL/6J MICE FOLLOWING SUB-CHRO/ED T-CELL RESPONSES IN Ca++-DEPENDENT AND INDEPENDENT MECHANISM/EXTRACELLULAR Ca++ AND Mg++ CONTENTS IN RAT/TS OF ORGANOPHOSPHATES ON C++ METABOLISM IN RAT BRAIN OREPIEPINEPHRINE RELEASE AND Ca++ STUDIES WITH QUIN2.-ATOTOXINS ELEVATE CYTOPLASMIC Ca++-ATase TO CYCLODIENE PESTICI/IVITY OF SYMPTOMS 1C404 INDUCES DAMAGE AND CYTOXICITY IN MAMMALIAN CELL CULTURES AND INFUX OF CADMIUM AND COPPER INTERACTIONS IN KIDNEYS OF THE SCALL CADMIUM AND METHYLMERCURY INHIBIT NEUROBONINE AGAINT CADMIUM AND SELENIUM INTERACTION ON DELTA-AMINE/EFFECT OF CADMIUM AND ZINC LEVELS IN MICE/ODUM SULFATE ON TISSUE CADMIUM AND ZINC.--1 AND METALLOTHIONEIN-II (MT-11) BY CADMIUM BINDING ASSAYS./F METALLOTHIONEIN BY SILVER AND CADMIUM CARDIOTOXICITY: EFFECTS ON CYTOPLASMIC AND MITO CADMIUM COMPOUNDS. I: CELLULAR / IN THE LUNG INDUCED BY CADMIUM COMPOUNDS./S FOR TESTING THE CARCINOGENICITY OF CADMIUM COMPOUNDS. II. PROTEASE/ IN THE LUNG INDUCED BY CADMIUM INDUCED CELL INJURY./ODULIN IN THE MECHANISM OF CADMIUM INDUCED STRUCTURAL AND /UM LOAD AND REVERSAL OF CADMIUM LOAD AND REVERSAL OF CADMIUM IND/REDUCED TISSUE CADMIUM ON KIDNEY AND URINE OF NONPREGNA/EFFECT OF OZAL CADMIUM ON PENTOSE CYCLE OF ADULT AND NEONATA/EFFECT OF CADMIUM TOLERANCE IN EARLY LIFE-CYCLE STAG/INDUCTION OF CADMIUM TOXICITY./ETAL AND STRESS PRETREATMENT ON ACUTE CADMIUM, MERCURY AND VANADATE ON THE GLUTA/INFLUENCE OF CADMIUM, VANADATE AND MERCURY I/N THE HEPATOXICITY OF CADMIUM-BINDING LIGANDS IN GREAT SALT LAKE BRINE SHRIMP CADMIUM-INDUCED CYTOXICITY IN RAT/RETMEN REDUCES CADMIUM./ADMIUM AND ZINC LEVELS IN MICE PRETREATED WITH
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760 DIKETONE NEUROPATHY./N IS A PATHOGENETIC STEP IN GAMMA-
1010 DIMERCAPTO/-1-PROPANESULFONIC ACID (DMS)/.G AGENT 2,3-
1009 DIMERCAPTO-1-PROPSNDRULIC ACID (DMS)/.G AGENTS 2,3-
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INACTIVATION OF PHENYL VALERATE HYDROLASES FROM HE/H/HEAT

INBRED MICE./ WITH NITROFURANTOIN (NF) IN 14 STRAINS OF

INBRED STRAINS OF MICE.-H/LOREDIBENZO-P-DIOXIN (TCDD) IN

INBRED STRAINS OF MICE./MAL METABOLISM OF PESTICIDES BY

INCLUSION BODY FORMATION IN RAT K/INFLUENCE OF Zn ON Pb

INCLUSIONS: STRUCTURE AND COMPOSIT/INTRANUCLEAR ARSENIC

INDOLES PRESENT IN BRASSICA OLE/SIS OF ANTICARCINOGENIC

INDOMETHACIN ON OZONE INDUCED CHANGES IN PULM/EFFECT OF

INDOMETHACIN PROTECTS AGAINST IN VIVO BENZENE INHIBITIO

INDUCERS AND INHIBITORS OF OXIDATIVE METABOL/EFFECTS OF

INDUCERS ON THE BILIARY EXCRETION OF /EFFECTS OF ENZYME

INDUCERS TO P450d./SPECIFIC BINDING OF

INDUCERS./TENE B4 (LTB4) AND LARVIC ACID (LA)/EFFECT OF

INDUCIBLE FORM OF RAT LIVER MIC/ROXYLATION BY THE FCN-

INDUCIBLE HEPATIC CYTOCHROMES P- FOR MULTIPLE STEROID-

INDUCTION ACTIVITIES OF TOXIC HA/R LEVELS ON THE ENZYME

INDUCTION AND TUMOR PROMOTION./ EFFECTS ON LIVER ENZYME

INDUCTION BY METHYLENEDIOXYPHENYL (MDP)/MONOOXYGENASE

INDUCTION FOLLOWING PERTREATMENT WITH TH/HEPATIC ENZYME

INDUCTION IN THE IN VIVO-IN VITRO MOUSE HE/DOES S-PHASE

INDUCTION OF Atherosclerosis IN / METHYLCHOLANTHRENE ON

INDUCTION OF CYTOCHROME P-450 ISOZYMES BY 2/COMPARATIVE

INDUCTION OF DNA REPAIR AND REPLICA/ION IN RAT HEPATOCYTE

INDUCTION OF HEPATIC METALLOTHIONEIN (MT) BY ALCOHOLS

INDUCTION OF HEPATIC METALLOTHIONEIN (MT) BY METAMIN/DAZOD

INDUCTION OF HEPATIC MICROSMAL /CHARACTERIZATION OF THE

INDUCTION OF HEPATIC PEROXISOMAL ENZYMES IN P344 RATS T

INDUCTION OF LIVER CARNITINE ACETYLTANSFERASE BY VALPR
INDUCTION OF METALLOTHIONEIN IN RAT HEPATOCYTE CULTURES

INDUCTION OF METHEMOGLOBINEMIA BY DINI/ISOMER-DEPENDENT

INDUCTION OF PEROXISOMAL ENZYMES /IVITY REQUIREMENTS FOR

INDUCTION OF PEROXISOME ENZYMES AND INCREASED LIPID PE

INDUCTION OF PEROXISOME PROLIFERATOR /IES SENSITIVITY TO THE

INDUCTION OF RAT HEPATIC PEROXISOM/IN VIVO AND IN VITRO

INDUCTION OF RAT LIVER CYTOCHROME P-450 ISOZYMES BY SUS

INDUCTION ON DIMETHYLNITROSAMINE T/EFFECTS OF IMIDAZOLE

INDUCTION WITH 3-METHYLCOLANTH/ A CYTOSOLIC FACTOR AND

INDUSTRIAL EXAMPLE OF A HEALTH HAZARD DETERMINATION /AN

INFERTILITY AND PARTIAL RECOVERY AFTER A SINGLE EXPOSUR

INFLAMMATION IN RESPONSE TO INHAL/LEASE OF MEDIATORS OF

INFLAMMATION IN THE INDUCTION OF POS/ROLE OF EPIDIDYMAL

INFLAMMATORY AGENT 5- (4-PYRIDYL)/ETABOLISM OF THE ANTI-

INFLAMMATORY AGENT 5- (4-PYRIDYL)/OKINETICS OF THE ANTI-

INFLAMMATORY REACTION IN THE LUNG INDU/TIME SEQUENCE OF

INFLAMMATORY REACTIONS IN THE LUNG IND/TIME SEQUENCE OF

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INFUX OF CADMIUM AND CALCIUM U/SUPERFICIAL BINDING AND

INFORMATIONAL SOURCES FOR THE HAZA/ENTIFY CHEMICALS AND

INFUSION STUDY WITH PIBENZIMOL (/CONTINUOUS INTRAVENOUS

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INHALANTS PRODUCE PENTOBARBITAL-LIKE DISCRIMINAT/ABUSED

INHALATION (AI) OR ENDOTrACHEAL /N BY NOSE-ONLY AEROSOL

INHALATION AND ORAL EXPOSURES /BY COMPARISONS BETWEEN

INHALATION EXPERIMENTS IN RODENTS FOR TESTING THE CARCI

INHALATION EXPOSURE OF P344/N RATS /EFFECTS OF A 4-WEEK

INHALATION EXPOSURE OF RATS AND /EFFECTS OF A 4-DAY

INHALATION EXPOSURE TO 1, 4-DICHL/TS FOLLOWING LONG-TERM

INHALATION EXPOSURE TO 1-NITRO/NE FUNCTION FOLLOWING

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INHALATION EXPOSURE TO PYROLYSIS/ONSES OF RATS TO ACUTE

INHALATION EXPOSURE TO STYRENE A/OTOXICITY DUE TO MIXED

INHALATION EXPOSURE./THYLENE DICHLORIDE (EDC) FOLLOWING

INHALATION EXPOSURES./THYL BROMIDE IN THE RAT FOLLOWING

INHALATION IN GUINEA PIGS: STEROE/CHRONIC COTTON DUST

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INHALATION IN GUINEA PIGS: STEROE/CHRONIC COTTON DUST

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INHALATION OF EMISSIONS OF PYROL/NTAL LUNG TUMORS AFTER

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INHALATION STUDIES./ERED MAN-MADE FIBERS FROM LONG-TERM

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INHALATION TOXICITY AND CARCINOGENICITY STUDY O/CHRONIC

INHALATION TOXICITY OF AEROSOLS OF T-2 TOXIN IN S/ACUTE

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INHALATION TOXICITY OF CHLOROTRI/UBACUTE AND SUBCHRONIC

INHALATION TOXICITY OF HEXACHLOROC/UBCHRONIC (13-WEEK)

INHALATION TOXICITY OF HYDROGEN CYANIDE./ACUTE
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INHALATION TOXICITY OF NICKEL SUBSULFIDE TO F344/N RATS

INHALATION TOXICITY OF NICKEL SULFATE TO F344/N RATS AN

INHALATION TOXICITY OF ORASOL NAVY/ACUTE AND SUBCHRONIC

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INHALATION TOXICITY OF RIBAVIRIN IN SUCKLING PERRETS.

INHALATION TOXICITY OF TETRAMETHOXYISILANE IN/SUBCHRONIC

INHALATION TOXICITY STUDIES OF B/UBCHRONIC MULTISPECIES

INHALATION TOXICITY, REPRODUCTION, AND TERAT/NINETY-DAY

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INHALATION./.578BL/6J MICE FOLLOWING SUB-CHRONIC BENZENE

INHALATION./.DY IN CD RATS EXPOSED TO METHYL BROMIDE VIA

INHALATION./DEVELOPMENT OF PULMONARY EDEMA FOLLOWING NO2

INHALATION./F344 RATS FOLLOWING METHYL ISOCYANATE (MIC)

INHALATION./IBENZO(c,g)CARBAZOLE AEROSOLS IN RATS AFTER

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INHALED ALDEHYDES./CROSS-LINKING IN THE NASAL MUCOSA BY

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INHALED ISOPRENE (2-METHYL-1,3-B)STUDIES OF THE RATE OF

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INHIBITION DURING PROLONGED DFP /VIVO PROTEIN SYNTHESIS

INHIBITION OF ACETAMINOPHEN GLUCURONIDATION BY DIETARY

INHIBITION OF CYTOCHROME P-450 MEDIATED METABOL/I IN VIVO

INHIBITION OF CYTOCHROME P-450-MEDIATED ARYL HYDROCARBO

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INHIBITION OF UTERINE GROWTH, ORNITHINE DECARBOXYLASE (EU-4867) IN BEAGLE DOGS/BACUTE TOXICITY OF AGE INHIBITOR OF GAMMA-GLUTAMYLTRAN/VENESS TO ACIVIN, AN QUINAPRIL/DRLY ANGIOTENSIN CONVERTING ENZYME INHIBITOR W-13 ON THE CELLULAR M/FECT OF THE CALMODULIN INHIBITOR./ BY LUNG CYTOCHROMES P-450 AND A PROSPECTIVE INHIBITOR. OF AN IRREVERSIBLE CHOLINE ACETYLTRANSFERASE INHIBITORS (BZAOI)./ CELL INJURY BY BENZYLAMINE OXIDASE INHIBITORS AND METHYLMERCURY (ME/TIONS OF MITOCHONDRIAL INHIBITORS OF OXIDATIVE METABOLI/PEFFECTS OF INDUCERS AND INHIBITORS ON THE TOXICITY AND P/ ALCOHOL DEHYDROGENASE INHIBITORS, ETHOPROPAZINE AND BW2/USE OF CHOLINESTERASE INHIBITORS./ INHIBITION OF TOXICITY BY SELECTIVE ENZYME INHIBITORS./CALCIOTROPIC HORMONES AND PHOSPHODIESTERASE INHIBITORS./TIVITY IN RABBIT AND RAT LUNGS USING ENZYME INHIBITS CYCLOOXYGENASE (AC) ACTIVITY IN CULT/OSONE (O3) INITIATING POTENTIAL IN RATS LIVE/STUDIES TO EXAMINE THE INITIATION AND PROMOTION OF LIVE/METERS ASSOCIATED WITH INOCULATION WITH DIVERSE CHEMICAL/KENS FOLLOWING INJU INSTOTROPIC EFFECT OF CYCLOPZIACONIC ACID (CPA) ON SMOOTH INSTOTROPIC/VASODILATING COMPOUND ON THE/F 94120, A NOVEL INSECTICIDE DETOXICATION IN BOVINE AND /ORGANOPHOSPHATE INSECTICIDE METABOLITE U-40481/. IONS BY THE FORMAMIDINE INSECTICIDES BY CONSTITUTIVE FORM/ABOLISM OF CYCLODIENE INSECTICIDES ON NTE, ACHE, AND GA/THREE ORGANOPHOSPHATE INSECTICIDES. SHOW SIMILAR EFFECTS /THREE ORGANOCHELORINE INSECTICIDES./TRANSDERMAL ABSORPTION OF ORGANOPHOSPHATE INSECTICIDES: RELATIONSHIP TO THE /CTIONS OF PYRETHROID INSPIRED CO2 FOR EVALUATION OF THE/APPROPRIATE LEVEL OF INSTILLATION (EI)./OR ENDOTRACHEAL NEBULIZATION (EN) OR INSULIN THERAPY./OTOCIN IN GUINEA PIGS: MODIFICATION BY INTERACTION BETWEEN PHENCYCLIDINE (PCP) AND 1-PHENYLICYC INTERACTION OF HALOGENATED HYDROCARBON MIXTURE/CHEMICAL INTERACTION OF ORGANOPHOSPHATE AN'/ PHOSPHOLIPIDS ON THE INTERACTION OF TRANSITION METALS AND ACTIVE OXYGEN IN D INTERACTION ON DELTA-AMINOLEVULIN/ CADMIUM AND SELENIUM INTERACTION WITH CARBON TETRACHLORIDE (CCl/CELIPROLOL'S INTERAKCTIONS IN KIDNEYS OF THE SCALL/CADMIUM AND COPPER INTERAKCTIONS OF DIETHYLPHENYLPHOSPHINE WITH PURIFIED , R INTERAKCTIONS OF MITOCONDRIAL INHIBITORS AND METHYLMERC INTERCELLULAR COMMUNICATION PROCES/RINATED BIPHENYLS ON INTERFERON INDUCES DECREASED HEMATOPOIESI AND AN/NOSE INTERFERON RESPONSE./O/VIRUS INFECTION AND VIRUS INDUCED INTERLEUKIN 1, 2 AND 3 PRODUCTION./INDUCED MODULATION OF INTERLEUKIN PRODUCTION BY DIMETHYLNITROSO/MODULATION OF INTERLEUKIN-2 RESPONSIVENESS./INDUCED DOWN-REGULATION OF INTERSCAPULAR BROWN ADIPOSE TISSUE/THOLOGY OF LIVER AND INTERSPECIES COMPARISON OF EYE IRRITATION. INTERSPECIES COMPARISON OF TIME COURSE, DOSE RESPONSE, INTERSPECIES COMPARISONS OF LUNG RESPONSES TO INHALED D INTERSTITIAL PNEUMONIA INDUCED BY TRIETHYLENE-TETRAMINE INTRACELLULAR PIGMENTATION IN MICE AND RATS FOLLOWING L INTRACOULAR INJECTIONS OF TUNICAM/ TRANSPORT INDUCED BY INTRATRACHEAL ADMINISTRATION OF EN/NEA PIGS TO REPEATED INTRATRACHEAL ADMINISTRATION./4 RATS FOLLOWING ORAL AND INTRATRACHEAL AND INHALATION MODES/ONS: A COMPARISON OF INTRATRACHEAL CATHETERIZATION AS A METHOD FOR/LONG-TERM INTRATRACHEAL DEPOSITION./PENTOXIDE FROM RAT LUNG AFT INTRATRACHEAL INSTILLATION OF MINE/PONE OF MICE TO THE INTRAVASCALLY TO RATS AND FERRETS/ACTANT ADMINISTERED INTRAVASCULAR HEMOLYSIS FOLLOWING THERMAL INJURY./ACUTE INTRAVENOUS INFUSION STUDY WITH PIBENZIMOL (/CONTINUOUS INTRAVENOUS INFUSIONS IN TOXICOLOGICAL STUD/EXPONENTIAL INTRAVENOUS INJECTION, OR DERMAL /RATS DOSED BY GAVAGE, INTRAVENOUS PRECLINICAL SAFETY ST/L VEHICLES FOR USE IN INTRAVENOUS SAFETY EVALUATION STU/MONAS IMMUNE GLOBULIN INTRAVENOUS TOXICITY EVALUATION OF METHIONIN/SUBCHRONIC INTRAVENOUS TOXICITY OF CI-937, AN ANTHIPYRAZOLE/ACUTE INTRAVENOUSLY TO RATS, GUINEA PIGS/E (MDA) ADMINISTERED INY-6202 IN THE RABBIT./SUBCHRONIC DERMAL TOXICITY OF
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ISOLATED HEPATOCYTES: STRUCTURE/NISTS TO SUSPENSIONS OF
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ISOLATED RAT HEPATOCYTES./ UNSCHEDULED DNA SYNTHESES IN
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ISOLATED RAT HEPATOCYTES./ORIDE (CCL4) AND DIGITONIN ON
ISOLATED RAT HEPATOCYTES./TY OF THE DICHLOROBENZENES IN
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ISOLATED RENAL PROXIMAL TUBULES TO HYPOXIA//RESPONSE OF
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ISOLATION AND PURIFICATION OF UR/ NEW TECHNIQUE FOR THE
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ISOMER-DEPENDENT INDUCTION OF METHEMOGLOBINEMIA BY DNI
JUNCTION (NMJ). APOTOSOMES & FROG SKELETAL NEUROMUSCULAR

KAINIC ACID NEURONAL RECEPTOR BINDING / INHIBITION OF KAINIC ACID. LUMBAR POTASSIUM IN AWAKE RATS TREATED WITH KAOLIN CYTOPATHIC EFFECT OF COAL ON SILICA AND KEPONE IN RATS FOLLOWING 4-AM/ROPIHENYL (6CB) OR 14C- KERATINIZATION IN THE HAIRLESS MOUSE AND ALTERATIONS IN KERATOINOCYTE CULTURE SYSTEM FOLLOWING A DIFFERENTIATED KERATOINOCYTE CULTURES. (RABP) IN PRIMARY RAT EPIDERMAL KERATOINOCYTE PROLIFERATION AND DIHT-INDUCED CHANGES IN KERATOINOCYTES IS MEDIATED BY DIFPE/DERMIS AND KERATOINOCYTES. AGE IN PRIMARY CULTURES OF RAT EPIDERMAL KERATOINOCYTES. LPDII (BCES) -INDUCED DNA CROSSLINKING IN KETOGENESIS BY METABOLITES OF PDA/INHIBITION OF HEPATIC KETOACIDURIA IN THE REDUCTION OF IC EFFICACY OF ALPHA- KETOGlutARIC ACID. CYANIDE-INDUCED LETHALITY BY ALPHA- KETONE (DIK) VAPOR RECEIVING 9 EXPOSURES OF DIISOBUTYL KETONE BY LUNG CYTOCHROMES P-450 OF METABOLISM OF PERILLA GONIOMOTORIS. LIVER INJURY BY METABOLITES OF METHYL ISOBUTYL KETONE. LIVER INJURY BY METABOLITES OF M ETHYL ISOBUTYL KETONE AND METABOLITES OF M ETHYL ISOBUTYL KETONE AND LIVER MITOCHONDRIA. LICYCIC ACID BY ISOLATED KIDNEY AND URINE OF NONPREGNAN/EFFECT OF ORAL CADMIUM OF KIDNEY ENDOPLASMIC RETICULUM C/OMALATE INHIBITS THE RAT KIDNEY LEAD-BINDING PROTEIN (P/ON CHROMATOGRAPHY OF RAT KIDNEY PBEP / PROTEIN (PBSP) IN BRAIN: COMPARISON WITH A KIDNEY SLICES/ DROQUINONE-GLUTHIONE CONJUGATES BY RAT KIDNEY 2N/METLALLOTHIONEIN (MT)/ATASE (ALAD) BY PURIFIED KIDNEY, LUNG AND BRAIN OF MICE. ENZYME SYSTEM IN LIVER, KIDNEY / MULTIPLE GSH CONJUGATE TRANSPORT SYSTEMS IN THE KIDNEY. NCE OF Zn ON Pb INCLUSION BODY FORMATION IN RAT KIDNEYS OF THE SCALLOP, PLACOPE/ COPPER INTERACTIONS IN KILLER CELL ACTIVITY IN C57BL/STYRENE OXIDE ON NATURAL KINASE (CAMP-PK) LEVELS AFTER /C CAMP-DEPENDENT PROTEIN KINASE AND SYNAPTOSOMAL UPTAKE/N CAMP-DEPENDENT PROTEIN KINASE C ACTIVITY IN EL4 CELLS/ 2,3,7,8-TCDD ON PROTEIN KINASE C ACTIVITY IN THE HEPAT/CAUSES INCREASED PROTEIN KINASE IN RAT LIVER./F CYTOSOLIC CAMP-DEPENDENT PROTEIN KINETIC EVALUATION OF CARRIER-MEDIATED TRANSPORT OF OUA KINETIC MEASUREMENTS IN RATS. ING NICKEL DOSIMETRY FROM KINETICS AFTER DERMAL EXPOSURE. CHLORDECONE KINETICS BY PERIPHERAL SITE LG/ASE (Che) CARBAMATE KINETICS IN RATS /A PHYSIOLOGICAL MODEL OF LEAD KINETICS IN SOMATIC AND SPERMAT/EGES IN RELATION TO CELL KINETICS OF INHALED METHANOL IN MALE FISCHER-344 RATS. KINETICS OF INHALED SUBSTANCES. ABSTRACTION AND ELIMINATION
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MI (1,2-14C) /yonal accumulation of radioactivity from
MI (2,3) /Teratogenicity does not /tion of 2-methoxyethanol /
Mechanism and locus of ethylene dibromide (EDB/possible
Mechanism for glycogen depletion/minophen as a possible
Mechanism of 2-bromohydroquinone-glutathione conjugate
Mechanism of antagonizing cyanide-induced lethality by
Mechanism of bromobenzene covalent binding
Mechanism of cadmium induced cell /e of calmodulin in the
Mechanism of carbon tetrachloride D protein adducts as a
Mechanism of chloroform stimulant/further studies on the
Mechanism of diabetes-induced enhancement of UDPGA synth
Mechanism of ethanol protection against acetalaminophen H
Mechanism of nitrosourea-induced hepatic/studies on the
Mechanism of nitrosourea-induced hepatic/studies on the
Mechanism of particle recognition/bers and particulates:
Mechanism of reversal of lead (Pb) inhibition of delta-
Mechanism of thyroid hyperplasia in /examination of the
Mechanism of toxic action /HeLENalin:
Mechanism of toxicity of 2,2,2-trifluoroethanol in rats
Mechanism of urototoxic activity of N,N-di/studies on the
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Medaka (oryzias latipes) liver /enzyme altered foci in
Medaka (oryzias latipes) /and survival of the Japanese
Medaka (oryzias latipes) /al parameters in the Japanese
Mediators of chemical toxicity I//I/derived oxidants as
Mediators of chemically induced /Se, dose response, and
Mediators of inflammation in res/ferences in release of
Medicine's new toxicology data NA/he national library of
Me(He) on spontaneous release /itors and methylmercury (en
MEK following acute exposure /ione urinary excretion by
Membrane composition and functi/duced perturbations in
Membrane damage /ated rat hepatocytes before detectable
Membrane lipids of rat livers /Dibromoethane to plasma
Membrane of the rat and guinea /y in the hepatic plasma
Membrane sodium channels /S intrinsic activity on nerve
Membrane structure /free-radical induced alterations of the
Membranes: use of 2,4,6-trinitro / in electroplax plasma
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Mercuric chloride and hexachlor/utathionuria induced by
Mercury (Hg2+) on neurotransmitter release /effects of
Mercury and vanadate on the GlutA/influence of cadmium,
Mercury compounds in mouse brain /uptake of
Mercury exposure in the rat /ions after in utero methyl
Mercury in mice /Epitoxicity of cadmium, vanadate and
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1022 METABOLISM OF n-OCTANE IN FISHER 344 RATS.
570 METABOLISM OF LEUKOTRIENE B4 (LTB4) /HEPATIC MICROSOMAL
114 METABOLISM OF PALMITIC ACID IN TCDD-TREATED RATS.
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833 METABOLISM OF PERILLA KETONE BY LUNG CYTOTOXICITY/POSITION OF
1022 METABOLISM OF PESTICIDES IN NIBRITION ON THE MICROSOMES.
1023 METABOLISM OF PICRIC ACID (2,4,6-ITY, DISTRIBUTION, AND
1205 METABOLISM OF PLASMA CHOLESTEROL/PHAME, ELIMINATION AND
540 METABOLISM OF PULMONARY SURFACTA/INDUCED CHANGES IN THE
52 METABOLISM OF PYRROLIZIDINE ALKAL/MONOOXGENASE IN THE
1049 METABOLISM OF SALICYLIC ACID BY ISOLATED KIDNE/IN VITRO
1049 METABOLISM OF THE ANTI-INFLAMMATORY AGENT 5-(4-PYRIDYL)
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102 METABOLISM OF TRICLOPYR BUTOXETHYL EST/TOXICITY AND
980 METABOLISM OF [3H]-2 TOXIN: COM/ANEOUS PENETRATION AND
1019 METABOLISM OF [HEXYL-2-14C]-DI[2-ETHYL THE ABSORPTION AND
1025 METABOLISM OF [U-14C]HYDROQUINONE IN FISCHER 344 RA/TH.
1270 METABOLISM OF 2,2',4,4',5,5'-HEXACHloro/RESPONSIBLE FOR THE
562 METABOLISM ON THE IRREVERSIBLE B/HIBITORS OF OXIDATIVE
336 METABOLISM PRODUCED BY DIOXIN 1 OF HEPATIC MICROSOMAL
620 METABOLISM TO A REACTIVE METABOL/CARBON DISULFIDE (CS2)
663 METABOLISM/PH CYTOCHROME C REDUCTASE ACTIVITY AND BaP
731 METABOLISM/METABOLISM IN THE RAT: CALCIUM AND PHOSPHORUS
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265 METABOLITE ANTIBODIES/EN USING SPECIFIC ANTI-HALOTHANE
53 METABOLITE OF R-(+)-NICOTINE/OXONICOTINAMON ION - A NEW
628 METABOLITE OF THE GENOTOXICANT 2/YL ALCOHOL (2ABAlc), A
324 METABOLITE U-404B1./IONS BY THE FORMAMIDINE INSECTICIDE
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1003 METABOLITES BY HPLC/ERMINATION OF SALICYLAMIDE AND ITS
1226 METABOLITES BY MASS SPECTROMETRIC/F QUATERNARY AMMONIUM
788 METABOLITES IN SPECIFIC BRAIN REG/F BIOPHYSIC AMINES AND
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1013 METABOLITES OF 2,4- AND 2,6-DIMETHYLANILINE IN /URINARY
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150 METABOLITES OF CIENT VIOLET IN CHEMOS/MUTAGENICITY OF
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449 METABOLITES OF METHYL ISOBUXYL KE/TATIC LIVER INJURY BY
1002 METABOLITES OF NICOTINE ENANTIOMER/TION OF POLAR URINARY
615 METABOLITES OF O-DIBROMOBENZENE (DBB). IN VITR/REACTIVE
464 METABOLITES OF PLASTICIZERS: STUD/EPATIC KETOGENESIS BY
686A METABOLITES ON THE IN VITRO HUMOR/HOM NAPHTHALEN (NA)
1157 METABOLITES OT ISOLATED SPERMATOC/ (DBCP) AND PROPOSED
630 METABOLITES TO DNA IN VITRO./BINDING OF BENZO(A)PYRENE
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149 METABOLITES/E AND ENAMINOSPECIFIC MUTAGENESIS BY AZIDE
583 METABOLITES/NA ADDUCTS WITH BENZO(a)PYRENE(BP) AND ITS
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203 METAL AND STRESS PREVENTION ON ACUTE IAD/INFLUENCE OF THE
1010 METAL BINDING AGENT 2,3-DIMERIC/BIOCONVERSION OF THE
1009 METAL BINDING AGENTS 2,1-DIMER/URINARY EXCRETION OF THE
425 METAL BINDING PROTEINS IN THE BLUE CRAB, CALLINECTES SA
344 METAL CATION TREATMENT./ELL FUNCTION IN VITRO FOLLOWING
1055 METAL GROUP FOR DEALKYLATION / ORGANIC CONSTITUENT AND
424 METALLOTHIONEIN (MT) BY ALCOHOLS./INDUCTION OF HEPATIC
422 METALLOTHIONEIN (MT) FOLLOWING ADMI/INDUCTION OF HEPATIC
193 METALLOTHIONEIN (MT)/ASE (ALAD) BY PURIFIED KIDNEY Zn-
426 METALLOTHIONEIN BY SILVER AND CADMIUM MIN/ESTIMATION OF
421 METALLOTHIONEIN IN RAT HEPATOCYTE CULTURES/INDUCTION OF
103 METALLOTHIONEIN IN RAT TISSUES./GES IN COPPER, ZINC AND
107 METALLOTHIONEIN IN THE RAT TESTES./E FOR THE ABSENCE OF
1080 METALLOTHIONEIN-I (MT-I) AND METALLO/NTIAL INDUCTION OF
1080 METALLOTHIONEIN-II (MT-II) BY CADMIU/ONEIN-I (MT-I) AND
408 METALLOTHIONEIN/KIDNEY LEAD-BINDING PROTEIN (PbBP) AND
430 METALLOTHIONEIN./ROUGH INCREASED SYNTHETIC CAPACITY FOR
423 METALLOTHIONEINS BY HIGH-PERFORMANCE/AND QUANTITATION OF
METALS AND ACTIVE OXYGEN IN INTERACTION OF TRANSITION METALS/EFFECTS OF NITRILOTRIACETIC ACID (NTA) AND HEAVY METALS./INHIBITION OF METABOLIC COOPERATION BY METAPHOSPHATE FIBER: A NOVEL MANMA/TY OF CALCIUM SODIUM METAPLASIA IN MOUSE BLADDER EPITHELIUM INDUCED/SQUAMOUS METASTASIS OF TUMOR CELLS IN MIC/E ON THE CLEARANCE AND METHACHOLINE IN ASTHMA/EXPOSE/SENSITIVITY TO INHALED METHACRYLATE (MA) ESTERS./TOXICITY OF ACRYLATE (A) AND METHANES, ETHANES, AND ETHYLENES/LITIRES OF HALOGENATED METHANOL IN MALE FISHER-344 RATS./KINETICS OF INHALED METHEMOGLOBINEMIA BY DINITROBENZENES/NDEUT INDUCTION OF METHIONINE-ENKEPHALIN (MET-ENK)/TOXICITY EVALUATION OF METHOD FOR DETERMINING INHIBITION OF IODIDE /IN VIVO METHOD FOR ESTABLISHING ACCEPTABLE EXPOSURE LEVELS (A/A) METHOD FOR RABBIT DERMAL TOXICITY TESTING EMPLOYING V/A METHOD FOR RAT TAIL VENOPUNCTURE AND USE IN PH/A SIMPLE METHOD FOR REPEATED ADMINISTRATIONAL CATHETERIZATION AS A METHOD FOR THE SERIAL DETERMINATION OF THE STOMACH CO/A METHYL BENZENOPHENE (MBA) IN RATS DO/ON OF 2-HYDROXY-4 METHYLCYCLOHEXENE (MCHE) IN MICE./VE DEVELOPMENT IN HAMSTERS INDUCED BY METHYLCYCLOHEXENE (MCHE) UPON THE HYPOTHALAMUS INHIBITION OF METHYLCYCLOHEXENE-INDUCED (MCHE) ALTERATIONS OF REPRODUCTIVE DE Methoxyacetoaldehyde AVOIDANCE OF THE FEMALE RAT BY METHOXYETANOL /METEROTGENICITY/O1 ATTENUATION OF 2 METHOXYFLURAN-INDUCED ENHANCEMENT OF ACETYL ALCOHOL DE Inhalation Toxicity of METHYLBROMIDE IN B6CF1 MICE./IC INHALATION OF METHYLBROMIDE IN THE RAT FOLLOWING INHALATION THE UPTAKE OF METHYLBROMIDE /PATHOLOGY OF METHYLBROMIDE VIA INHALATION .DY IN CD RATS EXPOSED TO METHYLCYCLIC IN MICE BY GLU/ACUTE RENAL TOXICITY OF METHYLCYCLIC INDUCED PREIMPLANTATION LOSS IN F-344 RATS BY METHYL ISOBUTYL KETONE./LIVER INJURY BY METABOLITES OF METHYL ISOCYANATE (MIC) IN F344/N R/TOXICITY OF INHALED METHYL ISOCYANATE (MIC) IN MAC/REPRODUCTIVE TOXICITY OF METHYL ISOCYANATE (MIC) INHALA/S IN F344 RATS FOLLOWING METHYL ISOCYANATE (MIC) TOXICITY INVOLVEMENT OF CYANIDE IN METHYL ISOCYANATE (MIC) VAPOR ON THE EYES OF EFFECT OF METHYL ISOCYANATE (MIC)./IN MALE F-344 RATS EXPOSED TO METHYL ISOCYANATE (MIC)/GENETIC TOXICITY OF METHYL ISOCYANATE IN MICE./IMMUNOTOXICOLOGY OF METHYL ISOCYANATE ON SMALL AIRWAYS I/INITIAL EFFECTS OF METHYL ISOCYANATE VAPOR INHALATION/RESPIRATORY EFFECTS OF METHYL ISOCYANATE./AND CLINICAL IMMUNOLOGIC RESPONSE TO METHYL ISOCYANATE./D DOSE TOXICITY STUDIES WITH INHALED METHYL ISOCYANATE: REPRODUCTIVE AND DEVELOPMENTAL TOXIC METHYL MERCURY EXPOSURE IN THE/TERATIONS AFTER IN UTERO METHYL PARATHION METABOLITES WITH REVERSE-P/ANALYSIS OF METHYL PCE2/-LIUM IN B6CF1 MICE TREATED WITH 15(R)-15- METHYL-1,3-BUTADIENE IN RATS MAY PRE/LED ISOPRENE (2- METHYL-BENOZATO FOLLOWING CHROMIC MINATION OF 4-BROMO- METHYL-MERCURY IN THE BALB/C MOUSE/SSUE DISTRIBUTION OF METHYL-N-AMINOLTRISAMINE FOLLOWING I.P./GEAL CARCINOGEN METHYL-T-BUTYL ETHER IN RATS./ION REPRODUCTION STUDY OF METHYLBENZYL TRIS-TP AN/XICITY OF SPECIFICALLY METHYLCHOLANTHRENE OR PHENOBARBITAL / INDUCTION WITH 3 METHYLCHOLANTHRENE OR PHENOBARBITAL / INDUCTION WITH 3 METHYLCHOLANTHRENE OR PHENOBARBITAL / INDUCTION WITH 3 METHYLCYCLOPENTADIENYL MANGANESE TRIC/TOXIC EFFECTS OF METHYLENE CHLORIDE (MEC)/AND RISK ASSESSMENT: EXAMPLE,
1048 Methylene Chloride (MEC). / IN VIVO METABOLISM 14C-
910 METHYLENE CHLORIDE IN MICE BY DO/N VIVO MUTAGENICITY OF
662 METHYLENE CHLORIDE: TUMORIGENIC IMPL/AcUTE TOXICITY OF
1028 METHYLENEDIAMINLINE (MDA) ADMINISTRATION TO RATS/G EFFICIENCY OF 4,4'-
1026 METHYLENEDIAMINLINE (MDA) IN RATS/.G EFFICIENCY OF 4,4'-
1027 METHYLENEDIAMINLINE (MDA)./N DERMAL DISPOSITION OF 4,4'-
627 METHYLENEDIAMINLINE (MDA)/.N DERMAL DISPOSITION OF 4,4'-
289 METHYLCYCLOPENTENONE (MeHg) IN THE RAT VI/ AND DISTRIBUTION OF
441 METHYLMERCURY (MeHg) N SPONTANEOUS/DRIAL INHIBITORS AND
288 METHYLMERCURY (MeHg): IT'S EFFECTS ON PROTEIN SYNTHESIS
607 METHYLMERCURY (MM) IN RATS/.N OF ENDONUCLEOS THIOLSE AND
444 METHYLMERCURY (MM)/.GLUTATHIONE-RELATED SULFHYDRYS AND
639 METHYLMERCURY IN THE MARINE ELASMO/LIARY ELIMINATION OF
199 METHYLMERCURY INHIBITION OF RAT BR/ AGAINST CADMIUM AND
445 METHYLMERCURY METABOLISM AND EXCRE/IC FACTORS AFFECTING
202 METHYLMERCURY ON LYMPHOCYTE MICROTUBUES/.EFFECTS OF
122 METHYLMERCURY ON SPERM G2 CONSUMPTION/.EFFECTS OF
496 METHYLMERCURY ON THE CYTOSKELETON OF EMBRYON EFFECTS OF
440 METHYLMERCURY/.E CEREBELLAR CELLS FOLLOWING EXPOSURE TO
856 METHYLPHOSPHONITE (QL).YL-O'(-2-DIISOPROPYLAMMINOETHYL)
1016 METHYLPHOSPHONITE /CARBONYL-14C] (4/ETICS OF 4-NITRO-N-
1011 METHYLPROPENE (DIMETHYLVINYLL CHLORIDE, DM/OP 1-CHLORO-3-
1098 METHYLXANTHINE TREATMENT AND ACTIVITY OF 2',5'-OLIGOADE
789 METOCLOPRAMIDE (MET) EFFECTS ON PROLACT/QUINAPZINE (QP) -
1162 METRONIDAZOLE ON PENTATHY, EPIDID/OF ORNITAZOLE AND
83 Mg++ CONTENTS IN RAT BRAIN./RAGANOPHOSPHATES ON CA++ AND
773 METHIONINE ACTIVITIES IN RAT BRAIN/ON NA+, K-, ATPase AND
309 MIC IN F344/N RATS AND B6C3F/HALED METHYL ISOCYANATE (MIC)
304 MIC IN MICE-.REDUCTIVE TOXICITY OF METHYL ISOCYANATE (MIC)
307 MIC INHALATION/.344 RATS FOLLOWING METHYL ISOCYANATE (MIC)
300 MIC TOXICITY./VEMENT OF CYANIDE IN METHYL ISOCYANATE (MIC)
308 MIC VAPOR ON THE EYES OF F3/E ACT METHYL ISOCYANATE (MIC)
303 IN MALE P-344 RATS EXPOSED TO METHYL ISOCYANATE (MIC).
312 MIC)./GENETIC TOXICITY OF METHYL ISOCYANATE (MIC)
902 MICROBIAL METABOLISM AND DETOXIFICATION OF 7,12-DIMETHYLB
1220 MICROENCAPSULATION PROCESSES ON CHEMICALS FOR/EFFECT OF
765 MICROPROBE ANALYSIS OF RAT SCIATIC NERVE UNDER NO/X-RAY
841 MICROPROBE FOR TRACE ELEMENT DETERMIN/SYNCHROTRON X-RAY
402 MICROSCOPIC STUDY OF CRANIOFACIAL D/A SCANNING ELECTRON
48 MICROSONAL CYTOCHROME C REDUCTASE ACT/THE INHIBITION OF
1103 MICROSONAL CYTOCHROME P-450/NDUCIBLE FORM OF RAT LIVER
569 MICROSONAL ENZYMES/RESIST-STERONE OXIDE-INDUCED HEPATIC
566 MICROSONAL METABOLISM IN THE ANE/IBLE EFFECT OF GOLD ON
1259 MICROSONAL METABOLISM IN THE RAT/ ANHYDRIE (B) HEPATIC
591 MICROSONAL METABOLISM OF LEUKOTRIENE B4 (LTB4)/HEPATIC
1032 MICROSONAL METABOLISM OF PESTICIDE/NOL CONSUMPTION ON THE
336 MICROSONAL METABOLISM PRODUCED B/E INDUCTION OF HEPATIC
334 MICROSONAL MIXED FUNCTION OXIDAS/VARIABLE INHIBITION OF
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1035 MICROSONAL OXIDATION OF ALLYL ALCOHOL/.NADPH-DEPENDENT
579 MICROSONAL OXIDATION OF PHORATE/.XENASE SYSTEM IN THE
1272 MICROSONAL TESTOSTERONE HYDROXYL/ROCLOR 1254 ON HEPATIC
14 MICROSONES AND ISOLATED LUNG CEL/ 1-NITROPYRENE BY LUNG
571 MICROSONES FROM FEMALE RATS TREA/ALTERATIONS IN HEPATIC
45 MICROSONES FROM FEMALE RATS/.O-4-NITROANILINE (DCNA) IN
618 MICROSONES./CONALDEHYDE FROM 14C-BENZENE IN MOUSE LIVER
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1005 MICROSONES./NAMIDE HYDROXYLAMINE BY RAT AND HUMAN LIVER
46 MICROSONES/.P-GLUCURONYLTRANSFERASE FROM RABBIT HEPATIC
951 MICROSONES./TRosoNORMONITRINE (NNN) MEDIATED BY RAT LUNG
643 MICROSONES./XIDE HYDROLASE (EH) ACTIVITY IN TROUT LIVER
764 MICROSPBULE AND SPINAL CORD NEURO/TION OF CHICKEN BRAIN
763 MICROSPBULE ASSEMBLY PROPERTIES ARE ALTERED FOLLOW/RAST
202 MICROSPBULES/.EFFECTS OF METHYLMERCURY ON LYMPHOCYTE
401 MIGRATION AS AN INDEX OF DELAYED TYPE/BONE MARROW CELL
991 MILK PRODUCES ENHANCEMENT OF /LOB AND DDE INTO MATERNAL
1169 MILK/.TER DIRECTIONS AND EXPOSURE T/DEEP VIA BREAST
23 MINERAL FIBERS IN MESTHESIOMA INDUCTION/SIGNIFICANCE OF
537 MINERAL FIBERS./CE TO THE INTRATRACHEAL INSTILLATION OF
NEPHROTOXIC POTENTIAL OF TRICHLOROETHYL STUDIES OF ACUTE
NEPHROTOXICITY AND RETENTION IN TH/ENTANE (TPM)-INDUCED
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NEPHROTOXICITY IN FEMALE C57BL MIC/OMYCIN (VAN)-INDUCED
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NEPHROTOXICITY IN RATS./ATE SUPPRESSION OF CIS-PLATINUM
NEPHROTOXICITY IN RATS./STEREOSPECIFICITY OF LYSINE
NEPHROTOXICITY IN SUBTOTALY NEPHRECTOMIZED RATS RECEI
NEPHROTOXICITY OF S-(2-CHLOROETHYL)-GSH (CEG), AND S-(2
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NEPHROTOXICITY./CHLOROPHENYL)SUCCINIMIDE (NDPS)-INDUCED
NEPHROTOXICITY./E CELLS AS AN IN VITRO MODEL OF MERCURY
NEPHROTOXICITY./HARACTERIZATION OF MALE RAT HYDROCARBON
NEPHROTOXICITY./QUINONE-GUTHATHIONE CONJUGATE MEDIATED
NEPHROTOXIN IN MALE RATS./IMETHYL PENTANE (ISOCTANE), A
NEPHROTOXIN N-(3,5-DICHLOROPHENYL)/PRETREATMENT WITH THE
NERVE MEMBRANE SODIUM CHANNELS/AS INTRINSIC ACTIVITY ON
NERVE UNDER NORMAL AND NEUROTO/ANALYSIS OF RAT SCATIC
NERVOUS TISSUE CALCIUM CONCENTR/EFECTS OF ACRYLAMIDE ON
NEURAL TISSUES AFTER TREATMENT/ENZYME ACTIVITIES IN HEN
NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF PREGNATAL /EARLY
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NEUROECTODERM./OSKELETON OF EMBRYONAL CARCINOMA-DERIVED
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NEUROFILAMENT CROSSLINKING IN RATS/DETECTION OF IN VIVO
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NEUROLEPTICS IN RATS./ANTATION INDUCED BY BUTYROPHENONE
NEUROMUSCULAR JUNCTION (NMJ)./APTOSOMES & FROG SKELETAL
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NEURONAL RECEPTOR BINDING BY DI/HIBITION OF KAINIC ACID
NEUROPATHOLOGICAL CHANGES IN MACAQUE/VE DYSFUNCTION AND
NEUROPATHY (OPIDN) IN CHICKENS./OSPHATE INDUCED DELAYED
NEUROPATHY (OPIDN) IN CHICKENS./SPHORUS-INDUCED DELAYED
NEUROPATHY (OPIDN) IN RATS./PHOSPHORUS-INDUCED DELAYED
NEUROPATHY./ON IS A PATHOGENETIC STEP IN GAMMA-DIKETONE
NEUROTENSIN AND HISTAMINE LEVELS./POSTRADIATION PLASMA
NEUROTOXIC CONDITIONS./ST SCATIFIC NERVE UNDER NORMAL AND
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NEUROTOXICITY OF A NEW STRUCTURAL ANALOG OF MTPP
NEUROTOXICITY OF ACETYL ETHYL TETRAMETHYL TETRALIN /THE
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NEUROTOXICITY: A MECHANISTIC APPRO/DITHIOCARBAMATE (DDC)
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NEUTROPHEL-DERIVED OXYGEN RADICALS.//ENCE OF A ROLE FOR
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NICKEL SULFATE IN MICE./IMMUNOTOXIC EFFECTS OF
NICKEL SULFATE TO F344/N RATS AN/INHALATION TOXICITY OF
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122 O2 CONSUMPTION /EFFECTS OF METHYLMERCURY ON SPERM
520 O2 TOXICITY AND ENDOGENOUS PROSTAGLANDINS /PULMONARY
516 O3 AND NO2 HEALTH EFFECTS DA /COMPREHENSIVE ANALYSES OF
250 ON SERUM CONCENTRATIONS OF VIT. A, C, AND /EFFECT OF
517 O3, NO2, AND COC12 /ON THE EDEMACGENIC EFFECT OF INHALED
985 OCCULSIVE BARRIER MODEL TO STUDY TOX/EFFICACY OF A NON-
846 OCHEART TOXICOSIS IN YOUNG BROILER CH/ID METABOLISM DURING
727 OCHRATEXIN A IN F344 RATS /CHRONIC TOXICITY OF
1247 OCTACHLORODIBENZO-P-DIOXIN (OCDD) IN RAT /DISPOSITION OF
836 OCTADECATETRAYNE-1,18-DIOL (COMPOUND B) /AND 5,7,11,13-
1020 OCTANE AND 2,2,4-TRIMETHYLPENT/SION OF DISPOSITION OF n-
1022 OCTANE IN FISHER 344 RATS /METABOLISM OF n-
365 OCTYL ACETATE IN RATS /TERATOGENIC EVALUATION OF OXO
958 OCULAR SAFETY EVALUATION STUDY OF CELIPROL0/A SIX MONTH
823 OCULOCARDIAC REFLEX /ITS POTENTIAL DIAGNOSTIC VALUE/ THE
1163 OFFSPRING /IONICS ON SEXUAL DEVELOPMENT IN THE MALE RAT
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ZINC-INDUCED RESISTANCE TO AKYLATING AGENT TOXICITY.
ZINC./-I) AND METALLOTHIONEIN-II (MT-II) BY CADMIUM AND
ZINC./D STRUCTURAL AND BIOCHEMICAL CHANGES IN CALVES BY
ZONISAMIDE./N IN RATS WITH THE NEW ANTIConvULSANT AGENT
ZnO./O HIGH PEAK LEVELS OF FRESHLY GENERATED ULTRA-FINE
Zn ON Pb INCLUSION BODY FORMATION IN RAT K/INFLUENCE OF
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