Preface

This second issue of the *Toxicologist* is devoted exclusively to the abstracts of the platform and poster sessions of the 21st Annual Conference of the Society of Toxicology, held at the Sheraton-Boston Hotel, Boston, MA, February, 22-26, 1982.

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1 BUTYLATED HYDROXYTOLUENE DOES NOT INHIBIT CIFRO-
FIBRATE AND DI-(2-ETHYHEXYL)PHTHALATE INDUCED
PEROXISOME PROLIFERATION IN RAT LIVER. N.D.
Lalwani, H. Hayashi, M.K. Reddy, C. Moehle, and
Med. Sch., Chicago, IL.

Evidence now suggests that hepatic peroxisome
proliferators as a class are carcinogenic. It is
hypothesized that persistent proliferation of
peroxisomes and increase in peroxisomal β-oxidation
initiates the neoplastic transformation of liver cells by increasing the intracellular produc-
tion of DNA-damaging H2O2 and other reactive
oxygen intermediates. In the present study we
have investigated the effect of antioxidant buty-
lated hydroxytoluene (BHT) to determine whether
free radical scavenging antioxidant could inhibit
peroxisome proliferation induced by hypolipidemic
drug ciprofibrate (2,2-[(2,2-dichlorocyclopro-
pyl)-phenoxy]-2-methyl propionic acid) and DEHP.
Ciprofibrate at 0.1% concentration in diet in-
creased peroxisomal enzyme activities in livers of
male F344 rats. Simultaneous feeding of BHT
at either 7000 ppm or 700 ppm in diet did not in-
hbit the peroxisome proliferation and peroxisomal
enzyme activities inducible by ciprofibrate.
Similarly, BHT failed to inhibit DEHP induced
peroxisome proliferation in rat liver. Additional
studies are required to ascertain whether BHT
inhibits the peroxisome proliferator induced
carcinogenesis in rat liver. Supported by NIH
grant GM 23750.

2 A MECHANISM FOR 6-METHYLCUMARIN PHOTODERMAT-
ITIES. M.N. Tabor, S. Kato, M. Ohno and R.R.
Suskind, Dept. of Environ. Hlth, Univ. of Cin-
cinnati Med. Ctr., Cincinnati, OH. Sponsor:
Dr. C. C. Smith.

Photoallergic dermatitis and allergic hypersen-
sitivity have been reported in persons using
commercial products containing 6-methylcoumarin
(6-MC) as a fragrance material. The purpose of
our study was to investigate the biochemical
basis of the photoallergic reactions of 6-MC.
We have examined the ultraviolet (UV) catalyzed
photochemical reactions of 6-MC with representa-
tive amino acids (lys, ser, gly, glu, cys, met,
phe, tyr, and trp) and glutathione (GSH) under
physiological conditions. These reactions were
used to model the photochemical reactions of
6-MC with proteins. The source of UV light
catalysis was either UVA of wavelengths from
310 nm to 400 nm or UVB of wavelengths from
280 nm to 370 nm. Samples were exposed for 1,
12 or 24 hrs, and then reaction products were
isolated by either silica gel TLC or sequential
extraction using a series of nonpolar to polar
solvents followed by silica gel TLC of the ex-
tracts. UVA was a more potent catalyst than
UVB for all reactions studied. For 6-MC alone,
the UV reaction yielded three products at 12
and 24 hrs. The production of the least polar
of these products was quenched by cys and GSH.
For the reactions of 6-MC with amino acids and
GSH, the UV reactions appeared to produce 6-MC
photoconjugates with lys, cys, and GSH, and to a
lesser degree with tyr, ser, trp and phe. Gly,
glu and met appeared to be unreactive. The re-
sults of this study clearly suggest the involve-
ment of a 6-MC protein or amino acid adduct in
6-MC photoallergenicity. (Supported in part by
the Research Institute for Fragrance Materials).

3 ALTERATION OF CALCIUM-DEPENDENT GABA RELEASE AND
THE ENZYME ACTIVITIES OF GLUTAMIC ACID DECARBOXY-
LASE (GAD) AND GABA-γ-KETOSGLUTARATE TRANSMANSE
(γ-KGT) IN THE STRIATUM AND CORTEX IN RESPONSE TO THE
ADMINISTRATION OF PHENCYCLIDINE (PCP). J. C. Norris,
Pharmacol. & Toxicol., Univ. MS Med. Ctr., Jack-
on, MS 39216.

The involvement of GABAergic system with acute
and chronic PCP administration was investigated by
noting alterations in calcium-dependent GABA
release in mouse brain slices and the activity of
the enzymes, GAD and GABA-T, of the whole mouse
brain. The chronic administration of PCP for the
release study was accomplished by osmotic minis-
pumps at a rate of 1 mg/day. The GABA release
for the chronic animal in both the striatal and
cerebellar slices was decreased while the time of
the change was different for the two discrete
areas. The striatal slices returned to the control
values after 4 and 6 days of continuous ad-
ministration whereas the cerebellum slices were
decreased only in the 4-day treatment group. The
tissues of acutely treated mice responded in a
manner dependent on the dosage level. The low
dose (10 mg/kg) of PCP increased Ca++-dependent
GABA release in striatum. The high dose (40 mg/
kg) of PCP, however, demonstrated a decrease in
GABA release in striatum. The enzyme activities
of GAD and GABA-T were measured in animals which
received 40 mg/kg, i.p., once daily for five days.
The chronically PCP treated animals demonstrated
a significant increase in GAD and GABA-T levels.
The acutely PCP treated animals showed a decrease
in the GAD activity and no change in the GABA-T
activity. Thus, it seems that the GABAergic sys-
tem is altered by the presence of PCP. (Supported
by research grant DA-01310 from NIDA and toxicol-
ogy training grant ES-07045 from NIEHS)

4 DRUG METABOLIZING ENZYMES IN C3Hl0T½ CELLS
EXPOSED TO POLYCHLORINATED BIPHENYLS (PCBs).
H.P. Chihla, G. Reddy, & B.H. Norback. Depart-
ments of Pathology, William S. Middleton Mem-
orial Veterans' Hospital and the University of
Wisconsin, Madison, Wisconsin, 53705.

We have shown the cytotoxic and oncogenic
effects of certain technical PCBs and PCB anal-
alogues on the C3Hl0T½ cell system. In order to
define possible mechanisms of pathologic
change, we studied basal drug metabolizing en-
zyme activities of this cell line and the re-
ponse to Aroclor 1260. Cells (1.5 x 10⁷) were
plated in T-75 flasks and, 24 hours later, were
continuously exposed to either 1 ppm PCB or 1% acetone.
On 6, 10 or 15 days, cells were col-
lected and microsomal and cytosolic fractions
were prepared. The specific activities of mi-
crosomal styrene oxide epoxide hydrolase (EH),
cytosolic glutathione S-epoxide transferase (GT)
and microsomal P-450-associated aldrin epoxidase
(AE) were assayed. Six day cultures demonstrated
basal activity for AE (4.3 pmol/kg/30 min) and
GAD and GT from preconfluent cultures (1.51 +/- 0.38 and

14.20 +/- 1.17 pmole/mg/min, respectively) declined approximately 50% after confuence had been reached (day 15). Exposure to PCB produced no change in EH activity at any of the time points. However, GT activity increased approximately 50% in confluent cultures (days 6 and 10) and approximately 10% in postconfluent cultures. These enzymes may play a role in the metabolism of PCBs and, thus, may alter the effects of PCBs on this cell culture system.

6 BIOCHEMICAL TOXICITY IN MICE OF SUBSTANCES PRODUCED BY Clostridium difficile.

M. Ehrich, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Clostridium difficile toxin is recognized as the etiological agent of importance in antibiotic-induced diarrhea and pseudomembranous colitis. Administration of toxin to mice causes grossly observable liver damage, which can be quantitized biochemically by elevations in certain serum enzymes. For example, glutamic pyruvic transaminase (SGPT) and isocitrate dehydrogenase (ICD) levels were raised to 291% + 20 and 297% + 20 of control levels, respectively (mean + SEM, N = 5, p<0.01), using a semipurified preparation containing both of the high molecular weight toxins (500,000-600,000 daltons) produced by this microorganism. Clearance of sulfobromophthalein (BSP) was delayed following iv administration of toxin, with only 91% of the dye cleared in 30 minutes versus 93% for control mice. The activity of liver microsomal enzymes, indicated by 0-demethylation of p-nitroanisole, was also reduced (to 69% + 6 of control). Toxic C. difficile culture supernatant inactivated by incubation with 0.4% formaldehyde had no effect on any of these biochemical parameters. Although a mechanism for the toxic action of C. difficile products has not yet been defined, we noted that toxic effects were reduced in the presence of the membrane-stabilizing agents triacinitone and chlorpromazine.

Supported by NIH Biomedical Grant 363183-4.


Chloramphenicol (CAP, RNYCOCHCl) has previously been shown to be activated by rat liver cytochrome P-450 aerobically to an oxamyl chloride (RNYCOOCocl) that either covalently bonded to microsomal protein (RNYCOO-protein) or reacted with water to form CAP oxamic acid (RNYCOOHOH). In the present study we have found that rat liver cytochrome P-450 also activates CAP anaerobically by another mechanism. When a mixture of 3H-(benzyl position) and 14C(dichloroacetamido carbons) labeled CAP (0.1 mM) were incubated anaerobically with rat liver microsomes (2 mg/ml) from phenobarbital (PB) pretreated rats and a NADPH generating system, both labels were bound irreversibly to microsomal protein to approximately the same amount (330 pmol/mg protein/30 min) and monodeschloro-CAP (DCl-CAP, RNYCOCHCl, 1228 pmol/mg protein/30 min) was formed. Under these reaction conditions, no nitro-reduction products of CAP were detected. The formation of covalently bonded product and DCl-CAP were decreased by over 70% when the incubations were conducted in the presence of SKF 525A (0.5 mM), CO (100%) or in the absence of a NADPH generating system. Moreover, less than 10% as much covalently bound product and DC1-CAP was detected when microsomes from control rats or rats pretreated with y-sphathoflavones were used. These results suggest that a PB inducible form of cytochrome P-450 reductively dechlorinates CAP into a radical intermediate (RNYCOCHCl) that either reacts covalently with microsomal protein or abstracts a hydrogen atom to form RNYCOCH2Cl.

8 HYPERMETHYLATION IN THE MSO-EPILEPTOGENIC BRAIN.

REVERSAL BY DILANTIN, PHENOBARBITAL OR ADENOSINE PLUS HOMOCYTEINE. R.A. Schatzl2, T.E. Wilens2, S.B. Tatter2 and O.Z. Seilinger2. 2Division Toxicology, Northeastern Univ., Boston, MA 02115 and 2MHI, Neurochem. Lab., University of Michigan, Ann Arbor, MI 48109. Administration of the convulsant L-methionine-d,l-sulfoximine (MSo) results in increases in the methylation of histamine, phospholipids and the carboxyl moieties of some proteins. Co-administration of equimolar doses of adenosine (Ado) + homocysteine (Hcy) elevates brain levels of S-adenosyl-l-homocysteine (AdoHcy), a potent endogenous inhibitor of transmethylation reactions. This increase in AdoHcy levels inhibits, to varying degrees, the aforesaid methylation reactions and prevents, at least partially, the MSO-induced increases in methylation as well as MSO seizures. More recently we have found that chronic administration of the clinical anticonvulsants Dilantin (DPh) of phenobarbital (PB) also increases brain AdoHcy levels although to a lesser extent than Ado+Hcy treatment. PB treatment decreased protein carboxymethylation (PCM) while DPh was without effect. Both anticonvulsants, however, prevented
the MSO-induced increase in PCM. These agents also increased the latency to, and decreased the incidence of, MSO seizures. In view of the proposed integral role of PCM in membrane associated phenomena (i.e. nerve signal transduction) it is likely that the MSO-induced increase in PCM is a reflection of increased neuronal activity. Whether or not this increased PCM is actually causative in MSO seizures remains to be determined, however, the findings that agents that inhibit methylation also prevent MSO-induced seizures as well as increases in methylation, lend support, albeit indirect, to this possibility. Supported by NINCDS, grant No. 06294 to OZS. Sponsored by D. Brown.


Citrinin is a nephrotoxic fungal toxin that may have an important role in various animal diseases and perhaps in some human nephropathies. Clearance experiments in anesthetized, Sprague-Dawley rats indicate that citrinin (an organic anion) is secreted. The citrinin:inulin clearance ratio is approximately 3. Probenecid is known to block the renal secretion of many anionic chemicals. Tune and others used probenecid to block cephalexin transport and nephrotoxicity. In the present experiments probenecid was tested for its effects on citrinin-induced mortality, citrinin-induced changes in renal transport, and the renal accumulation of citrinin. Rats were pretreated with probenecid (30-120 mg/kg) 30 min before the administration of 55 mg/kg of citrinin. Mortality measured at 72 hr was reduced significantly. Renal slice PAH and TEA transport was significantly enhanced at 72 hr by probenecid plus citrinin than with citrinin alone. Finally, a large dose of probenecid permitted kidney citrinin levels to reach over 50 µg/g compared to almost 200 µg/g in animals given citrinin alone. Finally, the urinary excretion of citrinin and its metabolites was reduced dramatically during the first 8 hr after citrinin administration to probenecid-pretreated rats compared to animals receiving citrinin only. These data taken together demonstrate that probenecid can block the renal transport of citrinin and that reduction of this transport is a significant factor in reducing nephrotoxicity. Hence to produce toxicity, citrinin or a metabolite utilizes anion transport to enter renal tissue. (Supported by ES 01643.)

10 ACETAMINOPHEN NEPHROTOXICITY AND METABOLISM IN FISHER 344 AND SPRAGUE-DAWLEY RATS. J.F. Newton, Jr., J.K. Howe-Baughman, D.E. Erickson, W.E. Baselton, Jr., M.W. Gemborys, G.H. Mudge and J.B. Hook. Department of Pharmacology and Toxicology, Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824 and Department of Pharmacology and Toxicology, Dartmouth College, Hanover, NH 03755.

Acetaminophen (APAP) produces renal cortical necrosis in the Fisher 344 (F) rat but not in the Sprague Dawley (SD) rat. Recently, we have reported that the F rat can metabolize APAP to the nephrotoxic p-aminophenol (p-AP). One possible explanation for the difference in APAP-induced nephrotoxicity between these two strains of rats may be differences in the generation of p-AP. Therefore, studies were carried out to investigate the difference between strains in p-AP generation from APAP both in vivo and in vitro. In vitro there was no significant difference between strains in APAP deacetylation by kidney and liver homogenates. In vivo APAP and its metabolites were excreted at equal rates by both strains at doses of APAP (250 and 500 mg/kg) that did not produce nephrotoxicity as measured by blood urea nitrogen (BUN). However, at a dose of 900 mg/kg APAP, BUN was increased (7%) and APAP excretion decreased in F rats. In addition, F rats excreted more p-AP (free and conjugated) than SD rats when p-AP was expressed as percent of total APAP plus metabolites excreted (F, 5.21 ± 0.82% vs S, 2.50 ± 0.37%). These studies demonstrate that there is no difference between the two strains in the excretion of APAP to the kidney after i.p. injection. However, the excretion of p-AP in relation to other metabolites in the F rat was that dose of APAP is greater than that in the SD. (Supported by USPHS Grant ES 00560.)


Acetaminophen, a widely used nonnarcotic analgesic drug, is known to be hepatotoxic in large doses. Previous studies have shown that acetaminophen is metabolised by cytochrome P-450 dependent mixed function oxidase (MFO) enzymes to reactive electrophilic intermediates which covalently bind to tissue macromolecules resulting in cell necrosis and death. We have investigated the effect of L-ascorbic acid (LAA) on this process. The covalent binding of [14C]acetaminophen metabolites to male and female mouse and male hamster hepatic microsomes was inhibited by reduced glutathione, cysteamine, L-cysteine and LAA. Although the sulfhydryl compounds were more effective inhibitors than LAA, the combination of LAA and any of the thiol agents resulted in additive inhibition of covalent binding. Investigations into the mechanism of inhibition of covalent binding of [14C]acetaminophen metabolites indicated that LAA probably acts by scavenging the reactive intermediates generated by MFO enzymes rather than by the inhibition of their formation. These results suggest that LAA, at concentrations found in rodent and human liver, may supplement the endogenous protective mechanisms (e.g. reduced glutathione) which operate in vivo to prevent the covalent binding of reactive acetaminophen metabolites and hence hepatic necrosis. (Supported by Beecham Products Research Department, U.K.)

12 ACETAMINOPHEN-INDUCED HEPATIC GLYCOGEN DEPLETION, Jack A. Hinman, JoAnn B. Mays and Alex M. Cameron, NCGR, Jefferson, Arkansas 72079

Acetaminophen(A-)induced hepatotoxicity is mediated by a reactive metabolite. In microsomal incubations the metabolite can react with gluta-
13 PREVENTION OF TESTICULAR DAMAGE BY DIETARY ALUMINUM IN RATS WITH DIETARY ZN AND CU DEFICIENCY - Jianye Liu, M.D. and Klaus L. Stemmer, M.D., University of Cincinnati Medical Center, Kettering Laboratory, Cincinnati, Ohio 45267

The role of Al in body functions is still unknown. Previous studies demonstrated that the interactions between Al and certain essential metals may be responsible for the expression of Al neurotoxicity in rats. To elucidate the effects of Al on the testicular damage caused by Zn or Cu deficiency, an experiment was performed on rats fed purified diet containing low Zn, low Cu, or sufficient levels of Zn and Cu, with or without addition of Al. The control group of animals were fed Purina Lab Chow. Observations were noted after 120 days.

Severe testicular atrophy was seen in rats fed either low Zn or low Cu diet, but the damage was more pronounced in the low Zn group. The testicular lesions included seminiferous tubular cell degeneration and necrosis, as well as reduction of spermatogenesis. All spermatogenic cell types were affected including Sertoli cells, but Leydig cells were spared.

When Al was added to the diet, testicular destruction was reduced. This indicated that the presence of Al in the diet protected the testes against the damage caused by Zn or Cu deficiency. Pituitary glands were also examined. Hypertrophy was more pronounced in Al treated rats with Zn or Cu deficiency than in rats fed Zn or Cu deficient diet alone. This suggests that the protection of Al on gonadal function may be due to its influence on the pituitary-testes axis rather than on the testes alone.

14 DISPOSITIONAL DIFFERENCES OF CADMIUM AND MERCURY IN RAT HEPATOCYTE PRIMARY CULTURES. R.J. Gerson and Z.A. Shaikh, Division of Toxicology, Univ. of Rochester, Rochester, N.Y. 14642

The in vivo administration of inorganic Cd and Hg has been shown to result in markedly different metal concentrations in rat liver. Primary cultures of rat hepatocytes were utilized to gain insight into the dispositional differences between these chemically similar metals. Hepatocyte monolayer cultures were exposed to varying concentrations of Cd or Hg (3, 10, 30, 100 µM) in serum-containing medium for 30 min at 37°C and 5% CO₂. The cells were then washed and incubated in fresh medium for the remainder of the experiment. In cells exposed to 3 µM Cd there was an initial efflux of Cd from the hepatocytes when placed in fresh medium, followed by a gradual reuptake of metal, concomitant with its binding to metallothionein (MT). This reuptake could be blocked with 10 µg/ml cycloheximide. Efflux of Hg from Hg-exposed hepatocytes was also observed upon addition of fresh medium but there was no reuptake. Measurement of lactate dehydrogenase (LDH) released into the medium demonstrated that Hg was cytotoxic at 100 µM whereas Cd showed toxicity at 10 µM. Elevations in LDH correlated with morphological signs of toxicity in the cultures. Hepatocytes exposed to 3 µM Cd contained eight times more Cd at 24 hrs than hepatocytes exposed to the same concentration of Hg. At a dose of 3 µM metal, 70% of the Cd and less than 1% of the Hg contained in the hepatocytes was bound to MT. In hepatocytes exposed to 30 µM Hg a maximum of 10% of the Cd was bound to MT. Hepatocytes exposed to 3 µM Cd had four times more metal bound to MT than hepatocytes exposed to 30 µM Hg (336 vs. 84 pmoles/mg protein in homogenate). These studies indicate that isolated hepatocytes differentiate between these two metals and preferentially accumulate Cd. (Supported by NIH Grants ES01247 and ES01448)

15 FLAVOR AVERSIONS INDUCED BY CADMIUM: IMPORTANCE OF ROUTE OF EXPOSURE. R. C. MacPhail, Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711. (Sponsor: L. W. Reiter)

These experiments further evaluated the utility of conditioned flavor averions for rapidly detecting the effects of toxicants. Individual rats were adapted to a daily restricted (30 min) water availability schedule. When intakes had stabilized, a 0.1% (w/v) sodium saccharin solution was substituted for water and was followed approximately 20 min after fluid availability by administration of cadmium. Cadmium (CdCl₂) was dissolved in normal saline solution and was administered intraperitoneally (i.p.), subcutaneously (s.c.) or by gastric intubation (p.o.). The dosage ranges were: 0.125-1.0 mg/kg (i.p.); 0.33-2.62 (s.c.); and 3.0-104.0 mg/kg (p.o.). Dosages were expressed as base, and represented 3.5, 7.0, 14.0 and 28.0% of the LD₅₀s reported by Kotsonis and Klassen (TAP 41:667, 1977). Each dose-effect determination included groups of 5-6 rats that received Cd, the saline vehicle, or no treatment. Three days later each rat was given simultaneous access to saccharin and water, and preferences...
16 ALTERATION OF MEMBRANE FLUIDITY AS A POSSIBLE MECHANISM OF CADMIUM TOXICITY. N.A. Amoruso, G. Witz and B.D. Goldstein, Dept. of Environmental and Community Medicine, CMDJU-Rutgers Medical School-Rutgers University Graduate Program in Toxicology, Piscataway, NJ

Preliminary studies from this laboratory showed that exposure of rat pulmonary alveolar macrophages and human granulocytes to cadmium in vitro both inhibits (10^-4-10^-4 M) and enhances (10^-9-10^-6 M) superoxide anion radical (O_2^-) production, findings which may relate to the toxic effects of cadmium in vivo. O_2^- is formed by a plasma membrane bound oxidase; hence a possible mode of action of cadmium could involve alterations of membrane fluidity by this metal ion. This possibility was explored in human erythrocyte ghosts using fluorescence polarization techniques. Membranes labelled with the fluorescent lipid probe 1,6-diphenyl-1,3,5-hexatriene (DPH) had increased DPH polarization values when treated with 10^-3-10^-7 M cadmium for 1 hr at 25°C. The DPH polarization at 25°C increased from 0.388+0.002 at zero Cd++ to 0.361+0.005 at 10^-4 M Cd++. Exposure of ghosts to Cd++ also increased the native protein fluorescence polarization. At 25°C, control ghosts had a protein polarization value of 0.154±0.001 while ghosts treated with 10^-3 and 10^-4 M Cd++ had values of 0.176±0.001 and 0.182±0.001 respectively. Erythrocyte ghosts treated with 10^-2 or 10^-3 M Cd++ had an increased native membrane emission compared to control ghosts. The increases were observed at temperatures ranging from 15-40°C. Temperature profiles with respect to polarization showed higher protein and DPH polarization values for 10^-2 and 10^-3 M Cd++-treated ghosts compared to control ghosts over the 15-40°C temperature range. These data suggest that interaction of cadmium with cellular membranes may alter membrane lipid and protein fluidity which may lead to abnormal cellular function.

17 CADMIUM CONCENTRATIONS IN AMNIOTIC FLUIDS.

There have been reports linking cadmium to both hypertension and cigarette smoking. This study involved analysis of amniotic fluid from women for which the history was known. A total of 85 individuals were evaluated. Cadmium concentrations were measured utilizing atomic absorption spectrophotometry with carbon rod atomisation after wet digestion of the samples. A correlation was seen between the range of cadmium levels and the incidence of hypertension. Also smokers showed higher levels of cadmium than non-smokers. Concentrations of cadmium in amniotic fluid ranged from 0.044 mg/dl to 0.981 mg/dl; with a mean of 0.243 mg/dl and a standard deviation of 0.198. An additional parameter considered in the study, did not show any correlation to the distribution of cadmium levels.

18 THE EFFECTS OF METHYLmercury BINDING TO MICROtUBULES. D. G. Vogel, R. L. Margolis, and N. E. Motter, Dept. of Pathology, Univ. of Wash. and Fred Hutchinson Cancer Research Center, Seattle, WA. Sponsor: M.E. Vuchau

Methylmercury (MeHg) is known to affect the integrity of microtubules (MTs) which perform a multitude of cellular functions. The interaction of MeHg with MTs is being investigated as one of many possible mechanisms for the observed human and animal teratogenic effects of MeHg. The direct interaction of MeHg and MTs was examined in vitro with tubulin purified from bovine brain by three cycles of assembly-disassembly and a crude tubulin purified from rat brain. Polymerization was done at 30°C and monitored at 350 nm. After 30 min polymerization the bovine brain MTs were 100% inhibited at 30 µM MeHg, 50% inhibited at 14.5 µM, and less than 20% inhibited at concentrations below 6 µM. After 30 min polymerization the crude rat brain MTs were 100% inhibited at 300 µM, 50% inhibited at 132 µM, and less than 30% inhibited at concentrations below 6 µM. Total protein concentrations (µmoles per ml) for the bovine and crude rat tubulin were twice the MeHg concentration needed to produce 50% inhibition of polymerization and approximately equal to the MeHg concentration needed to produce 100% inhibition of polymerization. This suggests the binding of MeHg to one site per tubulin monomer is sufficient to inhibit polymerization. Since MeHg binds to sulfhydryl groups of proteins, the binding of MeHg to free sulfhydryl groups of tubulin was examined using Ellman's reagent and monitored at 412 nm. There were 7 free sulfhydryl groups per tubulin monomer and binding MeHg to only one produced 100% inhibition of MT polymerization. The results are consistent with the proposed mechanism of MeHg interaction with MT to produce cellular disfunctions. (NIEHS ES 00677, ES 07032, and USPHS GM 28189).


The effect of sodium selenite on methylmercury poisoning was studied in the guinea pig. The compounds: CH_3_203 HgCl and Me_2 SeO_3 were given separately or simultaneously (per os) as a single equimolar dose (10 mg CH_3Hg/kg and 3.8 mg Se/kg). Tissue distribution, excretion, accumulation of inorganic and organic mercury were determined 1,3,7 and 13 days after dose. It was found that sodium selenite decreased retention of MeHg in various organs (µg/g tissue),

were determined. This procedure was repeated until Cd was administered, and flavor preference was assessed, on three occasions. Flavor aversions induced by Cd (i.p.) were apparent only at the largest dosage and after repeated saccharin-cadmium pairings. Flavor aversions induced by Cd (s.c.) were proportional to the dosage and became more prominent with repeated pairings. Flavor aversions induced by Cd (p.o.) were maximal at the smallest dosage and after one pairing. These results extend recent findings of flavor aversions produced by several toxicants, including heavy metals, and suggest further that route of administration is an important determinant of the magnitude of toxicant-induced flavor aversions.
mainly after 13 days (2.5 fold). Selenium accelerated accumulation of MeHg in the cerebrum and cerebellum specifically 1 day after administration, but on day 13, the level of methylmercury was lower than for the group given MeHg alone. The concentration of MeHg in both kidney and liver after simultaneous administration of methylmercuric chloride and sodium selenite were significantly lower than those for group administered only with methylmercury. Selenium had no significant effect on subcellular mercury distribution in the liver, kidney and cerebrum, other than that which could be accounted for by its effect on the whole tissue uptake. Sodium selenite changed the biotransformation of methylmercury in kidney, liver, cerebrum, cerebellum, red blood cells and plasma. Three days after dosing with MeHg and Se, it was found that the relative amounts of inorganic mercury were lower than for the group administered with MeHg alone. Studies on excretion of MeHg showed the mercury level excreted by urine was increased by selenium only 24 hr after administration, however mercury excretion via faeces was decreased during 13 days of observation.

20 INDUCTION OF DNA STRAND BREAKS BY NICKEL CHLORIDE AND CRYSSTALLINE NICKEL SULFIDE IN CHINESE HAMSTER OVARY CELLS. S.H. Robison and M. Costa, Div. of Tox., Dept. of Pharm., Univ. of Texas Med. School, Houston, Tx., 77025.

The effect of NiCl₂ and crystalline nickel sulfide (aNiS) on the DNA of Chinese hamster ovary cells was studied. Both compounds caused DNA strand breaks when the DNA was analyzed by the alkaline sucrose gradient technique. Cells were prelabelled with [3H] thymidine for 48 hrs and allowed to reach confluence. The cells were then exposed to the nickel compounds for the requisite amount of time, washed with ice cold saline, and harvested. Cells were resuspended in saline and gently layered onto a lysis solution which had been overlayered on a 5-20% alkaline sucrose gradient. When [3H] thymidine radiolabelled DNA from cells exposed to NiCl₂ at 1 µg/ml for only 2 hrs was analyzed, a high degree of DNA strand breakage was observed. Treatment with crystalline aNiS for 24 hrs also induced a significant number of DNA breaks at concentrations of 1 µg/ml. The breakage of DNA strands was dependent upon the concentration and time of exposure with respect to NiCl₂ and crystalline aNiS. Strand breaks are induced at nickel concentrations which normally had no detectable effect upon cell division. A concentration dependent effect upon size and number average molecular weight was observed with both NiCl₂ and crystalline aNiS. Since DNA strand breakage occurred at such low concentrations, these results suggest that nickel compounds which cause cellular transformation have highly selective and specific effects upon DNA structure. (Supported by grant #R808048 from the U.S. EPA and by contract #ER60016 from the Dept. of Energy and by PHS grant #CA06570 from the NCI.)


Microsomal heme oxygenase (HO) activity was measured by gas chromatographic quantitation of CO liberated in vitro by NADPH-dependent enzymatic degradation of heme. Microsomes were prepared from organs of male Fischer rats killed at specified intervals after sc injection of NiCl₂; control rats received similar injection of NaCl vehicle. HO activity (nmol CO/hr/mg microsomal protein) is reported as X ± SD [with no. of rats in brackets]. Time-Course Study: At specified hours after Ni-treatment (0.25 mEq/kg), renal HO activity was: 3 h, 4.8 ± 1.5 [5]; 6 h, 8.5 ± 1.8 [5]; 9 h, 14 ± 4 [5]; 12 h, 30 ± 8 [5]; 24 h, 26 ± 14 [5]; 48 h, 18 ± 9 [5]; 72 h, 20 ± 9 [5]; controls (72 h), 5.8 ± 1.2 [6]. Dose-Response Study: At 17 h after Ni-treatment the following dosedependent dosages, renal HO activity averaged: 0 mEq/kg (controls), 5.6 ± 1.6 [15]; 0.06 mEq/kg, 8.9 ± 2.8 [5]; 0.15 mEq/kg, 16 ± 4 [5]; 0.25 mEq/kg, 30 ± 8 [5]; 0.38 mEq/kg, 42 ± 9 [4]; 0.65 mEq/kg, 92 ± 15 [6]; 0.75 mEq/kg, 35 ± 7 [5]. Organ-Selectivity Study: At 17 h after Ni-treatment (0.25 mEq/kg), HO activity in other organs averaged: liver, 28 ± 7 [6] vs 9.2 ± 1.6 [6] in controls; spleen, 18 ± 4 vs 8.4 ± 2.4 [6] in controls; lung, 5.0 ± 1.2 [6] vs 3.6 ± 1.2 in controls; brain, 12 ± 1 [6] vs 10 ± 1 [6] in controls. Since maximal Ni-induction of HO activity occurred in renal microsomes at 17 hrs after sc injection of NiCl₂ (0.65 mEq/kg), these experimental conditions are currently being used to determine the effects of (a) hypoxia, (b) chelating agents, (c) actinomycin, cyclohexamide, and puromycin, and (d) glutathione on Ni-induction of microsomal HO activity. (Supported by DOE grant EY-03140 and NIEHS grant ES-01337).


Trimethyltin (TMT) is a potent neurotoxicant of increasing concern in the industrial environment. Light microscopy of adult animals has demonstrated selective injury to the hippocampus, amygdala, and pyriform cortex. We examined the neonatal mouse to determine whether TMT has similar regional specificity in the developing brain. Two day old BALB/c mice were injected s.c. with either 3 mg TMT/kg b.w. or 0.9% saline, and sacrificed at 4 hour intervals for the first day post treatment, and daily until day 25 for morphological evaluation. The earliest ultrastructural evidence to cell injury was observed at 8 hours, and consisted of mild dilation of the endoplasmic reticulum. Between 24 hours, and 7 days, increasing numbers of neurons in the pyriform cortex and hippocampus appeared dead. Degenerative changes were observed in both axons and dendrites. Light microscopical changes appeared first at 16-24 hours, and consisted of increased eosinophilia and nuclear pyknosis. Pathological alterations progressed in severity, peaking between days 2 and 7. The hippocampus and pyriform cortex were most extensively involved, although all areas involved and the caudate nucleus were involved to a lesser extent. After day 10, the number of degenerating neurons decreased rapidly, and by day 25, general decrease in neuronal density was seen in involved areas.
23 ABSORPTION AND TRANSPLACENTAL TRANSFER OF TIN DERIVED FROM DIMETHYLTIN DICHLORIDE AND STANNOUS CHLORIDE. E.A. Noland, P.T. McCaulay, R.J. Bull and K. Kelty, Health Effects Research Laboratory, U.S.E.P.A., Cincinnati, OH 45268

Dimethyltin dichloride (DMDC) is commonly used as a stabilizer in PVC pipe used for transport of potable water.

Learning deficiencies have been observed postnatally in pups from DMDC treated dams (Taylor, Noland and Bull, unpublished results, 1981). The present work was undertaken to determine whether these abnormalities could be reasonably attributed to an increase in the tin content of the brain during gestation.

Female Sprague-Dawley rats were exposed to distilled water as drinking water which contained 40 mg tin/l as either SnCl2 or DMDC from 2-weeks prior to breeding to parturition. Controls received only distilled water. Before first nursing pups were sacrificed and blood was taken by carotid artery bleeding and brains were removed. Dams were bled by syringe and needle from the abdominal aorta. All tissues were analyzed for tin by flameless atomic absorption. Blood from dams and pups exposed to DMDC in drinking water demonstrated more than 20x as much tin as either control or SnCl2 exposed rats (P<0.0001). Similarly, in DMDC exposed groups the pup's brains had nearly 3x the tin content of the other two groups (P<0.0001).

These data demonstrate that tin introduced as DMDC is absorbed much better than inorganic tin and crosses the placenta to the fetus. This resulted in a significantly elevated tin concentration in the pup's brain at birth. Therefore, it may be concluded that the learning deficits are accompanied by elevated tin concentrations in the brain.

24 THE EFFECT OF ZINC STATUS ON THE SYNTHESIS OF METALLOTHIONEIN IN VARIOUS TISSUES OF RATS. S. Onosaka and M.G. Cherian; Depts. of Pathology, Pharmacology and Toxicology; University of Western Ontario, London, Ontario, Canada

Metallothioneins (Mt) of various tissues contain exclusively bound zinc and any change in zinc status can alter its synthesis and tissue deposition. In the present study the changes in Mt levels and its inducibility in Zn injected and deficient rats are undertaken. Mt levels in eleven tissues of control and rats injected with three different doses of ZnSO4 (20 mg Zn/kg for 2, 4 or 7 times) were measured by Cd-hem method. A dose dependent increase in Mt levels was observed in pancreas, liver, kidney and small intestine after ZnSO4 injection - the highest amount being in the pancreas. A positive correlation was demonstrated more than 20x as much tin as either control or SnCl2 exposed rats (P<0.0001).

In pancreas, liver, kidney and small intestine a dose dependent increase in Mt levels was observed. In contrast to the results of feeding studies with TPA and DMT in which reduced weight gain, hematuria, bladder hyperplasia, and bladder uroliths have been observed.


Terephthalic acid (TPA) and dimethyl terephthalate (DMT) are extensively used in the plastics industry for the production of polyester fibers and films. The combined production of DMT and TPA exceeds 4 billion lbs annually. Adult male Sprague-Dawley rats and adult male Hartley guinea pigs were divided into three groups and exposed to 0 (control), 10 mg/m3 TPA and 15 mg/m3 DMT (6 hr/day, 5 days/week for 6 months). The "respirable" dust concentration for both TPA and DMT was 5 mg/m3. Serial sacrifices were conducted after 30, 60, and 140 exposures. Body weights of exposed animals remained similar to controls throughout the study. Organ weights (lung, liver, kidney, and spleen) and organ to body weight ratios similarly were not affected. Clinical chemistry parameters (SMA 12 screen) and routine urinalysis parameters indicated no differences between exposed and control groups. Gross and histopathologic evaluations of tissues from animals exposed to TPA and DMT were within normal limits. These data suggest that TPA and DMT do not induce renal or urinary bladder toxicity following low-level inhalation exposures at nuisance dust levels. This finding is in contrast to the results of feeding studies with TPA and DMT in which reduced weight gain, hematuria, bladder hyperplasia, and bladder uroliths have been observed.

26 DOSE–RESPONSE RELATIONSHIPS IN 2-HEXANONE–INDUCED POTENTIATION OF CHCl3 NEPHROTOXICITY. W.R. Hewitt and E.M. Brown. Department of Veterinary Anatomy–Physiology, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, MO 65211

Previous studies have demonstrated that several ketonic solvents, including 2-hexanone (Hx), could potentiate the nephrotoxic action of CHCl3 in rats. The purpose of this study was to determine the dosage range over which Hx potentiated CHCl3 kidney injury. CHCl3-induced nephrotoxicity was evaluated in male, Fischer 344 rats pretreated (po) with Hx in dosages ranging from 1 to 15 mmol/kg. After 18 hr a challenging dosage of CECl3 (0.5 ml/kg, ip) was given. Kidney damage was determined 24 hr later, using blood urea

hrs interval to control and Zn-D rats. Even though the distribution of Cd in liver, kidney and pancreas in both groups was similar, the Mt concentration in pancreas was significantly decreased in Zn-D. The plasma and tissue levels of Zn were also decreased in Zn-D rats following Cd injection. The decrease in both Zn and Mt levels was more prominent in pancreas of Zn-D rats than other organs. The results suggest that all of the organs studied, the induction of Mt in pancreas is most sensitive to Zn status and zinc may be the primary inducer of Mt in various tissues.

(Supported by grants from MRC, Canada and ILZRO).

The present study suggests that the neonatal animal is significantly more sensitive to TMT than the adult.
nitrogen (BUN) and creatinine (Cr) content and renal cortical slice p-aminohippurate (PAH) and tetraethyl ammonium ion (TEA) uptake. Renal damage was confirmed histologically. Maximum potentiation of CHCl₃ nephrotoxicity was observed in rats pretreated with 10 mmol/kg Hx. Although Cr content and slice PAH uptake were altered in rats receiving 1 mmol/kg Hx + CHCl₃, no effect on BUN content or slice TEA uptake was observed with this regimen. Thus the minimum effective dosage for Hx-induced potentiation of CHCl₃ renal injury was approximately 1 mmol/kg. Hx (10 mmol/kg) did not alter the nephrotoxicity of K₂Cr₂O₇ (5, 10 or 20 mg/kg, sc), suggesting that Hx did not potentiate CHCl₃ damage by nonspecifically increasing the susceptibility of the proximal tubule to toxic insult. However 10 mmol/kg Hx + CHCl₃ did not depress renal cortical glutathione content. Thus it remains unclear if Hx potentiates CHCl₃ renal injury by increasing proximal tubular bioactivation of CHCl₃. (Supported in part by USPHS grant 1 RO1 OH00986 02)

27 A COMPARISON OF THE EFFECTS OF VANADATE ADMINISTERED IN VIVO AND IN VITRO ON ORGANIC ION ACCUMULATION BY RENAL CORTICAL SLICES. J.H. Smith, W.E. Braselton, S.R. Tonsager and J.B. Hook, Department of Pharmacology and Toxicology, Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824.

Vanadium is a ubiquitous metal that can be detected in trace amounts in most mammalian tissues, primarily the renal cortex. Vanadate, an oxyanion derivative of vanadium, produces a profound diuresis and natriuresis when infused in rats that may be associated with its properties as a potent Na-K-ATPase inhibitor. Thus it was of interest to quantify the effects of vanadate on other membrane transport functions. Accumulation of the organic ions p-aminohippurate (PAH) and tetraethylammonium (TEA) in rat renal cortical slices was inhibited by vanadate in a dose-dependent manner at medium vanadate concentrations from 10⁻⁶ to 10⁻⁰ M. The slice content of vanadate was linear (7.5 to 325 µM v/g wet weight of tissue) at medium vanadate concentrations ranging from 10⁻⁶ to 10⁻⁰ M as determined by plasma emission spectrometry. Inhibition of PAH and TEA accumulation was reversible at vanadate concentrations less than 1.5 x 10⁻⁵ M and corresponded to a reduction of slice vanadate content. Injection of 1 or 5 mg vanadium/kg intraperitoneally produced a profound diuresis and natriuresis during the first hour. The ability of renal cortical slices from these rats to accumulate PAH was related to tissue vanadate concentrations. Increasing medium K⁺ concentrations potentiated vanadate inhibition of PAH accumulation which corresponded with inhibition of sodium pump activity, as determined by ⁴²K⁺ uptake. These results suggest that vanadate acts on proximal tubule transport of PAH via inhibition of Na-K-ATPase. (Supported by USPHS Grant ES 00560.)

28 EFFECTS OF AMINOGLYCOSIDES ON RENAL REABSORPTION AND LYSONOMAL DEGRADATION OF LOW MOLECULAR WEIGHT PROTEINS. C. Cojocel and J.B. Hook, Department of Pharmacology/Toxicology, Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824.

Aminoglycosides, gentamicin, netilmicin and tobramycin, were each administered (30 mg/kg/day) to male Wistar rats for 7 days. Clearances of inulin and cationic egg-white lysozyme were performed in the isolated perfused rat kidney. Unlabelled and ¹²⁵I-lysozyme were added to the perfusate to achieve concentrations of 80-120 µg/ml. Trichloroacetic acid-soluble radioactivity (TCA) and high performance liquid chromatography (HPLC) were used to quantify the release of tyrosine to the perfusate. Reabsorption of lysozyme averaged about 70% of the filtered load in control and netilmicin treated kidneys. Reabsorption of lysozyme decreased to about 50% and 57% of the filtered load in gentamicin and tobramycin treated kidneys, respectively. Tyrosine perfusate concentration increased to about 3 µg/ml in control and netilmicin treated kidneys, and to about 2 µg/ml in tobramycin kidneys after 120 min perfusion time. No increase in tyrosine concentration was detected in the perfusates from gentamicin treated kidneys.

This study reveals the greater nephrotoxic potential of gentamicin which impairs the endocytic reabsorption of lysozyme and almost completely inhibits lysosomal proteolysis. (Supported in part by USPHS grant ES00960. Dr. Cojocel is supported by a Fellowship grant from Deutsche Forschungsgemeinschaft 13-Co 114/1-1.)


SOPP has been reported to increase urinary tumors in rats (Hiraga & Fujii, Fd. Cosmet. Tox., 19, 303 (1981)). However, Opp (free base) and SOPP failed to produce mutations in 5 strains of S. typhimurium (2X10⁻⁴ M) and SOPP did not induce unscheduled DNA synthesis in rat hepatocytes (10⁻⁴ M). DNA alkylation was not detected after gavage with 500 mg/kg of OPP or SOPP (det. limit 1 alk/1⁶ nucleotides). These studies suggest little or no genotoxicity for these materials.

Animals consuming diets with 2% OPP or SOPP were sacrificed after 3, 7, 14, 30 and 90 days. Progressive hyperplasia of bladder epithelium was noted in the SOPP group, but was absent in the OPP group. Focal kidney lesions were observed in the OPP group but were largely absent in the SOPP group.

The metabolism of both OPP and SOPP was dose dependent. Below 150 mg/kg, 90-97% of the urinary metabolites were identified as sulfate and glucuronic conjugates, with 3-6% oxidation products apparently derived from microsomal (MFO) pathways. Following 500 mg/kg (gavage) or dietary administration (2%) of OPP and SOPP 20-30% of the urinary metabolites were oxidation products. Thus the production of reactive MFO metabolites may be correlated with the bladder toxicity observed in rats consuming diets containing high levels of SOPP but not observed after low levels of SOPP. The lack of genotoxicity of OPP and SOPP coupled with the dose dependent production of an oxidative metabolite suggests that the toxicity and carcinogenicity may be manifest only at high metabolically saturating doses.

30 PRECLINICAL TOXICOLOGY OF NITROSTAT, A STABILIZED INTRAVENOUS NITROGLYCERIN. J.A. Anderson, E.J. McGuire, J.R. Watkins, J.E. Fitzgerald, and F.A. de la Iglesia, Dept. of Toxicology, Warner-Lambert/Parke-
31 STUDIES OF THE INTERACTION OF BETA BLOCKING DRUGS
AND CALCIUM ANTAGONISTS. J.A. Vick, E.H. Herman
and T. Balazs, Food and Drug Administration,
Washington, D.C.
The increased use of beta blocking drugs and calcium antagonists in the treatment of cardio-
vascular disease prompted studies of their interactions. A series of 21 minipigs were used to study the possible toxic effects of widely used beta blocker, propranolol, and 2 increasingly popular calcium antagonists, verapamil and nifedipine. The minipigs were anesthetized with Na pentobarbital and instrumented to record arterial blood pressure, heart rate, EKG and respiration. In the first group of minipigs, verapamil 0.5 mg/kg was given by i.v. infusion over 10 minutes following 0.5 mg/kg propranolol i.v., and produced marked hypotension, severe bradycardia, A-V blockade and death in 8 of 8 animals. In contrast, nifedipine 0.5 mg/kg i.v. after the same dose of propranolol produced only modest decreases in blood pressure and heart rate, no A-V block and survival in 8 of 8 minipigs. Lethal doses (5-10 mg/kg) of nifedipine alone did produce profound decreases in arterial blood pressure and heart rate but no A-V block. All 5 minipigs died of what appeared to be cardiovascular collapse. The lethal effects of verapamil and propranolol combination and of high doses of nifedipine could be reversed with calcium chloride i.v. (150 mg/kg). These studies indicate that verapamil but not nifedipine increases the toxicity of propranolol, an effect which can be reversed by calcium.

32 THE EFFECT OF EXERCISE ON CARDIOTOXIC POTENTIAL
OF ADRIAMYCIN IN RHESUS MONKEYS. C.R. Hassler,
D.C. Thake, R.I. Hamlin*, and P. Feder, Battelle Columbus Labs., Columbus, OH 43201, *Ohio State Univ. College of Vet. Med. Sponsor: G.L. Fishbe An experiment was devised to determine the effects of post treatment exercise on the cardio-
toxic potential of adriamycin. Rhesus monkeys were utilized. Cardiac function was evaluated in these animals by echocardiography. They were treated with adriamycin until a significant devia-
tion of the velocity of circumferential fiber shortening (VCF), shortening fraction (SF), or systolic time interval (PEP/LVET) was observed. The average dose injected was 8.3 mg/kg, with a range of 6-11 mg/kg. Following treatment one half of the monkeys, including both treated and vehicle control animals, were trained to perform vigorous exercise in response to a food reward. Computer controlled exercise devices were designed to simultaneously exercise 24 monkeys. There were no detectable differences in mortality or myocardial damage between exercised and seden-
tary monkeys. Decrease in heart rate was positi-
vately correlated with exercise. SF and VCF generally decreased with drug treatment but de-
mere more in the sedentary animals. The PEP/ LVET ratio was less sensitive. Microscopic evaluation indicated primarily vacuolation, loss of myofibers, and myolysis in the most severely affected animals. Nearly all animals had some degree of myocardial damage. There was good correlation between histologic and echocardiographic data with dose. Extensive damage was observed in monkeys given 10-11 mg/kg. At do-
sages of 6-7 mg/kg there was generally minimal damage. Results from monkeys given 8-9 mg/kg were extremely variable. Supported by NCI Contract #NO1-CM-17365.

33 ISOPROTERENOL-INDUCED LETHALITY IN MALE MICE
FOLLOWING MANGANESE, SELENIUM, AND CADMIUM
TREATMENT. J. Modrak, R. Schnell, Department of Pharmacodynamics and
Toxicology, College of Pharmacy, University of
Nebraska Medical Center, Omaha, NE 68105.
Male, Swiss-Webster mice were treated intraperi-
toneally with manganese chloride (2 or 5 mg Mn**/kg), sodium selenite (2.4 mg Se**/kg),
cadmium acetate (1 mg Cd***/kg), or normal saline
(10 ml/kg), either alone or following a prior injection of Mn, at various time periods prior to receiving isoproterenol (ISO, 400 mg/kg, i.p.) and the total number of deaths was recorded 72 hours later. Mn (5 mg/kg) increased lethality when administered alone (9/11), but not 72 (3/11), hours prior to ISO as compared with saline treated animals (4/11). An additional study demonstrated that while Mn (5 mg/kg) administration alone 24 hours prior increased ISO lethality as compared to control animals (6/10 vs. 2/10, respectively), admini-
istration of Mn (2 mg/kg) three days prior to the higher dose resulted in protection against lethality (3/10). Mn (2 mg/kg) alone did not increase lethality (2/10). Se increased lethality when administered alone 24 (9/10), but not 72 (4/10) or 4 (5/10), hours prior to ISO as compared to saline treated animals (5/10). Interestingly, the administration of Mn (5 mg/kg) 2 days prior to Se protected against the increase
in lethality observed 24 hours following (5/10), Cd administered (1/10), but not 24 (7/10) or 24 (5/10), hours prior to ISO increased lethality as compared with control animals (6/10). The results of these studies indicate that Mn, Se, and Cd can enhance ISO-induced lethality in male mice. These results also suggest that the prior administration of Mn can block enhanced ISO-induced lethality following Se or Mn. (Supported by NIEHS Grant ES-02425)

**34 ELECTROCARDIOGRAPHIC FINDINGS IN DOGS WITH SUBCLINICAL PARVOVIRUS-RELATED CARDIOMYOPATHY.** S. A. Kutz, P. S. Struve, D. K. Detweiler, R. E. Cimprich, J. L. Robertson, P. J. DeBaecke, and C. S. Streett, Stuart Pharmaceuticals, a Division of ICI Americas Inc., Biomedical Research Department, Toxicology and Pathology Sections, Wilmington, DE and the School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA. Sponsor: L. E. Gongwer

Canine parvovirus is currently a problem for commercial dog breeders supplying animals for safety evaluation and toxicology studies. Puppies that survive the acute enteric and/or cardiac forms of the disease appear normal and later are sold by the breeder. These apparently healthy dogs may actually have irreversible subclinical sequelae from the acute disease. We found three beagle dogs purchased from a commercial breeder to have abnormal electrocardiograms. These abnormalities included multifocal ventricular ectopic beats, coupled ventricular ectopic beats, and fusion beats. The R/S amplitude ratios were less than 0.3 in lead V2, and were less than 0.9 in leads V2 and V3. Normal variants included slurred and notched P waves in limb leads, and low amplitude R waves (less than 0.6 mV) in leads V2, V3, or V4. Two of these three dogs were successfully identified by their abnormal electrocardiograms before treatment. The other dog had three sufficiently normal pretreatment electrocardiograms that it was assigned to a safety evaluation study. After oral intubation and gavage, almost all post-dose electrocardiograms contained several ventricular ectopic beats and other changes as described above. Serum was available from two of these three dogs and both showed a titer of 1:1280 to canine parvovirus. At necropsy, fine gray streaks were present on both ventricular surfaces of the heart in all three dogs. Myocardial fibrosis was confirmed by microscopic examination. If such affected animals are not identified and eliminated before treatment, the differentiation of compound-induced changes from pre-existent disease may be difficult.

**35 EFFECT OF LOW LEVELS OF n-HEXANE ON ISOLATED PERFUSED RABBIT HEART.** Raje R. R., Arnold & Marie Schwartz College of Pharmacy & Health Sciences, Long Island University, Bklyn, NY. Sponsor: Lawrence W.H.

n-Hexane is a widely used industrial solvent which is regarded as water immiscible. Its volatility and well documented neurotoxicity make it an occupational hazard. On determining the solubility of n-hexane in water by gas chromatography, it was found that water becomes saturated with n-hexane at 12 mg/l. In our laboratory it was found that n-hexane dissolves to a considerable extent (20 mg/100ml) in the blood of the rats following acute inhalation exposure. The level of solubility in blood is of sufficient magnitude to indicate the possibility of cardiotoxic effects. This study was undertaken to determine the toxic effects of n-hexane on isolated rabbit heart when perfused with Chenoweth solution containing n-hexane and using Anderson's coronary perfusion apparatus. Perfusate concentration of n-hexane which produced greater than 50% inhibition of inotropic activity after 60 minutes of perfusion was found to be 9.6 mg/l (0.11 mmol/l).

**36 FLUORESCENCE POLARIZATION STUDIES OF HUMAN ERYTHROCYTE MEMBRANES EXPOSED TO OZONE IN VITRO.** G. Witz, B.D. Goldstein, L. Myers-Basting, and M.A. Amoruso, Department of Environmental & Community Medicine, CMDNJ-Rutgers Medical School/ Rutgers University Joint Graduate Program in Toxicology, Piscataway, NJ.

Previous studies from this laboratory on intact red cells and red cell membranes showed that there is a dose-dependent relationship between ozone exposure and a number of red cell membrane parameters including lipid peroxidation and loss of native protein fluorescence. Studies on oxidant damage due to ozone were extended to investigations on membrane fluidity as measured by fluorescence polarization techniques.

Ghosts labelled with the fluorescent lipid probes all-trans-1,6-diphenyl-1,3,5-hexatriene (DPH) had significantly higher DPH polarization values (p<0.001) than control ghosts. The DPH polarization values, which ranged from 0.325±0.003 to 0.037±0.001 for control ghosts at 25° increased by 0.017 to 0.023 p units for ozonized ghosts. DPH bound to ozonized ghosts which had lost more than 50% of their native fluorescence exhibited a small decrease (~10%) in the excited state lifetime and a major decrease (~40%) in fluorescence intensity. Protein polarization values which ranged from 0.176±0.001 to 0.176±0.001 at 25° for control ghosts also increased significantly (p<0.001) after ozone exposure, the highest increase being 0.037 p units. In contrast to the DPH polarization, the increases in protein polarization correlated directly with decreases in native protein fluorescence. These data suggest that oxidative damage due to ozone results in membrane structural changes and decreases in membrane lipid fluidity, possibly due to cross-linking of membrane components by lipid peroxidation products formed after ozone exposure. (This work was supported by NIH grant ES00673.)
exposed head-only for 10-minute periods to atmospheres of each of the chemicals listed previously as well as the nasal tumorigens for H; 0, 400, or 3,000 ppm and for T; 0, 100, or 1,500 ppm. No treatment-related deaths were observed. Salient findings were:

- transient, dose-related behavioral signs during week 1
- no significant effect on body weight, hematology, or clinical chemistry, except, dose-related increase in serum alkaline phosphatase at week 26 (females only)

for H:

- increase in body weight at 1,500 ppm dose level (males only)
- no significant effect on hematology or clinical chemistry, except, dose-related decrease in blood glucose at week 26 (females only).

Conclusions: The absence of a pattern of toxic responses suggests that neither heptane nor toluene present a serious toxic hazard under common use conditions. The significance of the weight gain (T-exposed males) and of the isolated effects on alkaline phosphatase (H-exposed females) and blood glucose (T-exposed females) remains obscure.

Note: These studies were conducted under sponsorship by the American Petroleum Institute.


We have previously shown that B6C3F1 mice were more sensitive than F-344 rats to the sensory irritation effect of formaldehyde (CH₂O). The concentration at which the respiratory rate (f) decreased by 50% was 4.9 for mice vs. 31.7 ppm for rats. This species difference in sensory irritation may lead to a difference in the amount of CH₂O inhaled and its subsequent toxicity to the respiratory tract. To investigate this possibility, the CH₂O dose deposited in the nasal mucosa of mice and rats was assessed. Naive or CH₂O-pretreated (6 or 15 ppm, 6 hr/day, 4 days) mice and rats were exposed to 6 or 15 ppm CH₂O for 6 hr and f and tidal volume (VT) were measured. From these, the minute volume (V₉) was calculated. During exposure, the f of all groups was depressed without a compensatory increase in Vₑ. However, a near 20% depression in Vₑ was seen in all groups of rats, a greater reduction occurred in pretreated mice. The reduction was ~20% (naive) vs. 40% (pretreated) for the 6 ppm mice and ~35% (naive) vs. 50% (pretreated) for the 15 ppm mice. The dose of CH₂O distributed over the surface area of the nasal epithelium was then calculated and expressed as µg/min/cm². Thus, the calculated dose was similar for the naive and pretreated rats but lesser for the pretreated mice. The dose received by the 15 ppm-pretreated mice was ~50% less than that of the pretreated rats and comparable to the 6 ppm-pretreated rats. This calculated dose difference between mice and rats at 15 ppm was supported by CH₂O whole-body autoradiographic studies on similarly exposed rats and mice. Thus, by normalizing CH₂O dosimetry data to µg/min/cm², species differences in respiratory tract toxicity, including nasal tumor incidence, can be better compared.


We have previously shown that B6C3F1 mice were more sensitive than F-344 rats to the sensory irritation effect of formaldehyde (CH₂O). The concentration at which the respiratory rate (f) decreased by 50% was 4.9 for mice vs. 31.7 ppm for rats. This species difference in sensory irritation may lead to a difference in the amount of CH₂O inhaled and its subsequent toxicity to the respiratory tract. To investigate this possibility, the CH₂O dose deposited in the nasal mucosa of mice and rats was assessed. Naive or CH₂O-pretreated (6 or 15 ppm, 6 hr/day, 4 days) mice and rats were exposed to 6 or 15 ppm CH₂O for 6 hr and f and tidal volume (VT) were measured. From these, the minute volume (Vₑ) was calculated. During exposure, the f of all groups was depressed without a compensatory increase in Vₑ. However, a near 20% depression in Vₑ was seen in all groups of rats, a greater reduction occurred in pretreated mice. The reduction was ~20% (naive) vs. 40% (pretreated) for the 6 ppm mice and ~35% (naive) vs. 50% (pretreated) for the 15 ppm mice. The dose of CH₂O distributed over the surface area of the nasal epithelium was then calculated and expressed as µg/min/cm². Thus, the calculated dose was similar for the naive and pretreated rats but lesser for the pretreated mice. The dose received by the 15 ppm-pretreated mice was ~50% less than that of the pretreated rats and comparable to the 6 ppm-pretreated rats. This calculated dose difference between mice and rats at 15 ppm was supported by CH₂O whole-body autoradiographic studies on similarly exposed rats and mice. Thus, by normalizing CH₂O dosimetry data to µg/min/cm², species differences in respiratory tract toxicity, including nasal tumor incidence, can be better compared.


Male and female F344 rats and N.Z.W. rabbits were exposed to 0, 30, 100 or 300 ppm ethylene glycol monomethyl ether (EGME) vapor 6 hr/da, 5 da/wk for 13 wks. Some rabbits in the 100 and 300 ppm exposure groups died during the course of the exposures. Body weight gains of male and female rats in the 300 ppm group were depressed, and weights of thymus glands and testes of both rats and rabbits exposed to 300 ppm were significantly lower than for controls. Hematologic parameters, particularly white blood cell counts, were depressed in both rats and rabbits in the 300 ppm group. Histopathologic observations included diffuse, severe bilateral degenerative changes of testicular germinal epithelium in rats and rabbits exposed to 300 ppm. Less pronounced histopathologic changes occurred in testes of 3 of 5 rabbits in the 100 ppm group as well as in 1 of 5 rabbits exposed to 30 ppm. No testicular effects were found in rats in the 100 or 30 ppm groups. A follow-up evaluation of EGME-induced testicular damage in rabbits is currently in progress. Evaluation of the functional effect of EGME on fertility and dominant lethality in rats is discussed in a companion paper (Rao, et al.).


The chronic toxicity of ethylene oxide (ETO) and propylene oxide (PO), two industrially important epoxides, was evaluated in a two-year inhalation study (7 hr/day, 5 days/week). Male weanling
Fischer 344 rats and adult male cynomolgus monkeys were exposed to 0 (control), 50, 100 ppm ETO and 100 and 300 ppm PO. The purpose of the rat exposures was to conduct a rodent bioassay while the monkeys were utilized to evaluate effects of these epoxides on major organ systems. Multiple indices including pulmonary function, electrocardiography, clinical chemistry, hematology, urinalysis, gross and histopathology, neurophysiology, neuropathology and mutagenicity have been assessed to determine the toxic responses to ETO and PO. Body weights from rats exposed to ETO and PO at all exposure concentrations and body weights from monkeys exposed to 100 ppm ETO were significantly reduced ($p < 0.05$; t-test) compared to controls. A dose response effect was evident on rat survival with survival at 24 months ranging from 20% in the ETO 100 ppm group to 50% in controls. Eye irritation, consisting of tearing, and nasal discharge were evident in monkeys exposed to 500 PO. Clinical chemistry, hematology, and urinalysis results indicate no differences between exposed and control monkeys. Other indices (including histopathology) are currently being evaluated and statistically analyzed.

42 ETHYLENE OXIDE VAPOR TWO-YEAR INHALATION STUDY ON RATS: W.M. Snellings, C.S. Weil and R.R. Maronpot, Bushy Run Research Center, Export, PA

Fischer 344 rats were exposed to 100, 33, 10 or 0 ppm of ethylene oxide (ETO) for 6 hrs per day, 5 days per week for two years. Initially, 120 rats per sex per group were exposed, and every 6 months a portion of the animals was sacrificed to determine possible effects. For one or both sexes, inhalation of ETO resulted in a depression of body weight gain and an increase in mortality at 100 and 33 ppm of ETO but not at the 10 ppm level. At the 6-, 12-, and 18-month sacrifice intervals, there were no consistent patterns of association of histologically confirmed organ damage with any alteration in urinalysis, hematology, serum clinical chemistry, organ weight or skeletal muscle atrophy was present in both sexes of the 100 ppm exposure group at the 24-month sacrifice interval. Also, at this interval, histologic findings confirmed hematologic evidence that exposure to ETO resulted in an increased prevalence of mononuclear cell leukemia. The prevalence of this neoplasm in the females was treatment-related and increased for each of the three ETO-exposure groups. The prevalence of other neoplasms was increased as shown by the greater percentage of ETO-exposed rats with more than two neoplasms; this was noted also in each of the three exposure groups for the female rats. Furthermore, in both the 100 and 33 ppm exposure groups, the percentage of female rats with a malignant neoplasm was increased. The frequency of peritoneal mesothelioma was treatment-related in the male rats exposed to 100 or 33 ppm of ETO. While the incidence of mononuclear cell leukemia and peritoneal mesothelioma in the air-control rats was similar to those reported in the literature, the possible contribution of sialoadenitis viral outbreak (which occurred during the 15th exposure month) to the ETO exposure-related tumors is uncertain. (Sponsored by consortium of worldwide ETO producers)

ERYTHROCYTES IN HUMANS AND IN DORSET SHEEP, A MODEL WITH AN ERYTHROCYTE G-6-PD DEFICIENCY, E.J. Calabrese, G.S. Moore, and P. Williams, Division of Public Health, University of Massachusetts, Amherst, MA.

It has been hypothesized that individuals deficient in erythrocyte G-6-PD activity may be at increased risk to the effects of inhalation of elevated levels of ambient ozone. This study evaluated the effects of three proposed toxic ozone intermediates (i.e. methyl oleate hydroperoxide, methyl linoleate hydroperoxide, and methyl oleate oxonide) on red cell metabolism and oxidative stress (i.e. increase in methemoglobin levels, decreases in glutathione levels) in normal human erythrocytes and in human and sheep erythrocytes which are G-6-PD deficient. The results indicated that human G-6-PD deficient red cells were markedly more susceptible than normal human red cells to each stressor agent, with respect to methemoglobin formation. The results of the sheep responses were sufficiently different from the human deficient to discourage its usefulness as a model to predict responses of human G-6-PD deficient erythrocytes.


Nine-month old female A/J mice were inoculated intraperitoneally from the same suspension of Plasmodium berghei on day zero (0) of this study. The infected mice were randomly allocated to one of two stainless steel exposure chambers maintained at 72 + 3°F and relative humidity of 34 ± 1%. Mice were then exposed to either filtered air or filtered air with 0.30 ± .01 ppm O3 for 3 hour intervals every third day for 18 days. After each exposure period blood smears were obtained from tail blood and stained with Giemsa. Slides were subsequently stained on a double blind basis for the number of parasitized cells. Mice in the treatment group exposed to 0.30 ± .01 ppm O3 showed statistically significant increases in number of parasitized cells ($p < 0.03$ to $p < 0.005$) during the study. Further, the treated mice died an average of 3.5 days earlier than control mice.

45 ALVEOLAR PERMEABILITY TO SERUM PROTEIN AFTER FIVE HOURS OF 2 PPm OZONE EXPOSURE IN RATS SELECTIVELY BREED FOR SUSCEPTIBILITY OR RESISTANCE TO SALT-INDUCED HYPERTENSION. S.N. Scharfank, D.L. Costa, R.W. Weber & E. Jellett, Medical Dept., Brookhaven National Laboratory, Upton, NY 11973.

Sponsor: R.T. Drew

Sprague-Dawley rats susceptible (S) to salt-induced hypertension suffered higher mortality when exposed daily to 2 ppm ozone than did hypertension-resistant (R) rats. To examine this differential sensitivity to ozone, alveolar permeabilities to serum albumin were measured in both ozone-exposed and control S and R rats. Five to seven week old male rats maintained on a low-salt (0.4% NaCl) diet were injected intravenously with 1.25 bovine serum albumin, (28 microcuries/ml, 10 mg albumin/ml) at 45 microcuries/kg of body weight. The rats were then exposed to either 2
ACUTE CARBON MONOXIDE TOXICITY IN RESTRAINED VS. UNRESTRAINED RATS. R.T. Drew, Medical Department, Brookhaven National Laboratory, Upton, NY 11973.

The 60 minute LC-50 for carbon monoxide (CO) is significantly lower in restrained vs. unrestrained rats (1900 ppm vs. 4000 ppm, respectively. The Toxicologist, Vol. 1, p. 39, 1981). This observation is important in view of the increasing use of restrained rats for acute and chronic inhalation studies. These studies used naive animals which were subjected to a single acute exposure to CO. Under these conditions the observed increase in mortality in restrained rats could have resulted from the added stress imposed by the restraint. To test this hypothesis, two groups of 20 rats were acclimated to nose exposure tubes or to individual exposure cages over a 3 month period. After 2 weeks, the duration of exposure to air in a standard 1.4 m³ chamber was 6 hr/day, 5 days/wk under both restraint and individually housed conditions. After the first week or so, the rats readily entered the tubes and it took only about 20 minutes to load the chamber. After 3 months the rats were divided into subgroups of 5 animals each and were subjected to a single acute 1 hour exposure to CO. In 4 different exposures with CO concentrations ranging from 3000 to 4000 ppm, only 2 of the 20 caged rats died. While only 1 restrained rat died in 3 different trials at concentrations below 3200 ppm, at 3400 ppm 5 of 5 restrained rats died. One week later the surviving rats were subjected to a second CO challenge at 3000 ppm for 1 hour. In this case, 6/6 rats restrained for the first time in Modified Bowman cages survived, 4/6 rats restrained for the first time in nose exposure tubes survived, and 5/6 conditioned restrained rats survived. These results suggest that 3 months of conditioning did not completely, mitigate the enhancement of CO toxicity caused by restraint.

Requirements for successful implementation of a control, monitoring and data handling system at an Inhalation Toxicology Facility. D.M. Bernstein & D.G. Dimmler, Brookhaven National Laboratory, Upton, NY 11973. Sponsor: R.T. Drew.

Operation of an inhalation toxicology facility requires the ability to perform well controlled reproducible experiments with large numbers of animals and often with minimal technical interaction. To satisfy those requirements at Brookhaven National Laboratory, a system was developed for monitoring and control of animal exposures to exposure chambers and barrier exposure room integrity; for automated animal weighing; and for complete data storage, management and analysis. The need for high reliability, fast response and minimal user intervention resulted in the use of a distributed microprocessor based system with each processor being functionally independent from one another. Each program which runs in the system is also independent of every other program with all interactions between programs accomplished through shared memory and high speed communication links. Both hardware and software were modified through an iterative process to meet the needs of the user rather than the user having to adapt to the programs. User interaction is accomplished through video displays and keyboards with the response of the programs being faster than the user to avoid frustration. Access to the system is hierarchical through unique user identification numbers with operational modifications and data editing limited to supervisory personnel. The system maintains a complete record of user access, data entry and alarm conditions. Graphics and documented records...
are available to the user either automatically or upon request. Designed to meet the requirements of the toxicology user, the system has allowed the safe, reliable operation of a multichamber inhalation facility with minimal personnel.

*Research supported by the U.S. Dept. of Energy under contract #DE-AC02-76CH00016.


The hepatocarcinogen dimethylnitrosamine (DMN) is metabolized by rat liver preparations to methanol and formaldehyde and recent evidence suggests the involvement of multiple enzymatic pathways in this process. In this study we have compared the properties of the enzymes(s) responsible for hepatic DMN metabolism with cytochrome P-450 dependent mixed function oxidase (MFO) enzymes, monoamine oxidase (MAO, EC 1.4.3.4) enzymes and the Ziegler mixed function amine oxidase enzyme (EC 1.14.13.8). The treatment of rat hepatic microsomal preparations with the chaotropic agent KI progressively destroyed cytochrome P-450 and associated MFO activities. However, the metabolism of DMN to formaldehyde and MAO activity were less affected. Similarly, in storage stability studies with post-mitochondrial supernatant fractions DMN metabolism and MAO enzymes were stable under conditions where MFO enzymes were not. In inhibitor studies the enzyme(s) responsible for DMN metabolism had different sensitivities to MFO enzymes and the Ziegler enzyme catalysed N-oxidation of N,N-dimethylaniline. The treatment of rats with cobaltous chloride resulted in an inhibition of DMN metabolism, MFO and MAO enzymes, but had little effect on N,N-dimethylaniline N-oxidation. These results indicate differences between the hepatic enzyme(s) catalysing the degradation of DMN and a) cytochrome P-450 dependent MFO enzymes and b) the Ziegler mixed function amine oxidase. The apparent similarity between DMN metabolism and MAO enzymes may suggest the involvement of an N-oxidase enzyme in the metabolism of this nitrosamine. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food).


The onset of functional acrylamide (ACMD)-induced neuropathy has been reported to occur sooner in 11-week old male Holtzman rats vs 5-week old rats (Kaplan & Murphy, Toxicol. Appl. Pharmacol. 22, 259-68, 1972). To determine if changes in distribution of ACMD to tissues (especially nervous tissue) could explain this observation, 5- and 11-week old male Holtzman rats (2 groups each age, n=5) were dosed once daily with 50 mg/kg ACMD ip. Length of dosing period (5 or 7 days, one of each age group per period) corresponded to an 11-week compared to 5-week old deficit observed by Kaplan and Murphy. On day 5 or 7, animals were dosed with 50 mg/kg ACMD containing 50 μCi/kg 2,3-14C-ACMD, then killed 20 hrs post-dosing. Selected tissues (brain, spinal cord, sciatic nerve, lung, liver, kidney) were sampled, oxidized to 14CO2, and quantified by scintillation counting. (Counts obtained were converted to tissue concentration of ACMD expressed as mmoles/gm, for statistical analyses.) Within dosing periods (5 or 7 days), concentrations of ACMD (as 14C) were significantly greater (p<0.05, Student's one-tailed t-test) in 11-week old vs 5-week old animals, excepting sciatic nerves within the 5-day groups. Analysis of data via two-way ANOVA revealed that 80% of the variation in brain and spinal cord ACMD concentration, and 61% of the variation in sciatic nerve ACMD concentration could be explained using age as a factor and body weight as a covariate. Dosing on a per kg body weight basis exposed tissues of 11-week old rats to disproportionately higher amounts of toxin relative to 5-week old rats, possibly explaining the earlier onset of toxic signs seen by Kaplan and Murphy in the older animals. (Supported by 5-T32-ES07091 and ES-82130.)

51 BIOTRANSFORMATION ENZYMES (CYTOCHROME P-450 DEPENDENT MONO-OXYGENASES) IN THE NASAL EPITHELIAL MEMBRANE AND TURBINATES OF SEVEN SPECIES, A. R. Dahl, W. M. Hadley and J. M. Benson, Lovetace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185

The nose is a major point of entry for xenobiotics and since the way in which xenobiotics are metabolized often influences their toxicity, the biotransformation enzymes of the nasal cavity tissues are of great importance to understanding toxic effects to those tissues. We have isolated the microsomes (endoplasmic reticulum) from the nasal epithelial tissue and turbinates of the dog, rat, mouse, Syrian hamster, guinea pig, and rabbit. The nasal cavities were opened by splitting the skinned head along the midline and the relevant tissues were removed using forceps. Assays included measurement of the reduced carboxy adduct at 450 nm, para-nitroanisole O-demethylase, alyl hydrocarbon hydroxylase and aniline hydroxylase activities. Levels of monoxygenase activity and cytochrome P-450 content comparable to that of liver on a per mg protein basis were found in all species. Pigmented animals were found to be particularly high in cytochrome P-450 and this may be related to the more sensitive sense of smell reported for this phenotype. (Research conducted under DOE Contract No. DE-AC04-76EV10131.)

52 EFFECT OF STRUCTURE ON THE METABOLIC RELEASE OF CYANIDE FROM NITRILES. T. Hassan, M. Hassan, S. Kuttab, and E. H. Silver, Toxicology and Medicinal Chemistry Programs, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA. Sponsor: D.R. Brown

Nitriles are extensively used in the manufacture of plastics, synthetic fibers, dyes and pharmaceuticals. Several reports indicate that nitriles may metabolically release cyanide (CN'). We have conducted structure-activity studies to investigate the metabolism of saturated and unsaturated nitriles to CN'. Male Sprague-Dawley rats were given a nitrile (0.75 mmol/kg) po, ip, or iv. Urine was collected for a 24 hr
period and analyzed for thiocyanate (SCN⁻). Administration (po) of the saturated nitriles, aceto-, propio-, n-butyro-, n-pentane-, n-hexano-, iso-butyro-, and trimethylacetone resulted in 11.5±2.4% (mean S.E.), 65.3±2.9%, 64.7±2.7%, 43.5±2.5%, 32.4±1.2%, 74.0±2.6%, 0.1±0.04% of the dose excreted as SCN⁻ in the urine, respectively. Administration (ip) of the same nitriles resulted in 4.2±0.5%, 56.8±1.1%, 55.0±1.3%, 27.8±2.7%, 33.7±1.6%, 49.1±1.4%, and 0.2±0.1%, respectively. The unsaturated nitriles, acrylo-, croto-, and 3-butenes, yielded 37.3±1.9%, 5.6±0.6%, and 28.9±1.4% of the dose as SCN⁻ after po and 4.5±0.4%, 4.6±0.8%, and 17.7±2.9% after ip administration, respectively. Administration (iv) of propionitrole resulted in 44.6±3.7% of the dose excreted as SCN⁻ compared to 0.1±0.04% for acrylonitrile. The time course of SCN⁻ excretion was similar for propionitrile and acrylonitrile. These results suggest that length of the carbon chain, presence of substituents at the α-carbon, position of double bonds, and, for some compounds, route of administration, are important factors in the release of cyanide from nitriles. (Supported by Northeastern University's Research and Scholarship Development Fund.)

53 QUANTITATIVE PRECIPITATION OF FORMIC ACID FROM METHANOL. G.G. Berg, Toxicology Div., Dept. of Radiation Biol. and Biophysics, University of Rochester Sch. of Medicine, Rochester, NY 14642.

A convenient method for the separation of radioactively labeled methanol from its metabolites was developed for tissue dosimetry in concurrently reported toxicity tests. It consisted of fixation and extraction in a methanol-formaldehyde-formic acid fixative designed to stabilize labeled trace compounds, followed by selective precipitation of formic acid with barium from a mixture of the extract with tetrahydrofuran. Solution A: 12.5 ml formic acid, 37.5 ml methanol to make 75 ml. Solution B: 12.5 ml formic acid, 25 ml water to make 250 ml. Fixative: mix A and B; for standards, add C-14 labeled methanol or formic acid at 10 nCi/ml. Ba reagent: colloidal suspension of 0.20 M Ba(OH)₂ in methanol (mag. mixing).

Extraction: tissue sample into 2 ml of chilled fixative; add vol. of Solution A equal to tissue weight; homogenize with Polytron; rinse probe with 2 changes of 2 ml fixative; pool; let stand overnight; centrifuge. Separation: to 1 vol. of supernatant or standard and add 1.2 vol. Ba reagent (5 min.); add 4.4 vol. tetrahydrofuran, stir, chill (15 min.); centrifuge.

Assay: by scintillation counting of last supernatant in Aquasol-2 (NEN). Recoveries of methanol in the final supernatant averaged 97.6% (S.D. = 1.16%, n = 7). Removal of formic acid from supernatant averaged 91.7% (S.D. = 1.26%, n = 7) and remained complete up to a 6:1 stoichiometric ratio of formic acid to Ba(OH)₂.

(Metabolism supported in part by NIHES Grant ES01247 to the Environ. Health Sciences Center.)

54 METABOLIC DISPOSITION OF DIPYRITHIONE. C. Mitoma, T. Steeger, J. Rogers, D. Thomas and J. H. Wedig*, SRI International, Menlo Park, CA; Olin Corporation, New Haven, CT.*

The metabolite disposition of the MgSO₄ adduct of dipyrithione (Omadine™ MDS, a broad spectrum antimicrobial used in shampoo as an anti-seborrheic agent and in cosmetics as a preservative) was studied in several species. After i.v. injection of 2-3¹⁴C-Omadine™ MDS, the radioactivity was excreted predominantly in urine. Urinary metabolite patterns in rat, rabbit, rhesus monkey and swine were quite similar. One major metabolite accounted for over 80% of the metabolites. This was identified as the β-glucuronide of 2-methacryloxyphenyl N-oxide.

Three transient and one, more persistent, metabolite were detected in rat plasma by HPLC analysis. The persistent metabolite is 2-methylsulfonylpyridine (2MSP). The three transient metabolites were tentatively identified as 2-methylthiophenpyridine N-oxide, 2-methylsulfinylpyridine N-oxide and 2-methylsulfinylpyridine from their mass spectra. Approximately 8 hours after injection of Omadine™ MDS, the only plasma metabolite present was 2MSP. The plasma half-life of 2MSP was approximately 68 hours for rats, 37 hours for rabbits and 22 hours for rhesus monkeys.

Twenty-four hours after dermal application of Omadine™ MDS in a shampoo vehicle on the back (clipped, occluded) of rats twice daily for 4 days, the plasma levels of 2MSP were 33-7 ng/ml and 254-31 ng/ml for doses of 2 mg/kg and 20 mg/kg, respectively.

These studies suggest that in the species comparison, (1) the major urinary metabolites are identical; (2) the persistent metabolite in blood is identical, with the half-life: rats > rabbits > rhesus monkeys.

55 METABOLISM OF A MILKWEED CARDENOLIDE, USCHARIDIN. BY MONARCH BUTTERFLY LARVAE, DANAUS PLEXIPPUS. M.A. Marty and R.I. Krieger, Department of Environmental Toxicology, University of California, Davis, CA, and Department of Veterinary Science, University of Idaho, Moscow, ID.

Larvae of the Monarch butterfly utilize milkweeds, Asclepias spp., which contain cardenolides (CD) as their sole food source. Certain CD are retained in tissues of larvae, pupae, and adults. Exposure of CD may be a chemical defense against predation. We investigated the role of microsomal monooxygenases in the metabolism of a milkweed cardenolide, uscharidin, isolated from Asclepias curassavica. Homogenates of larval gut and fat body demonstrated a low level of monooxygenase activity as measured by p-chloro-N-methylaniline N-demethylation. Uscharidin was used as a substrate with portions of these homogenates: Extraction and TLC (E.M. Silica Gel 60 with concentrating zone; developed 3X CHCl₃; MeOH:OGCNH, 90:6:1 or 2X Ethyl Acetate; MeOH 97:3) revealed a "biotransformation" of uscharidin. The products cochromatographed with two cardenolide standards, calotropin, and calactin. HPLC also confirmed their identity. Uscharidin biotransformation was not inhibited by CO, sesamex, or piperonyl butoxide. Subsequent subcellular fractionations revealed the highest activity is in the soluble fraction. Larval monooxygenase is apparently not involved in uscharidin biotransformation. The reaction appears to be mediated by a pyrazole-insensitive NADPH-dependent dehydrogenase. Supported in part by NSF Grant DEB 7514266.)
57 METABOLISM OF 5-AMINO-1-NAPHTHOL IN THE RAT.


The absorption, distribution, metabolism and excretion of 1,3-diphenylguanidine (DPG), a chemical used extensively as an accelerator in the rubber industry, was studied in the male Fischer rat. DPG was completely absorbed after oral doses of 1.52, 15.2 and 152 µmol/kg body weight. The tissue distribution of DPG was not affected by the dose or route of administration, oral or intravenous (tail vein). Liver and muscle were the major depots of DPG and contained up to 60 to 70% of the radioactivity in the body. Skin, kidneys and adipose tissue were also sites for DPG deposition. DPG was excreted in approximately equal amounts in the feces, via the bile, and the urine. Total excretion accounted for approximately 75% of the total dose in 24 hours and close to 100% in 72 hours. Bile duct cannulations resulted in 50% excretion of DPG in the bile within 2 hours and up to 75% in 6 hours. Urine and bile were analyzed for DPG metabolites using High Pressure Liquid Chromatography. All DPG-derived radioactivity in the bile was in the form of a single major metabolite whereas approximately 60% of the radioactivity present in urine co-chromatographed with the parent compound. The remainder of the radioactivity excreted in urine consisted of one major (30%) and two minor metabolites. In summary, DPG is readily absorbed, metabolized and excreted by the rat.

58 METABOLISM OF 5-Amino-1-naphthol in the rat.

P.M. Enriquez and G.D. DiVincenzo, Health, Safety, and Human Factors Laboratory, Eastman Kodak Co., Rochester, NY 14650.

The metabolic fate of 5-amino-1-naphthol (5AIN) is of interest because of the association of certain aromatic amines with carcinogenesis in experimental animals. This association frequently involves a hydroxylation step which leads to the formation of electrophilic intermediates. Knowledge of the metabolicism of 5AIN is potentially of use in predicting its capacity to form active metabolites. 

[14C]5AIN was administered by gastric intubation to Charles River CD rats at doses 1, 37 and 135 µmol/kg body weight. In a separate experiment the rats were also dosed with 150 mg/kg of unlabeled 5AIN daily for four consecutive days. Between 74 and 85% of the administered dose was excreted in the urine. Over 98% of the urinary radioactivity was characterized. In addition to unchanged 5AIN, 5-acetamido-1-naphthol (5AA1N) and conjugates of glucuronic and sulfuric acids were identified as metabolites. Unchanged 5AIN and 5AA1N accumulated for less than 3% of the dose. The amount of 5AIN converted to 5AA1N varied inversely with the dose (36 to 64%) and both compounds were excreted in the urine predominantly as glucuronide and sulfate conjugates. The ratio of glucuronide to sulfate conjugates for both compounds increased directly with the dose. Two minor metabolites were not characterized. Rats dosed repeatedly with 150 mg/kg of 5AIN showed no significant change in the metabolite excretion pattern compared to rats dosed singly. These findings show that in the rodent model the metabolism of 5AIN was dose dependent, and occurred predominantly by phase II reactions (conjugative pathways) involving N-acetylation and conjugation with glucuronic and sulfuric acids. There was no evidence for the formation of hydroxylated metabolites over the dose range studied.

59 METABOLISM OF NITROTOLUENES BY RAT CECAL MICROFLORA.


An important step in the production of toxic metabolites from aromatic nitro compounds may be reduction of the nitro group. The hepatocarcinogenic dinitrotoluene isomers require reduction by rat intestinal flora for optimum production of a metabolite which covalently binds to macromolecules. The formation of methemoglobin following exposure of rats to nitrobenzene is dependent on reduction by cecal microflora. The purpose of this investigation was to determine the relative rates of reduction of three isomeric nitrotoluenes by cecal microflora. Cecal microflora were collected under low oxygen tension and incubated anaerobically with 100 µM 2-, 3- or 4-nitrotoluene (2NT, 3NT or 4NT). Metabolites were separated by reverse phase HPLC using a C-18 column and a methanol/phosphate buffer gradient. 3NT was more rapidly metabolized than either 2NT or 4NT (620 versus 240 and 550 mmoles/g cecal com-
METABOLISM OF TOLUENE IN RAT ISOLATED HEPATOCYTES. Male Fischer 344 rats were killed at Univ. of Arizona, Tucson, AZ 85721.


The in vitro metabolism of toluene was investigated to validate the use of isolated hepatocytes as a rapid metabolic screening technique for industrial chemicals. Toluene metabolism is generally thought to occur via oxidation of the methyl substituent, but recent evidence suggests that aromatic oxidation occurs to a small extent. This possibility was investigated using primary hepatocytes isolated from male Sprague-Dawley rats.

Cells were incubated in sealed flasks with 0.5, 5.0 or 25.0 µmol toluene for up to 1 hr. Analysis by HPLC showed that toluene is rapidly metabolized to benzoic acid (BA) and hippuric acid (HA). Benzoyl glucuronide (BG) was not formed to any appreciable extent even when formation of HA was saturated. In control cells the initial rates of metabolism were (nmol toluene/10^6 cells/15 min), for 0.5 µmol, 4.8 (3.4 HA, 1.4 BA); for 5 µmol, 14.0 (3.3 HA, 10.7 BA); for 25 µmol, 22.6 (0.7 HA, 21.9 BA). In cells from phenobarbital (PB)-treated rats the corresponding initial rates were; for 0.5 µmol, 12.5 (8.1 HA, 4.4 BA); for 5 µmol, 61.1 (6.8 HA, 54.3 BA); for 25 µmol, 80.3 (2.4 HA, 77.9 BA). Control or PB cells incubated with BA (0.1 or 0.5 mM) did not form BG but did produce HA. Cells incubated with 4-nitroanisole, however, rapidly produced glucuronide and this reaction was induced by PB treatment. Release of alanine aminotransferase was not increased and glutathione concentrations were only slightly depressed, suggesting that toluene is not hepatotoxic in vitro. No evidence was found for other toluene metabolites. Since metabolism in PB cells accounted for 98-100% of the toluene (0.5 or 5.0 µmol expts) in 60 min, aromatic oxidation can only occur to a very minor extent.

INTERACTIONS OF NITROBENZENES WITH HEPATIC MICRO­SOMES: EVIDENCE FOR CYTOCHROME P450 UNCOUPLING. Levin, A.A., Bus, J.S., and Dent, J.C. Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

Nitrobenzene exposure (300 mg/kg) induces methemoglobinemia, testicular necrosis and hepatic cellular change in rats. This study investigated the interactions of nitrobenzene (NB) with hepatic microsomes isolated from Fischer-344 rats. NB (0.1 mM) was metabolized aerobically by microsomes to a rate of 0.02 nmol/min/mg protein as determined by HPLC analysis of products. By comparison aniline was metabolized at 1.5 nmol/min/mg prot. Addition of NB to microsomes produced a Type I P450 binding spectrum, indicating that the lack of extensive metabolism of NB was not due to the absence of P450 binding. NB (0.1 mM-1.0 mM) stimulated microsomal oxygen consumption and NADPH oxidation. The V for the cofactor utilization was in the range of 12-16 nmol/min/mg prot., which was 600 to 800-fold greater than the observed rate of metabolism. NB-stimulated oxygen consumption and NADPH utilization were inhibited by CO and SKF-525A, indicating a role for P450. NB stimulated production of superoxide (O_2^-) by microsomes as determined by monitoring adrenochrome formation. The rate of NB stimulated O_2^- production was similar to the increases in NADPH and O_2 utilization produced by NB and was inhibited by CO. Nitrobenzene stimulated adrenochrome formation was inhibited by superoxide dismutase. These data are consistent with a P450 mediated reduction of the nitrogroup to form a nitroanion radical which may be reoxidized to NB by molecular oxygen, thus generating O_2^- This mechanism of futile redox cycling and O_2^- production differs from that proposed for paraquat. In the latter the latter is dependent only on P450 reductase. The uncoupling of cytochrome P450 and release of O_2^- elicited by NB in microsomes may be related to the toxicity of the compound.


While little data is available regarding the disposition and metabolism of p-nitrotoluene (PNT), its structural similarity to known hazardous chemicals indicates the need for such information. Male Fischer 344 rats were killed at times ranging from 5 min to 7 days after a single intravenous dose of 8 mg/kg 14C-PNT. Select tissues were removed and assayed for total 14C by oxidation to 14CO_2. Parent PNT was determined for certain tissues by an extraction procedure demonstrated to be selective for parent compound. 14C-PNT was rapidly metabolized and cleared from whole blood with approximately three percent of dose remaining at two hr, and less than 0.2% of dose as parent compound. Peak tissue levels occurred at 15 min, then rapidly decayed by four hr to approximately one percent in muscle and skin, and less than one percent in fat, kidney and liver. At four hr 73±13% (±SD) of the total activity in fat was parent compound; since other tissues assayed contained detectable amounts of parent PNT at this time point. 14C-PNT was rapidly excreted in the urine, with 57±22 appearing by one hr, 70±8% by four hr and 82±4% by 24 hr. At one hr the urine contained approximately three percent of the dose as parent PNT. While 30±1% of dose appeared in the bile of anesthetized rats by three hr, only 41±1% was found in feces by 24 hr suggesting that PNT undergoes significant enterohepatic circulation. By seven days total fecal excretion was 64±1.

These data demonstrate that after a single intravenous dose, PNT is rapidly metabolized and excreted, and has no major tissue storage depots. (Supported by NIEHS NO1-ES-8-2130.)
A COMPARATIVE STUDY OF HEPATIC MIXED-FUNCTION OXIDASES IN 3 RAT STRAINS FED DIETS CONTAINING 20% CABBAGE (BRASSICA OLERACEA) K. W. Miller and G. S. Stoesz and, Cornell University, Geneva, NY

Consumption of Brassica vegetables has been shown in this laboratory to inhibit tumor formation in rats challenged with a hepatocarcinogen; possibly by altering the activity of the hepatic microsomal mixed-function oxidase system (J. Envr. Path. Toxicol. 2, 399, 1978). This study focuses on the activity of different hepatic mixed-function oxidase enzymes, and organ weight changes in Fisher-344 (F), Long-Evans (LE), and Sprague-Dawley (SD) rats. Weaning male rats were fed either a purified or an isocaloric, isonitrogenous diet containing 20% cabbage. After 4 weeks, the LE and SD rats showed a significantly lower weight gain, corresponding with decreased diet intake and diet efficiency compared to controls.

Two-way analysis of variance linked cabbage diets to a significant depression of NADPH cytochrome C reductase activity in all strains. Aniline hydroxylation and p-nitrophenol O-demethylation levels varied markedly between strains, lowest levels occurring in F and SD respectively. Aniline hydroxylation was significantly elevated at 3 weeks and depressed at 4 weeks in LE cabbage-fed rats. The LE rats showed a pattern of early elevation and later depression for O- and N-demethylation as well. Excepting LE, aminopyrine N-demethylation activity tended to be elevated in the two other strains consuming the cabbage diets. Hepatic microsomal protein was significantly increased in cabbage-fed SD rats. Cabbage treatments increased testes weight in SD and thyroid weights in LE. LE and SD strains showed a significantly smaller thymus. This investigation shows that hepatic enzyme activities and organ changes due to consumption of cabbage are strain dependent.

DOSE DEPENDENT CHANGES IN ELIMINATION AND TISSUE DISTRIBUTION OF 14C DITHIOBIURET IN THE RAT. W. D. Atchison, J. Dickins, W. R. Porter, and R. E. Peterson, School of Pharmacy, University of Wisconsin, Madison, WI.

The aim was to compare elimination kinetics and tissue distribution of DTB-derived 14C in rats under various dosage regimens. To assess the effect of DTB dose rats were administered 1 or 25 mg/kg 14C DTB ip and killed 3, 6, 12 or 24 hr later. Ti/2 for plasma disappearance (8-10 hr) and cumulative fecal excretion (65-75% of dose) were similar in the low and high dose groups. The highest concentration of DTB-derived 14C was in the thyroid where it was 13x higher than plasma in the 1 mg/kg group but only 3x higher in the 25 mg/kg group. This difference may be due to saturation of the thyroid's ability to concentrate DTB. In other tissues the concentration of 14C DTB equivalents was 0.5-2.0x that of plasma and elimination of radioactivity from most of these tissues paralleled plasma. Exceptions were thyroid, stomach, skeletal muscle and brain in the 1 mg/kg group and brain in the 25 mg/kg group. At 25 mg/kg relatively less radioactivity was distributed to the thyroid, visceral fat and (3) plasma than 1 mg/kg, and more was distributed to other tissues particularly the lung. The lung concentration of 14C was 60x higher in the 25 than 1 mg/kg group.

When DTB was given daily (1 mg/kg x 7 days) elimination kinetics and tissue distribution of the 1st and 7th doses were similar. Also with continuous daily treatment there was an accumulation of 14C in all tissues except visceral fat. Tissues that accumulated 14C to the greatest extent were lung, stomach, thyroid, liver and skin. (Supported by NIH grant ES01906).

ELIMINATION, TISSUE DISTRIBUTION AND METABOLISM OF 14C AND 35S DITHIOBIURET IN THE RAT: EVIDENCE FOR DESULFURATION. K. D. Williams, W. R. Porter and R. E. Peterson, School of Pharmacy, University of Wisconsin, Madison, WI.

The objective was to compare 14C and 35S dithiobiuret (DTB) with respect to elimination kinetics and tissue distribution in rats. Rats were treated with 1 mg/kg of 14C or 35S DTB and killed 3, 6, 12 or 24 hr later. For both 14C and 35S the Ti/2 for plasma disappearance was about 10 hr and 70% of the doses were eliminated in urine in 24 hr. The concentration of DTB-derived radioactivity in the thyroid was 8-45x higher than plasma. Also the concentration of 14C and 35S DTB equivalents in the thyroid was similar as was their thyroid elimination kinetics. The concentration of 14C and 35S DTB equivalents in brain, gastrocnemius, sciatic nerve, lung and kidney was 0.5-1.2x that of plasma. Elimination of radioactivity from these tissues paralleled that from plasma and within each tissue elimination of 14C paralleled 35S. The only tissue where elimination of 14C and 35S was not parallel was the liver. Ti/2 for hepatic elimination of 14C was slower (15 hr) than 35S (10 hr). Thin layer chromatography (TLC) of the 24 hr urine sample from rats treated with 14C DTB revealed six peaks. One peak migrated with DTB (17% of dose), a second with monothiobiuret (11%), and a third with thiuret (8%). Of the three unknown peaks, two were more polar than DTB (9, 21%) and one was less polar (2%). TLC of urine from 35S DTB-treated rats demonstrated that sulfate was a metabolite. The results demonstrate that DTB undergoes metabolism in the rat and that desulfuration is one of the processes involved. (Supported by NIH grant ES01906).

SYNTHESIS, STABILITY IN SOLUTION, AND SEPARATION OF 35S-DITHIOBIURET FROM DECOMPOSITION PRODUCTS AND POTENTIAL METABOLITES. W. R. Porter, K. D. Williams and R. E. Peterson, School of Pharmacy, University of Wisconsin, Madison, WI.

Daily treatment of rats with 2,4-dithiobiuret (DTB) produces a delayed onset, flaccid, skeletal muscle weakness progressing from the hind limbs to the forelimbs, trunk, and head. DTB was labeled with 35S for metabolic studies. Exchange of 35S between DTB and hydrogen 35S-sulfide occurs within 2 hr in boiling 0.01 N HCl under a N2 atmosphere. 40-75% of the initial DTB was recovered in crystalline form. The DTB contained 10-90% of the initial radioactivity. When DTB was degassed, DTB solutions were stable for 24 hr at room temperature, but solutions stored in air at room temperature lose greater than 5% of the initial DTB within 24
67. Fate of 14C-carbon tetrachloride in rats following inhalation during a novel and standard workweek. D. J. Paustenbach, G. P. Carlson, G. S. Born and J. E. Christian, Schools of Health Science and Pharmacy and Pharmacal Science, Purdue University, West Lafayette, IN 47907.

Although the metabolism and toxic actions of CCl4 have been well studied, the pharmacokinetic parameters of excretion have not been thoroughly evaluated for all 3 excretory pathways. Male Sprague-Dawley rats were exposed in a closed-loop inhalation chamber to 100 ppm of 14C-CCl4 vapor for 8 hr/day for 10 of 12 consecutive days (standard workshift) or 12 hr/day for 7 (4+3) of 12 days (novel workshift). Rates and routes of elimination of 14c activity were followed for over 100 hr after termination of exposure. Following the standard workshift exposure, expired CCl4 and fecal 14c activity comprised 52% and 41%, respectively of the total 14c excreted. Following the novel workshift exposure, the values were 32% and 62%. In both cases the urine accounted for less than 6% of the total 14c excreted and exhaled CO2 for 2% or less. For both workshifts the excretion in the breath could be described by a 2-compartment 1st order pharmacokinetic model (r²=0.98). In the breath, the T½ for the 8 hr group for the 1st phase was 110 min and for the 2nd phase, 490 min. For the 12 hr group, the T½ for the 1st and 2nd phases were 80 min and 580 min. It is of interest that the feces constituted a significant pathway for the excretion of the highly volatile CCl4 and that 14c activity could still be detected in breath, urine and feces 104 hr after exposure. This study suggests that slight changes in dosing regimen may influence the rate and route of excretion of a toxicant and that this may need to be considered with respect to potential occupational health hazards in industries with novel workshifts.


The fate of two dichlorobenzidine-based pigments, chlorodiane blue (CDB) and pigment yellow 12 (PY 12), was studied in adult male Fischer 344 rats to determine whether these compounds were absorbed and metabolized. [14C]CDB and [14C]PY12 were synthesized from uniformly labeled [14C]dichlorobenzidine (11 µCi/µ mole). [14C]CDB or [14C]PY12 was administered via gastric intubation or dermally at doses of 1.24–2.65 µmol/kg of body weight (2.73–5.82 µCi/rat). Serial blood samples were taken during an 8 hr period after oral dosing; no radioactivity was detected in the blood of rats which received either compound. A 24 hr balance study indicated that orally administered [14C]CDB was not absorbed from the gastrointestinal tract, with the total dose found in the feces. Less than 0.03% of the dose could be detected in the liver 24 hr after oral administration of [14C]PY12; the balance of the unabsorbed radioactivity was in the feces. [14C]CDB or [14C]PY12 was applied dermally to the shaved backs of rats; no radioactivity was found in the blood or liver, with the entire dose detected at the application site. In vitro studies of CDB, with and without liver enzyme metabolic activation, in S. typhimurium TA100, TA98, TA1535, TA1537, and TA1538 showed that it was not mutagenic at all doses tested (25–1000 µg/plate). Only chemical reduction of CDB with dithionite produced a compound, presumably dichlorobenzidine, which was mutagenic with metabolic activation in strains TA1538 and TA98. Thus, the relatively insoluble pigments CDB and PY12 are neither absorbed nor metabolized in vivo, nor was CDB metabolized by liver enzymes in vitro.

69. Toxicokinetics and metabolism of a single oral dose of 0-ethyl 0-4-nitrophenyl phenylphosphonothioate (EPN) in chicks. M. B. Abeu-Donia, Y. M. Hernandez and N. S. Ahmed. Department of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The toxicokinetics and metabolism of a single 1 mg (2.7 µCi)/kg oral dose of uniformly phenyl-labeled [14C]EPN (O-ethyl O-4-nitrophenyl [14C]phenylphosphonothioate) have been studied in 1-week old chicks. One control and three treated chicks were killed at each of the following time intervals: 0.5, 2, 4, 8, and 12 days. Radioactivity was rapidly absorbed from the gastrointestinal tract and distributed in all tissues. 14C in tissues reached a peak of 16.9% of the dose after ½ day and decreased to 0.6% at 4 days. The tissues of the gastrointestinal tract had the highest concentration of radioactivity, followed by urinary bladder, bile and liver. Among nervous tissues, concentration of 14C was highest in the peripheral nerves. The spinal cord had the next highest concentration; while the brain had the least. By the end of 4 days 91.3% of the 14C had been eliminated in the combined urinary-fecal excreta. By the end of the experiment this percentage reached 93.1%. No 14C was detected in the expired CO2. Following the oral administration of [14C]EPN, a monophasic body level curve was observed. The half-life for the elimination of 14C from chick body was 16 hours, corresponding to a rate constant of 0.04 hr⁻¹. EPN and metabolites were identified by high-pressure liquid and sequential thin-layer chromatographic systems. Polar EPN metabolites accounted for most of the radioactivity identified in the excreta. (Supported in part by NIEHS Grant No. ES02717).
DESTRUCTION OF CYTOCHROME P-450 (P-450) AND OF HEME, AND FORMATION OF GREEN PIGMENT BY SEVERAL ALLYLIC INDUSTRIAL TOXICANTS HAS BEEN SHOWN IN HEPATIC MICROSOMES FROM PHENOBARBITAL (PB)-TREATED RATS [PATEL ET AL., FED. PROC. 40:636 (1981)]. IN THE PRESENT STUDY, WE EXAMINED THE EFFECTS OF ALLYL CHLORIDE (AICL) AND ALLYL BROMIDE (AlB) ON THESE PARAMETERS IN LIVER MICROSOMES AND SOLUBILIZED MICROSOMES FROM CONTROL AND PB- AND 3-METHYLCHLOROTHRANE (MC)-TREATED RATS. THE DESTRUCTION OF P-450, LOSS OF HEME, AND FORMATION OF GREEN PIGMENT AFTER INCUBATION WITH AlC1 AND AlB WERE 5- AND 4-FOLD GREATER IN MICROSOMES FROM PB-TREATED RATS THAN IN THOSE FROM CONTROL AND MC-TREATED RATS, RESPECTIVELY. WHEN MICROSOMES WERE SOLUBILIZED, THE LOSS OF P-450 AND HEME REMAINED IN THE SAME PROPORTION AS IN INTACT MICROSOMES, BUT THE FORMATION OF GREEN PIGMENT AFTER INCUBATION WITH AlC1 OR AlB WERE 1/10 OF THAT IN INTACT MICROSOMES FROM PB-TREATED RATS, AND COMPLETELY DISAPPEARED IN MICROSOMES FROM MC-TREATED OR CONTROL RATS. THE EFFECT OF AlCl REQUIRED NADPH IN ALL PREPARATIONS. ALTHOUGH AlB CAUSED DESTRUCTION OF P-450 IN THE ABSENCE OF NADPH, DESTRUCTION WAS MORE EXTENSIVE IN THE PRESENCE OF NADPH IN ALL PREPARATIONS. ADDITION OF GLUCURONIDE OR OF PROTOPORPHYRIN IX DI NOT PROTECT P-450 OR HEME FROM DESTRUCTION BY AlC1 OR AlB IN ANY PREPARATION. (SUPPORTED IN PART BY USPHS GRANT OH-00316)

COMPARISON OF URINARY METABOLITES OF 14C DI-N- AND MONO-N-BUTYL PHTHALATE IN HAMSTER AND RAT: POSSIBLE RELATIONSHIP TO EFFECTS ON TESTICULAR TISSUE. Foster, P.M.D., Cook, M.W., Thomas, L.V., Walters, D.G. and Gangolli, S.D., The British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey, SMS 4DS. U.K.

PREVIOUS STUDIES HAVE SHOWN RATS TO BE SUSCEPTIBLE TO TESTICULAR INJURY PRODUCED BY EITHER DI- (DBP) OR MONO-N-BUTYL PHTHALATE (MBP), WHEREAS HAMSTERS APPEAR RESISTANT. A STUDY WAS THEREFORE UNDER-TAKEN TO SEE IF THIS DIFFERENCE IN SUSCEPTIBILITY COULD BE AScribed TO ANY SPECIES DIFFERENCES IN THE METABOLISM OF THESE TWO COMPOUNDS. 14C-DBP AND 14C-MBP WERE ADMINISTERED WITH NON-LABELLED CARRIER AT DOSE LEVELS KNOWN TO PRODUCE TESTICULAR ATRIOPHY IN THE RAT (2G/DBP/kg body wt - 10µCi/kg body wt; 800mgMBP/kg body wt - 10µCi/kg body wt). URINE WAS COLLECTED OVER A 48HR PERIOD AND RADIO-ACTIVE METABOLITES ANALYSED USING REVERSED PHASE HPLC. IN BOTH SPECIES AND USING BOTH COMPOUNDS THE MAJOR METABOLITE WAS FOUND TO BE MBP-GluconorIDE, AND NOT MBP AS PREVIOUSLY REPORTED. THIS METABOLITE ACCOUNTED FOR APPROXIMATELY 70% OF DOSE FOUND IN URINE. INTERESTINGLY THE AMOUNTS OF UNCONJUGATED MBP WERE QUITE DIFFERENT WITH VALUES OF APPROXIMATELY 18% AND 6% RESPECTIVELY FOR THE RAT AND HAMSTER. ALL THE METABOLITES FOUND AFTER ADMINISTRATION OF 14C-DBP COULD BE ACCOUNTED FOR IN THE 14C-MBP EXPERIMENTS CONFIRMING THE NEED FOR ESTERASE ACTIVITY PRIOR TO ABSORPTION. GUT ESTERASE ACTIVITY IN THE TWO SPECIES WAS FOUND TO BE COMPARABLE. THE OBSERVED LOWER URINARY EXCRETION OF MBP IN THE HAMSTER COMPARED TO THE RAT, INDICATING A LOWER CIRCULATING LEVEL OF THE FREE MOESTER MAY BE A POSSIBLE EXPLANATION FOR THE RESISTANCE OF THIS SPECIES TO DBP-INDUCED TESTICULAR INJURY. (SUPPORTED BY THE U.K. MINISTRY OF AGRICULTURE, FISHERIES AND FOOD).

Previous studies in our laboratory have shown that human liver microsomes can metabolize 2,2', 3,3',6,6'-hexachlorobiphenyl, but apparently not 2,2',4,4',5,5'-hexachlorobiphenyl, to hydroxylated metabolites. Since the para-position is usually the preferred site of hydroxylation, we investigated if human hepatic microsomes could metabolize 4,4'-dichlorobiphenyl (4,4'-DCB), a congener of low chlorination, but substituted in both para positions. Human hepatic microsomes were prepared from livers obtained during resection and were characterized with respect to cytochrome P-450 (0.35±0.13 nmoles/mg), NADPH-cytochrome c reductase (137±32 nmoles/mg/min), benzphetamine-N-demethylase (0.73±0.3 nmoles/mg/min) and biphenyl-4-hydroxylase (0.94±0.15 nmoles/mg/min). Aqueous extractable metabolites were formed when 14C-4,4'-DCB (58 mCi/mole) was incubated at 37°C under air with these microsomes. The reaction was linear with respect to protein concentration (0.25 to 1.0 mg/ml) and time (0-90 min). The apparent Km and Vmax for formation of aqueous extractable metabolites was 0.4 µM and 1.2 nmoles/mg/min, respectively. HPLC analysis (C18 reverse phase with 14C:44:5:1 MeCN:H2O:EtOH:acetic acid) of the metabolite fraction revealed at least five peaks of radioactivity, which probably represent distinct metabolites of 4,4'-DCB. The major peak of radioactivity co-eluted with synthetic 4,4'-dichloro-3-hydroxybiphenyl, which is also the major metabolite produced by the monkey and dog in vivo. These results indicate that an unsubstituted para position is not essential for hydroxylation of PCBs by human hepatic microsomes. (Supported by ES-82130 and 5-T32-ES07091.)


Trichloropropane (TCP) is an intermediate in the manufacture of pesticides and rubber and exhibits structural resemblance to some pesticides of recognized toxicity, e.g., dibromo-chloropropane. The tissue distribution, excretion, and metabolism of 14C-TCP were studied in male Fischer 344 rats following an i.v. dose of 3.2 mg/kg. Within 1 hr 65% of radioactivity in the blood was metabolites, which disappeared with an initial half-life of 3 hr and a terminal half-life of 8 days. TCP distributed rapidly into fat and muscle; at 15 min, these tissues contained 30 and 20% of the dose, respectively. At 4 hr, however, these tissues each contained <10% of the dose. Liver and kidney reached maximum values at 1 hr of 8 and 3% of the dose, respectively. Except for fat, all tissues contained primarily metabolites by 4 hr. Excretion was virtually complete after 2 days: 40% in urine, 20% in feces, and 25% in expired air. Direct measurement of radioactivity excreted in bile showed 10% of the dose excreted within 3 hr. A fraction of the metabolites excreted in bile were reabsorbed, since radioactivity excreted in the feces by 2 days was only 60% of that found in intestinal contents at 2 hr. Urine and feces contain no detectable unmetabolized TCP. Small amounts of unchanged TCP were found in intestinal contents at 15 and 30 min, but these were reabsorbed, since no unchanged TCP appeared in the feces. The major urinary metabolites are probably mercapturic acids because 1) 85-100% of the radioactivity in acid, neutral, or basic urine was not extracted by ethyl acetate, and 2) thin-layer chromatography of urine revealed compounds that gave positive reactions with ninhydrin. (Supported by NIH Grants ES82130 and 5-T32-ES07091.)


The disposition and metabolism of drugs and other xenobiotics is known to be altered in the aged. Since the toxicities of xenobiotics are often linked to their metabolism, this study focused on the effect of senescence on the bioactivation and covalent binding of aliphatic halides to microsomal protein and lipid. Studies were performed using hepatic microsomes isolated from Fischer 344 rats aged 4, 11, and 27 mo. The 14C labeled aliphatic halides studied were 1,2-dibromoethane, carbon tetrachloride, trichloroethylene, and 1,1,2-trichloroethane. The 27 mo old rats were found to have significantly less hepatic cytochrome P-450 (~35%), cytochrome b5 (~32%), NADPH cytochrome C reductase (~31%), and ethylphenol-N-demethylase activity (~43%) when compared to the younger age groups. The hepatic glutathione transferase activity was not different between the different age groups but microsomal UDP-glucuronosyltransferase activity was elevated (+136%) in the 27 mo group. The hepatic microsomal bioactivation and covalent binding of the aliphatic halides increased slightly between 4 and 11 mo; however, the microsomal bioactivation and covalent binding decreased 50-75% in the 27 mo group. As reported by others, we found senescence decreased the reactivity of hepatic microsomal biotransformation enzymes. In addition, the microsomal bioactivation of xenobiotics appears to be suppressed indicating a possible decreased potential for the expression of toxicities requiring bioactivation. (Supported in part by NIH Grant AG01289.)

77 CHARACTERIZATION OF THE UPTAKE OF ESTRADIOL-17β-D-GLUCURONIDE AND TAUCRCHOLIC ACID IN ISOLATED RAT HEPATOCYTES. W.J. Brock and M. Vore, Grad. Center for Toxicology and Dept. of Pharmacology, University of Kentucky, Lexington, KY.
Studies from this laboratory have demonstrated that estradiol-17β-3-D-glucuronide (E217G), but not estradiol-17β-3-D-glucuronide (E23G), causes a dose dependent, reversible cholestasis, and a decreased bile acid secretion after its iv administration in the rat (JPET 214:87, 1980). Infusions of taurocholate (TC; 80 µmol/hr) completely protected against the cholestasis induced by E2-17G (11 µmol/kg, iv). These studies suggest that E217G and TC compete for transport at a single organic anion carrier site. This investigation was designed to: 1) characterize the uptake of E217G, and 2) determine if E217G or the non-cholestatic E23G competitively inhibit the uptake of taurocholate using isolated rat hepatocytes as a model system. Hepatocytes were prepared from female Sprague-Dawley rats (200-260g) by a collagenase digestion of the liver and were judged to be 85-95% viable by trypan blue exclusion.

The rate of [3H]-E217G uptake was linear for approximately 2 min at concentrations of 5, 10, 50, 100 and 150 µM; kinetic parameters were $V_{\text{max}} = 2.940.3$ nmol/min/mg protein and $K_m = 316.727$ µM. The uptake of [3H]-TC was linear for approximately 3 min at concentrations of 2, 5, 10, 20, 50 and 200 µM; kinetic parameters were $V_{\text{max}} = 0.940.2$ nmol/min/mg protein and $K_m = 16.41.4$ µM. However, Dixon plots indicated that neither E217G nor E23G, at concentrations of 1-300 µM, inhibited TC uptake. These data therefore indicate that although E217G and TC are both taken up readily by hepatocytes, they appear to be transported by distinct organic anion carriers. (Supported by HD12520.)

78 INDANOL DEHYDROGENASE (ID): SOME PROPERTIES OF THE ENZYME FROM HUMAN PLACENTA. A. P. Kulkarni, B. Strom and W. Houser. Toxicology Program, Dept. of Env. & Ind. Hlth, The University of Michigan, Ann Arbor, MI

Some characteristics of ID isolated from full term placenta of non-smoker women were investigated under in vitro conditions. ID converts 1-indanol to 1-indanone in the presence of either NAD$^+$ or NADP$^+$. The rates of generation of reduced pyridine nucleotides was monitored spectrophotometrically at 340 nm as an increase in absorbance at 300 µM with time. The amount of 1-indanone formed in the incubation media was estimated by gas liquid chromatography. Preliminary experiments on the subcellular localization indicated ID to be exclusively associated with the microsomal fraction. Low activity observed with mitochondria and cytosolic fractions may be due to contamination of microsomes. Therefore, washed microsomes were used in the subsequent experiments. Narrow substrate specificity of ID is evident from the fact that only 1-indanol but not 2-indanol can serve as a substrate. Although ID can utilize either NAD$^+$ or NADP$^+$ as a cosubstrate, the activity is 20-60% higher in the presence of NAD$^+$ than NADP$^+$. The range of specific activity of ID (nmol/min/mg protein) varied between 9 to 12 for NAD$^+$ and 4 to 7 for NADP$^+$ in different samples examined. The rates of NAD(P)H and 1-indanone formation were linear with respect to time, protein and substrate concentration. The kinetics of ID to establish $K_m$ values for 1-indanol, NAD$^+$ and NADP$^+$ was found to be complex and the experiments in progress may shed some light on this problem. The purification of ID from human placental microsomes and evaluation of substrate specificity is planned in the future to establish its possible in vivo significance. This work supported in part by NIEHS 5 T32 07062 and 5 D04 AH01661.


Although suspensions of isolated hepatocytes have been used in a variety of studies on the metabolism and toxicity of foreign chemicals, it is generally believed that the viability and functional integrity of such cell suspensions decline within a few hours. These studies were undertaken to assess the stability of several liver functions in hepatocytes isolated by collagenase perfusion. Hepatocytes were isolated from adult male Sprague-Dawley rats and were incubated at a density of 1.0 x 10$^6$ viable cells/ml of hormone supplemented Waymouth's medium in 5% CO$_2$-95% O$_2$. During a 6-hour incubation period, the viability (trypan blue exclusion) decreased from 86% to 77%. However, rates of urea synthesis, gluconeogenesis and protein synthesis did not change significantly. The cytochrome P-450 content of the hepatocytes also remained stable from the start of the experiments to 6 hours, 28.3 ± 7.6 pmoles/µg DNA and 24.3 ± 5.5 pmoles/µg DNA, respectively. The addition of cofactors (NADP$^+$ and NADPH generating system) to the suspension medium did not stimulate the N-demethylation of p-chloro-N-methylaniline (PCMA), indicating that the majority of cells had intact membranes even immediately after isolation. The rate of metabolism of PCMA did decrease about 30% during the incubation, but the rates of O-demethylation and conjugation of p-nitroanisole and the metabolism of benzoyleurea remained relatively constant. Preliminary data suggest that hepatocytes prepared by biopsy perfusion, a method which can be used to isolate cells from larger species, had similar characteristics. This work was supported by NIH GM2058-01.

80 COMPARATIVE RATES OF BENZENE METABOLISM IN PRIMARY HEPATOCYTE CULTURES. S.A. Knadle, C.B. Salocks, J. Nakashima, and J.L. Byard, Department of Environmental Toxicology, Univ. of California, Davis, CA 95616.

Since benzene is thought to be metabolically activated in the liver, a comparison of metabolism rates in primary hepatocyte cultures from humans and laboratory animals may explain species differences in susceptibility to bone marrow toxicity. Hepatocytes were obtained by biopsy perfusion and cultured in chemically defined, hormone-supplemented media in collagen-coated glass culture vessels sealed with Teflon-silicone discs. These cultures were found to metabolize and covalently bind a single dose of $^{14}$C-benzene to the same extent as cultures in unsealed plastic dishes. Furthermore, these cultures oxidatively metabolized glucose at the same rate as unsealed cultures. Non-cytotoxic doses of $^{14}$C-benzene were injected after 24 hrs of culture. The dose partitioned between air and medium with a coefficient of 0.23 (v/v) at 37°C. Time points from 2-50 hours after benzene addition were collected, adjusted to pH 12, and unmetabolized benzene was extracted with ethyl acetate. Hepatocytes from
male S-D rats converted 20% of 2.3 µg and 0.23 µg doses (12.5 µg DNA/culture) to water-soluble metabolites in 48 hrs by first order kinetics. Cultures from a C-D1 mouse (4.3 µg DNA/culture) metabolized only 3.3% of these doses in 48 hrs, also by first order kinetics. Triplicate cultures (7.9 µg DNA/culture) from one human liver biopsy metabolized 36% of a 2.3 µg dose and 66% of a 0.46 µg dose of benzene to water soluble metabolites in 48 hrs. These preliminary results suggest that in humans benzene is metabolized more rapidly and the metabolism becomes saturated at a lower dose than in mice and rats. (Supported by a Monsanto Fund Fellowship and NIAMS Training Grant PHS (ES07059-03).


Existing data on the transfer of parathion to fetuses and nursing offspring are limited. Studies with pregnant guinea pigs failed to demonstrate the transplacental transfer of parathion. Furthermore, other investigators were unable to demonstrate the presence of parathion in the milk of dairy cows exposed to this chemical. However, since neonatal rodents have been suggested to possess increased susceptibility to this compound, we have studied the transfer and disposition of 14C-parathion in pregnant and lactating mice. Parathion was administered IP to 18 day pregnant or nursing Sprague-Dawley mice at a dose of 3 mg/kg in corn oil. Animals were sacrificed at various times posttreatment and 14C activity determined in maternal, fetal and suckling tissues. Maximal 14C activity in fetuses was observed at 0.5h following maternal treatment, reaching levels of 0.075µg 14C-equivalents/g fetal tissue. Activity was still measurable 24h posttreatment and was higher than in maternal levels were 5.4, 0.039 and 0.055 µg/g in the corresponding tissues. Transfer of parathion or its metabolites to the mouse fetus or via milk may be significant due to both acute toxicity and increased susceptibility of newborns to parathion as well as to its proposed effects on perinatal development. Supported by HD 13591 and MOD 15-9.


Senecio vulgaris is a common perennial in the western United States which contains low macrocyclic pyrrolizidine alkaloids (PAs), all hepatoxins and suspected hepatocarcinogens. Unfortunately, obtaining uniformly 14C-labelled macrocyclic PAs for metabolic studies has been difficult as synthetic methods for some macrocyclic PAs are not presently available. To obtain uniformly 14C-labelled macrocyclic PAs from S. vulgaris, a CO2 plant growth chamber was constructed. The CO2 chamber has a capacity of 80 3" pots, each containing 4 plants. At 4 weeks plants were placed in the CO2 plant growth chamber, allowed to acclimate for 1 week and exposed to 14CO2. 14CO2 was also provided, and the amount of incorporation of each carbon was determined using 13C-NMR. To determine the optimum PA production by S. vulgaris a number of plants were removed at selected intervals over a 3½ week period. The PA concentration appeared to increase with the plants matured.

In a following experiment, 25 mci of 14CO2 was given to 4 week old plants over a 6 hour period. Pyrrolizidine alkaloid production, total 14C activity and specific activity of each PA were determined over a 3 week period. The highest 14C incorporation in the PAs occurred 4 to 5 days post exposure to 14CO2. The greatest amount of PA produced is senecionine which exhibits a specific activity between seneciphylline and retrorsine. Senecionine appears to be a possible precursor of retrorsine as its per cent in the plants decreases as retrorsine increases.

Using the above data, 800 mCi of 14CO2 were used over a 12 day period to maximize specific activity and yield. The average specific activity of the isolated PAs was 3.5 mCi/mM. (Supported by NSF 7806924.)

83 THE IN VITRO COVALENT BINDING OF SENECIONINE AND SENE CIPHYLLINE TO MOUSE LIVER MICROSOMES AND DNA. D. P. Eastman and H. J. Segall, Dept. of V. M. Physio. Sci., Univ. of Calif., Davis, CA 95616

Pyrrolizidine alkaloids (PAs) are natural plant hepatotoxins and carcinogens. Plants and food stuffs containing PAs are consumed inadvertently and intentionally by both humans and animals. This study examined the in vitro covalent binding mediated by the cytochrome P-450 mixed function oxidase system of two PAs, 14C-senecionine (SN) and 14C-seneciphylline (SY) to female BALB/c mouse liver microsomal macromolecules, added calf thymus DNA and deoxyguanosine.

Microsomal incubations followed standard procedure. Covalent binding was measured by the radioactivity remaining in the 105,000 x g pellet or the precipitated DNA after exhaustive washing with methanol, diethyl ether and chloroform. Covalent binding as measured in the complete system (including microsomes, DNA, cofactors and room atmosphere) was 606 pmole SY per mg DNA, 802 pmole SN per mg microsomal macromolecules, 347 pmole SN per mg DNA, and 509 pmole SN per mg microsomal macromolecules. In incubations using boiled microsomes, an absence of NADPH generating system or an N2 atmosphere the covalent binding to microsomal macromolecules and DNA was reduced to 6 to 22% of the complete system. Incubations with 10-3 M KCN had no effect on the binding of the PAs to either DNA or microsomal macromolecules. Incubations with 14C-PAs and [1',2'-3H]-deoxyguanosine substituted for DNA produced one peak and a possible second peak on a C18 reverse phase HPLC system which could be adducts.

These experiments indicate that the covalent binding of PAs to liver macromolecules seen in vivo is probably mediated through activation of the PAs by the cytochrome P-450 mixed function system. (Supported by NSF 7806924.)
IRREVERSIBLE BINDING OF ISOLATED PYRROLIZIDINE ALKALOID (SENECIONINE) METABOLITES TO MOUSE LIVER MICROSOMAL PROTEINS. L. J. Willis, H. J. Segall and D. F. Eastman, Dept. V.M. Physio. Sci., Univ. of Calif., Davis, CA 95616

Pyrrolizidine alkaloids (PAs) are natural plant toxins found in a wide variety of plant species. Many of these are consumed by both humans and domestic animals and are considered a public health hazard. Seneconine, a PA native to Senecio vulgaris plus other plants, causes liver necrosis and is a suspected carcinogen. This study examined the in vitro covalent binding of Seneconine to various microsomal proteins to determine if PAs bound to specific proteins.

In an in vitro experiment, 14C-seneconine (Sp. act. 3.5 mCi/mM) was incubated with female BALB/c mouse liver microsomes plus an NADPH generating system. After centrifugation at 105,000 x g for 1 hour, the pellet was washed 5x with methanol and 3x with diethylether. The protein binding patterns of the seneconine metabolites were examined by SDS-polyacrylamide slab gel and disc gel electrophoresis. The protein binding was measured as the percent of radioactivity relative to protein intensity.

All results indicate that there was a uniform binding to microsomal proteins with a possible increase of radioactivity binding at lower molecular weights (<10,000 M.W.). Using a variety of acrylamide disc gels (10%, 12%, 15%) or slab gels produced the same effect. Boiling of the samples for 5 minutes with glycerol, mercaptoethanol and SDS prior to loading the sample on the gels did not appreciably alter the binding pattern.


The peroxidation of hepatic mitochondrial and microsomal membrane lipids produces malondialdehyde (MDA) which undergoes metabolism in vivo and in mitochondrial preparations in vitro. The purpose of this investigation is to assess the role of hepatic aldehyde dehydrogenases in the metabolism of MDA. The cytosolic fraction contained the highest MDA metabolizing capacity as determined by MDA disappearance and by the rate of MDA stimulated production of NADH. Two cytosolic aldehyde dehydrogenases, one with a Km for MDA of approximately 10 µM and another with a Km value of approx. 800 µM had Vmax values of 0.88 and 0.59 µMol NADH produced/min/mg cytosolic protein, respectively. Minimal activity of either enzyme was observed using NADPH. MDA stimulated production of NADH from NADPH was completely abolished by the addition of the aldehyde dehydrogenase inhibitor disulfiram (10^-3 M). Mitochondria contained an enzyme with Km values for MDA of 7 mM and 31 mM using the cofactors NADPH and NADPH, respectively. The Vmax activity per g liver of the mitochondrial enzyme was approx. 70 times less than either of the cytosolic enzymes when NADPH (1 mM) was used as the cofactor. The findings suggest that MDA is metabolized in vitro by lower Km cytosolic aldehyde dehydrogenase(s) which require NADPH. Supported by NIADD grant-03257.

STUDIES ON PHENOL-O-METHYLTANSFERASE AND THIOL-S-METHYLTANSFERASE. P. T. Lin and W. C. Dauberman Toxicology Program, Dept. Entomology, N. C. State University, Raleigh, NC

Phenol-O-methyltransferase from rat liver was studied with regard to the stabilization and solubilization of this enzyme. Enzyme induction of phenol-O-methyltransferase by phenobarbital was demonstrated. A substrate specificity study using 22 monophenolic substrates showed no positive correlation between structure and activity.

A comparative study of thiol-S-methyltransferases present in the microsomes and the soluble fraction of mouse liver was conducted. Enzyme activity in both fractions was partially purified and some kinetic parameters were investigated.

SODIUM AZIDE: BIOAVAILABILITY AND METABOLISM IN RATS. E. W. Lee, Biomedical Science Department, General Motors Research Laboratories, Warren, MI 48090

Our previous investigation (Toxicologist, 1:21-22, 1981) reported the absence of azide in blood and the lack of toxicity of the daily dose of 23 mg/kg sodium azide (NaN3) in drinking water for 90 or 147 days in laboratory rats. This contrasts sharply with the lethal effects of a single dose of 42 mg/kg administered perorally. The bioavailability and in vivo metabolism of NaN3 was therefore investigated to identify the mechanism responsible for the absence of toxicity of azide in rats. Although azide was detected in the plasma as early as 5 min after the oral administration of 42 mg/kg, no azide was identified in both plasma and tissues 24 hours later. During the same period, no azide was excreted in the feces or exhaled in the air and only 7.5 µg of azide was excreted in the urine. Rat liver was found as the organ responsible for the deactivation of azide since (1) in the in vitro study, the liver homogenate rapidly destroyed NaN3, whereas homogenates of other tissues did not, and (2) in the in vivo study, the azide absorbed from the gastrointestinal tract was metabolized as soon as it reached the liver. At the maximum reaction rate, one milliliter of 20% liver homogenate metabolized 1.3 µg NaN3 per min. A peak level of azide in plasma, 49 µg/ml, was detected after a 40 mg/kg dose but no azide was found in the plasma with a bolus dose of 0.38 mg/kg. This indicates that a single oral dose of 0.38 mg/kg does not exceed the metabolic capacity of the liver. Also, since the oral administration required less than 10 sec for the appearance of azide in plasma, the calculated rate of sodium azide absorption from the gastrointestinal tract after intake of 23 mg/kg/day was equivalent only to 0.0027 mg/kg/10 sec. This intake rate is well below the metabolizing capacity of the rat liver tissue, and it is therefore not surprising that no azide was detected in the plasma and no pathological or histopathological abnormalities were observed in the experimental animals with this dose level.

DMA is a gas used in a number of diverse industrial applications. The current recommended threshold limit value for DMA (10 ppm) produced no measurable toxicity in F-344 rats (6 hr/day, 5 days), but focal ulceration and necrosis of the respiratory epithelium were induced at 175 ppm using the same treatment schedule (Swenberg, J. A., and Barrow, C. S.; unpublished observations). No information has been reported on the disposition and metabolism of DMA after inhalation. Male F-344 rats were exposed to 14C-labeled DMA gas in head-only exposure chambers for 6 hours at concentrations of either 10 ppm or 175 ppm. At each dose, the excretion of DMA-derived radioactivity into urine, feces, and CO2 was determined during a 72 hour post-exposure period. The disposition of radioactivity into various tissues was also investigated. The majority of recovered radioactivity appeared in the urine (76% at 10 ppm; 86% at 175 ppm) and feces (approximately 5 to 10% for each group). Less than 4% of the recovered radioactivity appeared in the expired air as CO2 suggesting only slight oxidative metabolism. Approximately 7% of the label remained in the tissues and carcass 72 hours post-exposure. Concentrations of radioactivity were highest in the tissues of the respiratory tract (nasal mucosa, trachea, and lung) as well as the liver, kidney, and intestine. The nasal mucosa contained the highest deposition of radioactivity, most probably due to the high water solubility of DMA and the fact that the nasal mucosa is the initial point of entry of the gas into the rat. Results suggest that in the rat saturation of metabolism and excretion is not achieved at 175 ppm DMA, and that oxidative metabolism of DMA is minimal.

**IN VIVO METABOLISM OF 3-METHYLCHOLANTHRENE (MC) IN PREGNANT C57BL/6 AND DBA/2 MICE.** J.D. George, J.M. Manson, S. Vater, and D. Warshawsky, Dept. Envir. Hlth., Univ. of Cincinnati, Cincinnati, OH. Sponsor: P.B. Hammond

MC has been linked to carcinogenesis in laboratory animals and their transplacentally exposed offspring. Activation of MC to reactive intermediates is catalyzed by microsomal aryl hydrocarbon hydroxylase (AHH), the activity of which correlates positively with tumorigenesis. This study compares the in vivo metabolic profiles of MC in the maternal and fetal tissues of C57BL/6 (high AHH) and DBA/2 (low AHH) mice. Maternal liver, placenta, and whole fetuses from pregnant mice exposed to 14C-MC were evaluated for extractable and bound 14C activity. HPLC analyses of ethylacetate extracts revealed no qualitative differences in the spectrum of metabolites found in the tissues of either strain of mouse; seven metabolites, in addition to MC, have been identified. In both strains, the placenta appears to be a reservoir for the parent compound. The free MC metabolite profiles of the two strains of mice differ quantitatively for all tissues studied. Tissues from DBA/2 mice contained higher relative levels of MC, which is consistent with the lower AHH activity of this strain. Two additional regions of 14C activity have been tentatively identified through HPLC and alumina column chromatography as conjugates of the free metabolites. In the non-extractable tissue residue from both strains, the 14C activity was primarily associated with the lipid and protein fractions, although some activity was associated with RNA, DNA, glucosaminoglycan, and carbohydrate. Findings to date indicate that the major difference in the metabolic profiles of MC in C57BL/6 and DBA/2 mice is in the level of metabolism and not in the types of metabolites produced. (Supported by NIEHS Grant ES07073 and The Ohio Coal Laboratory Research Association).


Low dietary protein levels (5%) decrease the hepatocarcinogenicity of aflatoxin B1 (AFB1), in part due to a decrease in hepatic microsomal mixed function oxidase (MFO) activity, which activates AFB1 to its ultimate carcinogen. Although low levels of dietary protein are consumed by certain populations, others may consume protein above that required for maintenance. This study was designed to ascertain the effects of increasing levels of dietary protein on the metabolic activation of AFB1. Male, weanling Sprague-Dawley derived rats were fed the AIN-76 semi-purified diet with casein levels of 5, 10, 20, 30 and 40% for 10 days. Body weight increased until protein levels reached 20% and then became asymptotic. Liver weights were higher only in the 5 and 10% groups due to lipid infiltration. Microsomal protein increased with dietary protein up to 20% and then became asymptotic. N-demethylation of ethyl-morphine (EM) increased until dietary protein levels reached 30% and then became constant. Cytochrome c reductase activity and cytochrome P-450 levels followed a trend identical to EM metabolism. In vitro metabolic activation of AFB1 to a product(s) capable of covalently binding DNA increased until a level of 30% protein was reached and then became asymptotic. In vivo covalent binding of an AFB1 metabolite(s) to hepatic DNA, RNA and protein followed the same trend as the in vitro studies. Inappropriately low levels of dietary protein may be protective in respect to AFB1 carcinogenicity, but high levels may increase risk. (This study was supported by Grant IN-105E from the American Cancer Society)

**IS THERE A LOW-DOSE THRESHOLD FOR HEPATIC MACROMOLECULAR BINDING OF AFLATOXIN B1?** B.S. Appleton, M. P. Goetchius and T.C. Campbell, Div. of Nutritional Sciences, Cornell University, Ithaca, NY.

The possibility of a glutathione dependent dose threshold for rat liver macromolecular binding of aflatoxin B1 (AFB1) was investigated. In Experiment I, mature rats were given 6 dosage levels of [3H]-AFB1 to determine if a dose threshold for macromolecular binding would be
observed. In Experiment II, immature rats were used to determine whether or not the extent of macromolecular adduct formation at low doses could be modified by depleting hepatic glutathione with diethyl maleate pretreatment. Nanogram per kilogram doses of radiolabeled AFB1 produced measurable covalent binding of aflatoxin to DNA, RNA and protein, and the extent of this binding increased linearly over a dose range of 0.01-1.00 µg/kg. Macromolecular adduct formation was observed at the lowest dose used (10 µg/kg) which is within the human exposure range. Rats whose liver glutathione was decreased from 5 mg/g of liver to 2.3 mg/g of liver by diethyl maleate pretreatment showed only a small and inconsistent increase in macromolecular adduct formation. Moreover, this increase was independent of AF1 dose and was more associated with the amount of AF1 found in the liver homogenate rather than with the intracellular events presumably involved in glutathione conjugate formation. These results indicate that macromolecular binding of AF1 is essentially a linear function of dose at low exposures and that hepatic glutathione activity has little or no role in that binding. (Supported by American Cancer PT 104 and USPHS PO1 CA 26755.)

92 SUBCHRONIC DIETARY TOXICITY STUDIES ON 2-CHLORO-6-(2-FURANYLMETHOXY)-4-(TRICHLOOROMETHYL)PYRIDINE, (DOWCO 444), A DEVELOPMENTAL FUNGICIDE, IN RATS AND DOGS. Jersey, G.C., Gorzinski, S.J., Barne-Lloyd, T., Quast, J.F., Tollef, J.T. and Schwetz, B.A., Health & Environmental Sciences, Dow Chemical U.S.A., Midland, MI 48640 and Lake Jackson, TX 77566.

To assess systemic toxicity, groups of male and female rats were fed diets supplying DOWCO 444 at dose levels of 0 (control), 1, 5, 15 or 30 mg/kg body weight/day for 13 weeks (12/sex/group) or for 16 weeks (8/sex/group). Those fed for 16 weeks were held on recovery for an additional 8 weeks. No effects attributable to treatment were found in rats fed 1 mg/kg/day for 13 weeks, or in any experimental group after the 8-week recovery period. In rats fed for 13 weeks, microscopic changes (increased hepatocellular cytoplasmic microvacuolation and centrilobular swelling) were found in males and females at 15 and 30 mg/kg/day and in some females at 5 mg/kg/day. The hepatic changes were reversible based on the 8-week recovery results.

DOWCO 444 was also fed to male and female dogs (6/sex/group) at dose levels of 0, 0.05, 0.5 or 5 mg/kg body weight/day for 6 months. Effects of treatment, found only in dogs fed 5 mg/kg/day were: increased liver weights in males and females, increased alkaline phosphatase levels in males but not females, increased relative kidney weight in females, and decreased RBC count in males. No histopathological lesions clearly corresponding to the increased liver or kidney weights were found. Based on these studies, the liver is considered to be the primary target organ in rats and dogs. The no-observable-effect level (NOEL) in rats was 1 mg/kg/day and in dogs 0.5 mg/kg/day.


Fenvalerate is a synthetic pyrethroid used for broad spectrum insect control on cotton, fruit, and vegetable crops. Groups of 50 male and female B6C3Fl mice were fed dietary concentrations of 10, 50, 250, or 1250 ppm Fenvalerate for two years. Two groups of control mice, 50 per sex, per group, received basal diet only. Mortality was increased and body weight was significantly decreased in male and female mice in the 1250 ppm treatment group. Mean body weight of female mice in the 250 ppm group was also generally lower than controls after the 60th week of feeding. Decreased ALB and increased GOT levels in mice fed 1250 ppm Fenvalerate were the only effects observed in the hematology and serum chemistry parameters evaluated. Mean organ weights and organ/body weight ratios of brain, heart, liver, kidneys, and adrenal glands were not affected by treatment. The only non-neoplastic pathology observed in the study was multifocal granulomatous lesions in lymph nodes, liver and spleen of 1250 ppm male mice and 250 ppm and 1250 ppm female mice. Less severe granulomatous changes were present in mesenteric lymph nodes of 50 and 250 ppm male mice. No statistically significant differences were observed in either the number or type of neoplasms in mice fed Fenvalerate diets when compared to concurrent controls. Thus, Fenvalerate was found not to be carcinogenic in B6C3Fl mice at a maximum tolerated dose under the conditions of the test.


Captan increases the incidence of duodenal tumors when chemically administered at relatively high concentrations in the diets of CD-1 mice. DNA was isolated from various organs of male CD-1 mice removed 24 hrs. after oral administration of 14C-captan, to determine if a difference in binding to DNA occurred between target and nontarget organs. Captan was administered at doses of 1600 mg/kg (specific activity 1.9 and 0.19 mCi/mmole) and 156 mg/kg (56 mCi/mmole). DNA was quantitated by absorption at 264 nm. 14C-radioactivity was quantitated by scintillation spectrometry. No radioactivity was detected associated with the DNA isolated from the duodenum and testes of mice administered 1600 mg/kg at either specific activity. Radioactivity was detected associated with DNA isolated from the duodenum and testes of mice administered 1600 mg/kg at either specific activity. Radioactivity was detected associated with DNA isolated from the duodenum, nonduodenal intestine, liver, stomach, kidneys, and testes of mice administered 156 mg/kg (specific activity 56 mCi/mmole). The number of trichloromethyl carbon atoms per DNA nucleotide was calculated and ranged from 5 x 10-6 (testes) to 4.2 x 10-5 (liver). DNA was subjected to neutral dialysis. Up to 90% of the associated 14C-radioactivity was dialyzable depending upon the DNA

*Trademark of The Dow Chemical Company.
95 A DERIVED CHRONIC TOXICITY-ONCOGENICITY STUDY
OF MYCELIAL MONENSIN SODIUM IN THE RAT. L.C.
Howard, M.N. Novilla and E.R. Adams (Sponsor,
J.L. Emmerson). Toxicology Division, Lilly
Research Laboratories, Greenfield, IN.

Groups of 100 male or female Wistar rats,
derived from parents given diets containing
monensin sodium (MS), were maintained for two
years on diets containing 0, 33, 50 and 80 ppm
MS activity as mycelial MS (1.4-5.0 mg MS
activity/kg/day). The reproductive capacity of
the parents, including fertility, litter size,
gestation length and survival, and progeny
survival and sex distribution was unaffected.
Body weight gain was decreased by MS treatment
for both parents and their offspring during
postpartum development. In the two year study,
body weight gain was decreased during the
initial two weeks of the study. Survival was
increased in a dose-related manner. Observations
of physical signs of toxicity and
evaluation of hematology, clinical chemistry,
urinalysis and organ weight parameters did not
reveal effects related to MS administration.
Inflammatory and degenerative conditions were
found to occur randomly in control and MS
treated rats. The latency and prevalence of
benign and malignant neoplasms were similar in
control and MS treated rats. In conclusion,
the continued exposure of rats to diets
containing up to 80 ppm MS activity during in
utero development and throughout their lifetime
produced neither chronic toxicity nor carcino-
genicity.

96 ONCOGENIC BIOASSAY OF 3-TRICHLOROMETHYL-
5-ETHOXY-1,2,4-THIADIAZOLE, ETRIDIAZOL, IN
CD-1® MICE. E. F. Erker, L. J. Slaughter, F.
Sperling and W. L. West, Depts. of Pharmacology
& Pathology, Howard University, Med. Sch., Washing-
ton, D.C. 20059 & J. Wedig, Olin Corp., New Haven,
CT 06511.

The fungicide, 3-trichloromethyl-5-ethoxy-1,2,4-thia-
diazole, Etridiazol, was bioassayed using individually
housed CD-1® mice. Approximately 60 post weaning
mice of each sex were begun in each of the following
groups: Naive and Vehicle Control (Corn Oil added)
and 320, 640, 1280 ppm Etridiazol in the diet. Six
and 12 month protocol sacrifices were performed. At
18 months, one half of the mice were sacrificed. The
remaining mice were fed on naive control food for
three months and were sacrificed. GLP was
followed. Reversible dose related increases in Liver
weight and organ/body weight ratios were observed in
the absence of gross or microscopic lesions. Alveo-
genic adenomas (11.4%) and carcinomas of the lungs
(28%), the most commonly occurring tumors, were
first observed in significant numbers at 18 months.
Cystic endometrial hyperplasia (78%) and splenic
extramedullary hematopoieses (56%) were present
from puberty onward. The tumor incidence for all
groups was within the range of published data. The
time course of development of these lesions was
similar in control and test groups. Based on the
results of this study, it was determined that Etri-
diazol was not carcinogenic in CD-1® mice.

97 THE ROLE OF HYDROGEN PEROXIDE IN MICROSO-
MAL LIPID PEROXIDATION. L.A. Morehouse, M.
Tien, J.R. Bucher and S.D. Aust, Dept. of Biochem-
istry, Michigan State University, East Lansing, MI 48824

Aerobic organisms, during enzymatic hydroxyla-
tions and oxidations of numerous endogenous sub-
strates and some xenobiotics, generate reactive and
potentially toxic, partially reduced forms of oxygen.
Accumulation of these potentially toxic species, which
could result in peroxidative damage of cellular mem-
branes, is prevented by enzymes such as superoxide
dismutase, catalase, and glutathione peroxidase, which
catalyze either the further reduction or the dismuta-
tion of the potentially toxic biproducts.

Hydrogen peroxide can react with reduced transition
metals generating the highly reactive hydroxyl
radical (·OH), most often proposed as the predominant
initiating species of microsomal lipid peroxidation.
In order to assess the potential involvement of ·OH,
generated from hydrogen peroxide, in microsomal lipid
peroxidation, we have altered the concentration of
microsomal hydrogen peroxide and measured the result-
grates of lipid peroxidation. Hydrogen peroxide
congestion in microsomes was changed by adding
exogenous catalase, washing to reduce endogenous
catalase and glutathione peroxidase activities, and
inhibiting endogenous catalase activity with azide in
either the presence or absence of exogenous hydrogen
peroxide. In only one instance was the rate of lipid
peroxidation, as measured by malondialdehyde forma-
tion, affected; exogenous hydrogen peroxide added to
microsomes, previously incubated with azide, inhibited
lipid peroxidation, the opposite effect from that
predicted if ·OH, generated from hydrogen peroxide, is
actually the major initiating species. Neither these
results, nor the inability of known ·OH traps to inhibit
microsomal lipid peroxidation, support the prevalent
theory for the initiation of microsomal lipid peroxida-
tion. (Supported in part by NSF Grant No. PCM-79-
13528)

98 THE CYTOCHROME P-450 DEPENDENT MONOOXY-
GENASE SYSTEM FROM THE LIVER OF UNINDUCED
MICE. P. Levi, G. Bissette, and E. Hodgson. Toxicology
Program, Department of Entomology, North Carolina
State University, Raleigh, NC.

Most previous studies of purified cytochrome
P-450 used as starting material the livers of animals
pretreated with a variety of inducing agents. The present study utilized the liver
from uninduced mice. Liver microsomes were
solubilized with sodium cholate and purified by
column chromatography using phenyl sepharose,
DEAE cellulose, and hydroxyapatite. At least
two forms were purified to a specific content of
18-20 mmoles/mg protein, and it is apparent from
ion exchange chromatography and SDS gel electrophoresis that there are more than two forms.
NADPH cytochrome P-450 reductase, molecular
weight 79000, was purified by column chromato-
graphy using DEAE cellulose and 2'SADP Sepharose
4B. The cytochrome P-450 monoxygenase system
was reconstituted by combining these components
with phospholipid. Current studies involve the
activity of the reconstituted system towards
various substrates, changes in the cytochrome
P-450 profile after induction by specific
inducers, e.g., isosafrole, and the inhibition of
cytochrome P-450 by methylenedioxyphenyl
compounds.

99 A COMPARATIVE KINETICS STUDY OF MONOCHLORAMINE
AND CHLORINE IN RAT. M.S. Abdel-Rahman, D.M. Waldrum, and R. J. Bull, CDMNJ-New Jersey Medical
School, Newark, N.J. 07103

The problem of trihalomethane formation now
exists with the use of chlorine (HOCl) as a dis-
inf ectant in drinking water. Monochloramine
(NH₂Cl) may be considered as an alternative to
HOCl as a disinfectant in public water supplies.
This study was conducted to compare the kinetics
between NH₂Cl and HOCl in rats. Radioactiv-
ity was absorbed from the gastrointestinal tract
following the administration of NH₂Cl (1.9 µCi)
or HOCl (0.7 µCi) orally and the peak plasma
levels of 36Cl occurred at 4 and 2 hrs. for
NH₂Cl and HOCl respectively. The distribu-
tion of NH₂Cl and HOCl was highest in plasma
followed by whole blood, testes, skin, bone
marrow, stomach and kidney, while the lowest
activity was observed in the fat. Subcellular
distribution in liver fractions after 24 hrs.
from the administration of NH₂Cl and HOCl revealed
that about 72% of 36Cl was detected in the
cytosol fraction. The half-life for 36Cl was re-
duced to 38.8 and 44.1 hr⁻¹ for
NH₂Cl and HOCl respectively. The distribu-
tion of NH₂Cl and HOCl in both treatments, how-
ever 7 and 29% of the total 36Cl excreted in
diuresis were observed after NH₂Cl and HOCl admini-
nistration. No 36Cl was detected in expired
air throughout the 120 hrs. studied. (Supported by U.S.E.P.A.)

101 EFFECTS OF POSTNATAL TRIETHYLTIN (TET) ON RADIAL-
ARM-MAZE BEHAVIOR IN THE JUVENILE RAT. D.B.
Miller and L. W. Reiter, Neurotoxicology Division,
Health Effects Research Laboratory, US Environ-
mental Protection Agency, Research Triangle Park,
North Carolina 27711.

Postnatal exposure to TET results in permanent al-
teration in brain function as evidenced by hyper-
activity in both the juvenile and adult rat. In
the present experiment the effects of postnatal
TET on the learning and memory capabilities of
the juvenile rat were assessed with an automated
radial-arm-maze. The maze consists of a center
area from which 8 arms radiates; a food pellet is
available at the end of each arm. The most effi-
cient performance consists of entering an arm
only once to obtain the pellet; any further arm
entries are considered as errors. Photocells in
various maze locations provide an index of ac-
tivity. Long-Evans hooded rat pups were injected
i.p. on postnatal day 5 with 0, 3, or 6 mg/kg of
TET bromide; all 8 pups in a litter received the
same dose. Beginning at 37 days of age rats were
given a daily 10-min session in the maze for 15
consecutive days. Rats were allowed to make arm
selections throughout the session. TET-treated
rats initially made more errors but were at con-
trol levels by the end of the 15 days. On day 1
of training the TET-treated rats did not differ
from controls in the number of photocell counts
but by day 3 were more active; this hyperactivity
persisted for the remainder of testing. This
hyperactivity is consistent with our previous
findings. In addition, the observed accuracy
differences suggest that postnatal TET adminis-
tration affects learning capability.

102 MICROWAVE INDUCED CHANGES IN CANALICULAR MEMBRANE
FUNCTION OF THE RAT. D.G. Lange, M.E. D'Antuono,
and J.M. Fujimoto. Research Services, VA Medical
Center, Wood, WI, and Dept. of Pharmacology and
Toxicology, Medical College of Wis., Milwaukee,WI.

Acute exposure of phenobarbital anesthetized male
Sprague-Dawley rats (225-350g) to 2.45 GHz micro-
wave irradiation (120mW/cm², 30 min) produced a
30 rise in core body temperature, and signifi-
cantly increased (22.7%) the amount of 3H-sucrose
recovered from the biliary tree following its in-
fusion into the biliary tree by a segmented retro-
grade intrabiliary injection (SRII). Similarly,
the recovery of 14C-mannitol administered by SRII
decreased by 11.7%. Animals exposed to an equiva-
 lent thermal load from a radiant heat source had
increases in both 3H-sucrose (70.7%) and 14C-mann-
itol (31.7%) recoveries following SRII. These
changes in SRII recoveries returned to control
values when the animals returned to normothermia.
Chronic exposure (4 days) to the above thermal
loads, by microwave irradiation and radiant heat,
resulted in irreversible changes in canalicular
membrane function, in normothermic animals. In
chronic microwave exposed animals, \(^{3}H\)-sucrose recovery increased 95.1\% and \(^{14}C\)-mannitol recovery increased 50.0\%. In animals chronically exposed to radiant heat, only \(^{3}H\)-sucrose recovery increased (46.0\%). Following acute exposure to both microwave and radiant thermal loads, bile flow rate increased in a temperature dependent fashion. After chronic thermal exposure, bile flow rate decreased between 19.9 to 32.2\%. Thus, chronic exposure to thermal loads produced irreversible alterations in the function of the hepatic canalicular membrane function, which were not a result of changes in bile flow rate. In addition, the chronic microwave exposure produced greater changes in canalicular membrane function than an equivalent thermal load from a radiant heat source. Supported by NIEHS Grant ES02006.


It is now recognized that 1,2-dibromo-3-chloropropane (DBCP) can produce liver and kidney injury in animals, as well as testicular damage and carcinogenesis. The following experiments were carried out to assess the hepaticorenal injury potential of the chemical when it is present as a water contaminant. DBCP was added to the drinking water of adult male S.-D. rats in concentrations of 5, 50, 100 and 200 ppm for up to 60 days. Water consumption and body weight were monitored weekly. Hepatic damage was assessed by measuring changes in morphology and in levels of the enzymes arginine succinate lyase, ornithine carbamyl transferase (OCT), sorbitol dehydrogenase, glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase in serum. Nephrotoxicity was assessed by measuring BUN, renal cortical slice transport of organic ions, and excretion of malatase and alkaline phosphatase (AP) in urine. Substantial decreases in water consumption and body weight were seen in animals drinking water containing 200 ppm DBCP. Depression in body weight gain was initially manifest 3 weeks into the study. Increases in levels of OCT in the serum were observed as early as one week in animals consuming 100 or 200 ppm DBCP. Significant changes in OCT were also observed at the lower dosages after one month of the regimen. Kidney injury, as reflected by inhibition of transport of organic ions in renal cortical slices, was seen within 1 week of consumption of water containing 100 or 200 ppm DBCP. No significant changes in levels of AP or maltase in the urine were detected during the initial two weeks. Findings to date indicate that DBCP, even when ingested in very small amounts in divided doses, can produce adverse effects in the liver and kidneys. (Supported by EPA Contr. 68-01-4839 & NIEHS ES07090)


Our laboratory has previously shown that dimethylphthalate (DMP), a widely used insect repellent, produced a positive dose-related mutagenic response in the Ames TA100 bacterial strain. The response could be eliminated by a rat liver microsomal preparation (S-9) independent of the addition of S-9. To explain this activating phenomenon the metabolism of DMP by rat liver was examined by means of a TLC-spectrophotometric assay. Since skin is a potential target organ for this topically applied repellent, metabolism by this organ was also studied. Monomethylphthalate (MMP) was the predominant phthalate metabolite formed from DMP incubation with liver S-9. MMP was found to be non-mutagenic in the Ames assay, thus, a mono-esterase activity of S-9 would appear to be the cause of the inactivation of the mutagenic response of DMP. When the in vitro rates of MMP formation by liver and skin were compared on a mg wet weight basis, there was a 60-fold greater rate in the hepatic tissue. Additionally, methanol was assayed by means of a modified Nash procedure and was found to be produced in equimolar amounts to MMP. Many mutagens have been shown to lead readily to liver and skin. Since liver and epidermis have markedly different capacities to metabolize DMP to non-mutagenic forms, the in vitro binding of carbonyl \(^{14}C\)-labelled DMP was compared in these two tissues. MMP bound to epidermal macromolecules at a rate of 0.98 amoles per mg TCA precipitate, a rate that was 5 fold greater than that in liver. Thus, the lower rate of de-esterification of DMP in the skin relative to liver is correlated with the higher rate of binding to DMP in the epidermis and thus suggests a mutagenic/carcinogenic hazard of this compound in skin. Supported by NIEHS grants 00454 and 07067.


Glutathione-S-transferases (GSH-Trs) comprise a group of multifunctional enzymes involved in the biotransformation/detoxification of a broad spectrum of xenobiotics. They are present in the cytosol of most cells and in some cases microsomal fractions. Recently, attention has been drawn to the peroxidase (Px) activity associated with the cytosolic GSH-Trs; this activity has been implicated in the protection of membranes from damage due to lipid peroxidation. However, lipid peroxidation occurs in the hydrophobic vicinity of membranes and these areas are not readily accessible to the cytosolic enzymes. For this reason, membrane associated GSH-Px activity may be of special importance. We have monitored the GSH-Px activity in pure microsomes isolated from rat and guinea pig tissues. Of the various tissues screened, liver microsomes exhibited significant GSH-Px activity; guinea pig liver having approximately 3 times greater activity and only marginal activities were observed in kidney microsomes. A protein exhibiting both GSH-Tr and GSH-Px activity was isolated from guinea pig liver microsomes and purified to homogeneity by DEAE-cellulose ion exchange and s-hexyl glutathione-sepharse 6B affinity chromatography. Separation of several forms was accomplished through the use of chromatofocusing and CM-cellulose chromatography. Different forms of GSH-Trs from guinea pig liver
were characterized by determining their substrate specificity, isoelectric points and SDS gel electrophoretic pattern. (Supported by EPA grant # R. 807746 and NIH grant # R01 ES02679-01.)

106 CHARACTERIZATION OF THE FLUOROACETATE DEFLOURINATION ACTIVITY IN MOUSE LIVER. Andrew I. Sosiefer and Paul J. Kostyniak. Department of Pharmacology and Therapeutics SUNY at Buffalo, School of Medicine, Buffalo, N.Y. 14214 Sponsor: T. R. Clarkson

Recent studies have identified a defluorinating system in the 105,000g fraction of rat liver which required glutathione (GSH) for the liberation of fluoride from fluorooacetate (FAc). The present investigation reports an analogous defluorinating system in the 105,000g supernatant fraction of mouse liver with three to five times the specific activity seen in the rat. The defluorinating activity was dependent upon GSH concentration in the assay, with peak activity observed above 5mM GSH. The reaction was further characterized as having a pH optima of 8.2 and an apparent Km of 5mM with FAc as substrate. In Tris-HCl the defluorinating activity was found to be labile when stored at 4°C. The addition of dithiothreitol, GSH, or 2-mercaptoethanol (2-ME) stabilized the activity, with the latter providing concentration dependent protection for greater than 150 hours. In comparing the time courses for sulfhydryl oxidation and defluorination activity there was a loss in defluorination when the concentration of reduced sulfhydryl fell below 500 µM. Protection of the defluorinating activity fell off to 360 hours was found when the Tris buffer was replaced by phosphate. This protection was enhanced by the inclusion of 1mM 2-ME in the buffer. Initial purification steps demonstrated that the defluorinating activity separated out with an anionic proteins in DEAE-Sephadex ion-exchange chromatography. This technique also separated the defluorinating activity from 90% of the glutathione S-transferase activity measurable with 1-chloro-2,4-dinitrobenzene. Defluorination activity did not bind to a GSH affinity column which selectively separates it from a highly purified anionic glutathione S-transferase. This work was supported by a grant from NIEHS # ES02061.


Organisms must contend with highly toxic peroxides and other activated oxygen species. Illustrating potential toxicity, H2O2 has been found to interact in a deleterious fashion from a variety of organisms. Upon exposure to 0.1 to 1 mM H2O2 (or benzo peroxide) at room temperature and neutral pH, these proteins rapidly form covalent dimers and polymers detectable by detergent gel electrophoresis. Formation of cross-linked human hemoglobin with H2O2 does not occur in the presence of ascorbic acid, N-acetyl, thiourea, aniline or catalase. Nonheme proteins such as e-lactoglobulin do not show this cross-linking behavior alone upon exposure to H2O2 but do so in the presence of heme proteins including human hemoglobin or sperm whale myoglobin. If the heme proteins are treated with H2O2 in the presence of benzo[a]pyrene, the latter forms covalent adducts with the protein to a small extent. Under the standard conditions employed, on the order of 1 adduct is formed per 100 human hemoglobin polypeptide chains. Other oxidizable small molecules such as 1βestradiol are also bound to the heme proteins under these conditions. Using glucose oxidase and glucose to generate H2O2 in the presence of the heme proteins produces cross-linking and adduct formation. Free hemin has been found to catalyze the cross-linking and benzo[a]pyrene adduct formation of nonheme proteins in the presence of H2O2. This work emphasizes the biological need for peroxidases and catalases to protect oxygen carrying electron transport heme proteins and possibly non-heme proteins from interaction with peroxides of exogenous or endogenous origin.

108 XENOBIOTIC-INDUCED HYPOFUNCTIONAL PSEUDO-HYPERPLASTIC RENAL ENDOPOLASMIC RETICULUM IN RAT LIVER. C. Difonzo, R.A. Martin, G. Feuer, J.M. Sturgess and F.A. de la Iglesia, Warner-Lambert/Parke-Davis Pharm. Res. Sheridan Park, Miss., Ont. Canada, and Ann Arbor, MI; Dept. of Clin. Biochemistry, Univ. of Toronto, Toronto, Canada

To explore the hypothesis that hyper trophy of smooth endoplasmic reticulum (HSER) is a primary event in the pathogenesis of hepatic cholestasis, concomitant administration of cobalt chloride (CoCl2) and phenobarbital (PB) with or without lithocholic acid (LCA) has been studied in rats. Liver microsomes were assayed and quantitative microscopy was used to determine surface and enzyme loads of endoplasmic reticulum (ER) membranes. Hepatotoxicity was observed with all CoCl2, Pb compensated CoCl2 dependent hepatotoxicity; CoCl2 + Pb with or without LCA, increased microsomal protein and phospholipid, and decreased glucose-6-phosphatase (G6P) activity with no change in cytochrome P-450. Aminopyrine-N-demethylase (APDM) activity was decreased by CoCl2 + PB with LCA, but not by CoCl2 + PB. The total ER surface was unchanged by drug PB with and without LCA, but RER values were significantly increased and SER marginally decreased. Concurrent CoCl2 + Pb with or without LCA increased the ratios of microsomal protein and phospholipid per ER membrane area. G6P-ase activity/RER ratio was reduced by the combined treatments, but there were no changes in cytochrome P-450 or APDM activity/SER ratios. These morphofunctional correlations indicated that CoCl2 + Pb with or without LCA, did not induce HSER but caused functional impairment and resulted in an hypo-functional pseudo-hyperplastic RER.

109 METABOLISM STUDIES ON WR-158,122, AN ANTIMALARIAL DRUG, IN BILE DUCT-CANNULATED RATS. C. C. Smith, M.W. Tabor, and G.J. Wolfe, Dept. of Env. Hlth. Univ. of Cincinnati Med. Ctr., Cincinnati, OH.

The purpose of this study was to investigate the metabolic fate of the antifolate antimalarial compound WR-158,122, an amino-(naphthylsulfonyl)quinazoline, in bile duct-cannulated and unoperated control (intact) rats and rhesus monkeys. Previously we described the extraction of highly polar urinary metabolites of WR-158,122 from rodent and simian urine. Using a previously described method for preparing milled feces powders
we have developed a sequential extraction procedure for fractionating the 14C-Wr-158,122 metabolites in the feces of rats and monkeys given single oral doses of the compound. For the solvent sequence of benzene (Bz), ethyl acetate (EtAc), acetonitrile (ACN) and tetrahydrofuran (THF) we found that EtAc and Bz extracted > 90% of the 14C, EtAc accounting for the larger portion. When this extraction procedure was applied to feces from intact rats considerably more 14C appeared in the ACN and THF extracts than was found with the feces from bile duct-cannulated rats. This finding was confirmed using a silica gel TLC procedure. Next we developed an HPLC procedure for separating Wr-158,122 and its metabolites on Porasil using a linear gradient from 100% methylene chloride to 100% methanol. The HPLC results showed: 1) the EtAc extracts contained the unmetabolized parent compound for both animal groups; 2) the Bz extracts contained some parent compound for both groups, and relatively nonpolar metabolites in greater amounts in cannulated than in intact rats; 3) the ACN and THF extracts contained polar metabolites from intact rats and virtually no metabolites from the cannulated rats. (Supported in part by Army Contract DAMD 17-79-C-9196).

110 DISPOSITION OF TOPICAL AND ORALLY ADMINISTERED INDOMETHACIN IN PATIENTS UNDERGOING CATARACT EXTRACTION SURGERY. M.S. Bernstein, D.R. Sanders and M.A. Evans, Interdisciplinary Toxicology Program, Depts. of Pharmacology and Ophthalmology, University of Illinois Medical Ctr., Chicago, IL 60680.

Cystoid macular edema (CME), a postoperative complication of cataract surgery, is hypothesized to result from increased prostaglandin levels in the aqueous humour due to surgical trauma. CME is associated with ocular vascular incompetence which sometimes causes permanent macular damage. This study was designed to evaluate the effects and disposition of indomethacin (I) in the reduction of CME based on its inhibition of prostaglandin synthesis. Topical (I) was administered onto the cornea as drops of a 1% ophthalmic suspension during the 24 hours preceding surgery. Patients given the drug orally, received 25 mg capsules within the preoperative period. Topical application of (I) to the eye was shown to reduce the incidence of CME without clinical evidence of toxicity, as assessed by fluorescein angiography at 4 months postoperatively. Aqueous humour and plasma samples were taken from patients at the time of surgery for HPLC-fluorescence analysis of (I). Patients receiving oral (I) in the 24 H preoperative period, were found to have therapeutic amounts of drug in the plasma but no detectable levels in aqueous humour. Patients given (I) topically were observed to have levels ranging from 77 to 666 ng/ml in aqueous humour but no detectable levels in the plasma. These values of non-protein bound (I) in aqueous humour reflect what normally would be considered toxic levels of drug. This study demonstrates the critical role which disposition plays in relation to varying tissue sensitivities to indomethacin.


This laboratory has previously found evidence that prostatic androgen inactivation and egress involve cytochrome P450-mediated hydroxylation reactions. We here give the first report of a partial analysis of the C1903-metabolites produced by organ culture incubation of rat ventral prostate (RVP) with radioisotope-labeled testosterone. In two separate experiments, RVP explants (22 and 14 mg) placed on lens paper covering stainless-steel grids were incubated for 21 hr in surface contact with serum-free Trowell's 78 medium containing 1.7 µM 4-14C-testosterone. Metabolic disposition was determined autoradiographically after TLC. Of the total steroids recovered from the organ-culture media, testosterone comprised 39% and 48%, total C1903-metabolites 21% and 28% including 15% and 12% 5α-dihydrotestosterone, and C1903-steroids in four chromatogram zones 30% and 24%, respectively. The 2 most polar C1903-fractions were resolved by 2-dimensional TLC and HPLC. 5α-Androstan-3β,17β-triol and 3β,6α-dihydroxy-5α-androstan-17-one were identified as the major components of these fractions by reverse-isotope-dilution analysis; together, they accounted for 13% and 8% of the 2 radiosteroid patterns. The 38-hydroxy-5α-androstan configuration of the identified C1903-metabolites supports our previously-suggested pathway of prostatic testosterone metabolism which effects activation by 5α-reductase and inactivation and egress by the coupled 5α-3-oxosteroid reductase/5α-38-hydroxysteroid hydroxylase reactions.


The pharmacokinetic fate of allyl chloride (AC) when administered by three routes appeared to be dose-dependent and route-dependent. Following a single oral dose of 100 mg 14C-AC/kg body weight, the majority of radioactivity was excreted via the urine (polar metabolites) and expired air (either as CO2 or parent compound). A one-compartment bimodal absorption model adequately described the data for parent compound blood levels following oral administration, while a two-compartment model was used to model the data from intravenous dosing and inhalation exposures. The clearance half-lives of AC from the blood following inhalation exposure (at >100 ppm) increased with increasing exposure. At 10 and 100 ppm, AC was cleared quickly from the blood (half-lives <30 minutes). Immediately following a single six hour exposure of rats to AC, liver, kidney and lung non-protein sulphydryl (NPSH) were markedly depleted at 2000 or 1000 ppm, slightly depleted at 100 ppm, and not significantly different from control at 10 ppm. Rats exposed to 1000 or 2000 ppm of AC vapor for six hours showed treatment-related kidney cytotoxicity when sacrificed 48 hours post-exposure. No light microscopic changes indica-
The environmental and physiological disposition of toxaphene has only recently been investigated and is poorly understood. The present investigation was undertaken to better understand the disposition of toxaphene in the lactating rat and the transfer to the young.

Lactating Sprague-Dawley rats were orally dosed with approximately 10x10^9 dpm of 14C-toxaphene in olive oil. The dams and young were housed in glass metabolism cages for three days and their urine and feces collected daily. The three-day total urinary excretion of activity was 23.9% (S.D. 4.8%) of the administered dose and 40.8% (S.D. 3.7%) was excreted in the feces. Tissue residues in the dams were highest in the fat (23 ppm), kidney (1.6 ppm), uterus (5.0 ppm), adrenals (1.7 ppm), and liver (1.0 ppm). Comparable tissue levels in nursing pups (excluding fat and uterus) ranged from 0.5 - 0.9 ppm. The disposition of 14C-toxaphene in lactating rats follows that in pregnant or virgin animals but tissue residues in the lactating young were much higher than in fetuses.
4-nitroanisole, was dissolved in the blood and a 0.4-ml pulse dose was infused into the liver via a Teflon valve. Aliquots of the eluate fractions were analyzed for the parent compound and the concentration was plotted against the elution volume. Virtual absence of the compound at the void volume and subsequent appearance of a peak gave evidence of chromatographic behavior of these xenobiotics through the liver lobule in vivo. Factors affecting parameters of migration and implications of this phenomenon in assessing health hazards of xenobiotics were investigated.

RESPONSE TO ETHANOL CONTAINING LIQUID DIETS IN THE FISCHER-344 (F-344) AND SPRAGUE-DAWLEY (SD) RAT. N. Missbeck, D. Kim, I. Barbeau, T. C. Campbell and D. A. Roe, Div. of Nutr. Sci., Cornell University, Ithaca, NY.

A study was designed to compare the effects of strain and diet on the response of the rat to ethanol-containing liquid diets. Six week old male F-344 or SD rats were fed either a low fat (LF) diet (8% of kcals) or high fat (HF) diet (35% of kcals) containing ethanol (35% of kcals). A control group for each strain and diet was pair-fed a diet with dextrin-maltose replacing the ethanol, and an additional group was offered ad libitum the non-ethanol diets. Animals were sacrificed after 3 wks. Although food intake was similar for both diets, SD rats drank more per day (75 ml/day) than F-344 rats (60 ml/day). Weight gain among pair-fed animals was not significantly different. In SD rats liver triglyceride (TG) content was not significantly altered by ethanol for the LF animals, but was increased for the HF animals. Pair-fed SD control animals had significantly lower liver TG levels than did ad lib SD control rats (9.4 mg/g vs 25.4 mg/g liver). F-344 rats showed no increase in liver TG on ethanol diets. Aniline hydroxylase activity was increased and ethylmorphine-N-demethylase activity remained unaffected by ethanol feeding. Both cytochrome P-450 and cytochrome b$_2$ levels were higher in ethanol animals. SD animals tolerated the ethanol better than F-344 rats, but were more sensitive to the fat content of the diet. In conclusion, the effects of ethanol on the rat is strain and diet dependent. (Supported by USPHS PO1 CA 26755 and NIH-AM07158.)

EFFECT OF METABOLIC INDUCTION ON ACUTE TOXICITY OF n-HEXANE IN MICE. Raje R. R., Arnold & Marie Schwartz College of Pharmacy & Health Sciences, Long Island Univeristy, Bklyn, NY.

n-Hexane is widely used industrial solvent. Its volatility and well-documented neurotoxicity make it an occupational hazard. It has been reported that the neurotoxicity is attributed to n-hexane itself as well as its toxic metabolites. The purpose of this study was to determine the effect of metabolic induction on acute toxicity of n-hexane in female mice. Groups of 48 S/W mice each were pretreated daily for 3 days with toxaphene 50 mg/kg i.p., piperonyl butoxide 5 mg/kg i.p. and cigarette smoke inhalation for 1 minute respectively. A fourth group of 48 mice was an untreated control and a fifth one was pretreated with vegetable oil i.p. daily for 3 days to serve as oil-treated control. On the fourth day n-hexane was injected i.p., using 8 mice per dose, in six graded doses for each group. The 48 hour LD$_{50}$ values were significantly lower for toxaphene and cigarette smoke pretreated animals and higher for piperonyl butoxide pretreated animals than the controls.


Groups of male and female rats were administered butyl acrylate in the drinking water at approximately 0, 12, 73 or 84 mg/kg/day for males and 0, 15, 91 or 111 mg/kg/day for females. A satellite group of male and female rats was given 150 mg butyl acrylate/kg/day via gavage for 13 weeks. In a separate study, rats of both sexes were given drinking water solutions of methyl acrylate at targeted dose levels of 0, 1, 5 or 20 mg/kg/day.

Water intake was slightly decreased to a similar degree at all concentrations of butyl acrylate in both sexes, probably the result of unpalatability. Male rats given the highest dose level had slightly decreased body-weight gain. Rats given 150 mg/kg/day via gavage had statistically significantly increased relative liver weight ratios. No morphological changes were seen upon light microscopic examination of tissues by either route. Thus, only minimal indications of toxicity were seen in this study for dose levels of 84-111 mg/kg/day in the drinking water or 150 mg/kg/day via gavage.

The 20 mg/kg/day dose level of methyl acrylate produced decreased body-weight gain and water consumption in both sexes. Females at this dose level also had increases in urinary specific gravity and relative kidney weight ratio. Both sexes given the highest dose had an increased incidence of renal tubular dilatation and eosinophilic cast formation. Based on the data generated, the no-observable-effect level (NOEL) of methyl acrylate for both sexes in this study was determined to be 5 mg/kg/day.


The acute toxicity of acrylonitrile (ACN), as measured by lethality or decreased of tissue glutathione (non-protein sulfhydryl, NPSH) concentration was measured in male or female Charles River SD rats at 6-7 weeks of age. When dosed during the day, the acute oral LD$_{50}$ in Purina fed rats was: 124 mg/kg (male), 121 mg/kg (female). When inhalation exposure was done during the day, the acute 4 hr inhalation LD$_{50}$ in Purina chow fed rats was: ca. 300 ppm (male), >615 ppm (female). PCB enhanced lethality in females at 600 ppm. A lipotrope deficient, high
fat diet resulted in lower oral LD50 in both males and females. Inhalation values were lowered in females. Males were affected to a lesser extent. As measured by hexobarbital sleeping time, the synthetic diet increased mixed function oxidation activity. In rats fed Purina chow, NPSH concentration was higher in females than males (0.59 ± 0.09 vs. 0.31 ± 0.06 mg NPSH/100 g body weight, respectively). The high fat diet, irrespective of lipotrope deficiency, increased these values in brain and liver. Values in females were still elevated above the concentration in males. 14C-ACN binding after oral 14C-ACN minimized these effects (males not studied), while quantitatively different due to diet, did not differ qualitatively. Whole blood had significant amounts of bound 14C activity as well as the highest specific activity. Liver had the highest total activity as a % of oral dose. (Supported by NIEHS ES-01871, ES-00002, and ES-00260. RJJ is the recipient of an NIEHS RCDA. SS is the recipient if an NIAMD RCDA).

121 ACUTE AND SUBCHRONIC ORAL TOXICOLOGIC STUDIES OF HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) IN RATS AND MICE. L.C.K. Wong, J.M. Cholakis, C.B. Hong, H.V. Ellis, III, and J.C. Dacre, Pharmacology/Toxicology Department, Midwest Research Institute, Kansas City, MO, and U.S. Army Medical Bioengineering R&D Laboratory, Ft. Detrick, Frederick, MD.

Despite widespread military use of the high explosive RDX, only limited toxicological data are available. Some results of studies in progress with Fischer 344 rats and B6C3Fl hybrid mice are reported here. The military grade RDX used had the following composition: 2.2 ± 0.1% water, 88.6 ± 0.9% RDX, ca. 9% HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine). The oral LD50 values (95% confidence limits) in mg/kg were: 119 (110-128) in male rats, 119 (108-129) in female rats, 97 (82-116) in male mice, and 59 (25-139) in female mice. Ninety-day subchronic feeding studies were carried out as follows: six groups of both rats and mice (10 animals/sex/group) were fed RDX in the diet at levels of 0, 10, 14, 20, 28, and 40 mg/kg/day. A supplemental study with mice (12/sex/group) had four groups of 0, 40, 60, and 80 mg/kg/day for 2 weeks followed by 0, 320, 160 and 80 mg/kg/day for the remaining 11 weeks. In rats, the 40 mg/kg dose was toxic (decreased mean body weights, and food consumption in male rats only) while other specific toxic effects (e.g. decreased hematocrit and SGPT) were small and inconsistent. The 28 mg/kg dose produced no apparent toxic effects. In mice, the 320 mg/kg dose produced unscheduled deaths, hyperactivity, increased kidney weight with mild tubular nephrosis (males), and increased liver weight accompanied by portal hepatocellular vacuolization (males) and microgranulomas (females). No toxicologically important effects were seen in mice fed the lower doses of RDX. (Supported by the U.S. Army Medical R&D Command under Contract 17-78-C-8027.)

122 COMPARATIVE TOXICITY OF TNT IN RATS, DOGS, AND MICE. B.S. Levine, E.M. Furedi, D.E. Gordon, J.M. Burns, J.H. Rust, and P.M. Lish, Life Sciences Division, IIT Research Institute, Chicago, IL and J.J. Barkley, U.S. Army Medical Bioengineering R&D Laboratory, Fort Detrick, Frederick, MD.

The toxic effects of 2,4,6-trinitrotoluene (TNT) were examined in rats, dogs, and mice following subchronic dosing. Fischer 344 rats and B6D2Fl mice received TNT in the diet for 13 and 6 weeks, respectively. Beagle dogs were given daily doses of TNT in gelatin capsules for 26 weeks. Doses (mg/kg/day) ranged from 5-300 for rats, 0.5-32 for dogs, and 1-2000 for mice. Although experimental designs were different (in part a function of their purpose, e.g. rodent studies were conducted to select doses for chronic studies), the data allowed for qualitative interspecies comparisons. In general, sensitivity to TNT toxicity increased as surface area/body weight decreased, i.e. dogs were most sensitive followed by rats then mice. Major toxic effects observed for all 3 species included ataxia, decreased food intake with subsequent reductions in body weight gain, anemia (reduced hematocrit, hemoglobin, and RBCs), methemoglobinemia, hypercholesterolemia, splenomegaly with congestion and hemosiderosis, hepatomegaly with hepatocellular hypertrophy and Kupffer cell hemosiderosis, and testicular atrophy with degeneration of germinal epithelium. Compensatory responses to the anemic state included reticulocytosis, macrocytosis, and an increase in nucleated RBCs. Toxic manifestations limited to 2 species included increased kidney weights with hemoglobin-like casts, splenic extramedullary hematopoiesis, and neural tissue vacuolation. Additional toxic effects were species-specific, and reinforced the determination of major target organs based on results in at least 2 of the species studied. (Supported by the U.S. Army Medical Bioengineering R&D Laboratory under Contract DAMD17-79-C-9120.)

123 MUTAGENIC AND REPRODUCTIVE STUDIES OF HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) IN RATS AND RABBITS. J.L. Minor, R.D. Short, Jr., D.L. Van Goethem, L.C.K. Wong, and J.C. Dacre, Pharmacology/Toxicology Dept., Midwest Research Institute, Kansas City, MO, and U.S. Army Medical Bioengineering R&D Laboratory, Fort Detrick, Frederick, MD.

Studies were conducted with RDX to evaluate the mutagenic effects (Ames Salmonella/microsome test, dominant lethal assay) and reproductive effects (teratology, two-generation reproduction) in Charles River CD rats and New Zealand white rabbits. Mutagenic studies. RDX produced numbers of revertants similar to those in vehicle-treated controls at doses up to 1 mg/plate in all five strains of S. typhimurium, both without and with activation with the S-9 fraction (Aroclor 1254). Groups of male rats from the F0 reproduction study were fed RDX diets containing 0, 5, 16, or 50 mg/kg/day for 13 weeks and then mated with virgin females. There was no evidence of either a preimplantation loss of blastocysts or a post-implantation loss of embryos. RDX therefore is not mutagenic in the Ames and dominant lethal tests. Reproductive studies. Animals were given RDX suspensions by gavage at dose levels of 0, 0.2, 2.0, and 20 mg/kg/day; rats on days 6 to 19 and rabbits on days 7 to 29 of gestation. RDX was not teratogenic to rats or rabbits although maternal toxicity (neurotoxicity), lethality and embryotoxicity were seen in the high dose rats.
TOXICOLOGIC ASSESSMENT OF ISOPROPYL METHYLPHOSPHONIC ACID. F.J. Mecler and J.C. Dacre, Dept. of Toxicology, Litton Bionetics, Inc., Kensington, MD, and U.S. Army Medical Bioengineering Research & Development Laboratory, Fort Detrick, Frederick, MD.

Isopropyl methylphosphonic acid (IMPA) is the ultimate mammalian metabolite of diisopropyl methylphosphonate (DIMP). DIMP is a decomposition product of GB, a U.S. Army toxic agent. DIMP and IMPA have been found in the groundwater of the Rocky Mountain Arsenal. Since there are no published toxicological data on IMPA, the following studies were made: Acute oral LD50 in rats and mice; skin sensitization in guinea pigs; Ames mutagen assay, and subchronic toxicity over a period of 90 days in rats. Oral LD50 values (95% confidence limits) in mg/kg for sodium IMPA were: 7650 (6560-8920) in male rats, 6070 (4760-7740) in female rats, 5620 (4530-6990) in male mice, and 6590 (5140-8360) in female mice. Application of sodium IMPA to the intact and abraded skin of rabbits at doses up to 2.0 g/kg produced no signs of systemic toxicity but did produce mild skin irritation; rabbit eyes similarly treated produced no signs of irritation. Sodium IMPA did not induce dermal sensitization in the guinea pig nor did it exert a mutagenic effect in any of the five strains of Salmonella typhimurium in the Ames assay when tested with and without S9 rat liver microsomes. A three-generation reproduction study was conducted with ATMP at dietary concentrations of 300, 1000, and 3000 ppm. Each F0 group contained 12 male and 24 female Long-Evans rats. No adverse effects were observed on fetal, pup, or adult survival, or on parental and pup body weights. No adverse effects were noted in mating or fertility parameters measured for any test group during the study. Histopathologic evaluation of selected tissues from F3b pups indicated no treatment-related effects.

THE COMPARATIVE TOXICITIES AND CARCINOGENICITIES OF BENZIDINE AND DIRECT BLUE 6 IN RATS. J.H. Mennear and B.N. Gupta, National Toxicology Program, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709.

Direct Blue 6, a bisazoibiphenyl dye known to be metabolized to benzidine (B) produces hepatocellular carcinoma when fed to rats at dietary concentrations of 1500 and 3000 ppm. The rapid onset of carcinoma (4 weeks) suggested that the effect might be mediated through mechanisms other than simple metabolism to B. The objective of the present study was to compare the potencies of the dye and B with respect to general toxicity and carcinogenicity. Groups of 20 female F344 rats were exposed to drinking water containing either B-2HCl (100-400 ppm) or the dye (500-2000 ppm) for up to 90 days. The concentrations of B were selected to be equivalent to B exposure if dye treated rats completely metabolized the chemical. Based on water consumption, the average daily doses of B were 10, 21, 28 and 31 mg/kg while the B equivalents of the dye consumptions were 13, 22, 30 and 37 mg/kg/day. All rats exposed to the two highest doses of B died within 9 weeks while only 6 rats exposed to the high dye concentration died during the study. Among rats surviving to terminal sacrifice, both chemicals produced dose-related increases in serum gamma glutamyl transpeptidase with the magnitude of effect produced by the 21 mg/kg/day dose of B being similar to that produced by the highest dose of the dye. Additional dose-related effects produced by both chemicals included: thymic involution, increased liver weights, and grossly observable hepatic nodules. Hepatocellular carcinoma was diagnosed in some rats that died after receiving 28 days of B for up to 9 weeks. The results suggest that Direct Blue 6 is not more toxic than an equivalent dose of B.

In 3 trials, single or multiple doses of citrinin dissolved in 0.5% NaOH and adjusted to neutral pH with HCl were administered to rabbits by the oral or intraperitoneal route. The 72-hour LD50 was 50 mg/kg body weight by intraperitoneal administration and 134 mg/kg by the oral route. The primary clinical sign was fluid diarrhea commencing 8 hours after oral administration. Pathologic alterations were confined primarily to the kidney and consisted of necrosis of proximal convoluted tubules and straight segments. The earliest histopathologic change was vacuolation of tubular epithelial cells and was seen as early as 8 hours post oral administration. Seven days after citrinin administration, regeneration of tubular epithelium with slight tubular necrosis were observed. Rabbits given repeated sublethal doses of citrinin had mild tubular degeneration and necrosis and tubular regeneration.

129 THE FATE OF INHALED METHYL CHLOROFORM (1,1,1-TRICHLOROETHANE) FOLLOWING SINGLE OR REPEATED EXPOSURE IN RATS AND MICE. Schumann, A. H., Fox, T. R., and Watanabe, P. G., Toxicology Research Lab., Dow Chemical USA, Midland, MI 48640

Male F-344 rats and B6C3Fl mice were observed to metabolize inhaled 1,1,2-trichloroethane (TRI) (600 ppm/6 hr) to a greater extent (262%) than male Osborne-Mendel rats. Mice were also observed to metabolize more (332%) inhaled TRI to a hepatic macromolecular binding metabolite in vivo than rats. Oral administration of TRI resulted in treatment-related hepatocellular cytotoxicity in repeated dosing trials in the mouse. Hepatic effects observed in mice treated with 2400 mg/kg/day TRI for 3 days were primarily centrilobular swelling with focal necrosis and increased DNA synthesis (220% of control). Treatment of mice with TRI for a 3 week period (5 days/week) resulted in a dose-related increase in hepatocellular swelling with giant and mineralized cells present in 2400 mg/kg/day dosed animals. Contrasting the mouse data, rats appeared to be less sensitive to TRI, displaying enhanced hepatic DNA synthesis levels (175% of control). An estimate of the extent of TRI interaction with hepatic DNA in vivo was also determined. Only a very low level of TRI-DNA interaction was observed in mice administered a carcinogenic dose level of 1200 mg/kg TRI (max. est. = 0.62 ± 0.43 alkylations/10^6 deoxynucleotides). When coupled with the weak or negative responses of pure TRI in vitro mutagenesis assays, these data indicate a relative lack of genotoxic potential. These data in toto suggest an epigenetic mechanism of tumor formation in the B6C3Fl mouse. However, a tumor-specific response to TRI exposure in these animals would only be evident upon chronic administration of high, cytotoxic dose levels of TRI.
Previous studies from this laboratory have shown that pretreatment with chlordecone (CD) results in potentiated hepatotoxicity and lethality of CCl₄ in rats. The objective of these studies was to investigate whether pretreatment with CD affected the hepatotoxicity of two agents structurally and mechanistically dissimilar to CCl₄. Male Sprague-Dawley rats (300-400 g) were exposed to 0 or 10 ppm CD, mirex (M), or 225 ppm phenobarbital (PB) in the diet for 15 days. On day 15 the animals received ip injection of trichloroethylene (TCE; 5 or 10 mmole/kg) or bromobenzene (BB; 1, 3 or 5 mmole/kg) in corn oil vehicle. Controls received the vehicle alone. Twenty-four hr later, animals were surgically prepared under pentobarbital anesthesia for hepatobiliary function tests using phenolphthalein glucuronide (PG). Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaline transaminase (SGOT) and isocitric dehydrogenase (ICD) were determined from blood samples. Liver samples were processed for histopathology. Hepatobiliary function tests, serum enzymes and histopathology results indicate that CD and M did not potentiate the hepatotoxicity of TCE. BB hepatotoxicity was mildly potentiated by CD and M treatments. PB treatment caused much greater potentiation of BB hepatotoxicity and the interaction was lethal at 3 and 5 mmole/kg doses. These results indicate that the capacity of CD to markedly potentiate the hepatotoxicity and lethality of CCl₄ is specific to halomethanes and that marked potentiation by CD is not a generalized phenomenon affecting structurally and mechanistically divergent hepatotoxins. (Supported by ES 01369.)

It has been demonstrated that dietary pretreatment (10 ppm) with chlordecone (CD) for 15 days markedly enhances the hepatotoxicity and lethality of CCl₄. The objectives of these experiments were: to determine if potentiation of CCl₄ hepatotoxicity by CD could be demonstrated by administering a single oral dose of CD equivalent to that previously used in the 15 day dietary protocol; and to define the time course of CD-induced sensitivity to CCl₄. Groups of 4 male Sprague-Dawley rats (250-350 g) were given a single oral dose of CD (10 mg/kg) in corn oil. CCl₄ (0.1 ml/kg) was given ip at 6, 12, 18, 24, 36, 48 and 72 hr after CD. Control animals received corn oil (1 ml/kg) alone in place of the CD and CCl₄ treatments. Rats were killed 24 hr later, liver sections were fixed in buffered formalin and stained with H&E-PAS. Blood samples were taken and three serum enzymes (SGPT, SGOT, and ICD) were measured. A rapid rise in all three enzymes occurred starting at 6 hr after exposure to CD, with a plateau at approximately 24 hr. Histopathological assessment of liver damage parallels the rise in serum enzymes. These results indicate that the potentiation of CCl₄ hepatotoxicity by CD can be demonstrated after a single oral dose of CD equivalent to that previously used in the 15 day dietary protocol. Maximal potentiation of CCl₄ hepatotoxicity is observed 24 hr after CD. These results are consistent with the CD induction of the CCl₄ bioactivation system. (Supported by ES-01369 and ES-07045.)

The metabolism of trichloroethylene (TCE) was studied using hepatocytes isolated by collagenase perfusion of livers from Osborne-Mendel rats. Previous work from our laboratory using microsomal and reconstituted cytochrome P-450-containing mixed function oxidase systems indicated that an oxygenated TCE-cytochrome P-450 transition state and not the epoxide was probably an obligate intermediate in TCE metabolism. TCE-oxide was detected in microsomal but not in hepatocyte systems. TCE metabolites detected in hepatocytes included: chloral, trichloroethanol, trichloroethanol glucuronide, trichloroacetic acid, glyoxy-
lic acid and carbon dioxide. Chloral, trichloroethanol and trichloroethanol glucuronide were the major metabolites. Only chloral, glyoxylic acid and TCE-oxide were detected in rat liver microsomal systems. Hepatocyte viability was greater than 80% for at least six hours with 1 mg/kg TCE. There was no detectable decrease in glutathione levels during this time period. A glutathione conjugate was tentatively identified at very low levels. Kinetic studies in hepatocytes support the proposed scheme that TCE is metabolized by the mixed function oxidase systems to chloral which is subsequently metabolized by the cytosolic dehydrogenases to trichloroethanol and trichloroacetic acid. A time lag exists between chloral and trichloroethanol formation and between trichloroethanol and formation of its glucuronide in hepatocytes. (Supported in part by USPHS Grants ES02205 and GM 07628).

135 RELATIONSHIP BETWEEN TRICHLOROETHYLENE METABOLISM AND ITS HEPATOTOXICITY. J. A. Buben and E. J. O'Flaherty, Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267
SPONSOR: P. B. Hammond.

This study was undertaken to establish the relationship between the metabolism of trichloroethylene (TCl) and its hepatotoxicity when high doses of TCI are administered. Male Swiss-Coax mice were treated by gavage with 100, 200, 400, 800, 1600, or 2400 mg/kg TCI per day for 6 weeks. Urine was collected at various times throughout the dosing period and the urinary metabolites, trichloroethanol and trichloroacetic acid, were quantitated by gas chromatography and used as an index of the amount of TCl metabolized. The following effects on the liver were seen: the liver weight/body weight ratio increased with dose, and was 18%, 31% and 61% greater than control at the 400, 800 and 1600 mg/kg doses respectively. Glucose-6-phosphatase activity was decreased by less than 10% at the 800 mg/kg level, but by 30 and 36% at the two highest dose levels. Slight increases in SGPT were also seen at the highest two dose levels. The metabolism of TCI was found to be linear throughout the dose range examined, of the dose administered being accounted for by the urinary metabolites. Saturation of metabolism cannot account for the increased toxic effects seen above 800 mg/kg TCl. This work was supported by NIEHS Toxicology Training Grant ES 07073 and by funds from the Monsanto Company.

136 STEREOCHEMISTRY OF THE METABOLISM OF 1,2-DICHLOROETHANE TO ETHYLENE. J. Livesey, M. Anders, P. Langvardt, C. Putzig, and R. Reitz, Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455 and (2) Health and Environmental Sciences, Dow Chemical Co. USA, Midland, MI 48640.

Recent studies have demonstrated that 1,2-dibromoethane and 1,2-dichloroethane are metabolized to ethylene (Drug Metab. Dispos. 7 (1979)199). This biotransformation is catalyzed by hepatic cytosolic enzymes, requires glutathione (GSH) and is specific for vicinal dichloro- and dibromoalkanes. Two mechanisms have been proposed for this reaction: the first involves Sn2 conjugation with GSH to form S-(2-haloethyl)glutathione followed by a sulphydryl-promoted E2 elimination of halogen from the conjugate. Alternatively, GSH may act as a base and form ethylene and the sulfenyl halide of GSH by a direct E2 reaction. We have differentiated these mechanisms by using meso-1,2-dideutero-1,2-dichloroethane as a substrate. The substrate was incubated with GSH (20mM), sodium phosphate buffer (20mM, pH 7.4) and liver cytosol prepared from male Long-Evans rats. d5-Ethylene was analyzed for stereochemical configuration by FT-infrared spectroscopy. meso-1,2-Dideutero-1,2-dichloroethane was metabolized exclusively to (Z)-1,2-dideuteroethylene. Since E2 eliminations in non-ordered systems require an anti-periplanar orientation of leaving groups, this result suggests the involvement of a substitution-elimination sequence and is consistent with the intermediate formation of ethylene-S-glutathionyl-episulfonium ion. Furthermore, this result supports the concept that this episulfonium ion is a reactive intermediate involved in the mutagenic action of 1,2-dihaloethanes. In contrast, previous studies have shown that meso- and dl-2,3-dibromobutane undergo a direct E2 elimination reaction to yield (E)- and (Z)-2-butenes, respectively. (Supported by USPHS grants ES01082 & GM07397)

137 EFFECT OF HYPOXIA ON CCl4 HEPATOTOXICITY. N.W. Anders and E.S. Shen, Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Halothane undergoes reductive bioactivation, and hypoxia is known to enhance the hepatotoxicity of halothane. Since CCl4 undergoes reductive bioactivation, the effect of hypoxia on CCl4 hepatotoxicity was studied. Male rats were exposed for 2 hr to CCl4, and differing O2 concentrations in Leach-type chambers. Chamber CCl4 and O2 concentrations were monitored by gas chromatography and polargraphy, respectively. SGPT activities were measured 24 hr after exposure. Exposure of rats to 4852 ppm CCl4 and 100, 21, 12, and 6% O2 resulted in SGPT activities of 489, 420, 3768, and 1788 I.U./1, respectively. Exposure of rats to air and 0, 1243, 2523, 4842, and 7565 ppm CCl4 resulted in SGPT activities of 35, 32, 69, 420, and 2188 I.U./1, respectively; when 12% oxygen was used, the corresponding SGPT activities were 32, 665, 691, 3768, and 4200 I.U./1, respectively. Conjugated diene formation, measured immediately after exposure to CCl4, was not altered by hypoxia. Hepatic microsomal cytochrome P-450 concentrations were decreased immediately after exposure to CCl4, but were the same when rats were exposed to CCl4, in the presence of 12 and 21% O2. Hepatic chloroform concentrations were higher in animals exposed to CCl4 (5095 ppm) under normoxic than under hypoxic conditions, and hepatic CCl4 concentrations were the same in animals exposed to 12 or 21% O2 and 5095 ppm CCl4. The covalent binding of metabolites of CCl4 to microsomal lipids was higher under hypoxia than under normoxic conditions. These results show that hypoxia increases markedly the hepatotoxicity of CCl4, and that this increase in hepatotoxicity is accompanied by an increase in the covalent binding of CCl4 metabolites to hepatic microsomal lipids and proteins but not by an increase in lipid peroxidation or the destruction of cytochrome P-450. Supported by NIH Grant ES 00953.

We have recently reported (The Pharmacologist 23,113(1981)) the trapping of electrophilic chlorine or bromine with 2,6-dimethylnaphthalene (DMP) to form 4-halo-2,6-dimethylnaphthalene during microsomal metabolism of CCl4, CBrCl3 and CBr4. As the first step in mechanistic studies of this reaction, we have examined the origin of the trapped chlorine with CCl4 (99 atom %) and Na37Cl (92 atom %). Liver microsomes (2 mg protein/ml) were incubated in the presence of NADPH (1 mM), DMP (1 mM) and either CCl4 (natural abundance, 5 mM), CCl4 plus Na37Cl (100 mM), C35Cl4 (5 mM) or C37Cl4 plus Na37Cl. The incubation mixtures, after extraction with ether and careful concentration, were analyzed by capillary gas chromatography mass spectrometry in the selective ion mode (m/z 156 and 158). Analysis of the incubations with CCl4, with or without added Na37Cl, revealed the natural abundance ratio of chlorine 35 and 37 isotopes (3:1) in the trapped chlorine product. In contrast, trapped chlorine from incubations with C2Cl5, with and without added Na37Cl, showed better than 99% of the 35Cl isotope in the product. These studies show that CCl4 is the source of the electrophilic chlorine. They also indicate that the electrophilic chlorine does not result from oxidation of chloride ion through a chloroperoxidase mechanism nor does it rapidly exchange with chloride ion.

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139 ETHANOL POTENTIATION OF CCl4, HEPATOTOXICITY: A POSSIBLE ROLE OF ACETALDEHYDE DEHYDROGENASE (ALDH) INHIBITION. M. Kenel and A. Kulkarni, Toxicology Program, Sch. of Pub. Hlth, The University of Michigan, Ann Arbor, MI

Liver microsomes of adult male Sprague-Dawley rats were incubated at 37° in the presence of 1 mM NADPH and/or CCl4 (1 µl/ml protein). NAD(P) dependent microsomal ALDH (relative) specific activities were then estimated after reseeding with NADPH alone decreased NAD dependent ALDH activity by 46%, 54%, and 70% after 5, 10 and 20 min incubations, respectively. CCl4 plus NADPH caused an even greater loss as NAD dependent ALDH decreased by 83%, 89%, and 94% after 5, 10 and 20 min incubations. Inclusion of 0.2 mM EDTA, a blocker of lipid peroxidation, prevented loss of ALDH activity due to NADPH alone, but only partially in the presence of NADPH + CCl4. This suggests that covalent binding of an active intermediate is primarily responsible for decreased ALDH activity. At least equal depression of mitochondrial ALDH (I + II) was noted when microsomes and mitochondria were similarly incubated; however, no inhibition of mitochondrial ALDH was observed when cytosol was then added. This may be due to inhibition of lipid peroxidation. Microsomal and mitochondrial NAD(P) dependent ALDH in ethanol-treated (3 g/kg p.o.) rats remained unchanged.

The CCl4-treated (1 ml/kg i.p.) group exhibited a decrease in activity. Rats receiving ethanol 18 hr prior to CCl4 had an even greater decrease. NAD(P) dependent cytosolic ALDH activities were not changed. SGPT values for CCl4, ethanol, and ethanol + CCl4 were 443%, 147%, and 2063% of control values, respectively. Thus, CCl4 metabolism appears to inhibit microsomal and mitochondrial ALDH in vivo, this may lead to high levels of aldehydes which may, in part, contribute to the potentiation of CCl4 hepatotoxicity by ethanol.


Previously, authors reported that the Plantago asiatica semen exhibited significant liver-protective activity against the hepatic damage in mice produced by CCl4 intoxication. Aucubin, an iridoid glucoside compound, was isolated from the Plantago asiatica semen and its potential liver-protective activity was evaluated in mice intoxicated with CCl4.

1) Effect of liver microsomal enzyme system; the administration of aucubin (45mg/kg/day for 2 days) reduced markedly the duration of sleep induced by hexobarbital after CCl4 intoxication(0.2ml/mouse/day for 2 days) in comparison with those of CCl4 alone treated mice.
2) Serum transaminase activities; the administration of aucubin prevented the elevation of serum GOT and GPT activities in mice received CCl4.
3) Liver RNA and protein syntheses, the compound appeared to inhibit the incorporation rates of 3H uridine and L-(4,5(n)-3H) isoleucine into liver RNA and protein. The mode of RNA inhibition appeared to be additive with Actinomycin D.
4) Histological examination; liver in mice received aucubin after CCl4 intoxication appeared to be significantly improved in comparison with those of CCl4 alone treated group.

The results obtained from experimental criteria in the above indicated that the aucubin appeared to be one of biologically active ingredients responsible for liver-protective activity in plantago asiatica semen. (Supported by International Foundation Grant #318R, Sweden)

141 ASSESSMENT OF THE EMBRYOTOXIC AND TERATOGENIC POTENTIAL OF NONOXYNOL-9 IN RATS UPON VAGINAL ADMINISTRATION. H.S. Buttar, Bureau of Drug Research, Health Protection Branch, Ottawa, Canada.

The effects of nonoxynol-9 (N-9), the active ingredient of vaginal spermicides, were studied on the conceptus of Wistar rats. The day of finding sperm in the vagina was counted as day zero of pregnancy. A single dose (2.5 mg/100 g) of aqueous solution of N-9 was administered intravaginally (0.1 ml/100 g) only once to ether anesthetized rats on gestational days 1 to 10. Control rats were given an equal volume of distilled water. The vulval metallic clips, used to prevent leakage of the solution, were removed 24 h post-treatment. The uterine contents were examined on day 21 of gestation. No signs of maternal toxicity were observed in any of the N-9 treated dams. The incidences
of non-pregnancy and resorptions were high in dams treated on days 3, 4, 5 or 6 of pregnancy. The number of live fetuses was significantly reduced in dams dosed on days 4, 5, 8 and 9, whereas the average litter size of dams treated on gestational day 10 was similar to that of the controls. A significant reduction in fetal weight was observed in dams treated 5 days post-conception. N-9 did not cause any discernible visceral or skeletal malformations. The results suggest that single vaginal application of N-9 is embryolethal and fetocidal but non-teratogenic in the rat at a dose approximately ten times higher than that recommended for controlling conception in women.


TNF was administered as aqueous Methocel® suspensions at dosage volumes of 10 ml/kg orally via gavage to naturally-bred (day 0=plug) Crl:COBS® CD®(SD)BR rats. In pilot studies, 8 female rats per group were administered dosages of 0(vehicle), 150, 450, 1500 and 4500 mg/kg/day on days 6 through 19 of gestation. No deaths occurred, but brown urine was observed in rats administered all TNF dosages and black discoloration of the skin and fur was observed in rats administered dosages of 450 to 4500 mg/kg/day dosages. Maternal weight gain was inhibited by dosages of 150 through 4500 mg/kg/day and an increased incidence of early resorptions occurred at 1500 and 4500 mg/kg/day dosages. In the teratology study dosages of 0(vehicle), 60, 300 and 1500 mg/kg/day of TNF were administered on days 6 through 19 of gestation to 25 naturally-bred (day 0=plug) female rats per dosage group. No deaths occurred. Pregnancy occurred in 23, 20, 19 and 22 rats Caesarean-sectioned on day 20. Dosage-related inhibition of maternal weight gain and brown urine was observed in rats in all TNF-dosage groups. Black discoloration of the skin and fur occurred in rats administered 1500 mg/kg/day dosages of TNF. Fetal body weights were decreased in the high dosage group litters. No gross external malformations were observed. Based on these data, TNF does not appear to be a unique hazard to the rat conceptus at dosages which are maternally toxic.


Japanese quail from lines* selected for low (L) or high (H) response of plasma cholesterol to ACTH were compared to a random-bred (R) line for sensitivity to polybrominated biphenyls (PBBs) and induction of hepatic mixed function oxidases (MFOs).

ACTH had no effect on either plasma cholesterol or hepatic benzo(a)pyrene hydroxylase and hexabarbital hydroxylase activities in the L line. ACTH did cause the expected marked increase of plasma cholesterol in the H line, but did not alter the activities of hepatic MFOs. PBBs at 80 ppm in the diet reduced hatchability to 60% of the control value for the L line as compared to 80% of the control value for the H line. Thus, the L line appeared to be more sensitive to PBBs.

In a second experiment egg production, a reflection of the pituitary-gonadal axis, was reduced to 14% in the L line fed 80 ppm PBBs as compared to 38 and 49% in the H and R lines, respectively. Hatchability and subsequent growth of F1 chicks were less in the L and H lines as compared to the R line when parents were fed 80 ppm PBBs. Also MFO activities (benzo(a)pyrene hydroxylase and ammoprine N-demethylase) and cytochrome P450 (448) concentrations were induced by 80 ppm PBBs more in H and R parents than in those of the L line.

The data indicated that the L line of quail was more sensitive to PBB's toxicity based on reproductive parameters while induction of hepatic MFO activity was significantly less than in the H and R lines.

* USDA-SEA, Athens, GA: Drs. H. Siegel and H.L. Marks
THE REPRODUCTIVE TOXICITY OF CITRININ IN RATS.

Living things are more sensitive to the adverse influences of the environment during early developmental stages. Several fungal metabolites, produced in common food and feedstuffs, are teratogenic in experimental animals. Citrinin, a fungal metabolite produced by molds infecting wheat, rye, oats and mixed barley, is nephrotoxic. Teratogenicity and embryo- and fetotoxicity of citrinin were investigated using pregnant CD-1 Charles River rats. The compound was administered subcutaneously on days 3 and 6-15 of pregnancy in 5% NaHCO3. A reduction in weight gain and increased mortality of dams occurred with 35 mg/kg of citrinin. Treatment with citrinin during the period of major organogenesis had no detectable adverse prenatal effects externally and also no significant increase in resorptions of implanted embryos, but was associated with adverse effects on fetal growth and to some extent on survival. No gross, skeletal and visceral anomalies were observed. Although developmental defects were not produced as a result of treating the unborn rats with citrinin, it did exhibit fetotoxicity by decreasing average fetal body weight. Judging from the number of maternal deaths, 35 mg/kg of citrinin treatment on certain days of gestation probably approached the LD50. Distribution, excretion and metabolism of 14C-citrinin is under investigation in pregnant rats after subcutaneous administration of 35 mg/kg. The results of the study indicated that citrinin is not embryocidal or teratogenic but is fetotoxic when given to female CD-1 rats during pregnancy. (Supported by USPHS research grant ES 02191.)


The site of Ordam's antifertility effect in male rats was investigated. In part I, male rats received either 0, 12, or 60 mg/kg of Ordam for 5 days, then they were mated with 1 female per week for 10 weeks. There was a substantial reduction in male fertility during the third post-treatment week, indicating the late spermatid stage was sensitive to Ordam exposure. The no-effect level was 12 mg/kg. In part II, male rats were treated with either 0, 0.2, 4, 12, or 30 mg/kg of Ordam for 5 weeks, then they were mated with 2 females per week for 1 week. At terminal sacrifice, blood, sperm samples, and reproductive tissues were taken for evaluation. A comparison was made between dose, fertility, changes in plasma hormone levels (testosterone, FSH, LH, TSH, T3 and T4), sperm morphology, motility and viability and morphologic changes in the testes and epididymes. There were no measurable treatment-related changes in serum hormone concentrations which correlated with the reduction in male fertility. The dose-response relationship observed for male fertility could be correlated with changes in sperm viability, morphology, motility, and concentration. Electron micrographs indicate that the site of action may involve the sperm plasma membrane. Histological examination of the testes and epididymes revealed a slight increase in degenerating spermatids in small portions of a few tubules. A clear dose-response relationship was observed at 4, 12, and 30 mg/kg with 0.2 mg/kg being the no-effect dose level in this study. Protein RIA kits were obtained from NIAMDD, DR. A. F. Parlow.

TESTICULAR DAMAGE IN THE RABBIT RESULTING FROM SIMPLE CHEMICAL CUTANEOUS IRRITATION

Although the albino rabbit has long been regarded as the species of choice for studying the cutaneous irritation potential of substances, it has also been used to evaluate systemic toxicity from substances applied topically. Recent reports of testicular degeneration in rabbits treated topically with a wide variety of agents have been surprising. The purpose of this study was to determine whether testicular degeneration could have resulted simply from a response to cutaneous irritation. Ten adult male rabbits (2.2-2.7 kg) were exposed daily to 2 ml/kg of a 2.5% (w/w) solution of sodium hydroxide five days/week for four successive weeks. Ten sham control rabbits were run concurrently. Most of the treated rabbits showed marked skin irritation, reduced food consumption and decreased body weights during the study. No mortality or overt toxicity was observed. At necropsy, the testicular weights of the treated rabbits were significantly less (p < 0.05) than those of the controls. Histopathology revealed testicular degeneration in the treated animals characterized by the increased frequency of giant cell formation and the overall decrease in the number of seminiferous tubules displaying spermatogenesis. Therefore, simple cutaneous irritation can induce degenerative lesions in the testicles of adult rabbits. The basis for this response may be stress-related; however, these results do indicate a limited usefulness of the rabbit in evaluating the systemic toxicity of many chemicals.


Male and female Sprague-Dawley rats were exposed by inhalation to 0, 30, 100 or 300 ppm EGME vapor 6 hours/day, 5 days/week for 13 weeks. Following 13 weeks of exposure, each rat was mated with an unexposed rat of the opposite sex. Exposure to 300 ppm EGME resulted in complete infertility in male rats. No adverse reproductive effects were observed in males exposed to 30 or 100 ppm nor in females exposed up to and including 300 ppm EGME. There was no evidence
of a dominant lethal effect at 30 or 100 ppm; the absence of litter (infertility) precluded evaluation for dominant lethality at 300 ppm. In order to assess the reversibility of infertility, the males exposed to 300 ppm were rebred with unexposed virgin females at 13 and 19 weeks post-exposure. At both of these mating intervals 50% of the males previously exposed to 300 ppm were fertile, indicating partial recovery.

Based on these results it is concluded that exposure of rats to concentrations of EGME as high as 100 ppm for 13-weeks resulted in no adverse effects on reproduction or on dominant lethality.

149 IMPACT OF PRENATAL LEAD EXPOSURE ON BIOCHEMICAL MARKERS IN RAT LIVER AND HEART. K.L. Blackburn, R.J. Bull, and M.K. Smith, Health Effects Research Laboratory, USEPA, Cincinnati, OH.

The effects of prenatal lead exposure were evaluated in rat pups from dams treated with lead nitrate during gestation. Dams were dosed by gavage on days 6-17 of gestation with 50 or 500 mg/kg Pb(NO3)2. One-half of the litters were cross-fostered at birth onto control dams. Biochemical markers included cytochrome c oxidase activity in heart, liver and kidney and monoamine oxidase activity towards three substrates (tryptamine, phenylethylamine and 5-hydroxytryptamine) in heart, liver and kidney. Biochemical markers were followed from three to 90 days post-partum. In addition, dam weight gains during gestation, dam and pup blood leads, pup body and organ growth rates, litter size and sex ratio at birth and pup mortality were monitored. Pups were evaluated for EKG abnormalities and changes in frequency of norepinephrine induced arrhythmias.

In both dose groups a significant effect on sexual differentiation of liver enzyme patterns was established. Lead exposed male offspring exhibited enzyme profiles which were statistically indistinguishable from female controls. These effects occurred in the absence of effects on litter size, pup body weight at birth or subsequent growth rate, or increased mortality. In addition, these effects occurred in both cross-fostered and noncross-fostered groups suggesting that they resulted from prenatal interference with liver enzyme imprinting. Preliminary data indicate that the serum steroid levels of the pups were significantly disturbed.

150 ALTERATIONS OF THE MATERNAL BLOOD SUPPLY TO THE FETUS BY TOXIC MATERIALS. Bruce J. Kelman.
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Dynamic measurements were performed to evaluate the effects of nine diverse toxic materials on maternal blood flow to the placenta. The experimental system uses a perfusion technique based on an isolation of the fetal circulation of the placenta in situ and has been previously described. Materials were introduced into the maternal circulation by injection into a carotid artery and into the fetal circulation of the placenta by addition to the perfusing fluid. This system detects alterations of maternal blood flow to the placenta by detecting changes in the clearance of tritiated water from mother to fetus. In order to report the resulting data, maternal doses were expressed relative to the maternal plasma concentration measured at 30 minutes after dosing and doses administered from the fetal side were expressed as the concentration measured in the perfusing fluid. Maternally administered benzo(a)-pyrene (7.7 µM), cadmium (1.0 µM), methylmercury (200 nM), and inorganic mercury (40 nM) did not affect maternal blood flow to the placenta. Maternal administration of inorganic lead (20 nM) caused sporadic changes in maternal blood flow to the placenta and monomeric plutonium-239 (3 nM) reduced maternal blood flow to the placenta. Fetal administration (10 µM) did not affect maternal blood flow while ouabain (300 nM), cadmium (37 µM), and fluorocitric acid (300 µM) reduced the maternal blood supply to the fetus. Based on data obtained in this laboratory and others, it appears that toxic materials which disrupt the maternal supply of blood to the fetus tend to be toxic to the products of conception, but not necessarily teratogenic. (Supported by the U.S. Dept. of Energy Contracts DE-AC06-76RL0-1830 and E-60-1-GEN-242 and NICHD Grant HD00727).


In the adult rat β-ADR stimulation of cardiac growth parallels ODC activation, and ODC response to sympathetic nervous system (SNS) stimulation is used as an indicator of SNS control. To determine the effects of prenatal drug exposure on subsequent SNS development, it is necessary to define the normal postnatal alterations in basal ODC and responsiveness to direct β-ADR stimulation. ODC activity was measured in rat hearts on day 20 gestation (20DG) through day 22 postnatal (22RND) under saturating conditions of L-ornithine and pyridoxal 5-phosphate. Basal ODC activity fell from 4 U (nmol CO2/mg Prot-hr) at 20DG to less than 0.5 U at 18RND, remaining low until after 21RND. The β-ADR agonist isoprotrenol (IPR) at 10mg/kg s.c. consistently resulted in peak ODC stimulation at 4 hr postinjection in postnatal hearts; however, at 200G determinations were prevented by maternal mortality at 0.30mg/kg s.c. In dose-response studies IPR produced a maximal response at 10mg/kg s.c. in all postnatal ages (increase from control: 6PND = 1.2 U; 14PND = 1.3 U; and 21PND = 4.8U). With control ODC activity at 6PND>> 14 PND>> 21PND, these increases in the magnitude of IPR stimulation of ODC activity between 2-3 weeks after birth result in even larger increases in % change from control at older ages, indicating a greater sensitivity of the heart at these ages. Thus, this study defines the normal ontogenic patterns of ODC and responsiveness to direct β-ADR stimulation. It should now be possible to determine in prenatally exposed animals whether alterations in ODC response to SNS stimulation reflect changes in neural development, or in heart ODC sensitivity.
EVALUATION OF BEHAVIORAL TOXICITY IN NEONATES.
Robert Infurna & Bernard Weiss, Division of Toxicology & Environmental Health Sciences Center, University of Rochester School of Medicine & Dentistry, Rochester, N.Y. 14642, Sponsor: G.G. Berg

Animal models in Teratology employing sensitive measures of the functional integrity of the nervous system following perinatal exposure are required to unmask incipient behavioral toxicity in neonates. The task is a challenging one as the behavioral assessment of neural function among altricial mammalian neonates is restricted by their limited behavioral repertoire and underdeveloped sensory systems. We report the results of two behavioral paradigms employed as part of our protocol for the evaluation of the ontogenesis of sensorimotor function in animals. The initial paradigm evaluates a response that is both characteristic and fundamental to all mammalian species, Suckling Behavior. The diagnostic significance of suckling has been made apparent by the reports of suckling behavior anomalies among human infants and suckling rats exposed to ethanol, methanol and caffeine. The second paradigm studies the tendency of neonatal animals to orient and locomote to their nesting site when removed from it. The Homing behavior test has been shown to be sensitive to 1) the prenatal effects of methyl and ethyl alcohols, 2) the acute effects of food additives such as caffeine and tartrazine, 3) the psychopharmacological effects of amphetamine and, 4) the influence of circadian rhythms. These paradigms are relatively inexpensive to execute and many be used in Teratological studies with various mammalian species. Supported in part by grant MH-11752 from NIH, Grant ES-01247 from NIEHS, and Contract No. DE-AC02-76EV03490 with the U.S. Dept. of Energy.

THE PATHOLOGY OF SUBLETHAL EXPOSURE OF FATHEAD MINNOWS TO CADMIUM: A MODEL FOR AQUATIC TOXICITY ASSESSMENT. Stromberg, P. C. and Ferrante, J. G., Battelle Columbus Labs, 505 King Ave, Columbus, OH 43201. Sponsor: G. L. Fisher

Fathead minnows were exposed to an LC50 concentration of CdCl2 for 96 hours then removed to clear water for 21 days. Fish were removed at intervals from Day 1 to Day 21 and evaluated for histopathological lesions. Lesions consisted of epithelial necrosis in the skin, oral cavity, bronchial chamber, and gastrointestinal tract. There was a patchy lifting of the gill epithelium interpreted as cellular degeneration and branchial edema. Renal tubular necrosis was also noted. Epithelial lesions were most severe during the 96 hour exposure. Renal and gill lesions were observed up to 7 days after exposure was terminated. It is proposed that histopathological evaluation of LC50 fish is an inexpensive and more sensitive method of assessing damage due to exposure to toxic substances. In addition, this model allows assessment of reversibility and recovery. The protocol can be changed to include subchronic and chronic exposures. Microautoradiographic and radiotracr studies may contribute data on tissue localization, metabolism, and elimination of the substance.

154 AQUATIC TOXICITY AND BIODEGRADATION OF CAPROLACTAM
Loewengart, C., Allied Corporation, Morristown, N.J. 07960

Caprolactam, the monomer for the production of Nylon-6, is a major industrial chemical. The literature contains little information about the fate and effects of caprolactam in the aquatic environment. Areas lacking adequate data included toxicity to aquatic organisms, phytotoxicity, and biodegradation of caprolactam in surface waters.

Environmental tests that were incorporated into the program were: static acute toxicity with three fish and an invertebrate species, time-independent flow-through toxicity to a fish species, seed germination and seedling growth phytotoxicity tests with six terrestrial plants, growth inhibition tests with four aquatic plants, determination of the rate of biodegradation in natural waters measured both by disappearance of caprolactam and mineralization to CO2, and determination of the effects of temperature and substrate concentration on the biodegradation rate.

Based on the results of these tests, caprolactam is characterized as a chemical that has a low toxic potential to fish, invertebrates, plants and microbes tested and can be efficiently degraded by microbes in enriched surface waters. Primary degradation of caprolactam proceeded to an extent of more than 90% caprolactam disappearance within ten days. Under the same conditions, approximately 50% of the caprolactam underwent ultimate degradation to CO2. An induction period observed for ultimate degradation suggests a rapid first step followed by slower, rate limiting steps in the route to ultimate degradation.

It is concluded that low level release of caprolactam does not present an environmental hazard and the combination of physical, chemical, and biological properties would mitigate adverse environmental effects of spills.

155 TISSUE DISTRIBUTION AND BINDING OF HEXACHLOROCYCLOPENTADIENLE TO PROTEINS IN TROUT. P. Sinhaseni and R. Hartung, Dept. of Env. & Ind. Hlth, Sch. of Pub. Hlth, and G.W. Jourdian, Rackham Arthritis Res. Unit, The University of Michigan, Ann Arbor, Ml

Hexachlorocyclopentadienle (Hex) is relatively toxic to fish and other aquatic organisms. The high chemical reactivity of this compound suggests the possibility of its binding to biological components.

Rainbow trout (Salmo gairdneri), approx. 400 g, were exposed to 120 ppb 14C-Hex (specific activity 5.3 mCi/mg) under static conditions until moribund (approx. 4 hr). Of the 14C added, 23.4% was recovered in 80.1% of the total fish wet weight.

Ten organs were analyzed for 14C-activity. The highest concentration (dpm/mg wet weight) was found in liver (169) and gills (81). Muscle which comprised 59.5% of the total body weight contained the highest amount of 14C (41.9% of total recovered 14C-activity).

When muscle was treated with chloroform-methanol (2:1), 52.8% of 14C was found in denatured protein. 9.6% was found in the lipid enriched chloroform fraction and 26.1% was found in the water phase containing water soluble components.
156 THE EFFECT OF B-NAPHTHOFLAVONE (BNF) TREATMENT ON DOSE DETERMINATIONS FOR WATERBORNE 2,5,2',5'-TETRACHLOROBIPHENYL IN TWO SPECIES OF TROUT. M.J. Vodicnik, L.A. Lau, and J.J. Lech, Dept. of Pharmacology and Toxicology, Med. Co. of Wisconsin, Milwaukee, WI.

While inducers of the 3-methylcholanthrene (3-MC)-type have been shown to elevate the hepatic microsomal metabolism of substrates specific for cytochrome P-448 in fish, it has not been demonstrated whether this process is a result of de novo synthesis of hemoprotein(s) or an activation of microsome hydroxygenase activity. We therefore studied the effect of BNF on the in vivo incorporation of 35S-methionine into hepatic microsomal protein of rainbow trout. Animals (60-70 g) were injected IP with corn oil or 150 mg/kg BNF. They were each injected with 50 µCi 35S-methionine at 12 h and killed 36 h following exposure to the amino acid. Microsomal samples were taken for scintillation counting. Ethoxyresorufin-O-deethylase (EROD) was assessed and solubilized microsomes were electrophoresed. Gel tracks were sliced into 0.5 cm segments and counted. Duplicate gels were prepared for fluorography. The incorporation of 35S-methionine into protein was elevated in BNF-treated animals (22,732 ±3652 vs 42,643±5918 dpm/mg protein) and EROD activity resulted in the intensification of a band with a molecular weight of 57,000 and the 0.5 cm sections of the gel containing this band had 4-fold greater radioactivity than the corresponding region of gels from control animals. Fluorography of gels demonstrated the intensification of several proteins, whether this process is a result of de novo synthesis of hemoprotein(s) or an activation of microsome hydroxygenase activity.


In order to evaluate dose determinations of 14C-TCB made on transected brook trout (Salvelinus fontinalis) and rainbow trout (Salmo gairdneri) a mass-balance study was undertaken. The total weight of TCB removed by the gills from the respiratory water flow was calculated by determining mean respiratory volume (5-8 L/hr), mean TCB water concentration (0.1 µg/L), and mean TCB uptake efficiency of the gills (60-86%) over the 48-hr exposure period. The total calculated µg of TCB absorbed by brook trout (17.9) and rainbow trout (24.5) was therefore calculated using the actual body burden measurements of TCB for brook trout (17.4) and rainbow trout (25.6) the latter measurements also included excretory losses through the urine, feces, and across the gill surface. Approximately 1% of the total dose was excreted with 75% of that lost being in the urine. The agreement between the whole body burden measurements of TCB and the total calculated µg taken up was excellent in both species (within 10%). Species differences seen in total accumulated µg of TCB were due to a larger respiratory volume and to a higher gill uptake efficiency in the rainbows. The mass-balance measurements were converted to dose by dividing by the fish weight. Calculated and measured doses were 29.6 and 28.4 µg/kg/48 hr, respectively, for brook trout and 33.3 and 32.3 µg/kg/48 hr for rainbow trout. The ability to accurately calculate a water dose for an individual fish that could be directly compared to a mammalian dose was demonstrated.

158 A COMPARISON OF THE ACUTE TOXICITY OF THREE ORGANIC COMPOUNDS TO AQUATIC ORGANISMS. R. J. Buccafusco, M. A. Palmieri, and K. F. Pfeiffer, Department of Environmental Science, Allied Corporation, Morristown, N. J. Sponsor: G. V. Loewengart

Acetaldehyde (AAO), aldicarb oxide (ADO) and methylthioisobutyraldehyde oxide (HTAO) are intermediates used in the synthesis of agricultural chemicals. The toxicity of these compounds to the rainbow trout, the bluegill sunfish and the aquatic invertebrate, Daphnia magna, was determined under static conditions as part of an environmental hazard assessment. The results indicate that there is no apparent or predictable pattern of species sensitivity to these chemicals. ADO was more acutely lethal to the rainbow trout (LC-50=28 mg/L) than the bluegill (LC-50=301 mg/L) or the daphnid (EC-50=343 mg/L) but not on rainbow trout. The bluegill sunfish and the aquatic invertebrate, Daphnia magna, was determined under static conditions as part of an environmental hazard assessment. The results indicate that there is no apparent or predictable pattern of species sensitivity to these chemicals. ADO was more acutely lethal to the rainbow trout (LC-50=28 mg/L) than the bluegill (LC-50=301 mg/L) or the daphnid (EC-50=343 mg/L). However, the bluegill was the most sensitive species to AAO (LC-50=31 mg/L) and HTAO (LC-50=120 mg/L). Additionally, at sublethal concentrations of ADO (-158 mg/L) bluegill experienced an apparent central nervous system or neuromuscular effect that was characterized by a general loss of equilibrium and swimming ability. This immobilization is similar to the response observed after exposing fish to a low concentration of a general anesthetic such as tricaine methane sulfonate (MS-222). ADO had a similar effect on daphnids but not on rainbow trout. The bluegill and daphnids which experienced this effect subsequently regained their equilibrium and swimming ability when placed in water without ADO. The results demonstrate that chemicals with similar structures can have unique biological activity and that the sensitivity of aquatic organisms to closely related chemicals is not always predictable.
A major physical factor governing interactions between xenobiotics and biological macromolecules is the hydrophobicity of the agent. A frequently used measure of hydrophobicity is the logarithm of the chemical's octanol:water partition coefficient \( \log P_{(o:w)} \). However, shake-flask partition coefficients are subject to numerous errors. Measurement by high pressure liquid chromatographic (HPLC) techniques may minimize certain problems, but the available methods often have limited range and reproducibility or require special columns.

An HPLC method has been developed which affords accurate and reproducible octanol:water partition coefficients over the 0 to 7 range. Correlations between the literature octanol:water log \( P \) and the corrected HPLC elution volumes are better than 0.999 with high reproducibility for numerous organic chemicals. Unlike other HPLC methods, this procedure uses commercial RP-8 columns, flow rates above 1 m l/min, and dual solvents to improve peak shapes and speed elution. It may be completely computer-controlled, enabling rapid, routine log \( P_{(o:w)} \) determinations at costs comparable to the calculation of values, but with better results. Variables affecting the procedure and correlations of toxicological data with HPLC log \( P_{(o:w)} \) will be presented.

Measurements of various methylated and halogenated benzenes suggest that substituent constants are not additive above 4 log \( P_{(o:w)} \) units. Other findings indicate that second components, formed in the shake-flask, may cause errors in traditional log \( P \) measurement. This work was supported by ES 02193, BRSG S07 RR07030, HATCH 20-323, and the University of Illinois.

160 AN ASSAY FOR DETECTING ERYTHROPOIETIC DAMAGE IN RAINBOW TROUT (SALMO GAIRDNERI) USING \(^{59}\)Fe INCORPORATION. K. Cooper and B. Snyder, Graduate Program in Toxicology, Rutgers Univ., Piscataway, NJ 08854

Detection of erythropoietic damage in mice can be determined by monitoring the level of \(^{59}\)Fe uptake into developing red blood cells (RBC) (Lee et al., 1974, Appl. Pharmacol. 27, 431-436). We developed an assay for trout (80-100 g) based on the above work and that of Walker (Dissertation, 1975, Mich. State Univ.). We injected trout ip for three consecutive days with either benzene (440 mg/kg) in corn oil, or corn oil as a control. On day 4 fish were injected ip with 0.5 \( \mu \)Ci \(^{59}\)Fe and sacrificed 11 days later. \(^{59}\)Fe content was measured in blood from the caudal vein, and in samples of liver, spleen, head kidney, pyloric caeca, intestine, gill and skeletal muscle. Compared to control values, benzene pretreatment resulted in significant decreases of \(^{59}\)Fe incorporation in RBC's (70%), and in the three organs involved in hematopoiesis in fish: liver (71%), kidney (43%) and spleen (23%) (p < 0.02 for all values). Using the same protocol, we injected toluene (440 mg/kg, ip) and found no decrease of \(^{59}\)Fe uptake in blood or in any of the tissues. Further studies are currently being conducted into the comparative sensitivity for detection of hematopoietic damage by this method versus standard cell counting techniques. On the basis of these preliminary data, this assay appears to be useful for determining erythrotoxic environmental pollutants.

(Supported by NIH Grant RR000322.)

161 DOSAGE LEVELS ASSOCIATED WITH THE INDUCTION OF RESPIRATORY TRACT INFLAMMATION BY ACUTE EXPOSURES TO TITANIUM TETRACHLORIDE SMOKES. B. Ballantyne, Union Carbide Corporation, Charleston, Wv 25303.

Titanium tetrachloride (TT) vapor reacts with atmospheric water, producing oxychlorides and hydrogen chloride with the liberation of heat. The resultant smoke is known to cause injury to the respiratory tract, but the pathology produced and its dose-dependency are not well documented. In this study rabbits, rats, guinea pigs and mice were acutely exposed to TT smokes at the following dosages, expressed as TT in mg min \(^{-3}\): 14,800 - 58,800 (delivered over 30 minutes), 5,000 - 10,000 (over 10 minutes) and 400 (over 1 or 10 minutes). Dosages in excess of 5,000 mg min \(^{-3}\) produced acute inflammatory effects as follows. Congestion, neutrophil infiltration and focal necrosis of laryngeal and tracheal mucosa; scattered foci of necrotizing bronchiolitis; alveolar capillary congestion with scattered areas of intra-alveolar haemorrhage and edema. These histopathological features were most severe in the 24 hours following exposure, and resolved in the subsequent 7 to 14 days. In general the severity of inflammatory change was dose-related. No specific histopathological features were seen in the respiratory tract of animals exposed to TT smoke at dosages of 400 mg min \(^{-3}\) over a 1 or 10-minute exposure period. In contrast with the severe irritation seen following liquid or high vapor contamination of the eye, no adverse ophthalmic effects were observed at the dosages of TT employed in this study.

162 HEXAMETHYLPHOSPHORAMIDE (HMPA) NASAL CARCINOGENESIS: SELECTIVE RETENTION OF \(^{14}\)C-HMPA RADIOACTIVITY IN TARGET TISSUES AND ITS INHIBITION BY METYRAPONE. R. W. Rickard and P. J. Gillies, E. I. du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 07111. Sponsor: G. L. Kennedy, Jr.

The role of metabolism and disposition in the induction of nasal turbinate squamous cell carcinomas was investigated in the rat. Rats were administered \(^{14}\)C-HMPA via nose only inhalation or oral gavage. The maxilloturbinate (MT), nasal turbinate (NT), and ethmoid (ET) regions of the nose contained 7- to 7-fold more radioactivity than other tissues in the body 72 hours after inhalation exposure; quantitatively similar results were obtained at 168 hours. The retention of radioactivity in these regions may be a unique property of the nose since even after oral administration the MT, NT and ET regions exhibited 3- to 7-fold more radioactivity than other tissues. To investigate the possibility that this retention depended on the metabolism of HMPA to a reactive species, similar studies were conducted in rats pre-treated with metyrapone. Metyrapone virtually eliminated the retention of radioactivity in the MT, NT and ET regions. To ascertain whether metyrapone inhibits HMPA metabolism in the nose as opposed to the liver, the
164 EXPERIMENTAL LUNG FIBROSIS IN THE MOUSE: LONG-TERM MORPHOLOGIC AND BIOCHEMICAL FEATURES. W.M. Haschek, A.J.P. Klein-Szanto, J.A. Last,* K.H. Rieser,** S. Lock, W.E. Dalbey and H.P. Witschi. Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830 and*Department of Internal Medicine, University of California, Davis, CA 95616

Both the early inflammatory and the late proliferative phases of the adult respiratory distress syndrome can be produced in mice with the antioxidant, BHT, and 70% oxygen. The present study followed the morphologic and biochemical progression of these lung lesions for 1 year. Young adult male Balb/c mice were injected ip with 400 mg/kg BHT dissolved in corn oil, or corn oil alone, and immediately exposed to 70% oxygen, or air, for 6 days. Mice were killed at various time intervals up to 1 year after BHT treatment and the lung changes were evaluated by light and electron microscopy, and by determination of total lung hydroxyproline levels and changes in collagen types. In animals treated with BHT and oxygen there was initially a diffuse interstitial pneumonitis. With time the inflammatory changes subsided but degenerative, emphysematous changes became apparent. Elevated lung hydroxyproline levels present 2 weeks after treatment persisted up to 1 year. The percentage of type III collagen was initially decreased but returned to normal within 1 year. In animals treated with BHT alone the lung changes were similar but less severe. Preliminary data indicate that lung compliance is decreased in mice 3 months after treatment with BHT and oxygen. Thus long-lasting morphologic and biochemical alterations were produced as a result of potentiation of lung injury by oxygen.

165 STUDIES ON THE DEPOSITION AND DISTRIBUTION OF CATECHOL FROM WHOLE CIGARETTE SMOKE IN BC3F1/Cum mice. K.K. Hwang, O. Sonko, D.R. Dansie, R.E. Kouri, and C.J. Henry, Dept. of Experimental Oncology, Microbiological Associates, 5221 River Road, Bethesda, MD 20816-1493

Tritiated-catechol has been used to follow the pharmacokinetics and metabolic fate of inhaled catechol in cigarette smoke in BC3F1/Cum mice. The presence of [3H]-catechol in the smoke was verified by silica gel chromatography, and gas chromatography/mass spectrometry. Mice were exposed to 10% (v/v) 2R1 cigarette smoke on the Walton Horizontal Smoking Machine under standard conditions of 35 ml puff volume, 2 sec/puff, 10 puffs/cigarette. The deposition and distribution of inhaled catechol were determined in all internal tissues, urine and feces. Data showed that clearance was occurring during the 10 minute smoke exposure period. Immediately after this exposure period, 56% of the radioactivity was found in the blood, with 8% found in the lung, 12% in the respiratory tract, 13% in the liver, and 14% in the kidneys. By 120 minutes after exposure, less than 0.6% of the total dose was found in the lung and over 94% of the inhaled radioactivity was found in the urine. Less than 10% of these radioactive urinary metabolites were extractable by chloroform-methanol (CM) or ethylacetate (EA) solutions. Upon treatment with 5-glucuronidase and arylsulfatase, at least 95% of the radioactive urinary metabolites were extractable by CM or EA solutions. These organic extractable fractions were purified, analyzed, and three chemicals positively identified: catechol, hydroquinone and 4-methylcatechol.

We conclude that catechol in smoke is rapidly absorbed, redistributed, conjugated, and excreted in urine from mice exposed to whole cigarette smoke.

166AUTORADIOGRAPHIC ANALYSIS OF DNA SYNTHESIS IN PULMONARY TISSUES OF MICE EXPOSED TO WHOLE CIGARETTE SMOKE. K.K. Kanagalingam, S.M. Reed, D.R. Dansie, W.C. Hall, R.E. Kouri, and C.J. Henry, Dept. of Experimental Oncology, Microbiological Associates, 5221 River Road, Bethesda, MD 20816-1493

DNA replication, as measured by estimation of Labeling Index (LI), was determined in BC3F1/Cum mice after daily exposure to whole cigarette smoke over a 12 month period. Immediately following the last smoke-exposure, lung DNA was labeled with 1.0 hr. pulse of tritiated thymidine (3H-TdR) and...
Heavy metals and polycyclic aromatic hydrocarbons co-exist in polluted air in the respirable particulate fraction. The metabolism of p-nitroanisole (NA), a model substrate for mixed function oxidases, was studied in a perfused lung preparation following a single 2-hr exposure of rats to aerosols of NiCl₂ (450 or 130 µg Ni/m³), CoCl₂ (300 or 125 µg Co/m³), or CdCl₂ (450 or 200 µg Cd/m³). Three hr post-exposure to the high and low NiCl₂ concentrations, NA O-demethylase activity was inhibited by 52 and 43% respectively. At 48 hr, the low dosed rats were nearly recovered, while activity in the high dosed rats was still inhibited by 42%. Inhibition was related directly to the Ni content of the lungs. Inhibition of activity was also observed 3 hr following exposure to CoCl₂ aerosols, but was maximal 24-48 hr post-exposure (38 and 25% following exposure to the high and low level, respectively). Recovery was complete 72 hr post-exposure. Following exposure to 200 µg Cd/m³ no immediate effect was measured, but at 72 hr O-demethylase activity was 54% greater than controls and remained elevated by 25% 168 hr post-exposure. Thus, the effects of individual metal salts on pulmonary NA O-demethylase are unique and these results suggest that inhaled metals may alter the metabolic and distributional profiles of respirable organic pollutants as well. (Supported by EPA Grant R806337, NIH Grant ES01859, and a Fellowship from CIIT.)

In vivo dose-response for dissolved zinc, selenium, vanadium, or nickel has been evaluated utilizing functional assays with pulmonary macrophages from Balb/c male mice. Pulmonary macrophages were lavaged and attached to glass coverslips to obtain a pure cell population. After rinsing nonadherent cells, the attached cells were exposed to the test material in media for twenty hours. At the end of the incubation period, viability was evaluated for each group, and phagocytic capacity was determined after challenge with carbon-coated latex microspheres for one hour in fresh media. Comparable in vitro exposures using bovine alveolar macrophages (BAM) were also done. In vivo dose-response with several compounds (Na₂SeO₃, ZnO, V₂O₅, As₂O₃, or Ni₃S₂) was studied in mice that had been exposed by intratracheal instillation. Function of pulmonary macrophages was measured on cells harvested from these animals two weeks after exposure. Comparison of the in vivo cytotoxicity with the in vitro toxicity data demonstrates that macrophage cytotoxicity is not predictive of the whole animal toxicity. This phenomenon is most clearly shown with selenium and zinc. In vitro dose-response and metal interaction studies with BAM using relatively insoluble particles of nickel, manganese, zinc and soluble selenium and vanadium were also performed. Synergism was displayed with nickel and manganese. Selenium did not change the functional response of cells to nickel but was antagonistic when coexposed with vanadium. Zinc also appears to be antagonistic to nickel exposure. This work demonstrates the utility of the BAM system in in vitro evaluation of metal interactions. Supported by the Electric Power Research Institute (Contract #RP1639-2).

Particles of crystalline αNiS are avidly phagocytized by mammalian cells, are potently carcinogenic and induce morphological transformation in Syrian Hamster Embryo (SHE) cells in vitro; while particles of amorphous NiS of similar size and elemental composition and having similar low water solubility are not phagocytized and lack carcinogenic and transforming activity. Observations of NiS particle binding to charged filters which indicated that there exists a difference in particle surface charge between the amorphous and crystalline particles were confirmed by subsequent zeta potential determinations: amorphous and crystalline NiS exhibit surface charges of +9.17 and +27.01 mV, respectively at pH 7.2. X-ray photoelectron spectroscopy also revealed differences in surface composition between the two particle types. Crystalline NiS particle surfaces had a Ni/S ratio of 2.1 compared to a ratio of 1.5 for the amorphous NiS particles. The sulfate/sulfide ratio was 0.5 for crystalline NiS and 2.0 for amorphous NiS. The


168 EFFECTS OF IN VIVO AND IN VITRO METAL EXPOSURES ON PULMONARY MACROPHAGES. K.L. McNeill, C.J. Democko, and G.L. Fisher, Battelle Columbus Laboratories, Columbus, Ohio 43201

POTENTIATION OF PHAGOCYTIC UPTAKE OF NONCARCINOGENIC AMORPHOUS NICKEL SULFIDE PARTICLES RESULTS IN MORPHOLOGICAL TRANSFORMATION OF SHE CELLS. J.D. Heck, M.P. Abraccchio and M. Costa, Div. of Toxicol., Dept. Pharmacol., Univ. of TX Med. School, Houston, TX. 77025

Particles of crystalline αNiS are avidly phagocytized by mammalian cells, are potently carcinogenic and induce morphological transformation in Syrian Hamster Embryo (SHE) cells in vitro; while particles of amorphous NiS of similar size and elemental composition and having similar low water solubility are not phagocytized and lack carcinogenic and transforming activity. Observations of NiS particle binding to charged filters which indicated that there exists a difference in particle surface charge between the amorphous and crystalline particles were confirmed by subsequent zeta potential determinations: amorphous and crystalline NiS exhibit surface charges of +9.17 and +27.01 mV, respectively at pH 7.2. X-ray photoelectron spectroscopy also revealed differences in surface composition between the two particle types. Crystalline NiS particle surfaces had a Ni/S ratio of 2.1 compared to a ratio of 1.5 for the amorphous NiS particles. The sulfate/sulfide ratio was 0.5 for crystalline NiS and 2.0 for amorphous NiS. The
170 RESPIRATORY TRACT DEPOSITION AND RETENTION OF INHALED ULTRAFINE PARTICLE ASSOCIATED BENZO(A)-PYRENE.

The present study demonstrates that rendering the surfaces of noncarcinogenic amorphous NiS particles more negative by chemical reduction potentially controls their phagocytic uptake and their reduction of morphological transformation of SHE cells to levels similar to those seen with carcinogenic crystalline aNiS. Thus it appears that the selective phagocytosis of crystalline aNiS particles by cells rather than a physicochemical feature unique to their crystalline structure compared to the amorphous form accounts for their potent transforming activity. (Supported by NIHES training grant E507009, and EPA grant #R806048.)

171 TOXICOLOGICAL AND CHEMICAL CHARACTERIZATION OF THE PROCESS STREAM OF AN EXPERIMENTAL LOW BTU COAL GASIFIER. J. M. Benson, S. L. Gunsett, L. L. Hanson and G. J. Newton (Sponsor: R. O. McClellan), Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185

The process stream of an experimental low BTU coal gasifier and combustion products of the clean gas were characterized as to their mutagenic properties and chemical composition. Samples were obtained at selected positions along the gasifier process stream using a sampling train. Mutagenicity was assessed using the Salinella mammalian microsome mutagenicity assay (TA-98, with and without rat liver S-9). Chemical analysis was chiefly by gas chromatography and mass spectrometry. All materials required metabolic activation to be mutagenic. Raw gas had a specific mutagenicity of 6.7 revertants/µg (50,000 revertants/1 process stream). Polycyclic aromatic compounds, including methylnaphthalenes, phenanthrene, fluoranthene and chrysene and nitrogen-containing compounds were positively identified in highly mutagenic fractions of raw gas. Gas cleanup by humidifier-tar trap system and Venturi scrubber led to a small reduction in specific mutagenicity of the gas (4.1 revertants/µg but, due to reduction in mass loading, a significant reduction in overall mutagenicity was achieved (2200 revertants/1). Chemical composition of this material was similar to that of raw gas. Combustion products had a specific mutagenicity of 1.1 revertant/µg (22 revertants/1) and also contained several polycyclic aromatic compounds. Results indicate that process stream material is potentially harmful to workers exposed emissions through fugitives during maintenance or repair of the gasifier. Combustion products have relatively low biological activity compared to raw gas. (Research performed under DOE Contract DE-AC04-76EV01013.)

172 MUTAGENICITY OF PARTICULATE EXHAUST FROM DIESEL AND SPARK IGNITION ENGINE CARS. C. R. Clark, Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185, D. E. Seizinger and T. M. Naman, Bartlesville Energy Technology Center, Bartlesville, OK 74003

Extracts of particulate exhaust collected from diesel and spark-ignition engine equipped cars are mutagenic in bacterial test systems. Differences in gasoline and diesel fuel composition and exhaust particle characteristics warrant investigation into the chemical characteristics of the particle-associated mutagens. Exhaust particles were collected from four diesel and four spark-ignition engine cars operated on a climate-controlled chassis dynamometer. Dichloromethane (DCM) extracts of the exhaust particles were fractionated on silica gel to yield DCM and methanol (MEOH) eluates and the DCM eluate further fractionated by high pressure liquid chromatography (HPLC). Distribution of mass into various HPLC fractions was similar for the 4 diesel samples with 76-80% of the mass eluting in nonpolar, aliphatic and polycyclic aromatic hydrocarbon (PAH) fractions. These fractions demonstrated only weak or no mutagenicity in Salmonella strain TA-98. Most of the mutagenicity (75-95%) eluted in a moderately polar fraction which constituted 9-11% of the total mass of the extracts. This fraction has been shown to contain oxygenated and nitrated PAH. Mutagenic potencies ranged from 10-50 revertants/µg compared to 1-7 rev/µg for crude extracts. The mutagenicity of the silica gel MEOH eluate (10-19% of the mass) ranged from 1-3 rev/µg. In contrast, only 25-36% of the mass of spark-ignition engine exhaust particle extracts eluted in HPLC nonpolar fractions while more polar constituents eluting in the MEOH fraction comprised 38-49% of the mass. Moderately polar HPLC fractions had % mass values similar to the diesel samples. (Research supported in part by DOE Contract No. DE-AC04-76EV01013.)
173 MUTAGENIC PROPERTIES OF COKE OVEN EMISSIONS AND DIESEL PARTICLES J-S. Siak and T. C. Pederson, Biomedical Science Department, General Motors Research Laboratories, Warren, MI 48090. Sponsor: J. J. Vostal

Both coke oven emissions and diesel exhaust particles consist of a carbonaceous core with adsorbed hydrocarbons and carcinogenic effects of coke oven emissions have been proposed as a model to predict the health impact of inhaled diesel emissions. However, the chemical composition of both adsorbed fractions is different, and the benzo[a]pyrene content in coke oven emission extract (COE) is much higher than in diesel particle extract (DPE). Nitro-PAH have been identified in diesel particles as the major contributors to the mutagenic activity and are not detectable in coke oven emissions. In this study, we used the Ames assay and thin-layer chromatography (TLC) to analyze and compare extracts of COE and DPE. Particulate samples were collected on Pallflex filters and extracted with dichloromethane. The extracts and their TLC fractions were assayed for mutagenic activity by Salmonella typhimurium strain TA98. Of the two extracts, DPE elicited a direct-acting response from TA98, whereas COE had weak direct-acting response and required rat liver S9 preparation to exhibit a stronger response. When chromatographed, COE and DPE each displayed distinct UV fluorescent patterns. No distinct pattern was observed in the direct-acting mutagenic activity of TLC fractions of COE. In contrast, DPE exhibited a strong response in the monosubstituted PAH and polar fractions. For COE, most of the S9 activated mutagenic activity was found in the polar and PAH fractions. The data indicate that COE and DPE have different biological and chemical profiles. The mutagens in DPE are mostly direct-acting and attributed to monosubstituted aromatic compounds, whereas the mutagens of COE are poly cyclic aromatics and polar compounds which require mammalian enzyme activation. Therefore, COE should not be used as a model for the assessment of the diesel particle activity in the living organism.

174 MUTAGENIC ACTIVATION OF NITRO-PAH BY MICROSOMAL AND CYTOSOLIC ENZYMES T. C. Pederson and J-S. Siak, Biomedical Science Department, General Motors Research Laboratories, Warren, MI 48090. Sponsor: J. J. Vostal

Rat liver S9 microsomal and cytosolic enzyme activation of nitro-PAH compounds in the Salmonella mutagenesis assay was examined using the nitroreductase-deficient strains TA98NR and TA98NR/1,8-DNP. Mono-nitro-PAH compounds such as 2-nitrofluorene, 1-nitropyrene and 6-nitrochrysene were activated by NADPH-dependent microsomal enzymes when using strain TA98NR, but not TA98NR/1,8-DNP. Other mutagens such as daunorubicin and S9-activated Bl[a] P were mutagenic in both strains. The lack of mutagenic activity by microsomal mononitro-PAH metabolites in TA98NR/1,8-DNP is probably attributable to the absence of bacterial enzymes required for conversion of the microsomal metabolites to ultimate genotoxic forms. The effects of S9 enzymes on the dinitropyrenes are distinctly different than those observed with mononitro-PAH. NADPH-dependent microsomal enzymes markedly reduced the mutagenicity of 1,3-, 1,6-, and 1,8-dinitropyrene. In contrast, nitropyrene activity was increased using the unfractionated S9 preparation, but the cytosolic fraction increased the activity of all three dinitropyrenes in the presence of either NADH or NADPH. The increased activity was partly attributable to cytosolic enzymes, but also included a cytosol-independent activation by reduced pyridine nucleotides — presumably the result of a direct non-enzymatic reduction. The dinitropyrenes, as well as mononitro-PAH, are believed to be among the bacterial mutagens detected in extracts of diesel exhaust particulate, and their activation as well as deactivation might occur in mammalian tissues. The liver microsomal enzyme-catalyzed increase of mononitro-PAH mutagenicity in the Salmonella assay is apparently dependent on further bacterial enzyme activation.


In previous work [J. Appl. Tox., 11(4), 1981] we have reported the prompt response of the alveolar Type II pneumocyte during inhalation of 6000 µg/m³ of diesel emission exhaust. The present investigation analyzes further the chronic changes in the rat lung seen after exposure to diesel particulates at a level of 1500 µg/m³ for up to 2 years. Again, the presence of increased numbers of Type II cells and striking increases of intra-alveolar phospholipids, a product of these cells, is clearly evident. This material, along with the high burden of particulate, is ingested by the alveolar macrophages. As a result, macrophages become foamy in appearance and tend to accumulate and agglomerate at first in alveoli near terminal bronchioles, and later, more diffusely. By electron microscopy, needle-like structures probably representing cholesterol are seen within alveolar macrophages. At a later stage these structures increase in size and can also be seen extracellularly within alveolar spaces. With this change, mast cells appear within alveolar septa in which also increased collagen is laid down. Occasionally, the focal accumulations of cholesterol and fibrous tissue form a so-called "cholesterol granuloma," a condition that derives from many causes including exposure to other particulates such as Sb₂O₃, marihuana smoke, antineoplastic drugs, and even a supposed viral disease in the feral mongoose. Collagen fibers, are generally distributed diffusely within the alveolar septa, allowing for preservation of the alveolar architectural integrity. Such a benign fibrotic change is probably a common lung response to the long-term inhalation exposure to high levels of non-injurious particles, representing more a non-specific reaction of the defense system to the presence of inert particles, rather than necessarily related to the nature of the diesel particle per se.

176 EFFECT OF PROLONGED EXPOSURE TO DIESEL EXHAUST ON THE PULMONARY CLEARANCE OF INHALED DIESEL PARTICLES T. L. Chan, P. S. Lee, and W. E. Hering, Biomedical Science Department, General Motors Research Laboratories, Warren, MI 48090. Sponsor: J. J. Vostal

The effect of diesel exhaust exposure on the pulmonary clearance of diesel particles was studied in Fischer 344 rats. Test animals were first exposed to diluted diesel exhaust in exposure chambers at nominal particulate concentrations of 0, 250, and 6000 µg/m³. The exposure regimen was 20 hrs/day, 7 days/week, lasting from
Effects of Repeated Exposures to Aerosolized Diesel Fuel.

*Biology Division and **Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN.

High concentrations of aerosolized diesel fuel are used as visual obscurants by the military. The effects of repeated exposure on Sprague-Dawley rats of both sexes is being investigated. Exposure variables include concentration (1.33 to 6 mg/l), duration of exposure (2 or 6 hours), and frequency of exposure (1/wk or 3/wk). Extensive chemical and physical characterization of the aerosol was performed during the exposures. Animals received nine exposures and were then tested 1-2 days and 2 weeks post-exposure for a variety of endpoints. These measurements included number and phagocytic activity of lavaged pulmonary cells, pulmonary function tests, neurotoxicity screening assays, blood cell counts, body and organ weights, clinical chemistry, and histopathology. The most pronounced effects occurred in the lungs and included dose-related increases in the number of alveolar macrophages, atelectasis, and changes in pulmonary function.

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Pulmonary Response of Fischer 344 Rats and CD-1 Mice to Exposure to Diesel Exhaust. R. F. Henderson, J. L. Maederly, R. O. McClelan, B. V. Mokler and H. C. Redman, Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuqueruqe, NM 87185.

To evaluate the effect on the lung of inhalation of diesel exhaust, Fischer 344 rats and CD-1 mice were exposed to diluted, whole diesel exhaust 7 hr/da, 5 da/wk for 6 months. Animals were exposed to air concentrations of 0, 300, 3300 or 6500 μg/m³ of particulate matter. Pulmonary response was evaluated by analysis of lung airway washings and enzymatic activity of the lung tissue. Parameters measured in the lavage fluid included total and differential cell counts, soluble protein and sialic acid content and the following enzyme activities: lactate dehydrogenase (LDH), glucose-6P-dehydrogenase (6PDPH), glutathione reductase (GR) and peroxidase, alkaline and acid phosphatase and β-glucuronidase. The enzyme activities were also measured in the lung tissues. Lavage fluid analyses indicated an inflammatory response in the mice at the medium and high exposure levels and in the rats at the high exposure level. Largest elevations were in GR and β-glucuronidase activities followed by lesser increases in LDH and acid phosphatase activities, sialic acid and protein content and leukocyte count. Tissue responses were smaller in magnitude than those seen in the lavage fluid but indicated an increase in lysosomal enzymes and GR activities. The mice also showed an increase in G6PDH activity. For all parameters, the mice showed a greater effect at a lower exposure level than did the rats. Additional animals will continue to be exposed and examined at six month intervals throughout the life span of the rodents to evaluate exposure time-effect relationships for diesel exhaust.

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180 COMPARISON OF TISSUE BURDENS FOLLOWING WHOLE-BODY AND NOSE-ONLY EXPOSURES OF FISCHER-344 RATS TO AEROSOLS. R. K. Wolff, L. C. Griffin, C. H. Hobbs and R. O. McClellan, Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185

Two groups of Fischer-344 rats were exposed to $^{67}$Ga$_2$O$_3$ aerosols (0.1 µm-volume median diameter) either in a whole body or nose-only exposure mode. Lung deposition was very similar for the two exposure modes: 15 ± 3% for whole body and 15 ± 7% for nose-only. Pelt activity was quite high for whole-body exposures: 2.1 times the initial lung burden as compared to 0.3 times for nose-only exposures. Pelt activity was primarily confined to headskin for nose-only exposures. Since the Ga$_2$O$_3$ particles were relatively insoluble, very little was absorbed systemically. Most of the excreted radioactivity was in feces and represented material initially deposited in the upper respiratory tract and on the pelt. Total activity excreted in feces expressed as percent of initial lung burden was 1.6 times greater following whole-body exposures than for nose-only exposures. Subtracting amounts expected to be cleared from the respiratory tract as determined from initial deposition measurements suggested that approximately 70% of pelt activity was ingested following nose-only exposures as compared to approximately 90% for nose-only exposures. The high gastrointestinal intakes of material and possible absorption for more soluble materials must be considered in inhalation exposures, particularly whole-body exposure, when assessing toxicological effects. (Research performed under DOE Contract Number DE-AC04-76EV01013.)

181 A SIMPLE COMPUTER INTERFACED INHALATION EXPOSURE SYSTEM FOR TOXIC GASES. R. E. Gregory, L. R. Wilson and J. A. Pickrell (Sponsor: R. O. McClellan), Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185

A simple computer-controlled and monitored small animal inhalation exposure system has been designed and constructed. Unlike other computer interfaced exposure systems which use individual stand-alone minicomputers, our system operates in a time-sharing configuration with the institute's main computer system. The exposure system is interfaced through a specially designed serial control unit (SCU) which uses a simple preprogrammed microprocessor in a multidrop configuration. This device allows on-line, real-time computer control and data acquisition without full-time computer commitment. The exposure system was designed to expose groups of rats to 0 ppm, 1 ppm, 5 ppm of nitrogen dioxide (NO$_2$) and 1 ppm with two spikes to 5 ppm per day, 7 hours/day, 5 days/week for the animals' life span. The computer, after initial operator interaction, controls the initiation of exposure, the administration of NO$_2$ spikes, chamber gas sampling and analysis and termination of exposure. The computer also continuously monitors and records (24 hours/day) chamber flow, pressure, temperature and relative humidity and sets alarms when these parameters exceed established limits. The use of the SCU in computer-controlled and monitored exposure systems will allow a large number of individual experiments to take place simultaneously while minimizing personnel time and computer costs. (Research performed under an interagency agreement between EPA (79-D-X5033) and DOE under Contract No. DE-AC04-76EV01013.)

182 CARBON MONOXIDE – NaCl INTERACTION IN THE DEVELOPMENT OF SYSTEMIC HYPERTENSION, R. N. Shibutaka and R. T. Drew, Medical Dept. Brookhaven National Laboratory, Upton, N.Y. 11973

The influence of carbon monoxide (CO) on the development of systemic hypertension was studied using Dahl rats selectively bred for susceptibility (S) and resistance (R) to NaCl induced hypertension. Forty "S" and 40 "R" female rats were maintained on a low (0.4%) salt diet until 5 weeks of age when 20 "S" and 20 "R" rats were transferred to a high (8%) salt diet. At 6 weeks of age, groups of 10 "S" and 10 "R" rats from the low and high salt groups were exposed to 500 ppm CO, 21 hours/day for 62-63 consecutive days. This exposure resulted in an equilibrium carboxyhemoglobin concentration of 42%. Carbon monoxide exposure and a high salt diet promoted the development of hypertension in "S" rats when compared to "S" rats fed an identical salt diet and exposed to air. Three of the 10 CO exposed "S" rats on the high salt diet died during the last week of exposures. All air exposed "S" rats on the high salt diet survived. No "R" rats nor "S" rats on low salt diet developed hypertension and all rats in these groups survived the study. Carbon monoxide induced a cardiomegaly of 36% in "S" rats on both high and low salt diets. The "R" rats exposed to CO exhibited a 29% and a 22% cardiomegaly on high and low salt diets, respectively. CO exposure also produced varying degrees of splenomegaly. There were no changes in whole body, brain, or adrenal weights attributable to CO exposure. The consistent hematologic response to this hypoxic challenge was elevated total hemoglobin (>77%) and hematocrit (>57%), irrespective of diet or line of rat. Thus, CO in conjunction with a high salt diet enhances the development of systemic hypertension in the susceptible line of Dahl rats.

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183 EFFECTS OF CADMIUM ON THE HEPATOTOXICITY OF BROMOBENZENE IN RATS. S. Chakrabarti and J. Brodeur, Dép. méd. travail et hyg. milieu, Fac. Méd., Univ. de Montréal, Montréal, Québec, Canada H3C 3J7.

The hepatotoxicity of bromobenzene (BB) is due to its metabolic activation to bromobenzene epoxide mediated by hepatic microsomal mixed function oxidase (MFO) system. Cadmium is known to inhibit the hepatic MFO system and hence, could affect the hepatotoxicity of BB. When male rats were treated with 0.2, 0.5 and 1 mg/kg (i.p.) of cadmium chloride (CdCl$_2$) 24 h prior to the i.p. injection of 2.5 mmole/Kg of BB and sacrificed 48 h after the BB dose, a significant decrease in the SGPT activity and a decrease (but not significant) in SGOT activity as well as a significant decrease in SGPT activity were observed in 0.2 mg/kg and 0.5 mg/kg of CdCl$_2$-treated rats respectively. Similarly a reduction in the activities of SGOT and SGPT (although not significant) from those of BB-alone treated rats was noticed when rats were given 1
mg/kg of CdCl₂ 6 h prior to BB (1 mmole/kg) or simultaneously with BB and sacrificed 24 h after the BB dose. In a separate study, different groups of rats were fed 25, 100 and 200 ppm of CdCl₂ in drinking water daily for four weeks prior to the administration of a single 2.5 mmole/kg dose of BB and were sacrificed 48 h after the BB dose. No reductions in the activities of SGOT and SGPT were noticed except that of SGPT was decreased close to significance in 200 ppm CdCl₂-pretreated rats. However the liver microsomal system in such chronic Cd-treated rats showed a significant decrease in a) liver weights, b) microsomal protein concentrations, and c) the activities of aniline hydroxylase and aminopyrine N-demethylase, but the levels of cytochrome P-450 did not change. Thus, among other factors, the depression of MFO system due to different Cd-pretreatments may not be sufficient enough to reduce significantly the metabolic activation of BB to its epoxide and hence its hepatotoxicity. (Supported by MRC Grant, MA-6159)

184 DEXAMETHASONE-INDUCED ALTERATION OF THE DISPOSITION OF Cd AND TRACE METALS IN DEVELOPING RATS. E.M.K. Lui, Dept. of Pharmacol. and Tox., The Univ. of Western Ontario, London, Ont., Canada Sponsor: C.M. Cherian

Recent studies have shown that glucocorticoid stimulates the uptake of Zn and Cd in hepatic tissues of adult rats. Since Zn contents as well as metallothionein levels are much higher in the newborn period than in adulthood, we have investigated the effect of dexamethasone (Dx) treatment on hepatic trace metal metabolism as well as the disposition of Cd in the neonatal rat. To assess the effect of Dx on trace metal contents in the liver, Dx (1 ug/gm) was administered twice daily by sc. injection to newborn rats on days 3, 4, 5, and 6 post-partum and killed 72 hr later. Dx treatment reduced the hepatic Zn levels by 50% but increased the hepatic Fe contents by 2-3 fold. In contrast, Dx treatment did not alter these two parameters in the kidney. Moreover, the hormone treatment resulted in a reduction of the increase in body weight as well as an increase in both the liver and kidney to body weight ratios in the neonates. To examine the disposition of Cd in neonatal rats, CdCl₂ (1 mg/kg, sc.) was administered on days 7 and 8 post partum and the neonates were killed 24 hr following the second injection. High Cd concentrations were attained in the liver; the total hepatic Cd contents represented 17% of the total dose of Cd. The Cd concentrations in extrahepatic tissues (kidney, heart, lung, gastrointestinal tract) were approximately 8-12% of those detected in the liver. However, brain tissues contained small amounts of Cd. Dc pre-treatment (days 3 to 6) reduced the hepatic Cd concentrations by 25%. This was accompanied by an increase in the Cd concentrations in extrahepatic tissues. These data suggest that Dc treatment i) altered metabolism of Zn and Fe in the liver and ii) reduced hepatic uptake of Cd in neonatal rats.

185 EFFECT OF SUBACUTE EXPOSURES TO CADMIUM, MERCURY, LEAD, COPPER AND ZINC ON THE PLASMA AND URINARY METALLOTHIONEIN LEVELS IN RATS. Y.H. Lee, C. Tohyama and Z.A. Shaikh, Division of Toxicology, University of Rochester, Rochester, NY.

We reported previously that metallothionein (MT) levels, measured by radioimmunoassay (RIA), increase in plasma and urine of rats upon Cd exposure (Fundam. Appl. Toxicol., 1:1, 1981). The purpose of this study was to investigate whether exposure to some other divalent metals will also result in similar elevation in MT levels. Female Sprague-Dawley rats (248±9 g) were injected subcutaneously with either saline, 5 mmole/kg/day of CdCl₂, HgCl₂, PbCl₂, CuSO₄, or ZnCl₂ for 5 days. Blood and urine samples were collected on days 0, 1, 3 and 5 and analyzed for MT by the RIA. The plasma MT levels were low and ranged from less than the detection limit (150ng/ml) to 320ng/ml. Rats injected with Cd or Hg had higher levels of MT in their plasma than rats injected with other metals or saline. The urinary MT level in control rats on day 0 was 0.85±0.17μg/g creatinine. There was no significant change in urinary MT level in rats given 5 injections of Pb or Cu or a single injection of Cd or Zn. Only Hg-injected rats showed a five-fold increase in MT level on day 1. In rats given 3 injections of Zn or Cd the urinary MT concentration increased about two- and four-fold, respectively. While the mean MT level in Hg-injected rats increased steadily during the five days to almost 21 times of the mean control value, those of the Cd- or Zn-injected rats did not change significantly after three days. In conclusion, it appears that the urinary MT levels are affected by exposure to not only Cd but also Hg and Zn. Also, the elevation in urinary MT level appears to be greater after Hg treatment than after Cd or Zn treatment. (Supported by NIH Grants ES 01247, ES 01448 and ES 07026)


Adult male Sprague-Dawley rats were exposed to 25, 50 and 75 ppm cadmium (C) mixed in their diet for 6 months in order to study the chronic effects of C on gluconeogenesis. Experiments also were conducted to determine the reversibility of the effects in the metal withdrawn rats. Body weight gain, serum protein (SP), serum glucose (SG), serum enzymes (SGOT, SGPT), hepatic and renal glucose-6-, phosphatase (G-6, Pase), fructose-1,6-, diphosphatase (FDFAse) and phosphoenol-pyruvate carboxykinase (PEPCK) were determined at 2, 4 and 6 months after treatment and 4 and 6 months after cessation of the treatment. A significant 20-30% reduction in body weight gain was observed in rats receiving 50 and 75 ppm C. Rats withdrawn from C showed normal weight gains. SG levels were significantly increased in rats fed on 50 and 75 ppm C. SP was not significantly changed from controls. Three-four fold increase in SGOT and SGPT levels were seen in rats fed on C at all time points. SG levels returned to near normal in rats withdrawn from C but SGPT levels stayed higher than normal in 50 and 75 ppm groups. All 3 gluconeogenic enzymes both in kidney and liver were significantly elevated in C treated rats except in 25 ppm at 60 days after treatment. The increase in enzyme activities was dose and time dependent. Rats withdrawn from C showed a reversal of effects with all 3 enzymes. G-6, Pase was,
however, significantly reduced 6 months after withdrawal. The significant increase in SG and gluconeogenic enzymes with C exposure and reversal of these parameters after withdrawal of the metal suggest that C may be altering the carbohydrate metabolism in rats. (Supported by PHS Grant RR-08169).

HEPATOTOXICITY AFTER ACUTE EXPOSURE OF RATS TO CADMIUM. R.E. Dudley and C.D. Klaassen, Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS.

While the liver is generally not considered a target organ of Cd toxicity, we observed severe injury after acute administration of Cd to rats. Therefore, we examined the effect of a high dose of Cd (3.9 mg Cd/kg) on the liver. Rats were injected iv with Cd and sacrificed 0, 1, 2, 4, 6, 8 and 10 hrs later. Plasma activities of alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (AP) were determined as were plasma concentrations of bilirubin (BIL) and glucose (GLU). In addition, heart, lung, duodenum, kidney, spleen, liver, skeletal muscle and testis were removed for light microscopic evaluation. ALT and AST activity rose from control levels of 58 and 47 SF units/ml to peak activities of 2500 and 2233 at 10 hrs. AP activity rose from 10.5 to 37.1 BL units/l while BIL concentrations increased from 0.20 to 0.81 mg/dl. These changes are similar to those observed with high doses of CCl₄ (1 ml/kg, ip). In addition, plasma GLU levels decreased significantly after Cd. Histopathological changes were not observed in tissues other than liver and testis. Pathological changes were already evident in liver 1 hr after Cd exposure and included parenchymal cell enlargement, eosinophilia, increased numbers of mitotic cells and occasional necrosis. At 4, 6, and 8 hrs, isolated parenchymal cell enlargements and eosinophilia persisted and the extent of necrosis was increased. Ten hrs after Cd, parenchymal cell necrosis was more extensive and was accompanied by fatty vacuolization. These changes were diffuse and not localized to a specific region (i.e. periportal vs centrolobular). These data indicate the liver is a major target organ of acute Cd toxicity. (Supported by USPHS Grants ES-07079 and ES-01142)


Differences in the kinetics and metabolism of arsenite and arsenate were studied in rats. Five minutes after IV administration of 4,8 nmole arsenite or arsenate to male Sprague-Dawley rats blood levels of arsenic were only 10% of the initial dose. Blood arsenic levels then rose; by four hours 60% of the initial dose of the arsenite and 30% of the initial dose of arsenate was in the blood compartment. The liver contained 16% of the dose five minutes after arsenite administration but this dropped to less than 3% by two hours. The liver contained less than 2% of the initial dose of arsenate at five minutes and two hours after administration. The metabolite, dimethylarsinic acid, appeared in the urine by one hour after administration of either form. Thus, there appeared to be a quantitative difference in the uptake of the two forms by the liver; this question and the possibility of a metabolic difference were examined by using isolated hepatocytes. The hepatocytes took up 14-33% of a dose of 2.4 nmole arsenite but less than 2% of a dose of arsenate. Furthermore, analysis of the medium showed that the hepatocytes rapidly metabolized arsenite, but not arsenate, to dimethylarsinic acid. When the dose of arsenite was increased to 12 or 24 nmole, the amount of metabolite also increased; at 120 nmole arsenite, the amount of metabolite dropped. Thus, the liver appears to be more important in the metabolism of arsenite than in the metabolism of arsenate. (Supported by NTH Grants ESO1247, ESO1448, ESO7026)


The initiation of NADPH-dependent lipid peroxidation (LP) by NADPH-cytochrome P₄₅₀ reductase is thought to occur either through an iron catalyzed Haber-Weiss reaction resulting in hydroxyl radical (•OH) formation, or through formation of a reactive ADP-Fe-oxygen complex. The two possible mechanisms were assessed in two model LP systems. The reductase, NADPH and the iron chelator were incubated with liposomes of extracted rat liver micromolar phospholipids, or with linoleic acid dispersed by the addition of 1% lubrol. LP, NADPH oxidation, and O₂ consumption by the reductase all require added EDTA-chelated iron (EDTA-Fe) and do not occur when all the iron present is chelated with ADP or in the absence of iron chelates. LP with EDTA-
Fe does not occur in the liposomal system, but does occur with linoleate dispersed with detergent. EDTA-Fe-dependent LP is inhibited by •OH traps (100 mM mannitol, 42%; 100 mM ethanol, 40%) and catalase (1 unit/ml, 37% inhibition), and formation of the •OH-DMP adduct is seen with EPR spectroscopy. In contrast, reductase-dependent LP with EDTA-Fe and ADP-chelated iron (ADP-Fe) is not sensitive to inhibition by •OH traps in the liposomal system (0-9% inhibition by concentrations of •OH traps sufficient to inhibit purely •OH dependent LP by 88-94%), but is somewhat sensitive in the detergent dispersed system (100 mM mannitol, 22%; 100 mM ethanol, 14%). In addition, LP is stimulated by the addition of ADP-Fe to EDTA-Fe. EPR spectroscopy shows no •OH-DMP adduct when the reductase is incubated with NADPH, EDTA-Fe and ADP-Fe, the system which catalyzes maximum rates of LP. The data indicate reductase-dependent LP with EDTA-Fe may occur though an •OH-dependent mechanism, but in the presence of ADP-Fe, the mechanism likely involves an ADP-Fe-oxygen complex. Supported by NSF PCM-79-15328.

191 AMELIORATION OF MANGANESE-INDUCED ALTERATIONS OF DRUG RESPONSE AND METABOLISM BY PRETREATMENT WITH SUBTHRESHOLD MANGANESE. M.J. Deimling and R.C. Schnell, Department of Pharmacodynamics and Toxicology, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68105

Acute treatment with manganese chloride at a threshold dose of 5 mg Mn⁺⁺/kg (i.p.) produces a significant prolongation of barbiturate-induced hypnosis in the male rat as a result of decreased hepatic metabolism by the microsomal mixed function oxidase (MFO) system (Pharmacologist 22, 1980). Additional experiments showed this was attributable to decreased levels of cytochrome P-450. Recent experiments have shown that the administration of a subthreshold dose of 1 mg Mn⁺⁺/kg (i.p.) to male rats three days prior to treatment with a threshold dose of Mn produces a significant protection against enhanced drug response as measured by the duration of hexobarbital hypnosis. Rats treated with a threshold dose of Mn slept significantly longer than the control group while animals treated with subthreshold Mn alone, or prior to receiving a threshold dose of Mn, awoke at time periods which did not differ significantly from the control group. Examination of aniline, ethylmorphine, and hexobarbital metabolism by the hepatic microsomal fraction in vitro revealed that pretreatment with subthreshold Mn could protect against decreased hepatic MFO activity which occurs following treatment with a threshold dose of Mn alone. Adsorptive experiments revealed that amelioration of manganese-induced decreases in hepatic MFO activity could result from protection against decreased levels of hepatic cytochrome P-450 which were observed in animals treated with a threshold dose of Mn alone but not in animals treated with subthreshold Mn alone, or in combination with threshold Mn. (Supported by NIH Grant # ES02511.)

Toxicity resulting from exposure to inorganic salts of manganese (Mn) is frequently characterized by extrapyramidal neurologic symptoms. In animals, numerous studies with Mn have reported changes in brain neurotransmitters, especially dopamine (DA), which may be related to these symptoms.

Injecting mice with the organic fuel-additive, methylcyclopentadienyl-Mn-tricarbonyl (MMT) on a subchronic schedule (80 mg/kg, sc, on alternate days for 3 weeks) resulted in a small, but significant increase in brain Mn and an accompanying 18-23% decrease in striatal and limbic DA concentrations. Additional experiments failed to produce these changes. The DA metabolite, dihydroxyphenylacetic acid, was also decreased after MMT. In addition, the pharmacologic effects of amphetamine which depends on the release of presynaptic DA for its effects, was also altered by MMT. The dose-response curve for amphetamine-induced stimulation of motor activity was shifted to the left in the subchronic MMT group, while the stimulant effects of the direct DA receptor agonist, apomorphine was not reduced. These results suggest that MMT produces a biochemical and functional deficit in brain DA synaptic activity, similar to the effects reported for inorganic Mn. (Supported by NIH Grant # ES02511.)

193 EFFECTS OF DIETARY PROTEIN CONTENT ON LOCOMOTOR ACTIVITY DURING CHRONIC LEAD EXPOSURE IN MALE AND FEMALE RATS. A.J. Verlangieri, J.J. Meyer, T.B. Barnes and J.C. Kapeghian, Dept. of Pharmacology, Sch. of Pharmacy, Univ. of Mississippi, Univ., MS 38677. Sponsor: W.M. Davis.

Nutrition has been reported to be a factor in lead-induced toxicity in rats. To examine this more fully, male and female Wistar rats were maintained on either a low, normal, or high protein-to-carbohydrate diet and received either 75 ppm of lead acetate in the drinking water or deionized water for 52 weeks. Locomotor activity was measured routinely by using a linear LC-33 running activity system in cages. Mean food and water consumption, body weights, and urinary delta-aminolevulinic acid (ALA) levels were recorded for each group. By week 52, the low protein diet alone resulted in significant reductions in body weights of both sexes while high protein produced no effect. Chronic lead ingestion further reduced body weights of both sexes in the low protein group but resulted in an elevation in this parameter in males on the normal diet. Water consumption was unaffected by diet or lead treatment. Food consumption was unchanged by diet but was significantly elevated by chronic lead ingestion in both sexes on low protein. Urinary ALA was reduced by low protein, while lead ingestion produced significant increases in this parameter by week 52 in all groups. The low protein diet produced significant increases in locomotor activity in both sexes while high protein decreased activity in males. Under a normal diet, females demonstrated a higher locomotor activity than males, however this was transient by week 52. Chronic lead ingestion significantly increased locomotor activity of both sexes on low protein. This response was also observed in males on high protein. Under normal dietary conditions lead exposure lowered locomotor activity in males and increased activity in females. Thus, the nutritional component in chronic lead exposure has been shown to interact in its toxicity profile.
LEAD EXPOSURE AND RENAL FUNCTION IN RATS: IS THE RAT A GOOD MODEL FOR LEAD INDUCED NEPHROPATHY?

W.D. Adams, E.J. O'Flaherty, and E. Taylor. Dept. of Environmental Health, University of Cincinnati, College of Medicine, Cincinnati, OH. 45267 SPONSOR: P.B. Hammond.

Lead nephropathy, characterized functionally by depression of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), is associated with prolonged occupational exposure to lead. Production of comparable lead-related renal functional deficits in rats has been difficult to achieve. Recently, Aviv et al. (Kidney International 17, 430-437 (1980) reported reductions in GFR and in ERPF in weanling rats given large amounts of lead in their drinking water. We have examined in rats some of the factors that might be expected to influence the likelihood of developing lead-induced renal functional damage. These factors include magnitude of lead exposure, age of the rat, diet, and occurrence of generalized lead toxicity as reflected in body weight loss. The relative importance of these factors has been evaluated using GFR (as inulin clearance) and ERPF (as para-aminohippurate clearance) as indices of renal functional competence. Diets examined were the NIH-07, AIN, and standard Purina Rodent Chow diets. (Supported by USPHS grants ES 07073 and ES 01872).


Much effort has been directed toward understanding the pharmacokinetics of lead in humans and in experimental animals, particularly to the similarities and interactions with calcium metabolism. Relatively little attention has been given to the subcellular distribution and kinetic behavior of lead. Experiments were conducted to characterize the steady-state kinetic behavior of Pb++ and to identify the biological structures associated with kinetic compartments. Hepatocytes were isolated from Sprague-Dawley rats by enzymatic perfusion, placed into primary cell culture, labeled with 210Pb as 3µM Pb(NO3)2 for 3 or 24 hours, and the kinetic parameters determined by analysis of desaturation curves. Extracellular compartments were defined by sensitivity to EGTA and LaCl3 and intracellular compartments identified by their behavior following exposure of the cells to KCN, Ouabain, 2,4-dinitrophenol, cAMP, Antimycin A, or phosphate. The kinetic behavior of lead can be described by a three compartment system. Since no kinetic pool was labile to chelation by EGTA or to displacement by LaCl3, it is concluded that unlike Ca++, hepatocytes do not contain a significant pool of Pb bound to the cell surface. The intracellular Pb is characterized by two small compartments (42 and 46 pmol/mg cell protein) with desaturation halftimes of 2 and 20 minutes respectively. The bulk (80%) of the cellular 210Pb is found in the kinetically deep compartment which contains 330 pmol/mg and exchanges with a halftime of 360 minutes. This compartment is believed to include mitochondrial 210Pb, based on its sensitivity to KCN, dinitrophenol, and phosphate. These findings indicate that the cellular metabolism of lead is very similar to the cellular metabolism of calcium in hepatocytes.

THE EFFECT OF LEAD ON THE RABBIT RETINA D.M. Talisma, K.L. Steemer, Univ. of Cincinnati Medical Center, Kettering Lab., Cincinnati, OH 45267

Evidence exists that the toxicity of lead may be related to its pro-oxidant effect on cellular components. The retina is susceptible to this type of stress, and the present study was performed to determine the oculary effects of systemic lead administration.

Rabbits were given drinking water with zero or 2000 ppm of added lead for periods of eight or twelve weeks. They were sacrificed following lead administration. Their retinas were examined ultrastructurally, Retinal catalase activity and malondialdehyde (MA) levels were also assayed.

Structural changes after 8 and 12 weeks included swelling of the retinal pigment epithelium (RPE), thickening of the RPE basal lamina, and an increase in lipofuscin within the RPE of exposed animals. Thinning of the photoreceptor nuclear layer also occurred.

Catalase activity was decreased in the exposed animals and MA levels were increased.

These findings point to an effect of lead on the oxidant status of the retina and further indicate the sensitivity of these organ to pro-oxidant stresses.

LEAD UPTAKE IN BRUSH-BORDER VESICLES FROM RAT KIDNEY CORTEX. W. Victery and B.A. Fowler, Laboratory of Pharmacology, NIEHS, Research Triangle Park, NC 27709

Lead accumulates rapidly and quite specifically in renal proximal tubule cells but the mechanism of this process is not well understood. In this study, isolated plasma membrane vesicles from rat kidney cells were used to evaluate the nature of the lead transport process. Vesicles prepared according to the technique of Beck and Sacktor were incubated with Pb-203 with carrier Pb acetate under a variety of conditions. At timed intervals (0-90 min), the reaction was stopped by addition of cold buffer and rapid Millipore filtration. Washing the vesicles with buffer containing 1 mM Pb acetate or 1 mM CaCl2, showed that Pb was more effective in displacing non-specifically bound label and hence all experiments were performed with 1 mM Pb acetate in the stop bath. Lead uptake was found to be time dependent and appeared to saturate at 100 µM Pb in the incubation mixture. To differentiate between Pb uptake and binding, Pb uptake was studied in vesicles whose volume was osmotically altered by sucrose addition to the incubation media. It was found that Pb concentration in the vesicles varied directly with vesicle volume indicating uptake. Vesicle incubation in either a NaCl or KCl gradient (extravesicular > intravesicular concentration) increased the initial rate of Pb uptake by approximately 50% but not the final equilibrium value. Incubation of vesicles at 0°C did not alter Pb uptake; however, incubation with either 5 mM EDTA or EGTA reduced Pb uptake to approximately 30% of control values. The data indicate that Pb is extensively bound to the brush-border membrane under in vitro conditions but also demonstrate that accumulation occurs within the membrane vesicles and that the rate of this process can be influenced by extravesicular ion composition. (Supported by NIEHS 1 F32 ES05213).
Increases in NSE are observed during postnatal neuronal maturation and following treatments which increase dopaminergic activity. Since perinatal lead treatment has been associated with delayed CNS development and altered dopaminergic function, the objective of this study was to determine the effect of lead on NSE levels and total enolase activity in the weanling rat.

Newborn Sprague-Dawley rats received lead acetate (10 mg/kg/day, i.p.) and littermate controls received equimolar sodium acetate from day of birth to postnatal day 20. Pups were sacrificed on day 21 by decapitation, trunk blood collected, and brains dissected into four areas; cerebrum, cerebellum, hippocampus and the rest of the brain (brainstem).

There was no change in the specific activity of total enolase in any brain region while NSE levels were unaltered except in the brainstem where an increase (18.8% p< 0.05) was seen. The ratio, µg NSE/unit enolase, was also increased (26% p< 0.05) in brainstem only.

These data indicate: (1) neuronal maturation as measured by brain NSE levels is not decreased at postnatal day 21 by perinatal lead treatment at doses which produce a decrease in body weight and (2) perinatal lead exposure increases the fraction of NSE isozyme in the brainstem. (Supported by USPHS NIH Grant No. ES 01638)

The presence of ultrastructurally discernible intranuclear inclusion bodies (INIB) in certain tissues is considered to be a characteristic feature of lead poisoning. INIB have been identified as lead bound to non-histone acidic proteins (Lab. Invest. 29: 488, 1973).

A previous study from our laboratory, we have shown the formation of characteristic INIB (Toxicol. Appl. Pharmacol. 56: 418, 1980) in rat kidney epithelial cells in primary cultures, on exposure to an equimolar complex of lead-glutamic acid (PbGlu).

Even though the induced formation of INIB due to lead exposure has been known for some time, its role in Pb induced nephropathy is not yet fully understood. The presence of Pb induced INIB could result in either cellular resistance or susceptibility to further exposure to Pb.

In order to elucidate their function in Pb toxicity, rat kidney cells were isolated and maintained in MEM-D-Val. to confluency. The resulting epithelial cell colonies were treated with 10⁻⁵M PbGlu for 2 days repeatedly to form INIB. These pretreated cells and control cells were again exposed to Pb-Glu (10⁻⁵M to 10⁻²M Pb) for 30 min. The cellular toxicity of Pb was measured using an inverted phase contrast microscope or by membrane leakage test using ³H Deoxy Glucose loading. The Pb pretreated cells showed significantly toxic effects of further exposure to Pb than the control cells both in morphology and in the membrane leakage test. The results suggest that INIB may have a protective role in Pb toxicity.

(Supported by NIH, ES 01535)
by 120 hours post-injection (p.i.) in the next four age groups. Kidney weights were unaffected at the younger ages, but were 15–50% higher than controls at the older ages. The treatment produced a transient polyuria by 48 hours p.i. at all ages. Urinary osmolality was lowered in all rats except those treated on day 1. This effect disappeared by 120 hours at the early ages, but persisted in the more mature animals. Urine pH was lowered in animals treated on days 8, 15 and 22, but was raised in those injected on day 29. Urine chloride content was reduced in MC exposed rats. Urinary protein content was elevated at all ages except the earliest, but the degree of proteinuria was more severe in the older animals. Glucosuria was detected in animals in the three oldest age groups at 24 and 48 hours p.i., and was most severe at the oldest age. Hence, the neonatal kidney is largely insensitive to MC toxicity. The renal response to MC increases sharply during the preweaning period.


A common early symptom of human methyl mercury poisoning is cutaneous paresthesia—distal hypaesthesia, numbness and tingling. These effects have not been studied in animals. Here we characterize the cutaneous deficits which follow acute exposure to this neurotoxic substance in rats using the method of reflex modulation to measure sensory dysfunction. Adult rats received a total body burden of 40 mg/kg(n=25); 13 mg/kg(n=6); or 0 mg/kg(vehicle; n=17) methyl mercury chloride in 5 days. At various times after poisoning (+1 day to +20 weeks) cutaneous sensitivity and reactivity were assessed. Weak shocks (60-220µA) were delivered to the tail 80 msec before an intense tone(120db) which elicited a startle reflex, and their sensory impact defined by reflex inhibition. On other days tail shocks of higher intensities (100-500µA) were given to elicit flinch responses. High dose rats showed a steady loss of cutaneous sensitivity for about 4 weeks, with substantial recovery at 20 weeks. High dose rats became hyperreactive at about 4-5 weeks after poisoning as did low dose animals at 20 weeks. Hyperreactivity must be based on a different structural deficit than cutaneous loss because it had a different temporal course and appeared in different animals. Structural damage will be assessed at the end of behavioral testing. These data demonstrated that reflex modification methods are useful in the analysis of sensory dysfunction following toxic exposure and that the rat model of methyl mercury poisoning is a reasonable analog for human exposure. Supported by NIHES grant ES02230 and ES05207.


The clinical syndrome of methylmercury intoxication frequently includes skeletal muscle weakness. The purpose of the present study was to determine if, and how, acute exposure with methylmercury altered neuromuscular transmission in vitro in a mammalian skeletal muscle preparation. Effects of methylmercury (4,20, and 100 µM) on postsynaptic potentials were assessed in the rat phrenic nerve-hemidiaphragm using conventional microelectrode recording techniques. At concentrations of 20 and 100 µM, methylmercury increased the frequency of miniature end-plate potentials (MEPPs) from control values of 0.3-0.6/sec to 1.5-10/sec. Increase in MEPP frequency occurred after 15-40 min of exposure to 20 µM, and 5-15 min after 100 µM methylmercury. At a concentration of 4 µM, methylmercury did not increase MEPP frequency, and, in fact, slightly decreased it. MEPP amplitude was not significantly altered by any of the above concentrations of methylmercury. End-plate potentials (EPPs) evoked under diminished quantal content were decreased in amplitude, and finally blocked by 20 and 100 µM, but not by 4 µM methylmercury. EPP after 30-40 min block with 20 µM and after 4-5 min with 100 µM methylmercury. At the time of EPP block, MEPPs were still observed. Resting membrane potential of muscle fibers was not affected significantly by any concentration of methylmercury. The observed effects of methylmercury were not reversible upon washing it out. These results are consistent with the hypothesis that methylmercury alters presynaptic function. Supported by NIH grants ES02320 and ES05207.

**204 CALMODULIN INHIBITORS PROTECT RATS AGAINST MERCURIC CHLORIDE-INDUCED NEPHROTOXICITY.** Jana L. Cox, R. C. Giles*, and S. D. Harrison, Jr., Graduate Center for Toxicology and Animal Disease Diagnostic Center*, Univ. of Kentucky, Lexington, KY.

Because prochlorperazine protects mice against nitrosourea nephrotoxicity (Pharmacologist 19: 236, 1977), we have studied a series of similar compounds in a different model. Young, adult male Sprague-Dawley rats received a single ip dose of HgCl2 (1 mg/kg) alone or in combination with one of 5 calmodulin inhibitors (CI, 4 or 18 µmol/kg/day ip daily for 5 days, day 1 = day of HgCl2): Prochlorperazine (PCP), trifluoperazine (TFP), perphenazine (PFA), fluphenazine (PFA), or chlorprothixene (CPT). On days 1, 3, 6 and 15, 24-hr urine samples were collected for measurement of creatinine (Cr), leucine aminopeptidase (LAP), and N-acetyl-glucosaminidase (NAG). Rats were killed on day 16, and their kidneys were weighed and fixed in 10% formalin (pH 7) for histopathologic evaluation. HgCl2 produced elevated urinary enzyme activity on day 1. Histopathologic evaluation of kidneys from representative rats confirmed tubular dilatation and necrosis. Rats treated with HgCl2 + CI exhibited less elevation of one or both of LAP and NAG on day 1. On day 1, NAG activity (U/mg Cr) after HgCl2 alone was 40 ± 7 (X ± SEM); NAG activity after HgCl2 + CI (4 µmol/kg/day) with: PCP, 33 ± 13; TFP, 13 ± 3; PFA, 17 ± 2; PFA, 32 ± 4; CPT, 19 ± 2. The higher CI dose (18 µmol/kg/day) resulted in more dramatic attenuation of enzyme activity by PCP, TFP, and PFA, but data for PFA and CPT suggested additive toxicity.
Cytoprotection by CI was confirmed histopathologically. The possible role of calmodulin in HgCl$_2$-induced nephrotoxicity seems to warrant further study.


The effects of triethyltin (TET) on the content of specific proteins from subcellular fractions of the central nervous system were assessed in the developing rat. Myelin (M), synaptic plasma membrane (SPM) and synaptosomal cytosol (SC) fractions were prepared from 22 day old Long-Evans rats that had received either saline or TET (3.0 & 6.0 mg/kg, i.p.) at 5 days of age. The day of birth was taken as day zero. In order to detect the presence of specific phosphoprotein markers associated with M and SPM, the endogenous phosphorylation of these fractions was assayed in vitro using $^{32}$P ATP as a phosphate donor. The protein composition of each fraction was examined by sodium dodecyl sulfate polyacrylamide slab gel electrophoresis. Specific protein and phosphoprotein content of each fraction was quantified by microdensitometry. The postnatal day-5 administration of TET caused a dose-related decrease in the concentration of two proteins associated with the M-enriched subfraction. At a dose of 6.0 mg/kg of TET, these proteins were depleted approximately 60% relative to control values. The affected proteins were of molecular weights (M.) 20,000 & 25,000, values that closely approximate those associated with the M basic and M proteolipid protein, respectively. The electrophoretic patterns of SPM and SC proteins were not affected by TET. Specific phosphoprotein markers associated with SPM were also not affected by TET, however, a phosphoprotein corresponding to M basic protein was markedly reduced in the M fraction. These results suggest that early postnatal administration of TET may interfere with myelogenesis.

206 TRIETHYLTIN REDUCES THE RESTING MEMBRANE POTENTIAL OF RAT SOLEUS MUSCLE IN SITU. W.R. Millington and G.G. Bierkamper, Laboratory of Neuromuscular Toxicology, The Johns Hopkins University, Baltimore, Md. (Sponsor: L. Pechter)

Muscle weakness is a prominent component of the toxic syndrome which results from prolonged exposure to triethyltin (TET). The etiology of this phenomenon includes, in part, an alteration of acetylcholine (ACh) release from motorneurons but there are also indications that TET causes a primary myopathy. We have found that chronic TET exposure (30mg/L. drinking water) causes a time-dependent reduction of resting membrane potentials (RMP) recorded from the soleus muscles of intact anesthetized rats. The RMPs of TET-exposed rats were significantly lower than those of paired control animals after four days (5.4 mV). They were further reduced by continuous TET exposure for 8, 14 and 21 days to a maximum of 11.2 mV less than control on day 28. TET caused a progressive hindlimb paralysis that paralleled the reduction of RMPs; both were completely reversible. Three weeks after rats were withdrawn from TET RMPs were restored to control values and the animals appeared to be motorically normal. TET had no effect on the frequency, amplitude or incidence of miniature endplate potentials. Spontaneous ACh release and its action on the postsynaptic membrane were not affected by TET suggesting that TET reduces RMPs by inhibiting the bioenergetic capacity of the muscle and that this myogenic toxicity is a significant factor in the development of muscle weakness following exposure.

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207 EFFECTS OF ORGANOTINS ON MAXIMAL ELECTROSHOCK SEIZURE(MES) RESPONSIVENESS IN MICE. S. V. Doctor and D. A. Fox. Div. of Tox., Univ. of Texas Med. Sch., Houston, Texas.

Organotin compounds are gaining commercial importance as biocides, catalysts and as polymer stabilizers. In this study the neurobehavioral effects of structurally related organotins were studied using the MES test. Male mice were injected (i.p.) with either 0, 3.5 or 17.5 µmoles/kg of trimethyltin(TMT) bromide, triethyltin(TET) bromide, tri-n-propyltin(TPT) chloride, tri-n-butylin(TBT) bromide, tricyclohexylin(TCT) bromide or triphenyltin(TPhT) acetate. Additional groups of mice were injected with 26.25 µmoles/kg of TCT or TPhT, 17.5 or 70 µmoles/kg of sodium bromide (NaBr) or 17.5 µmoles/kg of stannic bromide (Sn Br$_4$). Mice were seizure tested at 0.5, 4, 21-24 and 96 hrs following the injections. Mice exposed to TMT, TET, TPT or TBT exhibited dose dependent decreases in MES severity, assessed by seizure grade distributions and seizure phase durations. The tri-n-alkyltins, in order of decreasing ability in reducing MES responsiveness, were: TMT> TET> TPT> TBT. In contrast to the tri-n-alkyltins, TCT and TPhT did not produce a significant change in seizure grade distributions. However, mice treated with the two higher dose levels of TCT or TPhT exhibited an increased seizure severity, assessed by phase durations, at 30 min post-treatment. At 4 and 24 hrs post-treatment, exposure to the two higher doses of TPhT caused a decrease in MES severity followed by a recovery of normal seizure severity by 96 hrs. Conversely, mice exposed to the higher doses of TCT exhibited an increased MES severity at 4, 24 and 96 hrs post-treatment. No changes in seizure severity were observed in groups of mice treated with NaBr or SnBr$_4$. (Supported by NIEHS Tgr. Grt. #ES07090, NIOSH Grt. #800705 and U. T. Bio. Med. Res. Supp.)


Trimethyltin (TMT) is an organometallic neurotoxin which produces specific neuronal lesions in discrete brain regions. Damaged areas include the hippocampus, pyriform cortex, septal nucleus,
and amygdala. These lesions have been shown in the rat to be accompanied by hyperirritability and possibly increased agonistic behaviors.

The present study examines the toxicity of TMT in a wild-type rodent, Onychomys torridus, the grasshopper mouse. These carnivorous rodents not only display a diverse repertoire of social behaviors, but also show predatory behavior towards prey such as crickets.

Same-sex pairs of male and female Onychomys were exposed (p.o.) to 0, 0.5, and 1.0 mg/kg TMT weekly, for 3 weeks. The performance of the animals was monitored weekly on a variety of behavioral tasks both before, during, and following exposure. These tasks consisted of a measure of activity in running wheels, of the auditory startle response, cricket-killing as a measure of predatory aggression, as well as complex social behaviors in paired animals.

Results indicate sex- and dose-related disruptions of complex social, agonistic, and general activity behaviors of the mice, independent of effects of the toxin on activity and reactivity as measured by the tests of auditory startle response and running wheel performance.

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209 THE PULMONARY RESPONSE TO SIZED GLASS FIBERS IN RATS. D.M. Bernstein, R.T. Drew, M. Kuschner, Medical Dept., Brookhaven National Laboratory, Upton, NY 11973

A number of studies have suggested that both length and diameter of glass fibers are important parameters in determining their deposition and translocation in the lung and in the occurrence of a pathological response in the lung. To characterize the biological response to glass fibers, a study has been conducted to determine the translocation and ultimate fate of fibers of defined sizes after introduction into the respiratory tract of rats by instillation and inhalation. The fibers have geometric mean diameters of 1.5 µm ($\sigma = 1.1$) and lengths of either 5 µm ($\sigma = 1.49$) or 60 µm ($\sigma = 3.76$).

Serial sacrifices following intratracheal instillation of either 2 mg or 20 mg doses have shown the short fibers to lie primarily within mononuclear phagocytes in both the lung and lymph nodes. The majority of long fibers, however, cannot be totally engulfed by macrophages, nor are they cleared to the lymph nodes, although smaller fragments accompanying the long fibers may be so cleared.

The long fibers produce a striking foreign body reaction in the lung, particularly when impacted in the bronchi. Over 90% of the fibers deposited in the lung were cleared from the animals. Both long fibers and short fibers were found in the pleural cavity. At 18 months post exposure the surface area of both the long and short fibers were found to have been reduced by 25 to 50 percent through dissolution. The dissolution of these fibers may be an important factor in the lack of any observed fibrogenic or carcinogenic response to these fibers in the animals.

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210 DEPOSITION OF INHALED FIBERS IN THE BEAGLE DOGS, L. C. Griffis, S. J. McAllen and J. A. Pickrell (Sponsor: R. O. McClellan), Lovelace Inhalation Toxicology Research Institute, P.O. Box 5690, Albuquerque, NM 87185

The pulmonary deposition of inhaled asbestos and glass fibers has been predicted by others using models which incorporated the aerodynamic behavior of these materials. The purpose of these studies was to measure the deposition of very small asbestos and glass fibers inhaled by the Beagle dog. Crocidolite asbestos were radio-labeled with 14C by neutron activation. Young adult Beagle dogs from our colony were given 60-minute nose-only exposures to an aerosol of either asbestos or glass fibers which were generated in a fluid bed aerosol generator. Dogs were killed and tissue samples taken either 4 days or immediately after exposure. The mass of fibers in a sample were determined from the radioactivity in the sample and the specific activity of the fibers in the exposure aerosol. Four dogs exposed to a mean asbestos concentration of 71 µg/L deposited a total of 64 ± 6% (mean ± SD) of the inhaled fibers. Alveolar deposition was estimated from the activity remaining in the lung 4 days after exposure and was 17 ± 3%. Deposition normalized for tissue weight in the right apical and left diaphragmatic lung lobes was 20% higher and 13% lower than the mean lobar deposition with the other lobes between these values. The measured alveolar deposition for crocidolite asbestos in the dog is in excellent agreement with the predicted value (~17%) for deposition of fibers of this size in the human lung. (Research supported under DOE Contract No. DE-AC04-76EV00103.)


Acute inhalation studies were performed using a low density (.051 g/ml) amorphous silicon dioxide dust which is used as extending filler. A modified version of a NBS dust generator was used to aerosolize the material. Chamber concentrations were determined gravimetrically. Male and female Sprague-Dawley rats were exposed to 1.12 mg/l for 4 hours which resulted in 55% mortality by one day after the exposure. Values for mass median aerodynamic diameters (MMAD) were 7.8 and 5.8 um, with geometric standard deviations (og) of 3.25 and 2.16, respectively. Although this level is less than 1/4 of the EPA-prescribed concentration for acute inhalation testing, the cause of death was apparently suffocation by the dust and not due to any intrinsic toxicity. The surviving rats showed temporary clinical effects, but necropsy after 14 days showed no apparent adverse effects of the exposure. Rats were also exposed for 1 hour to 1.06 mg/l with a MMAD of 3.45 um and og of 2.72. The rats experienced temporary clinical effects, but there were no deaths and no adverse effects were found at necropsy 14 days later. Extensive modifications were made to the generation and sampling systems, and rats were exposed for 1 hour to 2.57 mg/l with a MMAD of 3.95 um
and of 1.80. Again, the rats experienced some temporary clinical effects, but there occurred no deaths, and no persistent adverse effects. The implications of acute inhalation exposures to required very high levels of low density dusts are discussed.


We studied the effect of zinc oxide aerosols, ZnO (CMD 0.05 µm, σg 2.0), generated by the condensation of supersaturated vapors, in the presence and absence of moisture and sulfur dioxide (SO2). We evaluated lung volumes, single breath diffusing capacity of the lungs for carbon monoxide (DLCO), dynamic compliance (Cdyn) and flow resistance (RF) in male Charles River Hartley guinea pigs immediately after exposure. Animals were exposed for 3 hrs to 1 ppm SO2 and 0.7, 1.4, 2.3, or 6.0 mg/m3 ZnO mixed in a furnace at 480°C. Water vapor was added either to the exposure chamber or to the furnace. SO2 and 1.4 mg/m3 ZnO mixed in a dry furnace and administered to animals in a humid chamber caused a 10% decrease below control values in vital capacity (VC), functional residual capacity (FRC) and total lung capacity (TLC) with no changes in the alveolar volume (VA) and DLCO. Cdyn was increased and RF was decreased. In animals exposed to SO2 and 2.3 and 6.0 mg/m3 ZnO generated in a humid furnace, lung volumes (VC, TLC, FRC) decreased by 12% and DLCO by 23% and 53% respectively. VA was decreased by 11% in the 6.0 mg/m3 ZnO exposure group. Cdyn and RF values did not differ from those of controls in both these groups. The nature of the response, decreases in lung volumes, VA and DLCO, suggests an effect in the periphery of the lung probably involving the closure or narrowing of small airways. Supported by NIEHS grant ES 02429-01.

213 PREVENTION OF CdO INDUCED IMPAIRED LUNG CLEARANCE BY SIMULTANEOUS ZnO INHALATION. Oberdorster, G., Hochrainer, D. and Oldiges, H. Dept. RBB, Medical Center, University of Rochester, Rochester, NY 14642 and Fraunhofer Inst. for Toxicology and Aerosol Research, 5948 Schmallenberg, W.-Germany. (Sponsor: Morrow, P.E.)

It has been shown in several studies that Zn can protect the organism against certain effects of Cd. Similarly, deficiency of Zn enhances the toxicity of Cd. In our study we wanted to find out if and to what extent the effect of Cd inhalation on lung clearance is prevented by Zn inhalation. Four groups of 20 rats each were used: one group was continuously exposed to CdO aerosols, 10 µg/m3 (mass median aerodynamic diameter 0.46 µm, σg = 1.5). A second group was continuously exposed to a combination of CdO (10 µg/m3) and ZnO aerosols (250 µg/m3). Two groups served as unexposed controls for the experimental groups. After 6 and 5 weeks of continuous exposure, a 1 hr nose-only exposure to 59Fe2O3 in all groups was performed where the inert iron oxide particles served as testaerosol for measuring lung clearance. This clearance was measured up to 70 days. It was found that 1) CdO inhalation increased long-term lung clearance of Fe2O3 significantly. 2) Combined exposure to CdO + ZnO did not influence this lung clearance. The biological half lives for the slow phase of Fe2O3 clearance from the lung in the control groups was 37 and 40 days, respectively. After Cd exposure, this was 82 days and after combined Zn and Cd exposure it was 32 days. The fast phase of Fe2O3 lung clearance was not affected by CdO or by the combined exposure.

Thus, simultaneous CdO-ZnO inhalation counteracts the impaired lung clearance following CdO inhalation alone. In the lung, too, Zn shows a protective effect on Cd toxicity.


This study deals with the hypothesis that the lymphatic uptake of particles from the lung parenchyma increases when phagocytosis by pulmonary macrophages is inhibited. An in vitro assay for phagocytosis of latex particles was used to quantify macrophage function. Lung clearance, lymphatic uptake, and macrophage function in vivo were assayed by exposing the animals to titanium dioxide (TiO2) dust. Cadmium chloride (CdCl2) was chosen as the toxicant to inhibit phagocytosis. Rats were exposed to CdCl2 aerosols at 2.5 mg Cd/m3, MMAD=0.35 µm, σg =1.43, or 5.0 mg Cd/m3, MMAD=0.42 µm, σg =1.60, for 30 minutes. Control animals received a saline aerosol. At various times post exposure, the lavageable macrophages were purified on an albumin gradient and assayed, in vitro, for phagocytic activity. Phagocytosis was depressed for 6 days in rats exposed to 5.0 mg Cd/m3. Macrophages from animals exposed to 1.5 mg Cd/m3 appeared to be activated immediately and at one day post exposure. To examine the effects on clearance and lymphatic uptake, rats were exposed to CdCl2 aerosols and TiO2 dust, 13-15 mg/m3, MMAD=1.03 µm, σg =2.29. Pre-exposure to 5.0 mg Cd/m3 decreased the initial deposition of TiO2 by 40 percent when compared to a saline aerosol preexposure. Although the overall clearance of the dust was not different in the cadmium-exposed animals, the lymph node burden was 2.7 times higher in the CdCl2-exposed animals than in the controls. Exposure to 1.5 mg Cd/m3 had no effect on lung clearance or lymphatic uptake. When TiO2 exposure preceded a 5.0 mg Cd/m3 exposure, the results were similar.
Insoluble crystalline NiS particles produce severe inflammatory reactions at sites of injection with the subsequent development of sarcomas. These particles have been shown to cause morphological transformation of Syrian Hamster Embryo (SHE) cells by a mechanism dependent on particle phagocytosis (Costa, M. and Mollenhauer, H.H., Science 209, 616, 1980). The purpose of this study was to further elucidate the subcellular events associated with NiS-induced transformation through the use of time-lapse Video Intensification Microscopy (VIM). Alternate use of fluorescent phase, and bright field VIM allowed for visualization of both NiS particles (bright field) and intracellular components such as lysosomes (phase microscopy). Cultivation of SHE and Chinese hamster ovary cells in a Dvorak chamber improved resolution of intracellular organelles at high magnification. Particle phagocytosis occurred primarily in regions of active cellular ruffling without the noticeable appearance of a vacuole. Following endocytosis, lysosomes moved toward and aggregated around the internalized particle. Lysosomes were repeatedly observed to contact the particle-vacuole complex. This lysosomal contact may participate in the dissolution of crystalline NiS particles. After 24 hours most particles were surrounded by lysosomes, and aggregated around the nucleus. It is possible that dissolution products Ni(2+) from endocytized NiS, released in proximity to the nucleus may be the ultimate agent causing cellular transformation. (Supported by grant #ES-02487 and #ES-070 90 from NIH and by grant #R808048 from the U.S. EPA).

**216 TOXICITY OF AIR CONTAMINANTS DETERMINED BY LAVAGABLE LUNG CELLS.** Oberdorster, G., Ferin, J., Pott, F. and Morehouse, B. Dept. RBB, Medical Center, University of Rochester, Rochester, NY 14642 and Med. Institute for Air Hygiene and Silicosis Research, University of Dusseldorf, 4 Dusseldorf, W.-Germany. (Sponsor: Shaikh, Z.)

It has been observed that some pollutants with known pulmonary toxicity (e.g., PbO, SiO2) do not suppress pulmonary defense against bacteria when tested by the "infectivity model". Using a number of compounds with various toxicity, including PbO and SiO2, we studied their effect on pulmonary cells. Peroxidase positivity has been linked to young alveolar macrophages (AM), making it possible to distinguish between old and new populations of macrophages. Peroxidase staining was used, therefore, to assess AM response. The following substances were instilled intratracheally into rats, after suspension or dissolution in water: CdS04 (100 µg), PbO (1 mg), SiO2 (500 µg), TiO2 (500 µg), urban air dust sample of the Ruhr valley (5 mg), H3O (0.3 ml). The instilled volume was kept at 0.3 ml. Twenty-four hours later, the lungs of the animals were lavaged and a differential cell count was performed. Compared to the control (H2O) rats, urban air dust, PbO and SiO2 led to an increase in total cell numbers, whereas CdS04 and TiO2 did not. Numbers of PMN's were increased in the urban air dust, PbO, SiO2 and CdS04 group, whereas macrophage numbers had decreased in CdS04, PbO, air dust and SiO2.

Numbers of peroxidase positive macrophages increased in the urban air dust, SiO2 and PbO group. Although macrophage response may also depend on lung dust burden, the inactivity of PbO and SiO2 in the bacterial challenge model may partly be explained by an influx of pulmonary macrophages.


Sensory irritation of fly ash from oil-fired (OF), fluidized bed coal (FB), and conventional coal (CC) combustion power plants, and from Mt. St. Helens volcano (VA) was studied in mice. The irritating sensation due to contact with ash was quantitated by observation of a characteristic contraction of the dorsal musculature of the mouse following intraperitoneal injection of the ash suspension. Dose-response curves and estimation of the 50% effective dose were based on the percentage of animals showing a positive response at various ash concentrations. Procedural modifications improved reproducibility; validation studies using 10 environmental chemicals confirmed that a positive correlation exists between this irritation test and the upper respiratory tract irritancy test of Alarie. The relative ranking of irritant potency was: Of and Of leachate>>FB<CC> VA. Other than OF fly ash, saline leachates showed no irritating effects. OF fly ash was about 160X more irritating than VA and 5X more irritating than a reference compound, sodium dodecyl sulfate. Study of chemicals present in fly ash or similar to those present indicated that acidic or basic compounds, heavy metal ions and metal oxides, and insoluble particles could all contribute to the irritancy of an ash. Leachable heavy metals appeared to be mainly responsible for the irritating effects of OF ash, while insoluble particles, including metal oxides, appeared to account for the irritancy of the other fly ash samples. The mouse peritoneal irritation test, shown previously to correlate with controlled human eye sting studies on shampoos, appears to be also relevant to study of environmental chemicals. It may be useful for samples difficult to aerosolize or for those available only in small quantities.


During the development of a test method for the toxicological assessment of inhaled combustion products, we measured both time-to-incapacitation (hind-leg flexure model) and lethality in Fisher 344 rats. Both time-concentration curves (time-to-incapacitation vs. mg/1*) and EC50 values (mg/1* which cause 50% of the rats to become incapacitated) were used to classify materials. Most of the animal exposures were 30 min; a few were allowed to continue until 100% were incapacitated. In those 30 min exposures where 2/6 to 5/6 animals were incapacitated, a statistical

The LC$_{50}$ (30 min exposure and 14 day post-exposure period) of PTFE thermal degradation products was established to be 0.045 mg/l* as compared to 22.8 mg/l* for Douglas fir in male rats. Because of the much higher acute toxicity of PTFE, a study was initiated to evaluate the physiological and pathological changes induced by PTFE thermal decomposition products. Groups of six rats were exposed for 30 min to a single concentration of products produced by decomposing PTFE at 595°C. The concentrations ranged from 0.005 to 5.025 mg/l*. Arterial blood, heart rate (HR) and direct arterial blood pressure (ABP) were measured in the exposure and at 12, 24, and 36 hrs PE from rats exposed to 0.045 mg/l*. Necropsies followed the PE monitoring. Respiratory impairment seen in rats exposed to 0.045 mg/l* during the exposure became more severe in the PE period as indicated by changes in blood CO, Hb, PO$_2$, PCO$_2$, and pH. Normal mean values + SE for these parameters were 93.7 ± 0.7, 91.8 ± 2.7, 30.4 ± 1.0, and 7.448 ± 0.011, respectively. By 36 hrs PE, the CO, Hb, PO$_2$, and pH decreased to 69.9 ± 8.5, 58.7 ± 5.9 and 7.347 ± 0.018. PCO$_2$ increased slightly to 32.9 ± 2.2. Bradycardia and depression of ABP were evident within the exposure and PE. By 36 hrs PE, HR decreased from 513 beats/min to 287 ± 59; normal systolic ABP decreased from 158 ± 2 mmHg to 94 ± 7 and diastolic ABP declined from 129 ± 1 to 80 ± 6. Histologic evaluation of tissues revealed pulmonary lesions and evidence of disseminated intravascular coagulation. The observed histopathology was both concentration and time related.

Boron trifluoride forms a liquid dihydrate in air containing as little as 50 ppm of water. It was, therefore, decided to evaluate the inhalation toxicity of boron trifluoride using an aerosol of the dihydrate. Using Fischer 344 rats, the four-hour inhalation LC$_{50}$ was 1.21 mg/l. The signs elicited by the exposures included symptoms of respiratory irritation as well as body weight depression. For the surviving rats, there was an apparent recovery. Increases in both liver and kidney weights were found in all treatment groups when compared to the control. Subsequently, a two week inhalation toxicity study was performed. Four groups of 10 rats were exposed to liquid aerosols 6 hours/day, 5 days/week to exposure concentrations of 0, 24, 66 and 180 mg/m$^3$. By the end of the first week, all rats in the 180 mg/m$^3$ group had died. There was no mortality in the two lower-level exposure groups although both groups showed symptoms of respiratory irritation and body weight gain depression. Lung weights and lung/body weight ratios were elevated in all exposed groups. Following the two-week study, a thirteen-week study was initiated. Groups of 40 rats were exposed 6 hours/day, 5 days/week to concentrations of 0, 2, 6 and 18 mg/m$^3$. Observations through the first 6 weeks of the study have not demonstrated overt signs of a toxic response; hematological and clinical laboratory parameters measured in all groups during Week 5 appeared normal. Measurements of urinary and serum fluoride levels showed increases in all treatment groups when compared to control values. Final results will be discussed.

Inhalation Toxicology Studies with Boron Trifluoride Dihydrate. G. M. Rusch, G. M. Hoffman, W. E. Rinehart

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The carcinogenicity of the pesticide Maleic Hydrazide (MH) was studied in C57BL/B6 mice. The free acid of MH was used (98.5% pure; containing 0.6 ppm of a hydrazine impurity). The carcinogenicity experiments with MH included: a) subcutaneous (s.c.) administration to 163 infant mice (5, 10, 20 and 20 mg MH per mouse on days 1, 7, 14 and 21 after birth, respectively); and b) oral administration (gavage) to 82 male and female mice, from weaning for life, at a weekly dose of 510 mg/kg bw MH. Groups of untreated and solvent controls were also available. The experiments were terminated at 120 weeks. The s.c. administration to infant mice resulted in high preweaning mortality when compared with the respective controls. Liver-cell tumours were observed in 7 males treated s.c. with MH. In the solvent-treated (tricaprylin) males, 3 liver-cell tumours were also found. One liver-cell tumour was seen in untreated male controls. No liver-cell tumours were observed in treated or control females. The difference in the incidence of liver tumours among the various groups was not significant. When MH was administered orally (weekly for life), liver-cell tumours were observed in 4 males and in 2 females of the treated group, and in 1 male and in 1 female of the solvent (olive oil) control group. In the respective untreated controls
1 male had a liver-cell tumour. The incidence of tumours at sites other than the liver was similar in all groups. These results partially confirm the negative finding of a recent parallel study in rats (Van Der Heijden, Toxicology, 18: 139-150, 1981).


A 24-month industry supported study was conducted in Sprague-Dawley, Spartan substrain rats to assess the chronic toxicity and oncogenicity of acrylonitrile (AN) following 24 months of inhalation exposure. Groups of 100 rats per sex were exposed to either 0, 20 or 80 ppm AN for 6 hours per day, 5 days per week for 24 months.

Evaluation of hematology, urinalysis, and clinical chemistry parameters did not reveal any alterations indicative of an adverse effect on bone marrow, kidney, or liver function. Decreased body weight, early mortality, unthrifty clinical appearance, and increased incidence of palpable tumors were noted in exposed rats, especially at the 80 ppm level. Early mortality in the exposed rats resulted in a decreased incidence of pathologic changes usually seen in aged rats.

A statistically significant (p<0.05) increased incidence of tumors in the central nervous system (CNS), ear canal gland (Zymbal gland), tongue, and small intestine was observed in the 80 ppm group of males. In the 20 and 80 ppm groups of female rats the tumor incidence was statistically significantly increased in the CNS and the mammary gland. In addition, in the 80 ppm female group the incidence of ear canal gland (Zymbal gland) tumors was increased. Tumors considered treatment related but not occurring at a statistically significant increased incidence were also noted in the stomach of 80 ppm male rats, the brain of 20 ppm male rats, and the nasal turbinate of 80 ppm female rats. An apparent decrease in tumors of the pituitary, adrenals, thyroid, pancreas, and testes was observed in exposed rats.


Promoting effect of phorbol esters, as represented by 12-0-tetradecanoyl phorbol-13-acetate (TPA), has been extensively studied on mouse skin, using a conventional initiation-promotion protocol. Since it has been shown that these tumour promoters exert a variety of biological and biochemical effects on the cells derived from various tissues other than mouse skin, it was of our interest to examine the promoting activity of TPA on internal organs as well as on skin. When fifty C57BL/B6 mice, 6 week old, were painted once with 3 mg of benzo[a]pyrene (B[a]P) and painted with 5 µg of TPA twice a week, 64% of mice developed papillomas by 30 weeks of treatment, whereas none of control groups, treated with B(a)P alone or TPA alone, developed papillomas. These results confirm that C57BL/B6 mice are reasonably sensitive to a conventional two-stage carcinogenesis protocol. However, when the same amount of B(a)P was injected subcutaneously to pregnant mothers and their descendants were painted on skin of the back with 5 µg of TPA twice a week from weaning for 93 weeks, no persistent papilloma was observed. Similarly, the transplacentally exposed mice were injected intraperitoneally with 5 to 10 µg of TPA twice a week. After 100 weeks, the number of mice that died with tumour (4/63) was not significantly different from that in the group without TPA injection (3/64). Taken together, these results suggest that under the experimental conditions used, a conventional mouse skin initiation-promotion carcinogenesis is demonstrable, but transplacental initiation-postnatal promotion of skin and internal organs has not been clearly observed so far in C57BL/B6. The experiment is being continued and all the surviving mice are scheduled to be sacrificed at 120 weeks of age.


Diesel particulates and dichloromethane extract of diesel particulates were tested to assess their potential as complete carcinogens and as initiators or promoters of carcinogenesis. The test agents were applied as suspensions to the dorsal skin of male C3H mice at the highest doses that were non-irritating and sufficiently flowable for application. Lower concentrations were also used to obtain information on the dose-response relationships. Acetone was used as the vehicle in all studies; diesel particles were applied in 10% or 5% concentrations, and dichloromethane extract was administered as 50%, 25%, 10%, or 5% suspension. Dosing was performed 3 times per week in the initiation and complete carcinogenesis studies, and 5 times per week in the promotion studies. Positive control groups received repeated application of 0.2% benzo[a]pyrene (B[a]P) for complete carcinogenesis, or a single application of 1.5% B[a]P, or of 10% particulates or 50% of particulate extract suspensions, followed by repeated application of 0.0001% phorbol myristate acetate (PMA) for the initiation studies. In promotion studies, repeated application of PMA was replaced by 10% suspension of diesel particulates or 50% and 25% of extract suspensions. The test agents were applied similarly in place of B(a)P in the complete carcinogenesis and initiation studies and in place of PMA in the promotion studies. At the end of two years of treatment, there was no substantial evidence for a positive effect in complete carcinogenic, initiator or promoter activity of either diesel particulate suspension or dichloromethane extract in any of the test groups. Statistically significant increases in tumor yield have been observed in both positive control groups, thus establishing the validity of the test system.

Groups of male weanling B.C.F strain of mice were fed a control semi-purified diet (C) or the same diet devoid of choline (CD). Some mice from each group were subjected to partial hepatectomy (HepX) after two weeks on diet. Sequential sacrifices of mice from each group revealed the following: after three months, no tumors in any mice although the CD mice had enzyme deficient and fat-free islands of proliferating hepatocytes; after six months choline deficient mice only had liver tumors; after nine months control mice, 0% tumors; C + HepX mice 20% tumors; CD mice 100% tumors and CD + HepX mice 40% tumors. Autoradiographic studies indicated that both HepX and CD increased DNA synthesis and liver cell proliferation at all periods examined. These data suggest that the liver of B.C.F mice is sensitive to agents or conditions which are not tumorigens themselves but which may promote tumors. (Supported in part by USPHS grant ES00597 and a grant from Eli Lilly Co.)

227 INITIATING AND PROMOTING EFFECTS OF METHAPYRILENE ASSESSED BY THE ENZYME-ALTERED FOCl BIOASSAY. D. Couri, S.R. Wilt, and K.M. Milks, Division of Toxicology, Dept. of Pharmacology, College of Medicine, Ohio State University, Columbus, OH. The once commonly used antihistamine methapyrilene was recalled by the FDA in 1979 after laboratory findings that the drug produced liver tumors when fed to rats. However methapyrilene was not mutagenic in the Ames assay nor was the drug active in transforming hamster embryo cells in culture. For these reasons, the initiating and promoting effects of methapyrilene were evaluated using the rat hepatic enzyme-altered foci bioassay. In this study, putative preneoplastic islands were identified by the focal occurrence of the enzyme γ-glutamyl-transpeptidase (GGT) in hepatic tissue. Male Sprague-Dawley rats were partially hepatectomized and twenty-four hours later were administered either methapyrilene (50 mg/kg), nitrosodiethyamine (30 mg/kg/mouse) or distilled water by oral gavage. Subsequently, the animals received drinking water containing either 500 ppm phenobarbital (a known promoter of hepatocarcinogenesis), 200 ppm methapyrilene, or control tap water ad libitum for eight weeks. Fresh frozen sections of liver were then prepared, stained, and scored for GGT-positive foci. Methapyrilene, when tested for initiating activity and promoted by phenobarbital did not produce foci above background levels. However, when substituted for phenobarbital as the promoting agent following nitrosodiethyamine initiation, methapyrilene enhanced enzyme-altered foci formation to an equal or greater extent than did phenobarbital. These results suggest that the carcinogenic effects of methapyrilene are related to its ability to promote liver cells already initiated by dietary or other environmental carcinogens.

228 COCARCINOGENICITY OF SACCHARIN TOWARD N-DIBUTYL-NITROSAMINE CARCINOGENICITY IN RAT LIVER AND URINARY BLADDER. M.A. Pereira, Health Effects Research Laboratory, U.S. EPA, Cincinnati, OH and Alfred L. Britt, Department of Laboratory Animal Medicine, University of Cincinnati Medical Center, Cincinnati, OH. Sponsor: R.J. Bull

Saccharin has been demonstrated to be a promoter of chemical carcinogenesis in the rat urinary bladder. Since other tumor promoters are also cocarcinogens, we evaluated the possibility that saccharin is also a cocarcinogen. Male Fisher 344 rats simultaneously received 0.02% dibutylnitrosamine (DBN) in their drinking water and 5% sodium saccharin in their diet for a total of 26 weeks. The liver of rats that received only DBN contained 16 hyperplastic nodules among 5 of 29 (17%) animals and 2 hepatocellular carcinomas in 1 of 29 (3%) of the animals. The simultaneous administration of sodium saccharin to the animals that received DBN, produced an enhanced hepatocarcinogenic response of 106 hyperplastic nodules in 17 of 21 (81%) animals and 81 hepatocellular carcinomas in 17 of 21 (81%) animals. The number of preneoplastic focal alterations in the liver that were induced by DBN, was also enhanced by sodium saccharin. The livers of untreated control rats and rats that received only sodium saccharin were free of preneoplastic foci, hyperplastic nodules and hepatocellular carcinomas. In the urinary bladder, treatment with DBN produced simple and nodule hyperplasia that was enhanced by simultaneous treatment with saccharin. Sodium saccharin did not result in either a preneoplastic hyperplasia or a neoplastic response in the urinary bladder of the rats. Sodium saccharin administered as 5% of the diet was demonstrated to be cocarcinogenic toward DBN carcinogenicity in the liver and urinary bladder of rats.

229 DRUG METABOLIZING ENZYMES IN THE LIVERS AND HEPATIC TUMORS OF RATS TREATED WITH POLYCHLORINATED BIPHENYLS. G. Reddy, H.P. Cihla, R.H. Weltman, & D.B. Norback, Wm. S. Middleton Mem. VA Hospital, Univ. of Wis., Madison, WI, 53705.

 Hepatic drug metabolizing enzyme activities in the livers of rats were investigated after chronic exposure to Aroclor 1260(PCB) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Sprague-Dawley rats of both sexes were fed diets containing PCB (100 ppm for 16 mo followed by 50 ppm) or TCDD (100 ppm for 9 mo followed by 5 mo period of intermittent exposure followed by 100 ppm). After 24 mo they were fed a normal diet until sacrifice. The hepatic microsomal enzyme activities of aldrin epoxidase (AE), styrene oxide epoxide hydrolase (EH), and cytosolic glutathione S-epoxide transferase (GT) and the quantity of cytochrome P-450 were measured. Livers from male rats fed HCB contained enzyme levels equal to or decreased from that of controls. Livers from PCB-fed male rats showed enzyme levels 1.3 to 4 fold higher than that of controls. Livers from female rats exposed to HCB showed enzyme levels equal to or decreased from control levels. Liver tumors or surrounding tissues of female rats fed PCB showed 2 to 10 fold higher enzyme activities and cytochrome P-450 content than controls. AE activity was slightly higher in surrounding tissues than in tumors, while EH activity was 1.5 times higher in the tumors than in the surrounding tissues. The levels of P-450 and GT in tumors and in surrounding tissue were nearly equal. In conclusion, increased enzyme activities and P-450 content persist after discontinuation of PCB exposure. These enzymes in tumor and in the surrounding tissue likely play a role in the metabolism of PCBs. (Supported by the VA and NIH grants CA22140 & 5-T32-Es07015.)
HEPATIC EFFECTS OF α-HEXACHLOROCYCLOHEXANE IN THE MOUSE. F. Iverson, L. Tryphonas, C. Miller, Toxicology Research Division, Foods Directorate, Health Protection Branch, Tunney's Pasture, Ottawa, Canada, KIA OL2.

Many cyclic chlorinated hydrocarbon compounds produce increased hepatic microsomal enzyme activity, enhanced DNA synthesis and hepatic nodules in mice. Pathological classification of these lesions is often disputed but several studies with rat liver systems suggest that hepatic nodules are precursors of hepatocellular carcinoma (HCC).

We gave hexachlorocyclohexane (HCH, α isomer) in the diet to male HBP black mice. 500 ppm α-HCH for 9 months or 250 ppm α-HCH for 23 months produced a 100% incidence of hepatic nodules. There was no evidence of HCC and control animals showed no spontaneous tumor formation. Flow of cellular alteration, commonly found in carcinogen exposed rat liver, were not observed in the mice.

Additional studies showed that 250 ppm and 500 ppm α-HCH at 4 weeks produced a significant but similar increase in liver weight and mixed function oxidase activities associated with an increase in the cytochrome with a CO binding peak at 450 nm. DNA synthesis was increased 2-fold with 250 ppm α-HCH and 4-fold with 500 ppm α-HCH. Enzyme activity in nodules from 9 month α-500 dosed animals was within 20% of the activity in non-nodular areas.

These studies suggest that a) hepatic nodules formed in response to α-HCH in this species of mouse do not progress to HCC b) that P450 dependent enzyme activity in nodules is not decreased over that in surrounding areas and c) that the early appearance of nodules with 500 ppm α-HCH is associated with enhanced DNA synthesis and not increased microsomal enzyme activity.

At 100 mg/kg, the levels of binding at 0.5, 1.5, and 4.5 hrs were 3.1 ± 0.3, 7.4 ± 0.4, and 43.9 ± 0.8 micromoles adduct/mole DNA phosphorus, respectively. Thus, the magnitude of adduct formation tends to increase with both time and dose. Furthermore, analysis of this DNA by buoyant density equilibrium centrifugation showed the radioactivity bound to the isolated DNA co-migrated with the unlabeled DNA at 1.7 g/ml. These results suggest that 2,4-TDA initiates hepatocarcinogenesis in rats by directly interacting with DNA.


Phorbol esters (PE) are potent tumor promoters which have been shown to alter specifically the binding of epidermal growth factor (EGF) to diverse cell types in culture. In GH4C1 cells, a clonal strain of rat pituitary cells, PE stimulate the synthesis and release of prolactin and inhibit growth hormone production after binding to specific PE receptors. GH4C1 cells also have specific membrane receptors for EGF, thyro­tropin-releasing hormone (TRH), and somatostatin (SRH). Treatment with PE decreased the binding of EGF, TRH and SRH to GH4C1 cells. 12-0-Tetradecanoyl-13-phorbol acetate (TPA) at 100 ng/ml decreased 125I-EGF binding to 15% of control within 1 h. By Scatchard analysis of 125I-EGF binding data, a 1-h pretreatment with TPA decreased both the apparent affinity (2- to 5-fold) and the number of 125I-EGF receptors (by 25 to 50%). After 4 h of treatment, TPA decreased 125H-TRH binding to 35% of control due to a decrease in affinity (2- to 5-fold) with no change in the number of TRH receptors. After 24 h, TPA also decreased 125I-Tyr3-SRH binding to 40% of control. Effects on peptide binding were temperature dependent, occurring at 37°C but not 4°C, and reversible. The potency order for decreased peptide binding was TPA > phorbol-12,13-didecanoate (PDD) > phorbol-12,13-dibenzoate, while phorbol and 4α-PDD were inactive, which parallels the activity of these compounds as tumor promoters. Thus, unlike findings reported in certain other cells, the actions of PE on peptide ligand binding in GH4C1 cells were not specific for EGF. Modulation of receptors for multiple regulatory signals may play a role in the mechanism by which PE promote neoplastic transformation.


We have previously shown that propylene is a major hepatic promoter in male Charles River CD-1 CB-6 Sprague-Dawley rats pretreated with polychlorinated biphenyl (PCB; Aroclor 1254, 100 mg/kg for 3 days). We now report that pretreatment of rats with phenobarbital (PB; 80 mg/kg for 4 days), 8-methoflavone (BNF; 60 mg/kg for 4 days) or a mixture of the two at the above doses failed to result in the elevation of serum sorbitol dehydrogenase (SDH) or liver/body weight ratios observed in PCB-pretreated exposed
animals. Likewise, there was no change in cytochrome P-450 levels from not-exposed controls. Pretreatment with isosafrole (75 mg/kg for 3 days) followed by a 4-hour exposure to 50,000 ppm propylene also failed to result in increases in SDH or liver/body weight ratios. No measure of cytochrome P-450 levels was made. The unique ability of PCB pretreatment to elicit hepatotoxicity from propylene suggests that PCB induces an enzyme or enzyme system distinct from those that PB, BNF, and isosafrole induce and that this system is responsible for propylene activation. In vitro incubation of hepatic microsomes from PCB-pretreated rats with 40% propylene and a NADPH-generating system resulted in a decrease in cytochrome P-450 ((+)-propylene: 1.98 ± 0.06 nmole P-450/mg protein vs. (-)-propylene: 2.43 ± 0.06 nmole P-450/mg protein). This effect was NADPH dependent. There was no decrease in P-450 in anaerobic incubations containing microsomes, NADPH-generating system, propylene and a glucose oxidase system (anaerobic: 1.95 ± 0.02 nmole P-450/mg protein vs. aerobic: 1.13 ± 0.07 nmole P-450/mg protein). This implies that oxidative metabolism of propylene is necessary for in vitro destruction of P-450 to occur.

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The stimulation of benzene (B) metabolism by fluoride (F) was studied in rat liver microsomes. Metabolites measured were phenol (Ph) and an unidentified aequous component (Ao). In microsomes from control (I), benzene (II) and beta-naphthoflavone (III) induced rats, in the absence of fluoride, Ph formation increased from 0.002-0.08 mmol B and plateaued from 0.8-2 mmol B; in phenobarbital induced microsomes (IV), no plateau was reached by 2 mmol B. Ph per mg protein was greatest in II, intermediate in I and III, and least in IV below 0.6 mmol B. At 2 mmol B, the rate in IV was higher than in I and III. F stimulation was dependent on F concentration; other halides had no effect. In I, II, and III, F stimulated at all B concentrations above 0.08 mmol B; in IV, stimulation occurred from 0.03-0.6 mmol B, but not at 2 mmol B. F did not affect the proportions of Ph and Ao. In IV, Ph was always less than 60% of the total, while in I, Ph was over 70%. In II and III, the percent Ph increased as B concentration increased. The percent Ao was greatest in IV. Thus the characteristics of B metabolism appeared to differ among the microsomal preparations. In I, II and III, metabolism was stimulated by F and saturated above 0.8 mmol B; the rate in II was much higher than in I or III. Loss of this activity was seen in IV. Another activity, not affected by F and not saturated at 2 mmol B, occurred in IV. Formation of Ao also appeared induced in IV. These data suggest that the array of microsomal benzene metabolizing enzymes was modified by these inducing agents.

(Supported by NIH Grant ES00322.)

235 ALTERED HEPATIC 5 AMINOLEVULINIC ACID DEHYDRATASE (ALAD) AND SULFHYDRYL (SH) CONTENT DUE TO PRE-SUMED BENZENE METABOLITES. B.S. Lallian, G.S. Rao, G. Witz and B.D. Goldstein, Dept. of Environmental and Community Medicine, CMU/Rutgers Medical School/Rutgers University Joint Graduate Program in Toxicology, Piscataway, N.J.

In the present study we have evaluated the effects of certain of the proposed toxic benzene metabolites on mouse hepatic ALAD activity, which has previously been shown to be decreased following injection of benzene in rats, and on hepatic SH content in six weeks old male CD-1 mice. In vitro studies revealed a dose-responsive inhibition of ALAD activity by 10^{-3}-10^{-4} M benzoquinone, m-coumaldehyde and hydroquinone, but not by catechol or benzene. Following incubation for 1 hour, benzoquinone, hydroquinone, m-coumaldehyde, and benzenediol decreased both hepatic protein and free SH by at least 50% at the highest concentration (10^{-3}M), while phenol, catechol and benzene produced only a 13-22% decrease at 10^{-3}M. However, there were no statistically significant decreases in hepatic or red cell ALAD activity in vivo after intraperitoneal administration of 0.45 mmole of these compounds. Certain of the benzene metabolites did produce statistically significant changes in hepatic SH content following injection. Benzenediol and m-coumaldehyde decreased free SH by 11% and 12% respectively but there was no change in protein SH content. Benzenediol produced a 12% decrease in protein SH but did not affect the free SH while benzene caused a 21% decrease in protein SH and a 13% increase in free SH. The toxicological significance of these small changes in hepatic sulfhydryl levels is questionable. The lack of confirmation of an effect of benzene on mouse hepatic ALAD activity in vivo previously reported for the rat may reflect species difference. (Supported by NIH grant ES 02538)


Sponsor: P.E. Morrow

Peroxidation of the membrane phospholipids is believed to be the mechanism of toxicity for many environmental chemicals. Lipid hydroperoxide, a major intermediary product of lipid peroxidation, is believed to undergo decomposition to generate free radicals capable of initiating further lipid peroxidation. Linoleic acid hydroperoxide (LOOH) was prepared enzymatically from linoleic acid with soybean lipoxygenase. LOOH alone was remarkable stable at 37°C, it was rapidly decomposed by FeSO4 at both pH 5 and 7.4. However, Fe^{2+} completely chelated with EDTA did not break down LOOH at either pH. Furthermore, EDTA-Fe^{2+} with or without LOOH produced no malondialdehyde when incubated with liposomes of extracted microsomal lipid. However, while Fe^{2+} could stimulate liposomal peroxidation the initial rate of peroxidation was higher when LOOH was also present. This potentiation of lipid peroxidation by LOOH was observed at pH 7.4, when Fe^{2+} was rapidly autoxidized, but not in acidic conditions when Fe^{2+} was stabilized. This data suggest that
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peroxidative damage. In these studies, we have
investigated the effects of dietary E and Se
synergistically to constitute an important anti­
oxidant defense mechanism to protect the cell from
free radical-initiated lipid peroxidation. The altered
membrane permeability as a result of free
radical attack polyunsaturated fatty acids, thus
enhancing the rate of lipid peroxidation.
However, Fe²⁺ at high effective
concentrations probably reduce this reactive
intermediate directly to the relatively stable
hydroxy product.

237 FREE RADICAL FORMATION, LIPID PEROXIDATION, ENERGY
METABOLISM AND INTRACELLULAR Ca²⁺, Na⁺ AND K⁺ IN
RAT CEREBRAL CORTEX SLICES EXPOSED TO OXYGEN AT
HIGH PRESSURE. R.C. Dirks and M.D. Faiman, Dept.
of Pharmacol. and Toxicol., Univ. of Kansas, Lawrence, KS 66045

It is proposed that during hyperbaric oxygen
(OHP) exposure, free radicals are formed leading to
the peroxidation of membrane lipids, altered
membrane permeability, and the disruption of cellu­
lar homeostatic mechanisms necessary for the re­
establishment of normal ionic gradients. It is
further proposed that these abnormal ion movements
play an important role in the initiation of O₂-in­
duced convulsions. In the studies to be reported, the
effects of OHP on free radical formation, lipid
peroxidation, ion movement, and factors affect­
ing ion transport were investigated in rat cere­
bral cortex slices. Rat brain slices, approximate­
ly 0.5mm thick, were cut with a Stadie-Riggs tis­sue slicer and placed in a bicarbonate-buffered
balanced salt solution containing 10 mM glucose.
The brain slices were incubated at 37°C for 90
min. with continuous oxygenation under oxygen
pressures from 1 to 14 ata. Using spin-trapping
techniques, free radical formation was demonstra­
ted beginning at 6 ata. At 9 ata, tissue malonal­
dehyde levels (indicator of lipid peroxidation)
were significantly increased when compared to non­
exposed control slices. Also at 9 ata, an increase
in intracellular Na⁺ and Ca²⁺ and a decrease in
intracellular K⁺ was observed. No change in brain
tissue Na⁺K⁺ ATPase activity was observed; however,
brain slice ATP content dropped significantly be­
ginning at 9 ata. It is concluded that OHP causes
changes in ion movement which may be due to de­
creased cellular energy production or, possibly,
altered membrane permeability as a result of free
radical-initiated lipid peroxidation. The altered
ion movements could then be responsible for the
O₂-induced convulsions. Supported in part by NIH
grant # T32-GM 07775 and NS-07797.

238 INHIBITION OF LIPID PEROXIDATION BY REDUCED
GLUTATHIONE: EVIDENCE FOR THE ENZYMATIC REGEN­
eration of Vitamin E in Liver Microsomes.
C. E. Thomas, C. G. Reddy, C-Y. Wang R. W. Scholz,
and E. J. Massaro,* The Pennsylvania State
University, University Park, PA.

Lipid peroxidation, initiated by a number of
atmospheric oxidants such as ozone and nitrogen
dioxide as well as endogenously generated
reactive oxygen species, has been implicated as a
causative factor in oxidative cellular damage.
However, vitamin E (E) and selenium (Se) function
synergistically to constitute an important anti­
oxidant defense mechanism to protect the cell from
peroxidative damage. In these studies, we have
investigated the effects of dietary E and Se
status on in vitro lipid peroxidation of liver
microsomal preparations obtained from male Long­
Evans Hooded rats fed chemically defined diets
containing adequate or documented deficiencies of
E and/or Se (+E, +Se; -E, + Se; +E, -Se; -E, -Se;). In both enzymatic and non-enzymatic
systems, lipid peroxidation, as determined by the
measurement of thiobarbituric acid reactive pro­
ducts, was markedly inhibited by reduced
glutathione (GSH) in E supplemented groups.
However, the inhibitory effect of GSH was not
observed in a non-enzymatic system containing heat
treated microsomes. It was also found that micro­

239 IN VITRO HEPATOTOXICITY OF THE GLUTATHIONE
DEPLETERS DIETHYLMALEATE, IODOACETAMIDE, AND ACETAMINOPHEN. D.B. Mitchell and D. Acosta,
College of Pharmacy, The University of Texas, Austin,
TX 78712.

We have developed a model of primary cultures of
postnatal rat hepatocytes to investigate the mecha­
nisms by which xenobiotics produce cellular injury.
Glutathione (GSH) is a unique tripeptide that has a
well-known role in the hepatic conjugation and detoxi­
cation of xenobiotics and is associated with the main­
tenance of normal cellular structure and function. In
order to test the hypothesis that GSH depletion may be
a primary pathophysiologic event in cell injury, we
chose to compare the toxic effects of 3 different
chemicals known to deplete cellular GSH pools: di­
ethylmaleate (D), iodoacetamide (I), and acetaminophen
(A). Treatment with I (10⁻⁶ M) depleted GSH levels by
90% within 1 hr. Cell viability decreased significantly,
LDH leakage was 500% of control, and lipid peroxi­
dation was 2 times control values. A higher dose of
D(10⁻⁶ M) was needed to produce similar toxic effects
as I. Whereas I and D caused significant GSH depletion
and toxicity by 1 hr, A required higher doses and longer
treatments to deplete GSH (24 hr, 65%). Toxicity
produced by A was not as pronounced as that observed
with I or D. The degree of toxicity observed with the
eight agents may reflect their interaction with GSH and
cellular constituents. The concurrent treatment of cells with A and D had an additive effect on
toxicity. The time lag prior to toxic effects caused by
A may be the result of cytochrome P-450 mediated
metabolism to the toxic intermediate that binds to
GSH. In general, GSH depletion that reached approxi­
mately 70% of control values resulted in irreversible
hepatocellular injury.
Thioacetamide is a much studied hepatocarcinogen which binds covalently to cellular macromolecules in vivo and after microsomal metabolism in vitro. Previous work in this laboratory has identified the major hepatic protein covalent adduct formed in vivo as being \( N^\epsilon \)-acetyllysine. Greater than 90% of the radioactivity which is bound to protein in vivo during microsomal metabolism of thioacetamide-S-oxide has been identified as the same adduct, \( N^\epsilon \)-acetyllysine. Acetamide, another product of thioacetamide-S-oxide metabolism by rat liver microsomes, and the protein adduct, \( N^\epsilon \)-acetyllysine, appear to be derived from a common intermediate postulated to be thioacetamide-S-dioxide. Addition of thiol-containing compounds to the in vitro incubation media decreased the levels of radioactivity covalently bound to protein to a small extent. Aliphatic amines decreased the level of binding to a much greater extent. Studies with \( 18^\text{O} \)-enriched water or gas indicated that the oxygen atoms of acetamide and the acetyl group of \( N^\epsilon \)-acetyllysine are derived solely from water. Chemical oxidation of thioacetamide-S-oxide in the presence of protein results in covalent binding of radioactivity to the protein. The adducts which are formed are highly \( p^\text{H} \) dependent with \( N^\epsilon \)-acetyllysine being the major adduct formed at \( p^\text{H} 7.4 \) and \( N^\epsilon \)-acetylaminodisine being the predominant adduct formed at \( p^\text{H} 10.4 \). (Supported by NIH grants ES 00075, ES 00267 and ES 07028.)

241 \( 7^\text{M} \)-METHYLGUANINE AND \( O^6 \)-METHYLGUANINE FORMATION IN LIVER DNA OF RATS, MICE, AND HAMSTERS TREATED WITH HEPATOXINS. R.A. Beeker and R.C. Shank, Dept. Comm. & Env. Med., University of California, Irvine, CA 92717.

Previous studies have demonstrated \( 7^\text{M} \)-methyguanine (7-MG) and \( O^6 \)-methylguanine (\( O^6 \)-MG) in liver DNA from rats treated with hydrazine. We have now quantitated 7-MG and in some cases \( O^6 \)-MG in liver DNA from rats treated with hepatotoxic doses of ethanol, carbon tetrachloride, phenobarbital, and puromycin. Neither 7-MG nor \( O^6 \)-MG was detected in comparable amounts of liver DNA from control animals. Kinetics of formation and removal of 7-MG and \( O^6 \)-MG in liver DNA were investigated in rats treated with 90 mg hydrazine/kg body wt. and killed 0.25-96 hr later. 7-MG and \( O^6 \)-MG formed rapidly and concomitantly; half-maximum methylation occurred within 1 hr after treatment. At maximum methylation (6-24 hr after exposure) liver DNA contained 870 \( \mu \text{mol} \) 7-MG and 80 \( \mu \text{mol} \) \( O^6 \)-MG per mol guanine. 7-MG was removed from DNA at a rate of approximately 50% in 47 hr, while the half-life of \( O^6 \)-MG in liver DNA was approximately 13 hr. Similar levels of 7-MG and \( O^6 \)-MG were found in liver DNA from mice and hamsters killed 1-24 hr after exposure to hepatotoxic doses of hydrazine. The molecular mechanisms responsible for liver DNA methylation during hepatotoxicity are under investigation. S-Adenosylmethionine (SAM) has been tentatively identified as the proximal methylating species. The ratio of 7-MG to \( O^6 \)-MG at maximum methylation is consistent with an \( S^\text{N} \)1 reaction, suggesting that during hepatotoxicity-induced disruption of cellular homeostasis, SAM may react non-enzymatically with nucleophilic sites in DNA. Whatever mechanism is ultimately found to be responsible for the methylation of DNA during hepatotoxicity, the consequence, genetic damage, may relate cytotoxicity to carcinogenicity. (Supported by U.S. Air Force Contracts 33615-75-C-5005 and 33615-80-C-0512.)


Many carcinogens, including dialkyl and alkylamines, are thought to induce cancer through covalent binding of derivatives to target organ DNA. Some evidence suggests that N-nitrosopyrrolidone (NP) and other cyclic nitrosamines are at variance with this mechanism (Cancer Res. 33:1634, 1974). Liver DNA was isolated 3, 6, 12, 18, 24, 36, or 36 hr after rats were given 900 mg NP/kg body wt. The DNA was subjected to neutral thermal hydrolysis in pH 7 buffer for 1 hr at 100°C, and hydrolysates were fractionated by strong cation exchange high pressure liquid chromatography. Elution of fractions was monitored by fluorescence spectrophotometry (excitation, 286 nm; emission interference filter, 343 nm). An unidentified fluorescent material was detected in all DNA hydrolysates from NP-treated rats, but not in hydrolysates from control animals. The putative adduct formed rapidly; the concentration of this material in liver DNA increased more than 3-fold from 3 to 12 hr following NP treatment, then began to gradually decrease over later times. The fluorescent material did not co-chromatograph with standard guanine derivatives methylated or ethylated in either the 7- or O'-position. The concentration of fluorescent material in liver DNA hydrolysates was dose-dependent in rats killed 12 hr after receiving 14, 28, 57, 113, 225, 450, or 900 mg NP/kg body wt. Neutral thermal hydrolysis is known to release 3-alkyladenine and 7-alkylguanine from DNA; alkylguanines fluoresce well, while alkyl­adenines do not. Hence, the putative DNA adduct in NP-treated rats may be a guanine derivative substituted in the 7-position. This evidence is consistent with the generalization that NP, as other chemical carcinogens, binds covalently to target organ DNA.

243 D(+)-GALACTOSAMINE CYTOTOXICITY IN PRIMARY CULTURES OF POSTNATAL RAT HEPATOCYTES. K.S. Santone and D. Acosta, College of Pharmacy, The University of Texas, Austin, TX 78712.

We have developed an in vitro system of primary cultures of postnatal rat hepatocytes to serve as an experimental model of chemical toxicity. Because the hepatotoxicity of galactosamine has been investigated extensively in vivo, we chose it as a model hepatotoxin to evaluate the specificity and sensitivity of our in vitro system for hepatotoxicity studies. To evaluate its potential cytotoxicity, we utilized commonly used parameters of in vitro toxicity, trypan blue viability test and cell protein levels, as well as more sensitive indices of toxicity developed in our laboratory—leakage of cytoplasmic enzymes and lactate to pyruvate ratios (L/P). Liver parenchymal cells were isolated by an in situ collagenase perfusion technique and cultured 24 hr prior to experimental treatments. The cultured hepatocytes were exposed to 0.25, 0.50, and 1.0 mM galac-
DECREASED RESPONSE TO PHORBOL ESTERS INVOLVES A TWO-STEP PROCESS INCLUDING RECEPTOR MODULATION.

Tumor-promoting phorbol esters (PE) act on cells via interaction with specific membrane receptors. Escape from PE-mediated actions, possibly including tumor promotion, have been documented. We have studied the mechanisms by which cells escape from PE effects using as a model system a clonal strain of mouse pituitary cells (D16) which synthesize and secrete ACTH. PE enhance ACTH release from D16 cells 2- to 5-fold above unstimulated control levels. ED50s for this response, -16 nM for phorbol dibutyrate (PDBu), -2 nM for phorbol myristate acetate (PMA), agree closely with the apparent Ks of these ligands for the PE receptor (-11 nM for PDBu and -2 nM for PMA), as measured by competition for binding with [3H]PDBu. Pretreatment with PE for 2 h resulted in a decrease in ACTH release with PE rechallenge to 23 ± 8% of that from cells not pretreated (desensitization). The 2-h pretreatment did not change the affinity or number of PE receptors nor did it decrease the responsiveness of the cells to other ACTH secretagogues (cAMP, isoprenaline). However, the continued presence of PE caused a gradual decrease in [3H]PDBu binding which was first detected at 5 h (70% of control) and was maximal by 24 h (50 ± 5% of control). Scatchard analysis indicated a decrease in the number of [3H]PDBu binding sites (down modulation). In these down-modulated cells, the magnitude of PE stimulation of ACTH release correlated well with the number of remaining PE-receptors. We conclude that decreased cellular responsiveness to PE occurs via a two-step process initially involving uncoupling of the receptor from the secretory response and subsequently to down modulation of the PE receptor.

TERATOGENICITY OF THALIDOMIDE BY DIRECT INJECTION INTO FETAL RATS.

The teratogenic effects of thalidomide (Th) were studied in rats, a species usually resistant to malformations induced by this drug. Four groups of day 15 Sprague-Dawley rat fetuses were directly injected with 2 μl dimethylsulfoxide containing 0, 25, 50 or 200 μg Th. Females were sacrificed on day 20 of gestation (positive vaginal smear day '0'). The percentage of dead or resorbed fetuses was 14% in control and in low and middle dose and 74% in high dose Th. Among the living young 0/48(0%) control, 1/64(1.6%) low, 5/65(7.7%) middle and 4/10 (40%) high-dose groups had limb deformities, including hemimelia, amelia and ectrodactyly. Since metabolic products, rather than the parental Th, are believed to be involved in the teratogenic process in sensitive species, an attempt was made to test the teratogenic activity of blood plasma from female cynomolgus (Macaca irus) previously treated with Th. Blood plasma samples were taken from one untreated and one Th-treated monkey and injected into the fetal rat bodies, 10 μl/fetus. Of 56 fetuses that received blood plasma from the treated monkey 66% were dead or resorbed while 10/19 (53%) living young had malformations (limb deformities as above). The figure in control fetuses was 32% dead or resorbed and 0/73(0%) malformed. Given the close similarity between monkeys and humans in terms of response to teratogenic stimuli, these results suggest an interesting approach for testing drugs that may adversely affect human conceptuses.

THE TERATOGENICITY OF THE AMINOPHENOLS (AP) IN SYRIAN GOLDEN HAMSTER (SGH).

This study was undertaken to evaluate the teratogenic potential of p-AP and the other isomeric forms, m-AP and o-AP. Timed pregnant SGH (LKV strain) were used. Day 1 of gestation is that day following the evening of breeding. On the morning of day 8 of gestation the SGH received an ip dose of 100-200 mg/kg of p-AP, o-AP, or m-AP. The dams were sacrificed on day 13 of gestation. p-AP and o-AP produced a significant teratogenic response. A dose-effect phenomenon was evident by a rise in the frequency of litters with one or more malformed fetuses. The total number of fetuses with one or more malformations also increased with dose, as did the resorption response. Frequently observed malformations were exencephaly, encephaloceles, eye defects, rib fusion, tail defects, spina bifida, limb defects, and umbilical hernia. Other rare malformations were also noted. Although conclusive evidence regarding the teratogenicity of m-AP was not obtained, the data support the conclusion that o-AP and p-AP are highly teratogenic in the SGH without compromising maternal health. Supported by USPHS grants ES00697 and ES07104.
We designed a computerized form generation system utilizing SAS programming via CMS terminals to: (1) generate preprinted data recording forms for various behavioral teratology tests; (2) calculate testing days; and (3) serve as data submission forms for statistical analyses. We use these computer-generated forms for the following behavioral/developmental tests for rats: pup weights, pinna detachment, surface righting, cliff avoidance, incisor eruption, eye opening, negative geotaxis, swimming development, open field activity, swimming maze, and operant visual discrimination learning. The major advantages of this system include: (1) elimination of manual data transcription; (2) elimination of human error in calculating testing dates; (3) the ability to spread parturition dates over several weeks, thereby equalizing the work load; and (4) providing more consistent data recording and scoring conditions. We have conducted two major behavioral teratology studies using the computer-generated forms. In the first study, 1 computer-calculated test date was overlooked. This resulted in the loss of 8 of approximately 5,696 test results and represents a human error rate of approximately 0.14 percent. In the second study, 8 test dates were missed. This resulted in the loss of 59 of approximately 11,680 test results and represents a human error rate of approximately 0.51 percent. We are currently conducting a third behavioral teratology study and have added a dynamic computer-generated daily activity schedule for use by the laboratory to prevent overlooking test dates. We also added an automatically-generated OS data set (database type file) for use by data entry personnel.


We conducted a uniformity study to evaluate the design and scoring method of several behavioral teratology tests. Ten pregnant HLA-SD rats approximately 13 weeks of age were dosed by intubation at 5 ml/kg wit distilled deionized water on days 6-19 of gestation. On postnatal day 3, a maximum of nine pups from each of the ten litters were randomly selected for behavioral testing. The following behavioral/developmental tests were evaluated: pinna detachment, incisor eruption, eye opening, surface righting, negative geotaxis, swimming development, and open field activity. Interpretation of statistical analyses of these data yielded the following recommendations and results: (1) pups should be randomly selected for testing by sex on postnatal day 1; (2) the use of more litters with fewer pups is preferred for analysis of treatment responses; (3) an all or none scoring method is acceptable for pinna detachment, incisor eruption, and eye opening; (4) female pups develop pinna detachment, incisor eruption, and eye opening faster than male pups; (5) surface righting, cliff avoidance, and grasp/holding should be timed for test completion and not timed to meet an arbitrary criteria for success; (6) the negative geotaxis test should be completed prior to any ocular light perception; and (7) female rats are more active than male rats in the open field test.

**249 FETAL DEVELOPMENT IN NEW ZEALAND WHITE (NZW) RABBITS TREATED IV WITH ETHYLENE OXIDE (ETO) DURING PREGNANCY.** C.A. Kimmel, J.B. Laborde, C. Jones-Price, T.A. Rayoux and T.A. Marks, Div. of Teratogenesis Research, NCTR/FDA/DHHS, Jefferson, AR, and Research Triangle Inst., Research Triangle Park, NC.

Previous studies on the iv administration of ETO to pregnant mice (Laborde and Kimmel, TAP 56:16-22, 1980) indicated a teratogenic effect following 150 mg/kg on days 6-8 of gestation. Studies were therefore conducted in rabbits to assess the teratogenic potential in a non-rodent species. NZW rabbits were administered 0, 9, 18 or 36 mg/kg ETO iv (in 5% dextrose) on days 6-14 of gestation or 0, 18 or 36 mg/kg iv on days 6-9 of gestation. Preliminary studies had indicated the maximum tolerated dose (MTD) to be approximately 40 mg/kg. Maternal toxicity was assessed throughout pregnancy and on day 30, dams were killed and uterine contents examined. A gross examination of surviving fetuses included external, visceral and skeletal evaluations. A significant trend toward decreased maternal weight gain was seen during treatment and throughout gestation after treatment either on days 6-9 or 6-14. No significant effects were seen in the fetal parameters examined after treatment on days 6-9 or 6-14. However, a significant increase in mean number and % resorptions/litter was noted in the 36 mg/kg dose group treated on days 6-14 of gestation. No other effects on fetal viability, weights or morphology were seen after the longer treatment period. Thus, ETO administration to pregnant rabbits does increase embryotoxicity, but only after treatment throughout organogenesis and at a dose that also produces maternal toxicity. Unlike the effect of ETO in mice, no teratogenic effects were detected in rabbits in this study.


Alterations in blood flow to the uterus and its contents during pregnancy have been suggested to account for the teratogenicity and/or embryotoxicity of several agents, including caffeine. Using the radioactiv microsphere technique (Bueke-Sam and Holson, 1980), blood flow to several maternal organs, including ovary, uterus, decidua and chorioallantoic placenta (CAP), was measured following a single dose of $0.120 \text{ mg/kg}$ caffeine by gavage to pregnant CD rats on day 12 of gestation. At 1 and 4 hrs after treatment, animals were anesthetized and $3^8$S-labeled microspheres (25u diam.) were infused via the right carotid artery. Whole body and tissue radioactivity were determined. Maternal cardiac output (CO) and absolute flow (f1; ml/min), relative flow (f2; ml/min/g tissue) and flow as %CO (f3) to each tissue were calculated. Maternal CO was not altered. Ovarian f1 was reduced at 4 hrs and f2 and f3 at 1 and 4 hrs after caffeine treatment. Uterine f1, f2 and f3 were reduced at both times. Decidual weight was reduced at 1 hr, f2 at 4 hrs, and f1 and f3 at both times. How-
ever, CAP weight and blood flow were not altered by caffeine treatment. Examination of embryos from these litters and from other animals at 24 h after caffeine treatment and fetuses at term did not reveal any effect of this dose of caffeine on viability, growth or physical development. Thus, a single dose of caffeine significantly reduced blood flow to the ovary, total uterus and decidua. In spite of this effect, conceptual development was normal. This may be attributable to maintenance of normal blood flow to the CAP, a functioning placental unit by day 12 of gestation.


Earlier teratology studies with ethylene oxide (ETO) in mice (LaBorde and Kimmel, TAP 56:16-22, 1980) reported teratogenicity of ETO at 150 mg/kg on days 6-8 of gestation. ECH, a reaction product of ETO and chloride ion, may be a residue in poorly degassed medical devices following sterilization with ETO. This study assessed the teratogenic potential of ECH (in 5% dextrose) administered iv to CD-1 mice (0, 60 or 120 mg/kg) on days 4-6, 6-8, 8-10 or 10-12 of gestation; and to NZW rabbits (0, 9, 18 or 36 mg/kg) on days 6-14 of gestation. The highest dose levels approximated the MTD in each species. Litters were examined on day 17 (mouse) or day 30 (rabbit) of gestation. No significant maternal or embryo-fetal effects were noted in the rabbit. In mice, a significant reduction was noted in maternal weight gain during treatment at all time periods. Maternal weight gain throughout gestation was significantly reduced following 120 mg/kg on days 8-10 and 10-12 of gestation. A significant increase was seen in mean resorptions/litter on days 4-6 and 8-12 at 120 mg/kg. Mean fetal weight/litter was significantly decreased at the high dose for all treatment periods. A significant increase in the mean number of malformed fetuses was observed only on days 8-10 at 120 mg/kg. Malformations were mainly skeletal, although a low incidence of cleft palate, open eye, microelia, and adactyly was recorded. Thus, ECH increased malformations and resorptions, but only following treatment that was toxic to maternal mice. Additionally, ECH was not teratogenic in the rabbit with the dosing regimen employed in this study.

**252 FURTHER STUDIES ON THE EFFECTS OF THE PHTHALATE ACID ESTERS (PAE) ON RAT MALE REPRODUCTIVE ORGANS. Karen A. Curto, Robert E. McCafferty, Michael P. Donovan and John A. Thomas. Dept. Pharmacol. & Toxicol., West Virginia University Medical Center, Morgantown, West Virginia 26506.**

While high doses of diethylhexyl phthalate (DEHP), and its principal metabolite monoethylhexyl phthalate (MEHP), reportedly cause gonadal zinc depletion and eventually some spermatogenic arrest, there is no evidence that levels of these PAEs that might be leached from plastic medical devices can exert such toxic actions. Using a series of dose regimens of DEHP or MEHP (ranging from 1 mg/kg to 100 mg/kg daily x 5-ip) that were confirmed not to decrease the total of endogenous zinc in rat testes or prostate glands, investigations were undertaken to study a number of zinc-related events. Gonadal alkaline phosphatase, an enzyme affected by androgens as well as being zinc-dependent, was not altered by either DEHP or MEHP in sexually mature rats. Likewise, no PAE-induced changes in prostate alkaline phosphatase activity were noted. Neither RNA nor DNA levels in reproductive organs were affected by levels of DEHP/MEHP that would be comparable to those encountered as a result of their being leached from plastic medical devices. The lack of changes in gonadal nucleic acids was correlated with normal morphology in the seminiferous tubules. The present findings reveal a wide range of no-effect levels of DEHP or MEHP in reproductive organs of the rat.

**253 FAILURE OF PHTHALATE ACID ESTERS (PAE) TO STIMULATE LIPID PEROXIDATION IN RAT REPRODUCTIVE ORGANS. Walter C. Brogan, III, Karen A. Curto and John A. Thomas. Dept. Pharmacol. & Toxicol., West Virginia University Medical Center, Morgantown, West Virginia 26506.**

Although dietary zinc deficiency and PAE-induced zinc deficiency reportedly enhance hepatic lipid peroxidation, no studies have examined this relationship in the gonads despite the fact that the testes are a primary site of toxic action of diethylhexyl phthalate (DEHP) and/or monoethylhexyl phthalate (MEHP). When rat testes or prostate were incubated (e.g. 60 min) with varying concentrations (viz., $10^{-5}$ to $5 \times 10^{-2}$M) of either DEHP or MEHP, there was no increase in the production of a thiobarbituric acid-reacting chromogen, malonaldehyde (MDA). Further, MDA levels were not increased when NADPH was added to the in vitro system. Testes obtained from rats previously injected with DEHP or MEHP (e.g. 48 or 100 mg/kg daily x 5-ip) failed to reveal any significant decrease in cytochrome P-450 content or aryl hydrocarbon hydroxylase activity. While additional studies are underway to determine whether these PAEs can affect gonadal steroidogenesis, the present findings do not indicate that lipid peroxidation is involved in DEHP/MEHP-induced gonadal toxicity.

**254 ACUTE OR CHRONIC DIETARY EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBs): EFFECTS ON MATERNAL METABOLIC HOMEOSTASIS AND ON EMBRYOTOXICITY CAUSED BY 5-FLUORODEOXYURIDINE (FUDR) IN MICE. F. Welsch, K.S. Hardyniec**
255 METHYLMERCURY EFFECT ON TRANSPLACENTAL TOXICITY with increasing MeHg level with the 2 highest levels of MeHg and NO₂-EU in the diet (0, 2.4, 3.2 and 4.8 g/kg) during gestation. Litter rate was not affected by either the level of MeHg or NO₂-EU but the effects did not consistently increase linearly with increasing level of MeHg for all parameters. Survival to weaning age decreased progressively less delay at the lower doses of X-ray. This dose response was evident in the pups' performance on the the righting reflex (days 1 to 14), reflex suspension (days 5 to 14) and spatial maze exploration (day 35) tests as well as a test in which the rats are placed on a hole board and the observer counts the number of times the rat pokes its nose into the holes (day 35). In addition, a marked effect was noted on the time of eye-opening with controls averaging 14.6 days of age and the pups exposed to 125 R averaging 16.2 days of age. There are two possible explanations for the development delay produced by X-irradiation: 1. the effects on behavioral development are reflecting the gross morphological damage to the CNS that is produced by X-irradiation on gestational day 15 (Mullenix et al., Exp. Neurol., 48: 310, 1975) or 2. since developmental delay is known to occur with thyroid deficiency (Eayrs and Lishman, Brit. J. Anim. Behav., 3: 17, 1955), possibly the X-irradiation is damaging the thyroid and producing functional hypothyroidism. (Supported in part by USPHS grants NS 16694 and ES 07079.)


A series of developmental behavioral tests was used to assess the performance of prenatally irradiated rat pups. Pregnant rats were irradiated on gestational day 15 with doses of 0, 25, 50, 75 and 125 R. Neurobehavioral testing was begun on postnatal day 1. Preliminary data indicate that pups exposed to 125 R show a marked developmental lag with progressively less delay at the lower doses of X-ray. This dose response was evident in the pups' performance on the the righting reflex (days 1 to 14), reflex suspension (days 5 to 14) and spatial maze exploration (day 35) tests as well as a test in which the rats are placed on a hole board and the observer counts the number of times the rat pokes its nose into the holes (day 35). In addition, a marked effect was noted on the time of eye-opening with controls averaging 14.6 days of age and the pups exposed to 125 R averaging 16.2 days of age. There are two possible explanations for the development delay produced by X-irradiation: 1. the effects on behavioral development are reflecting the gross morphological damage to the CNS that is produced by X-irradiation on gestational day 15 (Mullenix et al., Exp. Neurol., 48: 310, 1975) or 2. since developmental delay is known to occur with thyroid deficiency (Eayrs and Lishman, Brit. J. Anim. Behav., 3: 17, 1955), possibly the X-irradiation is damaging the thyroid and producing functional hypothyroidism. (Supported in part by USPHS grants NS 16694 and ES 07079.)

257 ASSESSMENT OF PERINATALLY INDUCED RENAL DYSFUNCTION. R.J. Kavlock and J.A. Gray. HERL, USEPA, Research Triangle Park, NC (Sponsor: N. Chernoff)

In order to ascertain the sensitivity of a series of tests we developed for the evaluation of renal function in neonatal rodents (The Toxicologist 1:82 (1981)), we administered four known or suspected renal toxicants to Sprague Dawley rats at critical periods of development and applied a diuresis test with and without antidiuretic hormone on postnatal day 3 (PD 3), a hypodrenia test on PD 6, and determined kidney weights, glomerular counts in mid-hilar cross sections and the specific activity of renal alkaline phosphatase on PD 3 and 6. Chlorambucil (CHL) was given i.p. on day 11 of gestation at 0, 3 and 6 mg/kg. Nitrofen (NT) was given p.o. on days 7-16 of gestation at 0, 4.17, 12.5 and 25 mg/kg/day, Benomyl (BEN) was given p.o. on PD 1 at 0, 37.5, 75, 150 and 300 mg/kg/day, and D.B. Stedman (Sponsor: J.I. Goodman), Dept. Pharmacol./Toxicol. and Ctr. Env. Toxicol., Mich. State Univ., E. Lansing, MI 48824.

The potential consequences of multiple low level exposure to chemicals on embryonal/fetal development are of great concern in prenatal toxicology. In this contribution we describe the effects of PCBs Aroclor 1254; 0,1,10 and 100 ppm for 90 days prior to breeding (=chronic) or beginning on day 6 of gestation (=acute) fed to ICR Swiss mice on the metabolism of xenobiotics whose fate is coupled to cytochromes P448 and PCBs. We have also measured the fetal body burdens of PCBs and correlated all observations with embryotoxicity induced by a single i.p. injection of FUdR (7.5 or 20 mg/kg; 260-263 hrs after mating). This teratogen can be activated in the embryo/fetus. The effects will be compared in ongoing experiments to those caused by cyclophosphamide which in mice relies solely on maternal activation. Liver P448 content was increased by 100 ppm PCBs both in acute and chronic mice. Aminopyrine demethylase activity was elevated in chronic 100 and 10 ppm animals, but acutely only at 100 ppm. Among the P448 mediated reactions aryl hydrocarbon hydroxylase and ethoxyresorufin-O-deethylation activity were induced by chronic and acute 100 ppm PCBs and also by chronic 10 ppm, but by none of the other exposure levels. While PCBs alone had no teratogenic action, FUdR alone was very effective (Welsch and Hardyniec, Teratology 23, 68A, 1981). The differing PCBs regimens caused a variety of alterations in the expression of teratogenicity including changes in frequency of malformations at specific sites. One example is an increase in polydactyly of the hind limbs among 7.5 mg/kg FUdR plus chronic PCBs exposed fetuses in comparison to FUdR alone. (Supported by NIH Grant ES 02461.)

255 METHYLMERCURY EFFECT ON TRANSPLACENTAL TOXICITY INDUCED BY NITRITE AND ETHYLUREA IN RATS. J.E. Nixon and J.P. Luebke, Dept. of Food Sci. & Tech. and Env. Hlth. Sci. Cntr., Oregon State University, Corvallis, OR 97331

In our lab 10 ppm methylmercury chloride (MeHg) increased the transplacental toxicity of sodium nitrite (NO₂) and ethylurea (EU) for reproduction and latency of neurogenic tumor development in rats. To determine whether the MeHg effect was dose dependent and effective with different levels of NO₂-EU exposure, 4 levels of MeHg (0, 5, 10 and 15 ppm) were fed from weaning age until parturition to female Wister rats in combination with 4 levels of NO₂ in the drinking water (0, 0.75, 1.00 and 1.50 g/l) and EU and the diet (0, 2.4, 3.2 and 4.8 g/kg) during gestation. Litter rate was not affected by either the level of MeHg or NO₂-EU and ranged from 88 to 100% for all treatments with 16 females per group. The combination of the highest level of MeHg and NO₂-EU produced the most severe effects on litter size, birth weight and survival (ca. 56, 90 and 60% of positive controls) but the effects did not consistently increase linearly with increasing level of MeHg for all parameters. Survival to weaning age decreased with increasing MeHg level with the 2 highest levels of NO₂-EU. A reproduction efficiency index computed from also correlated litter size and survival with units of number of pups weaned/female/treatment showed the best dose-response for MeHg at each level of NO₂-EU. Mortality and neurologic tumor incidence and latency period for 40 progeny, 20 of each sex, from each treatment will be presented. Completion of these studies will provide basic information on toxicity produced by the simultaneous exposure of several environmental contaminants and will help determine what effect MeHg has on potentiation of cancer induced by nitroso compounds. (Supported in part by USPHS Grants ES-00210 and ES-00040.)
Benzothiazolesulfenamides (sulfenamides) are used in the rubber industry as accelerators for vulcanization processes. Three commercial sulfenamides, N-cyclohexyl- (I), N-t-butyl- (II), N-morpholino- (III), were tested for teratogenic activity in Charles River CD® rats. Groups of 25 pregnant female rats were treated orally on days 6 through 15 of gestation with corn oil suspensions of test material at concentrations of: I - 100, 300, 500 mg/kg/day, II - 50, 150, 500 mg/kg/day, III - 100, 300, 1000 mg/kg/day.

Surviving animals were sacrificed on day 20 and uterine horns were examined for number of implantations, viable or nonviable fetuses, or resorptions. One-half of the total fetuses were examined for internal anomalies after preservation in Bouins solution; one-half were examined for skeletal anomalies after clearing and staining. Mean fetal body weight reduction at the high dose was associated with a significant decrease in maternal weight gain. Mean fetal weights were comparable to controls at all levels for II and III. No significant differences in mean numbers of implantations, resorptions, or viable fetuses were observed after treatment. No significant difference between control groups and groups treated with I, II or III were noted in external malformations, soft tissue malformations, or skeletal anomalies. All incidences for observed anomalies were within ranges expected from historical controls. No teratogenic response was observed in rats after treatment with these 3 substituted mercaptobenzothiazoles during the period of organogenesis.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a widespread environmental contaminant, is a known teratogen and potent inducer of mixed-function oxidase activity in rodents. This study was designed to examine the effects of TCDD on development in the ferret. Animals received 1,6,13.5,20,30 or 60 µg TCDD/kg body weight subcutaneously as a single dose on day 18 or as ½ the dose on days 18 and 20 of gestation. Laparotomies were performed on days 28,29 and 30 of gestation due to toxicity observed at the two highest doses. The average number of implant sites, live, dead, and resorbed fetuses, as well as fetal weight, crown-rump length, and specific malformations was recorded. In addition, S-9 homogenates were obtained from maternal and fetal liver and placenta, and levels of aryl-hydrocarbon hydroxylase (AHH) activity and cytochrome P-450 were determined.

Increases in the number of dead and resorbed fetuses, as well as retarded growth of viable fetuses was found in all test groups. In addition, several malformations, including unilateral and bilateral palatoschisis, open eyelids, anasarca, and brachygnya, were observed in fetuses which were exposed to TCDD, whereas none were present in control animals. Maternal and fetal AHH activity and cytochrome P-450 levels showed significant individual variation in both the liver and placenta. The data obtained indicate that ferrets may be "non-responsive" to hepatic MFO induction by TCDD.

In conclusion, TCDD was teratogenic and embryotoxic to ferrets at all doses tested with no demonstrated no-effect level.

formate in adult livers than in other tissues. Techniques reported here yield a rapid and convenient quantitation of tissue doses of methanol and its extractable and tissue-bound metabolites. Supported in part by Contract No. DE-AC02-76EV03490 with the U.S. Dept. of Energy.

261 TRANSPLACENTAL TOXICITY ASSOCIATED WITH GENETIC DIFFERENCES AT THE AH LOCUS. R.G. York and J.M. Manson, Kettering Lab., Univ. of Cincinnati, Cincinnati, OH. Sponsor: P.B. Hammond

The Ah locus controls the synthesis and inducibility of aryl hydrocarbon hydroxylase (AHH), one of the xenobiotic metabolizing enzymes of the P-450 monooxygenase system. Based on the inducibility of the Ah locus inbred strains of mice have been shown to be highly sensitive or resistant to toxicity when exposed as adults to polycyclic aromatic hydrocarbons (PAH). This study is an attempt to determine whether toxicity (carcinogenicity) caused by transplacental PAH exposure can be correlated with inducibility of the Ah locus. C57BL/6J (Ah inducible) and DBA/2J (Ah noninducible) mice were appropriately crossed so that fetuses with both phenotypes were found in the same litter. These fetuses were exposed in utero on days 15, 16 and 17 of gestation to 3-methylcholanganthrene (3-MC) given orally to the dam at doses of 7.21, or 63 mg/kg/day. Analysis of variance on 90 dams and their litters showed the high dose group significantly (p<.03) different from controls for percent pups surviving day 4, mean pup weight on days 7, 14 and 21, and number of pups weaned per litter. This weight depression in the high group was still significant (p<.05) at 90 days of age at which time the offspring were phenotyped by zoxazolamine paralysis time. The high dose group also contained statistically fewer inducible than noninducible pups. This may be due to the higher levels of toxic 3-MC metabolites in the inducible fetuses, resulting in more resorptions, stillbirths and neonatal deaths. At twenty weeks of age carcinogenicity and AHH activity in the lung and liver will be measured to determine if differences at the Ah locus did result in differences in transplacental carcinogenesis. (Supported by NIAMS Grant ES-07073 and the Ohio Coal Lab Research Association).


This study was conducted to determine the suitability of 0.1% Triamcinolone Acetonide Cream (Aristocort®) as a positive teratogen, when applied topically to shaved backs of pregnant Charles River COBS® CD® rats. Each rat was administered 0.03 ml of Triamcinolone Acetonide Cream or control material, Hydrophilic Plastibase, from gestation days 6 through 15. Each rat was fitted with an Agar (acetate) collar during the treatment period to prevent oral ingestion of the test or control materials. Prior to mating rats were conditioned with the collars. Each female was injected intraperitoneally throughout gestation and examined daily for signs of systemic toxicity and skin irritation. Dams were sacrificed on gestation day 20 by carbon dioxide inhalation and the location and number of viable and nonviable fetuses, early and late resorptions and corpora lutea were recorded. The fetuses were removed, individually weighed, sexed and examined for external, visceral and skeletal malformations and variations. A statistically significant decrease in maternal body weight was observed in the Triamcinolone Acetonide treated group when compared to the control group. One of 12 treated rats aborted on gestation day 15 and another died of maternal toxicity on gestation day 18. Skin irritation was minimal. Significant increases in post-implantation losses and a corresponding decrease in mean number of viable fetuses were observed in the treated group as well as decreased fetal weight. The number of litters with malformed fetuses was significantly increased following treatment. Cleft palate and omphalocele were the most frequently observed anomalies. Triamcinolone Acetonide Cream is a suitable positive teratogen when administered topically to rats.

263 TERATOGENICITY OF OCHRATOXIN A IN RATS. K. Mayura, A. W. Hayes and W. O. Berndt, Dept. Pharmacol. & Toxicol., Univ. of Mississippi Med. Ctr., Jackson, MS 39216

Teratogenic potential of ochratoxin A (a potent nephrotoxin and a teratogen, produced by various species of Aspergillus and Penicillium) in rats has been studied by determining dose response and time course studies. Crystalline ochratoxin A was injected single dose subcutaneously to the rats on one of gestation days 4, 5, 6, 8, 9 and 10. The dose levels tested were 5, 2.5, 1.75, 1.0 and 0.5 mg/kg. There was complete fetal resorption in rats given 5 mg/kg, whereas in rats given 1.0 and 0.5 mg/kg, much less fetal resorption occurred and the fetuses did not show any abnormalities with regard to gross, skeletal and internal soft tissue. There was an increased incidence of fetal resorption in rats given 2.5 mg/kg. Among the dose levels tried, 1.75 mg/kg ochratoxin A seems to be the minimum teratogenic dose in rats which resulted in decreased fetal weight and various fetal malformations like omphalocele, ectopia cordis, anophthalmia and internal hydrocephalus. Skeletal defects also were noted involving ribs, vertebrae, sternebrae and skull. Largest number of resorptions, greatest depression of fetal weights, and largest number of malformations occurred when ochratoxin A was injected on either day 5 or day 6. (Supported by USPHS research grant ES 02191.)

264 THE EFFECT OF URANIUM ON THE FEMALE REPRODUCTIVE SYSTEM OF THE RAT. H. K. Gardner, F. A. Smith, R. B. Baggs, Departments of Radiation Biology and Biophysics, and Laboratory Animal Medicine, University of Rochester, Rochester, NY.

Although uranium is an extensively studied toxin, it has been studied predominantly in males. The increasing environmental appearance of uranium hexafluoride and its hydrolysis products, uranyl fluoride and hydrofluoric acid, has necessitated a study in the female. A pilot project was therefore undertaken. Adult virgin Sprague-Dawley rats were intratracheally instilled with either 2.5 mg uranyl fluoride in tris buffer per kg body weight or with equal volumes of buffer,

Previous studies in our laboratory have shown that the volatile solvent 2,2,2-trifluoroethanol rapidly produces testicular damage following i.p. administration or inhalation. The fate of carbon-14 labeled trifluoroethanol (2-14C-TFE) in the male rat was investigated over the interval of 15 minutes to 48 hours after intraperitoneal injection of 100 or 5 mg/kg. Findings were similar for each dose. The disappearance of 14C activity from the plasma was biphasic, with half times of 2 hours and 48 hours. Testicular levels of radioactivity 15 minutes to 48 hours postinjection were found to be comparable to those in the liver, kidney, and lung. Thus, there is no evidence of testicular concentration of TFE, and the fact that this organ can be severely damaged by the compound reflects a genuine sensitivity of the testis to trifluoroethanol. The pattern of 14C excretion was: urine >> expired air >> feces. No 14CO2 was detected in expired air.

One hour following the administration of 14C-TFE to control rats, 3% of the radioactivity in the testis (on a mg protein basis) was bound to proteins, whereas 58% of the radioactivity in the liver was associated with proteins. Pretreatment of animals with phenobarbital did not significantly increase the levels of apparent protein binding in either organ. The levels of protein binding observed in these experiments are so low as to be of questionable significance as a possible mechanism of trifluoroethanol's toxicity.

Omadine MDS, a broad spectrum anti-microbial proposed for use as an anti-seborrheic agent in shampoos and a preservative in cosmetics, was evaluated in a series of studies to assess fertility and general reproductive, postnatal and postnatal function and teratogenic potential.

In one set of experiments, Omadine MDS was given orally to COBS-CD Rats by gastric intubation at dosage levels of 1, 3 and 7.5 mg/kg to males for 60 days prior to mating (with untreated females), to females for 14 days prior to mating (with untreated males) through lactation day 21, or to females from gestation day 15 through lactation day 29. Controls received distilled water at the same regimen. No adverse effects were noted at 1 mg/kg. Dosage levels of 7.5 mg/kg were toxic in these experiments. There were clinical signs of intolerance, reduced body weight gain and high mortality of parental animals. Further, marked motor impairment was observed in the animals, manifested by ataxia and hind limb paralysis. Offspring parameters of treated animals in the fertility study were comparable to controls at all dosage levels, however, in the perinatal study, neonatal body weights and survival during lactation were affected in the 7.5 mg/kg group. In experiments assessing teratogenic potential, Omadine MDS incorporated in a shampoo base vehicle, was applied dermally to shaved intra-scupular skin for two hours/day on days 6-18 of gestation to New Zealand white rabbits at dosage levels of 0.45, 1.5 and 5 mg/kg or on days 6-13 of gestation to COBS-CD Rats at dosage levels of 1, 10 and 30 mg/kg. Omadine MDS was not teratogenic in the rat. Preliminary examination of the rabbit data indicated no teratogenic capabilities.


Presently chemicals are tested for their potential to damage the reproductive system by observing the chemical's effects on fertility, litter size and survival of the new generation. Alternatives to these protocols are needed because these studies are time consuming, expensive, and insensitive. The majority of the protocols suggested as replacements are focused on adult males with little attention given towards females or developmental susceptibility. The present study was an attempt to develop a more comprehensive protocol. In this study male (M) and female (F) hamsters (H) and rats (R) were exposed to known reproductive toxicants (including DBP, Kepone, MSG, Cd) during sexual differentiation of the brain, hypothalamic maturation, puberty and as young adults. The age at puberty was determined by vaginal opening in FR, behavioral estrus in FH, preputial separation in MR and flank gland growth in MH. Estrus cyclicity was observed in FR and FH. Sexual behavior was observed in FH and MH. The animals were bred, dosed through gestation, and fertility, litter sizes, offspring growth and survival were measured. Following breeding MR and MH were necropsied and reproductive tissues were measured (including the testes, epididymis, seminal vesicle, flank gland) sperm concentration in the vas deferens was measured, testicular histology was evaluated and hCG stim-
ulated serum testosterone was measured. Results demonstrate that exposure to known reproductive toxicants using this protocol can be easily identified and the effects of exposure during sensitive developmental periods are often more severe and persistent than adult exposure.


Adult Sprague-Dawley male rats (65 days of age) received 10 daily treatments of 0, 200 or 400 mg Benomyl/kg/d by gavage. Body weight, tissue weights, total epididymal sperm count and sperm concentration from the vas deferens were measured 14 days after the last treatment. Testicular histology was evaluated in 0 and 400 mg/kg/d groups. A second study used prepuberal Sprague-Dawley male rats (33 days of age) which received 10 treatments of 0 or 200 mg/kg/d by gavage. At 3, 17, 31, 45 and 59 days after the last treatment, 8 animals per treatment group were killed and measurements similar to those in the first study were made.

Significant findings included 40-50% depressions in the total epididymal sperm counts and in the vas deferens sperm concentrations in adult animals treated with 200 or 400 mg/kg/d. Histological evaluations of testicular sections from 6 adult animals in the 400 mg/kg/d group indicated a slight to moderately severe hypoplastic testis in 2 animals and a slight to severe generalized hypoplastic testis in 2 animals. No treatment effects were found in body weight, liver, kidneys, testes, seminal vesicles, or epididymides weights in the adult animal. Results from the study using the younger animal indicated no significant treatment effects in body weight, tissue weights, total epididymal sperm count, vas deferens sperm concentration or histology at any of the observation times.

This data suggests that younger animals are less sensitive to 200 mg Benomyl/kg/d than adults receiving the same dose.


Eighty 70 day old male rats were injected (sc) with either 0, 1.6, 3.1, 7.4, 16, 33, 74, or 152 µM Cd (as CdCl2)/kg. Typically studies of this nature use Cd doses of 10 to 50 µM/kg or greater and it has been reported that testicular toxicity does not occur at 6 µM or below. Fourteen days post dosing all animals were killed and testes, seminal vesicles and epididymids removed and weighed. Sperm concentration was determined in semen samples from the vas deferens. Serum [T] was measured following maximal in vivo hCG stimulation. In selected groups decapsulated testes were incubated in vitro and T production assessed.

Reproductive assessment was not conducted in the groups receiving 74 and 152 µM Cd/kg due to the mortality of 10 and 60 percent, respectively. Testes, seminal vesicle and epididymides weights were reduced at least 40 to 50 percent at doses of either 16 or 33 µM Cd/kg, and sperm concentration in the vas deferens and hCG stimulated serum [T] were undetectable.

Doses of 1.6 to 7.4 µM Cd/kg did not produce tissue weight decrements, however, significant dose related decrements in vas deferens sperm concentration (p < 0.0001) and hCG stimulated serum [T] (p < 0.003) were observed. In vitro androgen production following in vivo hCG stimulation was unaltered in the two lower Cd doses.

These results demonstrate that acute Cd exposure at doses reported to have no observable effect result in a decrease of both spermatogenic potential and androgen secretory potential, and demonstrate the utility of a multivariate approach to reproductive assessment.


Ordram, a rice herbicide, was tested for effects on the fertility of male mice and rabbits. In study I, 100 male CD-1 mice of proven fertility were randomized into 5 dose groups; vehicle control, 2, 20, 100, and 200 mg/kg/day. The males were dosed daily by oral gavage for 7 weeks. Fertility was determined by mating each treated and control male with 2 untreated females during treatment and again after a 4-week recovery period. An interim sacrifice was performed on 5 males/group at the completion of dosing and a final sacrifice was performed on the remaining males after the end of the recovery period. Treatment-related antifertility effects were observed in the 100 and 200 mg/kg dose groups during treatment. There were reductions in the number of pregnancies but no increase in resorptions. The study clearly shows a no-effect level of 20 mg/kg for the observed antifertility effects in male mice. No antifertility effects were observed in the fertility test after the 4-week recovery period, demonstrating complete reversibility. There were no histological or macroscopic changes indicative of a compound-related effect on the testes, epididymides, thyroids, or pituitaries in any treatment group. In study II, 37 male Dutch-Belted rabbits of proven fertility were assigned to 4 dose groups. There were 10 males in the vehicle control group and 9 males each in the 2, 20, and 200 mg/kg dose groups. A similar study design was employed as described above for the mice. No evidence of impaired fertility was found. Also, there were no histological or macroscopic changes found in the testes, epididymides, pituitaries, thyroids, or adrenals which could be related to the administration of Ordram.

271 REPRODUCTIVE TOXICITY OF METHYL-(1-BUTYLCARBAMOYL)-2-BENZIMIDAZOLE CARBAMATE (BENOMYL) IN MALE RATS. T.B. Barnes, K.M. Hayward, A.J. Verburg-Wiener, and M.C. Wilson, Dept. of Pharmacol. Sch. of Pharmacy, Univ. of Mississippi, University, MS. Sponsor: W.M. Davis
The present investigation into the reproductive toxicity of Benomyl was undertaken to determine the biochemical basis and functional significance of the damage previously reported by this laboratory (presented at SOT, 1980) to the male reproductive system. The experiment was divided into two phases, a 70 day feeding phase followed by a 70 day recovery phase. Adult male Wistar rats were fed chow mixed with 0.02, 2.0, or 200 ppm Benomyl. Control animals received powdered chow for the 70 day treatment period. Following the feeding study, one-half of the animals per group entered the recovery phase. In order to determine the effects of this fungicide on the testes, mutagenic, behavioral, and reproductive tests were performed during both the feeding and recovery phases. At the termination of both phases sperm counts were evaluated and a complete necropsy was performed on all animals. At the termination of the feeding phase sperm counts were depressed in the highest dosage group (200 ppm). A bilateral decrease in the relative testicular weights of all dosage groups was also observed. This damage to the testes was not permanent however, since following the recovery phase no differences in the sperm counts or in relative testicular weights were observed between any of the dosage groups. The functional and biochemical significance of this damage is under further investigation.

Supported in part by the Research Institute of Pharmaceutical Sciences.


Nitrobenzene (NB) produces a number of toxic effects in rats including methemoglobinemia (MetHb). Intestinal microflora metabolize nitrobenzene to nitrosobenzene and phenylhydroxylamine, both potent inducers of MetHb. Induction of MetHb is dependent on the presence of gut flora, since NB fails to induce MetHb in axenic or antibiotic treated rats (Reddy et al., Biochem. Pharm. 25). The current study investigated the role of gut microflora in the etiology of NB-induced testicular necrosis. Fischer-344 rats were treated p.o. with neomycin sulfate (100 mg), bacitracin (50 mg) and tetracycline (50 mg) in 1% carboxymethylcellulose or vehicle alone twice daily for 2 days prior to gastric intubation with 300 mg/kg NB in corn oil. The antibiotic regime was continued twice daily for 3 days and once daily for 2 additional days. The rats were killed 5 days after exposure to NB. The dose of nitrobenzene used was the lowest single dose which was previously shown to induce testicular necrosis. Antibiotic treatment prevented NB-induced MetHb. At sacrifice NB-antibiotic treated rats had 2% MetHb vs 20% MetHb in NB treated rats. In vitro analysis of the metabolic activity of the cecal contents showed that antibiotic treatment completely inhibited the metabolism of NB while the vehicle treated rats metabolized NB at greater than 1 µmole/min/g cecal contents. Despite the inhibition of cecal metabolism, the seminiferous tubules of the testis from antibiotic treated rats had a similar incidence and severity of necrosis as vehicle treated controls. Therefore the induction of testicular necrosis by nitrobenzene appears to be independent of gut microflora metabolism.


Chronic administration of 2,5-hexanedione (2,5-HD) to animals causes azoospermia and histopathology in some central nervous system (CNS) areas. These studies were designed to assess the degree of CNS involvement in the testicular lesions seen in the rat after 6 wks of 2,5-HD consumption. Additionally, activity measurements were made of some enzymes found in specific testicular-cells types. 2,5-HD was fed to adult F-344 rats as a 1% solution in the drinking water. After 1, 3, and 6 wks of treatment, 6 treated rats, their pair-fed controls, and 6 ad lib controls were sacrificed. Serum levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone were not depressed at any point examined, indicating that 2,5-HD does not
cause testicular atrophy by decreasing release of LH and FSH. At 6 wks, the testes were azoospermic. This coincided with a rise in LH (mean ± SE difference, treated value minus pair fed value = 47 ± 13 ng/ml) and FSH (mean ± SE difference, 280 ± 30 ng/ml), while testosterone levels were unchanged. Hormonal values from pair fed rats were not different from ad lib group values. After 3 wks, the testes were histologically normal. However, the specific activity of the Sertoli cell enzymes, S-glucuronidase and y-glutamyl transpeptidase, decreased by 68 ± 3% and 20 ± 7%, respectively (mean ± SE, n = 6). Liver, which is not affected histologically by 2,5-HD, showed slightly decreased lysosomal enzyme activities (S-glucuronidase and acid phosphatase) at all times. The significant changes in Sertoli cell localized enzymes before the onset of azoospermia, and the lack of effect on LH and FSH, suggest a direct toxic effect on the testis.

**275 MORPHOLOGICAL MATURATION OF RAT TESTIS**


**Sponsor:** J. B. Hook

The immature testis has only recently been recognized as a potential selective target of a variety of chemical toxicants. Since this organ changes markedly during maturation, the response to toxicants may vary depending on age at time of exposure. It is therefore important to define specifically the morphology at differing stages of cellular development in testis. The object of this investigation was to identify critical stages of development at which effects of toxicants might be most profound. Testes from male Sprague-Dawley rats at 1, 3, 6, 9, 12, 15, 18, 24, 30, 36 and 45 days of age were fixed in Bouin’s, embedded in glycol methacrylate and sectioned at 2 μ for morphologic analysis. Based on the data obtained and structural and functional events observed by others, 4 critical ages were identified: day 6, both Sertoli cell precursors and gonocytes, primordial germ cells, were actively dividing and Leydig cells were of fetal origin; day 15, Sertoli cells were forming tight junctional complexes, a critical part of the blood-testis barrier, spermatogonia were dividing mitotically to produce primary spermatocytes and Leydig cell numbers were at their nadir; day 24, spermatids had formed, thus reflecting both mitosis and meiosis and adult Leydig cells were beginning to differentiate; day 45, the time of puberty, Sertoli cells had attained all adult characteristics, mature spermatogonia had developed and there was a full complement of adult Leydig cells. Thus, exposure of animals during these 4 time periods should allow detection of any differential susceptibility of the immature rat testis to chemical toxicants.

**276 EFFECTS OF PHTHALATE ESTERS ON RAT TESTICULAR CELL CULTURES AND ON SERTOLI CELL FUNCTION IN THE INTACT TESTIS.**


Testicular injury produced by phthalate esters is characterised by an early disruption of normal Sertoli cell-germ cell interrelationships, with shedding of spermatids and spermatocytes from the germinal epithelium. We have therefore examined the effects of some phthalates on Sertoli cell function. This was assessed in vivo by measuring secretion of seminiferous tubular fluid and androgen binding protein (ABP) after unilateral ligation of the testicular efferent ducts. For in vitro studies, mixed cultures of Sertoli and germ cells were prepared by trypsin and collagenase digestion of testes from 4 wk old rats. Fluid secretion and ABP production were measured widely inhibited within 3 hrs after a single dose of dipentyl- (2200 mg/kg) or mono- (2-ethylhexyl) phthalate (MEHP) (1000 mg/kg) but were unaffected even after 3 doses of diethylphthalate, an ester not causing testicular injury. Addition of MEHP to Sertoli-germ cell cultures accelerated detachment of germ cells from the Sertoli cell monolayer at concentrations as low as 10-9M. Studies with other phthalate monoesters showed a correlation between production of germ cell detachment and testicular toxicity in vivo. These results suggest that Sertoli cells may be a primary target of phthalates causing testicular injury and that testicular cell cultures may be useful for investigating mechanisms of action and for screening compounds likely to cause testicular injury by similar mechanisms to phthalate esters. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food).

**277 BEHAVIOR OF OFFSPRING OF DAMS GIVEN ETHANOL DURING GESTATION.**

C. Kruger-McDermott, and I. Rosenblum, I.E.P.T., Albany Medical College, Albany, N.Y.

This study has been conducted to determine whether ethanol, given to pregnant dams at a critical time during organogenesis of the central nervous system, will result in behavioral aberrations in the offspring. Ethanol (20 mg/kg of 20% ethanol) p.o. was given to dams on day 8 of gestation; control rats received normal saline. Rate of gain in body weight of dams during gestation and reproductive performance was observed. Offspring were examined postnatally for gross evidence of teratology. They were also examined at the time they were killed. Growth rates were recorded. Behavior was measured by a shuttle box in a passive avoidance situation.

The first behavioral test was done on the 25th post natal day. Analyses of data indicate that offspring of dams receiving ethanol required more trials to learn the task than did controls. By the second test (day 32), the difference was no longer evident. Offspring of ethanol treated dams showed no teratology but the rate of weight gain was slower when compared to controls.

It is concluded that ingestion of ethanol by pregnant dams results in a decrement in behavior of the offspring which is mitigated within 3 hrs. The decrement may be due to 1) delayed learning capacity 2) motor deficiency.

*Shell Fellow*
The metabolism and plasma kinetics of orally administered theobromine (TB) were studied in pregnant (P) and non-pregnant (NP) female Sprague-Dawley rats at doses of 5, 10, 50, 200 and 200 mg/kg using TB sodium acetate and [8-14C] TB as a radioactive tracer. No statistically significant differences were observed in mean TB plasma half-life, apparent volume of distribution and clearance in either P- or NP-rats after TB doses of 5-200 mg/kg. Plasma kinetic parameters of P-rats were also similar to NP-rats. Plasma radioactivity was >99% TB and <1% 6-amino-[N-methylformyla-mino]-1-methyluracil as shown by HPLC analysis. Analysis of urinary metabolites by HPLC and a radioactivity monitoring system (after oral TB doses of 5 mg/kg TB with 10 µCi [8-14 C] TB) revealed more extensive TB metabolism in P- than in NP-rats with 38% and 55% administered TB excreted as unchanged TB, respectively. Urinary metabolites identified were 6-amino-[N-methylformylamino]-1-methyluracil (18-24%), 3-methylxanthine (5-9%), 7-methylxanthine (3%), 7,7-dimethyluric acid (2-5%) and 7-methyluric acid (1%). The percent of the TB dose recovered in urine (0-48 hours) was 76.7% for NP-rats and 76.2% for P-rats.


Male Sprague-Dawley rats were fed with beryllium as sulfate (0,5,50,500 and 1000 ppm) in their diets, with and without magnesium. Changes in body weights and mortality rate were determined periodically up to 8 weeks. On the 4th and 8th week, rats were sacrificed to obtain internal organs, each organ weighed and then preserved for future histological studies. Rats fed on 5 ppm beryllium and above showed growth inhibition by the 2nd week in the absence of magnesium. Such inhibitory effects were overcome in the presence of Mg++. Rats fed on beryllium showed higher mortality rates in the absence of magnesium. Rats receiving 1000 ppm beryllium showed a significant reduction in thymus and spleen weights, both in the presence and the absence of magnesium. Beryllium caused an increase in the weights of liver and kidney. Histological, biochemical and immunological studies are in progress. *Visiting Professor, Dept. of Anatomy, UTCHS, Memphis, TN (Minority BioMedical Support Program, NIH RR-08179).

Studies on the Mechanism of Tolerance to Cadmium Toxicity. P.L. Goering and C.D. Klaassen, Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS.

Animals pretreated with a sub-lethal dose of cadmium (Cd) subsequently develop tolerance to a lethal dose of Cd. Two possible mechanisms for this tolerance are reduced absorption and a shift in the tissue distribution of Cd. Male Sprague-Dawley rats received a single sc pretreatment (2.0 mg Cd/kg) either 1, 2, 4, 8, or 16 days prior to iv administration of the challenge dose (3.9 mg Cd/kg). Mortality was 100% in control rats. Tolerance was evident following injection of the lethal dose in Cd-pretreated rats since no mortality was observed in any of the pretreated rats. Since Cd pretreatment induces synthesis of hepatic metallothionein, a low molecular weight Cd-binding protein, its induction might enable a greater percentage of the challenge dose to be sequestered in liver of pretreated animals and less distributed to target organs of toxicity (kidney, testes). To determine if a challenge dose of Cd distributes differently after Cd pretreatment, rats were dosed as follows: Group 1 (control) received a saline pretreatment and an iv challenge dose (2.0 mg Cd/kg), Group 2 received a sc pretreatment (2.0 mg Cd/kg) and the challenge dose, and Group 3 was pretreated with Cd and given a saline challenge. The distribution of the challenge dose of Cd in the Cd-pretreated group was estimated as the difference in tissue concentrations between Group 2 and Group 3. At 2 and 24 hrs following Cd challenge, the content of radioactive Cd was determined. No marked shift in the distribution of Cd due to Cd pretreatment was observed in control and pretreated rats. We conclude that differences in absorption or tissue distribution of Cd are unlikely explanations for development of tolerance to Cd. (Supported by USPHS Grants ES-07079 and ES-01142).

Absorption of metals by the small intestine of newborn rats has been observed to be greater than absorption by adult intestine. Factors that influence mucosal uptake and whole body retention have been identified; however, the mechanism involved in the elevated uptake has not been elucidated. Experiments have been performed to measure the removal of Cd from proximal jejunum of newborn rats. Sprague-Dawley rat pups of either sex were examined on days 14, 20, 22, and 28 after birth. Intestinal cell uptake was measured using single pass perfusion of 0.05, 0.1 and 0.2 mM CdCl₂ through 2-7 cm segments of proximal jejunum. The perfusate was collected over 5 minute intervals, samples drawn, and the radioactivity compared with the pre-perfusion solution. Reducing the perfusion flow rate from 0.4 to 0.1 ml/min resulted in an increase in Cd uptake in all age groups and concentrations. Both increasing concentration and age reduced Cd uptake. At each concentration, uptake was highest in 14 day old pups compared to all other age groups. At 0.02 mM CdCl₂, 0.50±0.13% Cd/mg (mean±S.D.) was removed from the intestinal lumen of 14 day old pups while 0.08% Cd/mg was removed by intestine of 28 day old animals. At each age, uptake was highest at 0.02 compared to 0.2 mM CdCl₂. On day 14, 0.50±0.13% Cd/mg tissue was removed during perfusion with 0.02 mM CdCl₂; while 0.20±0.08% Cd/mg tissue was removed at 0.2 mM CdCl₂. Thus, flow rate, Cd concentration, and age influence Cd uptake from solutions perfusing the jejunum of the newborn. This information may prove useful in elucidating the mechanism responsible for the high Cd uptake and absorption by immature intestine. (Supported by NIH grants ES00159 and ES07073, and the Natural Sciences and Engineering Research Council of Canada.)


Although various cell systems are used or have been proposed as prescreens for efficacy/toxicity assessments of new chelating agents, no one is using freshly isolated Fe-loaded cells for that purpose. Male Sprague-Dawley rats (200-300 g) were administered ip 50 mg Fe dextran/injection on 3 alternate days. Liver cells were isolated 48 hr later by a perfusion method. Loads of 7-14 µg Fe/10⁶ total cells (~95% hepatocytes), 15-fold higher than normal, were achieved. The cells were cultured in hormone-supplemented Waymouth's medium for 22 hr ± cholylhydroxamic acid (CHA) and ferrichrome (FC) ionophores for Fe; deferoxamine (DF), the test standard; 2,3-dihydroxybenzoyl-substituted poly(vinylamine-vinylsulfonate) (DHBP), a new synthetic Fe chelator intended for use in the gut or blood stream; and combinations of these. DF at 25 mM decreased Fe content in the cells by 23%. For sensitivity in detecting synergism, DF and DHBP were tested at 0.5 mM and ionophores at ≥ 10-fold lower concentrations. Results were (% Fe release relative to control cells, based on 10⁶ of each): DF, 0; DHBP, 0; FC (0.2 mM), 2; FC + DF, 19; FC + DHBP, 34. CHA (0.5 mM) with or without DHBP was the most effective in releasing cellular Fe but cell viability was reduced to 3-4%, indicating significant cytotoxicity. These results suggest that properly chosen ionophore-polymer combinations may be more effective than DF for reducing excess Fe load in target organs. Experiments to optimize CHA-chelator mediated Fe release at lower nontoxic levels and to examine other ionophore chelate combinations are in progress. (Supported by NIH Grant AM 25647-03).


The consequences of chronic Mn exposure in young organisms whose brains are undergoing rapid development has received little study. There is evidence, however, that neonatal animals may be more susceptible to Mn toxicity because Mn is not excreted prior to weaning and because it shows enhanced relative accumulation in the brain of immature organisms. A series of experiments were designed to examine the accumulation of Mn in the brains of animals exposed either prenatally or neonatally to MnCl₂.

Pregnant long Evans rats when given MnCl₂ in their drinking water (5, 10 and 20 mg/ml) show depressed maternal body weight gain only at the 10 and 20 mg/ml doses. Birthweight and litter size were depressed at the 20 mg/ml dosage but not at the 5 and 10 mg/ml doses. Measurement of brain Mn by flameless atomic absorption did not show any significant elevations in experimental subjects. Further studies with the radionuclide ⁵⁴Mn indicate that only small amounts (I-3%) of an acutely administered dose crosses the placenta.

Direct administration of MnCl₂ (0, 25 and 50 µg/g/day) to rats by intubation during lactation does not alter body weight gain. The highest dose leads to an increase in Mn levels in several brain regions as measured by flameless atomic absorption.

These results suggest that the placenta is an effective barrier for the prevention of Mn accumulation in the rat fetus and that the fetus is unlikely to be particularly susceptible to maternal manganese exposure. Neonatal exposure, results in accumulation of manganese in the brains of experimental animals and can therefore be used as a model for studying the interaction of Mn with the catecholamines in developing animals. Supported in part by NIH grants ES00454 and ES07067.

285 THE INFLUENCE OF CHRONIC MANGANESE ADMINISTRATION AND EDTA TREATMENT ON THE TISSUE LEVELS AND URINARY EXCRETION OF Mn, Zn, Fe, and Cu IN RATS. A.M. Scheuhammer and M.G. Cherian, Dept. of Pathology, University of Western Ontario, London, Ontario, Canada N6A 5C1

A neurological syndrome resembling Parkinson's...
Tissue distribution of lead in neonatal rats

The Pennsylvania State University, University Park, PA 16802.

Although there are efforts to control the dissemination of lead (Pb) into the environment, Pb toxicity continues to be a major public health hazard, particularly with children, who have the tendency to absorb a higher percentage (as high as 80%) of their Pb intake than adults (3-10%). However, little is known about the retention and tissue distribution of Pb in the newborn, especially during chronic exposure. Accordingly, we have investigated the pharmacokinetics of Pb metabolism in neonatal rats chronically exposed to Pb. Starting from day 6 of parturition on every third day, the pups were administered intragastrically with either 0, 50, 100, 150, or 200 mg Pb/kg as Pb acetate containing 100 µCi of radioactive 210Pb. On day 21, animals were sacrificed and tissue levels of Pb were measured. The highest concentrations of Pb were observed in the bone, stomach, kidney, intestine, and intestinal contents, with lesser amounts in blood, liver, and brain. There was no appreciable Pb in either heart, lung or spleen. A dose related depression in body weight gain was observed in Pb treated animals. It was also observed that the major target organ affected by chronic Pb exposure is the spleen as evidenced by a significant decrease in weight.

Delayed behavioral toxicity of lead with increasing exposure concentration: A systematic replication.

Little work has been directed towards lead exposure beyond weaning. We report a systematic replication of an earlier study of chronic post-weaning lead exposure on schedule-controlled behavior including biological measures of lead body burden. Exposure of male rats to 500 ppm sodium acetate or 50, 100 or 500 ppm lead acetate in drinking water began at 21 days of age. The lever press response was shaped in a standard operant chamber at 55 days of age. Next, a fixed-interval (FI) 60 sec schedule of food reinforcement was imposed.

Despite changes in the strain of rats, diet, FI value, and experimental laboratory, results clearly resembled those of the previous study. All three exposure concentrations again increased response rates. The higher the exposure concentration, the greater the number of sessions to maximal rate-increasing effect. Rate increases of 200-300% of control values occurred after 30, 60 and 90 sessions for the 50, 100 and 500 ppm groups, respectively. Such delayed effects at higher concentrations suggest the presence of physiological or behavioral compensatory mechanisms. Blood lead values of treated groups increased over the first 100 exposure days, then declined slightly, averaging 2, 12, 19.5 and 55 µg/dl for the 0, 50, 100 and 500 ppm groups, respectively, after 150 exposure days. These data further confirm the vulnerability of rats to lead intoxication, even from moderate dose levels, beyond the nursing period and may require some revision of current notions about sensitive periods. Supported by ES01247, ES01248 and ES05177 (NIEHS) and DOE contract No. DE-AC02-76EV03490.

The effects of dietary lactose on the absorption and retention of inorganic lead in suckling and weanling rats.

The ability of the milk sugar lactose to increase the absorption and retention of many divalent cations, including Ca, Fe, Zn, Mg, Cu, Mn, Mo, and V, suggests a nonspecificity of action which should not exclude toxic elements such as Pb. The present studies assessed the effects of lactose on ingested Pb in suckling and weanling rats. In tracer studies fasted rats were intubated with 4 µCi of 210Pb or 2 µCi of 203Pb, and 0, 1, 3, or 6 mg lactose per gram body weight at 14, 18, 22, 26, 34, or 52 days of age. After a second overnight fast, the rats were killed and the radioactivity in blood, GI tract (including feces), liver, kidneys, and femur was measured by gamma emission spectroscopy. Retention in each organ was determined as a percent of dose, while absorption was calculated by difference from the radioactivity remaining in the GI tract. Under these conditions, lactose increased the absorption and retention of Pb at 3 and 6 mg/g, and at 22 and 26 days of age, compared to water and glucose controls. Maltose and galactose did not affect the absorption or retention of Pb. The results indicated that lactose affects the excretion of intravenous 203Pb. In chronic feeding studies with nonradioactive Pb, suckling rats were offered lactose or glucose at 0, 80, or 160 mM and Pb at 0, 10, or 100 ppm in drinking water, and a...
semipurified diet containing either adequate (0.43%) or low (0.02%) Ca. Rats were killed as before and femur and kidneys were collected. Tissue Pb levels were assayed by flameless atomic absorption spectroscopy to assess retention of ingested Pb. Under these conditions, lactose reduced the retention of dietary Pb and protected against the increase of Pb retention caused by the low-Ca diet. (Supported by NIEHS Grant ES-05147-02).


Although alterations in development due to inorganic lead poisoning have been intensely investigated, little is known about the early toxicity of organic lead compounds. Assessment of developmental consequences due to triethyl lead (TEL) intoxication included (1) determination of the acute LD50 as 12.8 ± 0.9 mg/kg, and (2) detailed examination of early neurobehavioral sequelae. The offspring of 12 Fischer 344 dams were pooled on postpartum day 3 with 4 pups of each sex assigned per litter. On day 5 pups were administered either a sham-injection, 15% ethanol, 3 mg/kg or 6 mg/kg TEL via s.c. injection (20 µl).

Small, but significant, weight reductions for 3 (6%) and 6 (13%) mg/kg dosed pups were observed (days 14-30). Early sensory deficits of TEL pups indicated by decreased homing ability (day 7) and nipple attachment (day 9) accompanied alterations in motor control (day 10).

While these initial effects were transitory in nature, weekly activity evaluations demonstrated progressive development of dose-related hypoactivity (days 14, 22, 29). During 72 and 144 hr tests of passive avoidance retention (days 21, 25) performance effects—hypoactivity—were noted in TEL males in opposition to retention loss in low dose female pups. No significant effects of T on Pb level in these tissues were 26-37% lower in Pb+ T treated animals. Reduced the retention of dietary Pb and protected against the increase of Pb retention caused by the low-Ca diet. (Supported by NIEHS Grant ES-05147-02).

EFFECTS OF INORGANIC LEAD ON CARBONIC ANHYDRASE ACTIVITY AND REGIONAL BRAIN LEAD AND ZINC LEVELS. D.A. Fox and R. Ku. Div. of Tox., Dept. of Pharm., Univ. of Texas Med. Sch., Houston, Texas 77025

Experimental findings reveal that exposure to inorganic lead (Pb) produces both convulsant and...
anticonvulsant effects: the response being both
dose and age dependent. Similarly, acetazolamide,
a carbonic anhydrase (CA) inhibitor, produces both a
dose and age dependent alteration in seizure re-
sponsiveness. To determine if the effects of Pb
were due to alterations in CA activity, in vitro
CA activity was examined using PbCl₂ and NaCl. CA
activity was inhibited by 10⁻⁹ and 10⁻⁸ M PbCl₂,
while 10⁻⁷ to 10⁻⁵ M PbCl₂ increased CA activity
relative to NaCl controls. These results suggest
that alterations in CA activity may partially ac-
count for the dose dependent convulsant and anti-
convulsant effects of Pb. In addition, regional-
cortex, hippocampus (HIPP) and cerebellum—levels
of zinc (Zn) and Pb were analyzed at 21 and 100
days of age in neonatally Pb-exposed rats pre-
viously demonstrated to exhibit dose and age de-
pendent alterations in seizure responsiveness (Fox
et al., Neurotoxicology 1:149, 1979). At 21 days of
age a dose dependent increase and decrease in the
Pb and Zn concentrations, respectively, was observ-
ed in all brain regions. These brains, regardless
of treatment, showed a selective accumulation of
Pb in the HIPP. In the 100 days of age, almost three
months post-exposure, a dose dependent increase
and decrease in Pb and Zn concentrations, respect-
ively, still existed. However, only in the control
group was there a selective accumulation of Zn in
the HIPP. This latter effect may partially account
for the demonstrated age dependent alterations in
seizure responsiveness in neonatally Pb-exposed
rats. (Supported by NIEHS Trng. Grant #ES07090,
NIOSH Grant #OH07090 and U.T. Biomedical Research
Starter Grant).

DEMMETHYLATION OF METHYLMERCURY BY MOUSE GUT FLORA
IN VITRO - EFFECT OF AGE AND DIET. I.R. Rowland*
sterne Rd, Carshalton, Surrey, U.K. and Environ.
Health Sciences Center, Univ. of Rochester,
The rate of fecal Hg excretion after oral admin-
istration of methylmercuric chloride (MeHg) is
much slower in pre-weanling mice (7⁻²⁰ days) than in
post-weanling mice, which excrete Hg at the
adult rate (7 about 10 days). In addition, adult
mice maintained on an evaporated whole milk diet
excrete Hg slowly (7%= about 10% after a
single p.o. dose of MeHg. Differences in the in
vitro rates of demethylation of MeHg>90% absorbed
in the gut) to inorganic Hg (about 10% absorbed)
were investigated as a possible explanation for
the age and diet effects.

In cultured cecal and colon contents from 10 day
old (pre-weanling) mice, radiolabelled MeHg was
demethylated slowly (8% of added MeHg demethylated
in 48 hours) whereas the cultured cecal contents
from 20 day old (post-weanling) mice converted
over 50% of added MeHg to inorganic Hg in 48 hours,
a rate comparable to that in adult animals. Cecal
contents from adult mice fed an evaporated whole
milk diet or a pellet diet had values of about 70%.
Organic Hg accounted for 48% of the Hg in liver at 10 dpd
and 38% at 32 dpd. In the brain, the organic frac-
tion was 95% at 1 dpd and declined to 60% by 32
dpd. The percentage of Hg body burden in pelt
rose from 30 to 85%. At all time points, about 90% of the Hg in pelt was organic Hg.

These results confirm the initial high re-
tention of Hg in methyl-Hg treated neonatal rats.
During this high retention period, a large part
of the initial dose of Hg entered the pelt. In
all tissues except pelt, Hg content declined rap-
idly from a total of 30% of the dose at 10 dpd to
10% at 32 dpd. These results show that demethyl-
ation of methyl-Hg begins by 1 dpd and occurs
throughout the interval of slow Hg excretion.
Thus, demethylation of methyl-Hg and initiation
of clearance of Hg appear to be independent pro-
cesses. (Supported in part by USPHS Grant
ES-01104).

SEX DIFFERENCES IN MERCURY DISTRIBUTION AND EX-
CRETION BY METHYLMERCURY TREATED RATS. Fisher,
H.L.¹, L.L. Hall¹, D.J. Thomas², P. Mushak³, M.
Sumler.¹ USEPA/HERL, RTP, NC², Sch. Hyg., Johns
Hopkins Univ., Balt., MD², UNC Sch. Med., Chapel Hill,
NC³, NSI, RTP, NC².

Male and female Long Evans rats (56 days
old) received 1 µmole of methyl (²⁰³Hg) mercury/
kg sc and were sacrificed 1 to 98 days post
dosing (dpd). Total and organic Hg contents of
tissues and excreta were determined.

At 98 dpd, total body organic Hg body burden
(BB) was 7.5% of the methyl-Hg dose in males
and 5.8% in females. Percentages of total tissue Hg
contents present as organic Hg ranked from high-
est to lowest as pelt, muscle, blood, kidney,
Liver, and brain. Total organic Hg BB was dif-
f erent in male and female due to difference in
pelt and muscle. Total inorganic Hg BB peaked at
about 8% of the dose of methyl-Hg. Pelt and kid-
neys were the largest depots for inorganic Hg.
Inorganic Hg content of pelt declined more slowly
of the gut microflora which, in turn, affects the
elimination rate and body burden of Hg.
(Supported by NIEHS ES01247, ES01248, US/DOE
EY76CO223490).

DISTRIBUTION AND DEMETHYLATION OF METHYLMERCURY
IN NEONATAL RATS. Thomas, D.J.¹, H.L. Fisher²,
L.L. Hall², P. Mushak³, M. Sumler.¹ Sch. Hyg.,
Johns Hopkins Univ., Balt. MD¹, USEPA/HERL, RTP,
NC², UNC Sch. Med., Chapel Hill, NC³, NSI, RTP,
NC².

Seven day old Long Evans rats received a
single dose of 1 µmole of methyl (²⁰³Hg) mercury
per kg sc and were sacrificed 1,2,4,10 and 32
days post dosing (dpd). Whole body radioassay
indicated little if any whole body clearance of
Hg until 17 days of age when there was definite
initiation of a single exponential clearance pro-
cess. At 32 dpd about 70% of the dose of Hg was
retained. At all time points, greater than 80% of
the Hg in blood was in an organic form. Hg in
muscle ranged from 79 to 100% organic, except at
32 dpd when it had decreased to 66% organic. At
10 dpd about 56% of the Hg in kidney was in an
organic form but at 32 dpd it was only 8%. Thus,
the kidney is 7 major depot for Inorganic Hg pro-
duced by metabolism of methyl-Hg. Organic Hg ac-
counted for 48% of the Hg in liver at 10 dpd and
38% at 32 dpd. In the brain, the organic frac-
tion was 95% at 1 dpd and declined to 60% by 32
dpd. The percentage of Hg body burden in pelt
rose from 30 to 85%. At all time points, about 90% of the Hg in pelt was organic Hg.

These results confirm the initial high re-
tention of Hg in methyl-Hg treated neonatal rats.
During this high retention period, a large part
of the initial dose of Hg entered the pelt. In
all tissues except pelt, Hg content declined rap-
idly from a total of 30% of the dose at 10 dpd to
10% at 32 dpd. These results show that demethyl-
ation of methyl-Hg begins by 1 dpd and occurs
throughout the interval of slow Hg excretion.
Thus, demethylation of methyl-Hg and initiation
of clearance of Hg appear to be independent pro-
cesses. (Supported in part by USPHS Grant
ES-01104).
296 ROLE OF GUT FLORA IN MERCURY EXCRETION AFTER METHYLMERURY ADMINISTRATION IN THE MOUSE.
I.R. ROWLAND*, R.B. ROBINSON & R.A. DOHERTY.,
Adult mice fed a pelleted rodent diet (RMH 3000), evaporated whole milk, or a semi-synthetic liquid diet (GIBCO 116 EC), exhibit different rates of whole body Hg elimination (T½'s for GIBCO, RMH, and milk were 6, 10, and 20 days, respectively) and fecal Hg elimination after a single oral dose of methylmercuric chloride (MeHg). After 6 days, 36%, 22%, and 9%, respectively of the initial dose was excreted in the feces. The proportion of inorganic Hg in the feces (5%, 21%, and 34%, respectively) indicates that more demethylation occurred in the first two groups and that this may affect fecal elimination. To assess the contribution of the gut flora to in vivo demethylation and excretion of Hg, we gave antibiotics to mice on each diet to eliminate their intestinal flora before MeHg administration. Antibiotic treatment reduced fecal elimination to nearly zero in the GIBCO and milk groups, and the RMH group excreted only 6% of the initial dose in the feces after 6 days. Antibiotic-treated mice on the GIBCO and RMH diets had higher tissue Hg concentrations and higher proportions of organic Hg in the feces, cecal contents, liver, kidney, and gut wall than their non-treated cohorts. The antibiotic treatment had little effect on these parameters in the milk-fed mice. These results are consistent with the theory that demethylation of MeHg by intestinal bacteria is a major factor determining the rate of excretion of Hg and that modification of the gut flora by diet may influence the rate of demethylation.

297 EFFECTS OF DISODIUM TETRACHLOROPALLADATE ON ISOLATED RAT LIVER MITOCHONDRIA. R.E. Biagini and W.J. Moorman, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Experimental Toxicology Branch, Cincinnati, OH; and G.W. Winston, Mt. Sinai School of Medicine, Department of Biochemistry, New York, NY. Sponsor: T.R. Lewis.
The effects of Na2PdCl4 on isolated rat liver mitochondrial electron transport and energy transfer were studied in vitro. Concentrations necessary for half maximal inhibition of oxygen uptake (ECS0) were determined polarographically. The ECS0 for a NADH-linked substrate system (glutamate, malate, and maleate) was 18 µM while the ECS0 using succinate as substrate was 15 µM. At 64 µM, NADH-linked substrate oxidation was inhibited 88% while succinate oxidation was inhibited 80%. At concentrations of Na2PdCl4 sufficient to inhibit oxygen uptake, there was a concomitant decrease in the rate of ADP phosphorylation measured by proton absorption. Additions of uncoupling agents (carbonyl cyanide-m-chlorophenol phenthlydrazone, CCCP) had no effect on Na2PdCl4 inhibition. State IV respiration was not increased at low Na2PdCl4 concentrations and there was no activation of the Mg-ATPase. Data from these experiments indicate that Na2PdCl4 is a mitochondrial respiratory chain inhibitor in vitro.

298 FACTORS AFFECTING NICKEL SULFIDE TOXICITY.
D.A. McNeill, C.L. Chrisp, and G.L. Fisher, Battelle Columbus Laboratories, Columbus OH 43201
The toxicity of intratracheally instilled Ni3S2 particles of different size distributions was investigated in two mouse strains. The LD50 for strain A/J mice exposed to coarse (maximum diameter of 20 µm) particles of Ni3S2 was 50 mg/kg. In contrast, the LD50 for both strain A/J and B6C3Fl mice exposed to fine (maximum diameter of 2 µm) particles was 4 mg/kg, i.e. the fine particles were about 12 times more toxic than the larger particles. The results of multiple exposure of these two different sized particles were compared in another experiment. Strain A/J mice exposed once per week for four weeks to coarser particles had an LD50 of 2 mg/kg. Both strains of mice similarly exposed to the finer material resulted in an LD50 of 1 mg/kg. In this case, the fine particles were only twice as toxic as coarse particles of Ni3S2 with that mode of exposure. When those same two sized particles were incubated in horse serum at 37°C for 3 days, the fine particles were three times more soluble than the coarse material. These in vitro and in vivo studies indicate that both particle size and mode of exposure may affect the biological availability and therefore toxicity of intratracheally administered Ni3S2. Support for the Electric Power Research Institute (Contract Number RP1639-2)

Vitamin E (Vit E), as a free radical scavenger, and selenium (Se), as an essential component of glutathione peroxidase (EC 1.11.1.9) (GSH-Px) function synergistically to modulate free radical production and hydroperoxide tone of the cell. Thus, they have the potential to affect the enzymes of prostanooid biosynthesis and also the profile of products formed in the arachidonic acid (A.A.) cascade. Accordingly, we investigated the influence of altered Vit E and Se nutrition on
A. A. metabolism in tissues of Long-Evans Hooded rats fed chemically defined purified diets containing adequate or documented deficiencies of Vit E, Se or both (diets: +E, +Se; -E, +Se; +E, -Se; -E, -Se). Cyclooxygenase activity of lung and liver microsomal preparations was significantly increased in Vit E deficiency whereas PG dehydrogenase activity (a key enzyme in PG catabolism) was increased two-fold in Se deficiency. Similarly, the activity of GSH-s-transferases (a group of related enzymes involved in leukotriene biosynthesis) was markedly increased in liver, kidney and lung cytosolic preparations of Se deficient animals. The Se-independent GSH-Px activity of GSH-s-transferases was concomitantly increased in Se-deficient states, while Se-dependent GSH-Px activity was completely abolished. In addition, epoxide hydrolase (an enzyme that competes with GSH-s-transferases for Se-dependent GSH-Px activity) will either prevent Sr$^{2+}$ will either prevent Sr$^{2+}$; and trivalent ions, Y$^{3+}$ and Cr$^{3+}$. For quantitative estimates of toxicity for these ions we utilized statistical parameters of LC50 and R, the change in concentration between the LC2.5 and the LC97.5. A standard assay procedure using 30 animals was devised to quantify the effect of sex, genotype, temperature, seasonal variations, and developmental state on the response of Drosophila to metal ions in the diet. Statistically reliable estimates of sensitivity under a variety of conditions have provided a basis for interpreting the biochemical alterations observed in response to metal ions, notably Cd$^{2+}$. Six divalent cations were tested for their effect on Q(+)$t$RNAs. (Q=7-deazaguanosine with a cyclopentenediol at C-7 through a -CH$_2$-NH$_2$- linkage.) As an aid in evaluating mechanisms of action, 13 metal ions with diverse physicochemical parameters have been examined and has been reported to correlate with degree of myelination in developing rabbit spinal cord (Lanbne et al, J.Neurochem 18: 1113, 1971). Lactate dehydrogenase (EC 1.1.1.27), (MES) severity in adult rats (Fox et al., The Toxicologist 1: 45, 1981) and mice (Doctor and Fox, The Toxicologist 2: this issue, 1982). The mechanisms responsible for these changes are not known. This study was undertaken to examine the neuropharmacological basis of this anticonvulsant effect. Male mice were injected (ip) with specific post-synaptic antagonists of the noradrenergic (NA), dopaminergic (DA), serotonergic (5HT) and muscarinic cholinergic (MACH) systems or with a pre-synaptic depletor of NA, DM and SHT prior to dosing with TET. Mice were MES tested at 0.5 hr post TET injection. The MES pattern is characterized by 5 grades of severity: 1 (minimal) to 5 (maximal). Control and all drug treated mice had grade 5 seizures. 1 mg/kg of TET (TET-1) produced 20% grade 4 and 80% grade 5 seizures, while 5 mg/kg TET (TET-5) produced 90% grade 2 and 10% grade 3 seizures. Reserpine (RESRP), yohimbine (YOH), metergoline, haloperidol and scopolamine protected against the effect of TET-1, while prazosin did not. However, only RESRP and YOH afforded any protection against TET-5. This suggests that there is a dose related effect of TET on various neurotransmitter systems: at low doses of TET the alpha NA, DM, 5HT and MACH systems modify the anticonvulsant effect, while at high doses of TET only the alpha NA system is involved. Correlative neurochemical data will also be presented. (Supported by NIHES Trng. Grant #ES07090, NIOSH Grant #NH07085 and Univ.Texas Biomedical Research Starter Grant).

The exact mechanism whereby triethyltin (TET) causes decreased myelin synthesis in postnatal rat brain is unknown. TET is a useful neurotoxic tool for studying hypomyelinating conditions. The purpose of these experiments was to examine the effect of TET on the development of selected enzymes in the cerebral pentose phosphate pathway (PPP). TET has been shown to cause a selective effect on myelogenesis (Beuker, et al J. Neurochem 36: 44, 1981). Histochemical studies have indicated that many PPP enzymes are concentrated within myelinated tracks (Kaufman et al, J.Neurochem 19: 1, 1972). The PPP is a major source of NADPH which is required for lipid synthesis and appears particularly active in oligodendrocytes. 15 mg/l TET SO$_4$ was added to the drinking water of Sprague-Dawley dams from day 1 postpartum. Whole pup brains were taken on days 3, 5 and 15, perfused with cold saline and homogenized in 0.32 M sucrose. On day 15, a period of active myelination, the total protein per brain of test pups (N=5) was 63% and cyclic nucleotide phosphodies-
304  EFFECTS OF TRIMETHYL TIN INJECTIONS IN PIGEONS.

D.E. McM illan, M. Brocco, G.R. Wenger, and L.W. Chang. Dept. of Pharmacology and Interdisciplinary Toxicology and Dept. of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.

Male white Carneaux pigeons received intramuscular injections of trimethyl tin hydrochloride (TMT). Two birds receiving 3 mg/kg doses died within 48 hrs. Two others, given 3 mg/kg of TMT were alive 24 hrs after injection, at which time they were sacrificed. Three birds receiving 3 mg/kg TMT in divided doses two weeks apart (1 and 2 mg/kg doses) survived, but exhibited impaired motor coordination, abnormal body posture, and decreased food intake. One of these birds slowly recovered over several weeks time, but the other two birds remained impaired for two months before sacrifice. Three birds receiving 1.75 mg/kg TMT did not recover during a 2 month period, but was successfully retrained under the same schedule at that time. A dose of 1 mg/kg TMT produced no effects in any of the four birds that received the dose for the first time, but weekly or biweekly administration of this dose in two of these birds produced a gradual decline in rates of responding under the fixed-ratio schedule of food presentation. Under light microscopy, pathological changes of nerve cells in the hippocampus, the pyriform cortex, and the cerebral cortex could be identified 24 hrs after exposure to 3 mg/kg TMT. These pathological changes included chromatolytic changes (disappearance of Nissl substance and eccentric nuclei), accumulation of eosinophilic hyalin material in neuronal cytoplasm, and neuronal shrinkage.

305  TWO ACUTE HUMAN POISONING CASES DUE TO EXPOSURE TO DIAZINON TRANSFORMATION PRODUCTS IN EGYPT.

S.A. Soliman, W. Sovocool, A. Curley, N.S. Ahmed, S. El-Fiki and A. El-Sebae. US Environmental Protection Agency, Research Triangle Park, NC and Alexandria University, Alexandria, Egypt. This is a report of two cases of high acute toxicity of diazinon in spraymen working in public health occupations in Alexandria, Egypt. Symptomatology was similar to that previously reported for exposure to paraathanon or other organophosphorus insecticides. Plasma and RBC cholinesterase (ChE) activity values were determined in blood samples obtained from both cases at different times after the incident. Cholinesterase activity showed a marked reduction up to 18 days after exposure. In case 1, blood cholinesterase activity was recovered to about 90% of the normal level of activity 28 days after the poisoning incident. This activity recovered to about the same level in case 2 but after only 20 days from the poisoning date. Experimental results suggested that this acute toxicity was due to unsuitable storing conditions of the emulsifiable concentrate (EC) formulation of diazinon. The used sample of diazinon that was applied was stored in "tin" containers (made of tin plated sheet steel). The EC (60%) was not in compliance with the WHO standard specifications regarding the emulsion stability tests and due to the presence of crystals in the EC. A sample of this crystalline material was subjected for analysis. Gas chromatographic analysis combined with mass spectrometric techniques failed to identify intact diazinon in samples of that material. The sample represented virtually complete conversion of diazinon into transformation products. Sulfo-tepp, dithionotetramethylpyrophosphate and monothionotetramethyl pyrophosphate were two of the identified products in the sample. These products are highly toxic compared with diazinon.

306  TRIMETHYL TIN INDUCED PATHOLOGICAL CHANGES IN THE BRAIN STEM NEURONS. L.W. Chang, T.M. Tiemeyer, K.R. Reuhl*, D.E. McM illan and G.R. Wenger. Departments of Pathology and Pharmacology, University of Arkansas for Medical Sciences, Little Rock, AR and Research Council of Canada*, Ottawa, Canada. Trimethyltin (TMT) has been reported to be a potent neurotoxicant. Besides the hippocampal neurons, some large neurons in the brain stem were also found to be affected by this toxic com-
MANGANESE CHLORIDE EXPOSURE ALTERS HIGH AFFINITY RECEPTOR BINDING AND DRUG-INDUCED ACTIVITY IN MALE RATS. J.M. Gerhart and H.A. Tilson, Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. (SPON: Richard Mailman)

Adult male Fischer-344 rats were exposed for up to 30 days via their drinking water to either 0, 10, 20, or 40 mg MnCl₂/ml dH₂O. The average daily dose for 10 mg MnCl₂/ml dH₂O-exposed rats was 166 mg MnCl₂ per day resulting in a cumulative dose of 5.0 g MnCl₂ over 30 days. No significant changes in body weight or water consumption occurred at this dose level. Rats exposed to either 20 or 40 mg MnCl₂/ml dH₂O had significantly decreased body weights within a 7 day exposure and were excluded from further testing. Exposure to 10 mg MnCl₂/ml dH₂O produced slight, but not statistically significant increases in whole brain Mn levels, as determined by atomic absorption spectroscopy. However, Mn levels were significantly increased in sera and adrenal glands. Activity responsiveness to a pharmacological challenge with harmine, but not apomorphine, was altered in Mn-exposed rats. Neurochemical alterations were seen in neurotransmitter high-affinity receptor binding assays. [³H]-spiroperidol binding was significantly increased in the striatum, but not in the frontal cortex. [³H]-muscimol binding was significantly decreased in the hippocampus but not cerebellum. [³H]-diazepam binding was not altered in the hippocampus.

Because of the projected increase in the use of diesel passenger cars, the possible health effects of their exhaust emission are being investigated. The exhaust particles from diesel passenger cars were collected, and the associated organic chemicals were extracted using dichloromethane as solvent. The resulting extract had low mutagenicity toward Chinese hamster ovary (CHO) cells in culture (~1/200 of benzo(a)pyrene). The extracts, however, had definite comutagenic effects. When CHO cells were treated with a diesel exhaust particle extract in combination with a mutagen [N-methyl, N′-nitro, N-nitroso-guanidine (MNNG) or benzo(a)pyrene (B(a)P)] the mutant frequency induced was 200-300% of that expected from the mutagenicity of each agent alone. This co-mutagenicity was observed for all diesel exhaust particle extracts (from cars of 5 different manufacturers) tested, using mutation at the hypoxanthine-guanine phosphoribosyl transferase gene locus, mutation at the Na⁺-K⁺-ATPase gene locus, and induction of sister-chromatid-exchange as endpoints. The interaction between diesel exhaust extract and B(a)P is of particular importance since B(a)P is a known environmental pollutant, and experimental mutagen and carcinogen. Our data therefore suggest the possible enhancement of the activities of environmental mutagens/carcinogens by the chemicals associated with diesel exhaust particles. This enhancement effect should be one of the factors considered in the health-risk analysis of the diesel exhaust emission. (Research performed under DOE Contract No. DE-AC04-76EV01013.)
COMPARISON OF THE MUTAGENIC AND CARCINOGENIC ACTIVITIES OF ASPHALT AND COAL TAR PAINTS USED IN CONTACT WITH POTABLE WATER. M. Robinson, R.J. Bull, D. Cmehil, R. Slater and J.R. Moler, Health Effects Research Laboratory, U.S.E.P.A., Cincinnati, OH 45268

Tanks and pipes used for storage and transport of drinking water are often coated with paints which contain either coal tar or asphalt pitch. We have begun a comparison of the potency of a series of products containing these materials utilizing the Salmonella/microsome assay (Ames test) and mouse skin initiation/promotion studies. Eight enamels were chosen for testing: 4 asphalt-based (AP) and 4 coal tar-based (CTP).

Initiation/promotion studies were conducted in the SENCAR mouse (40 females per group) utilizing single topical applications. A 20-week promotion schedule was used applying 1 ug of TPA (12-0-tetradecanoylphorol-13-acetate) 3 times weekly. Ames test results with AP were negative at up to 10 ul/plate. A very high mutagenic activity was found with CTP with rat liver S9 fraction. Significant tumor initiating activity was observed with AP as well as CTP. The potency of AP was considerably less, requiring approximately 200 ul/mouse to obtain an average of 0.5 tumors/animal at 20 weeks vs. 0.2 ul/mouse required of the CTP. These data correspond to a potency equivalent to approximately 15 to 60 mg of benzo-(a)pyrene [B(a)P] per ml of coal tar-based product. Estimates of B(a)P equivalence in AP would be 3 orders of magnitude lower. Total B(a)P and B(2)P content of CTP ranged from 1.9 to 3.4 mg/ml; although total indicated pyrometric hydrocarbon concentrations ranged from 29 to 60 mg/ml. Since the bulk of these compounds are weak or inactive as carcinogens, the analyses to date account for a relatively small fraction of the biological activity present.

DECREASED TIME OF ONSET AND INCREASED BRAIN DISPOSITION OF PHENOBARBITAL (PB) AFTER MICROWAVE IRRADIATION (MIR). E.B. Benson, J.M. Fujimoto and D.G. Lange. Research Services, V.A. Center, Wood, WI, and Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI.

MIR has been reported to increase cerebral blood flow and blood-brain barrier permeability. PB is a drug whose pharmacodynamics would likely be affected by these parameters. Male Swiss Cox mice, maintained at an ambient temperature of 23° C, were exposed for 10 min to near-field MIR at 2.45 GHZ power density 10 mW/cm², to give a 1.2° C increase in rectal temperature. An incandescent bulb (L) was adjusted to give an equivalent increase in rectal temperature. Within 1 min after irradiation, sodium PB, 150 mg/kg was given iv. Onset of anesthesia was the time from injection to when the mice could no longer right themselves for a 5 min period. Exp. I: Onset of anesthesia (min) and corresponding brain concentration of [PB, nmol/g] ± S.E. were for control (C) = 17.4 ± 1.6 [926.4 ± 7.5]; L = 13.3 ± 0.6 [905.4 ± 29.6]; MIR = 8.3 ± 0.3 [900.2 ± 11.2]. MIR decreased onset relative to C and L groups. Since all 3 groups had the same brain [PB] at the onset of anesthesia, the brain sensitivity was not changed. Exp. II: Brain concentrations of [PB, nmol/g] measured 5 min after PB, iv, were C = [603 ± 20.4]; L = [666.1 ± 14.3]; MIR = [734.1 ± 19.8]. Thus, MIR increased the disposition of PB to the brain, thereby decreasing its onset of action. Ambient temperature affected the times of onset for control vs. MIR groups: at 19°C, 27.5 ± 3.0 vs. 15.5 ± 2.6; at 27°C, 14.0 ± 1.5 vs. 11.7 ± 15 min. Supported by ES02006 and 5T32 ES07043.

A fire in a PCB-filled transformer produced widespread contamination of an 18-story building in Binghamton, NY, with soot-like material containing 2 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin, >47 ppm 2,3,7,8-tetrachlorodibenzofuran, 0.5% total PCBs in addition to numerous other PCDF and PCDD congeners. The bioavailability of the highly toxic components of the soot was investigated because of the possible high adsorbancy of the soot matrix. Female Hartley guinea pigs (350-550g), n=6, were administered a single oral dose of either 1-1250 mug crude soot in 0.75% aqueous methyl cellulose (MC)/kg body weight or a benzene Soxlet extract in MC equivalent to 4-1000 mug crude soot/kg. Activated carbon served as control. Body and organ weights were determined and histopathology and hematology were conducted only for animals which received up to 500 mg soot/kg and survived 40 days. A significant (p<0.05) increase in the absolute and relative (to brain) spleen weights was observed only at 10 mg soot/kg. A decrease in the absolute and relative thymus weights was observed at 100 and 500 mg soot/kg and a decrease in the absolute and relative kidney weights at 500 mg soot/kg. A decrease in the WBC count occurred only at 100 mg soot/kg. Body weight loss was observed at 500 mg soot/kg and above. The 14- and 21-day LD50s for crude soot were 1025 and 692 mg/kg, respectively, and the 14- and 21-day LD50s for soot extract were 625 and 282 mg soot-equivalent/kg, respectively. These results indicate that in an acute exposure to guinea pigs, the organic solvent extractable toxic components of the soot are approximately 40-60% bioavailable.


PCP-T is a widely used wood preservative and general biocide. We previously reported highly significant enhancement of tumor growth and depression of cell-mediated immunity in mice chronically exposed to PCP-containing diets. The present studies examined the humoral immune response in mice exposed to 50, 100, 250 or 500 ppm PCP-T in the diet for 9-11 weeks. No overt toxicity was apparent at these exposure levels. The T-cell dependent antiSRBC splenic antibody response was quantitated in Swiss-Webster mice using the Hemolytic Antibody Isotope Release (HAIR) assay. The T-cell independent antiDNP-Ficoll response was quantitated in C57Bl/6 mice by Cunningham et al.'s plaque assay. Serum antiDNP antibody levels were measured by passive hemagglutination. Following
exposure to 250 or 500 ppm PCP-T, the primary IgM response to SRBC was reduced to 30 and 19% of the control response and the secondary IgM response was delayed by 1 day and suppressed to 54 and 45% of control, respectively. The secondary IgG response was also delayed 1 day and the peak response suppressed to 82 and 68% of control. Exposure to 50 ppm PCP-T slightly delayed the IgG response but did not affect its magnitude. The IgG response was not altered by 50 ppm PCP-T. A direct suppressive effect of PCP-T on the number of antibody-forming cells (AFC) in the spleen was observed following immunization with DNP-Ficoll. The number of IgM AFC/10^6 spleen cells was reduced to 57, 54, and 33% of control in animals exposed to 50, 100, and 250 ppm PCP-T. In addition, peak serum antibody titers to DNP were delayed and dose dependently suppressed in PCP-T exposed mice. Comparable spleen weights and cell recoveries following immunization suggested that cells from PCP-T animals proliferate but are inhibited in the production of antibody, particularly IgM antibody.

**COMPARATIVE HAZARDS OF METHANOL, ETHANOL, ISOPROPYL ALCOHOL AND GASOLINE ALONE AND IN MIXTURE AS FUELS FOR VEHICLES.** H.S. Posner, NIEHS, NIH, P.O. Box 12233, Research Triangle Park, N.C. 27709 Sponsor: J.A. Moore.

It is important that severe health problems not be created in switching to new fuels. Though gasoline has been the predominant fuel for vehicles in the U.S.A., plans are in progress to substitute methanol, ethanol or mixtures of gasoline or diesel fuel with lower alcohols.

Review of the literature shows that gasoline toxicity is slight after ingestion during siphoning mainly by adults or inadvertent swallowing by children, unless inspiration into the lungs occurs (serious and mainly seen in children); after inhalation in confined spaces by children and adults, and sniffing or huffing mainly by children and teenagers, which have resulted on occasion on death by sedation or sudden death believed to be by effect on the heart. In most cases recovery occurs with rest and supportive care. Alkyl lead has added additional toxicity.

Between methanol (M), ethanol (E) and isopropyl alcohol (I), M has the highest vapor pressure (100, 40 and 33 mm Hg, at about 20°C, resp.), the lowest metabolism (0.3 and 27 mg/100 ml/hr for M and E, resp.), a high skin absorption (0.145 mg/cm²/min after 15 min on the forearm, increasing to 0.220 then decreasing to 0.185 mg/cm²/min after 35 and 60 min, resp.); the lowest minimal oral lethal dose (75, 300-400 and 240 ml, resp.); and the longest latent period before symptoms (up to 72 hr, an hour and several hours, resp.). The M in a mixture of 155 M in diesel fuel was also absorbed 3 times as fast on the forearm as pure M. Treatment for M poisoning, on the whole, has been the most intensive and most prolonged. M and less so I have been abuse problems as substitutes for E.

M could be more safely used in closed systems such as turbines, fuel cells, etc. rather than in large quantities in the open environment. (Sponsored by Dr. John A. Moore.)


The detection of short-patch DNA repair by scintillation spectrometry (SS) in the primary rat hepatocyte/DNA repair system has been difficult due to the small number of bases inserted, variability in the level of scheduled DNA synthesis and nonspecific thymidine binding. This report demonstrates that suspension cultures of hepatocytes are valuable for detection of short-patch repair if steps are taken to reduce the level of semi-conservative DNA replication and to remove nonspecifically bound thymidine. Isolated hepatocytes from Sprague-Dawley rats were placed into suspension culture with hydroxyurea (HU) and after preincubation were exposed to dimethylnitrosamine (DMN), ultraviolet irradiation (UV) or methylmethanesulfonate (MMS). H-thymidine was added to each culture and incubation was continued. Cells were processed for SS after preparation of whole cell homogenates, an 800xg. fraction or nuclei isolated by discontinuous sucrose gradient centrifugation. Specific activity of DNA obtained from each step was determined and comparisons were made between treatments. 10 mM HU reduced 3H incorporation in control cultures by 40%-50% at all points in the nuclear purification procedure. DMN produced dose-dependent UDS at concentrations of 0.1 - 1.5 mM. This repair was apparent only in the 800xg. fraction and isolated nuclei. Incubation for 4 hrs was necessary to detect UDS at DMN concentrations less than 1.0 mM. Repair of UV-induced damage was apparent in all three steps of nuclear purification. In all cases the fold difference in 3H incorporation was greatest when comparing isolated nuclei of DNA damaged cells to controls. Preparation of an 800xg. fraction did not increase the sensitivity of UDS detection for UV compared to whole cell homogenates.

Methods currently utilized to measure tissue metallothionein (MT) either rely on non-routinely available substances (RIA), or lack the sensitiv­ity to measure low levels of MT. Omosaka et al. (ISEI Kagaki 24:128, 1978) described a method for measuring MT in tissues which utilized guinea pig blood hemolysate to scavenge exogenous 109 Cd not bound by MT in the sample. We have modified and evaluated this method for estimating MT. Aliquots of heat-denatured 15000 x g homogenate supernatant are incubated with 109 Cd at a final Cd concentra­tion of 1.0 µg Cd/ml. Cd not bound to MT is re­moved by addition of 2% hemoglobin solution fol­lowed by heat denaturation and centrifugation to remove the precipitate and excess Cd. The supernatant is then quantitated for Cd by gamma counting. Utilizing this procedure on Sephadex G-75 and DEAE A25 column chromatography fractions of liver homogenates from Cd and Zn treated rats, Cd remained in the supernatant only in fractions where MT elutes. Tissue levels of MT were eval­uated with this assay in Zn (200 µmol/kg), Cd (10 µmol/kg), Hg (5 µmol/kg) and non-treated rats. MT levels in non-treated rats were low but measur­able (1.6 and 10.1 nmol Cd bound/g of liver and kidney, respectively), whereas Zn, Cd and Hg in­duced liver MT was 441, 252 and 10 nmol Cd bound/g tissue, respectively. The limit of sensitivity for this assay is 0.5 nmol Cd bound/g of tissue. The coefficient of variation (COV) of replicate assays of a given supernatant dilution are less than 0.2. However, measurement over several dilutions re­sults in greater variation, with a COV of about .10. The sensitivity of this assay allows quanti­tative determination of MT in small amounts of tissue from organs other than liver and kidney, such as lung, spleen, testis, placenta and fetal liver.


Ethylenthiourea (ETU) has been shown to produce various teratological, toxicological and tumorigenic effects in rats and mice. The objective of the present study was to ascertain whether thyroid changes elicited by the inclusion of ETU in the diet of rats were reversible. Groups of four randomly selected, weanling, Caesarian-derived, Sprague-Dawley male littermates (obtained from BioBreeding, Ottawa) were fed diets containing 0 (32 litters), 75 (64 litters) or 150 (64 litters) ppm ETU for periods of 7 to 82 weeks. Upon discon­tinuation of the ETU diet, one littermate from each of 4 control and 16 treated litters were killed to assess the extent of thyroid lesions elicited by ETU. The remaining three littermates were then fed control diet for 9 to 42 weeks during which time the second, third and fourth littermates were killed to assess the thyroid lesions.

Parameters monitored included body weight, feed consumption, some absolute and relative thyroid weights and histo­pathology. A scoring system for the pathological lesions was established and a proposed general statistical procedure for assessing reversibility was applied to the results. The severity and extent of reversibility of the histopathological lesions were found to be a function of the duration of exposure to ETU.


Earlier work in our laboratories suggests that interference with brain lipid metabolism during the critical period of rapid brain growth in the neonatal rat can result in the development of chronic, epileptiform activity later in life (Brain Res. 150:543, 1978). Administration of Ul8,666A (10mg/kg,i.p.) an inhibitor of cholesterol biosynthesis, to neonatal rats induces a recurrent spontaneous epileptic condition by 10 weeks of age as assessed by flurothyl seizure threshold test­ing and electrocorticography. The objective of the present study was to re-examine the character­istics and time-course of Ul8,666A-induced seizures and to test similar analogs, AY9944 and 20,25-diazacholesterol for potential epileptogene­rity. Rats treated with Ul8,666A from birth (but not from 30-60 days of age) exhibited recurrent, stereotypic seizure patterns consisting of 3-5s bursts of high voltage spiking identical to our previous report. Administration of AY9944 (10mg/kg.s.c.) to neonatal rats every 6 days for 7 wks resulted in spontaneous epileptiform activity in the adult similar, though more frequent than the Ul8,666A seizures. Epileptiform activity was an­alyzed by computer and plotted as the power spec­tral density for comparisons. Neither acute ad­ministration of Ul8,666A, AY9944 nor chronic exposure to 20,25-diazacholesterol (10mg/kg.s.c., qod for 5 wks) caused any evidence of recurrent seizures. Induction of epileptiform activity by Ul8,666A and AY9944 may be correlated to changes in brain lipids. It is concluded that neonatal exposure to neurotoxic substances may lead to per­manent alterations in brain function in the adult.

3-METHYLCOLANTHRENE-IRON DEXTRAN INTERACTIONS. L. Brooks and M.A. Gallo, Dept. of Environmental and Community Medicine, CMDNJ-Rutgers Medical School/Rutgers Univ. Joint Graduate Program in Toxicology, Piscataway, New Jersey.

The combined effects of two hepatic enzyme inducers on subcellular hepatic systems were studied. 3-Methylcholanthrene(3MC) is a potent inducer of mixed function oxidase activity (MFO) and Iron Dextran(ID) has been shown to induce lysosomal systems.

Sprague-Dawley rats were injected i.p. with 3MC (20 mg/kg/day) for three consecutive days, followed by i.p. injection of either ID (125 mg/
kg/day) or saline for three consecutive days. Appropriate control groups were maintained.

Treatment with 3MC resulted in significant increases (p 0.05) in MFO activity in terms of production of 4-OH Biphenyl (approximately 300%), production of 2-OH Biphenyl (approximately 750%) and measurement of cytochrome P450 (approximately 200%). When treatment with 3MC was followed by administration of ID this induction was attenuated. Administration of iron, regardless of pretreatment, resulted in significant increases in membrane lipid peroxidation. Induction by iron of lysosomal enzymes in the post-mitochondrial supernatant and post-lysosomal supernatant was observed to be greater in the presence of 3MC.

Microscopic examination of stained liver preparations suggested that iron exacerbates previously reported sinusoidal distention induced by 3MC.


It has been found that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits growth in culture of certain human keratinocyte lines derived from squamous cell carcinomas and that this inhibition is antagonized by hydrocortisone. In the present work, we have examined modulation by hydrocortisone and vitamin A of cross-linked envelope formation, a distinctive marker of keratinocyte differentiation. Cells of the carcinoma line SCC 13 were grown on a lethally irradiated 3T3 feeder layer in Dulbecco-Vogt Eagle's medium supplemented with 5% fetal calf serum and examined soon after reaching confluency for competence to form ionophore-inducible envelopes. Under these conditions, 10% or fewer of the cells were competent to form envelopes, whereas 40% to 60% of the cells grown with added hydrocortisone (0.4 µg/ml) were competent. When the cells were grown and passaged in medium supplemented with charcoal extracted fetal calf serum, however, 60% of the cells were competent. These results are interpreted to mean that low concentrations of hydrocortisone in untreated fetal calf serum suppress cellular competence to form cross-linked envelopes, and that hydrocortisone is capable of antagonizing this effect. Since TCDD was not inhibitory to the growth of cells competent to form envelopes, this work emphasizes that target cell physiology is a critical factor in response to toxic agents and must be considered in exploration of TCDD toxic mechanisms.

322 STIMULATION OF MURINE SPLENIC LYMPHOCYTES AFTER SKIN PAINTING WITH A TUMOR PROMOTER. R.A. Rahof and C.S. Baxter, Dept. of Environ. mental Health, Univ. of Cincinnati Medical Center Cincinnati, OH. Sponsor: P.B. Hammond.

The tumor promoter 12-O-tetradecanoyl phor­ bol-13-acetate (TPA) synergistically enhances the response of murine lymphocytes to T-cell mitogens but has very little mitogenic activity alone in vitro. In order to examine the effects of TPA in vitro, its interaction with Staph. enterotoxin A (SEA), a recently reported in vivo T-lymphocyte mitogen, was investigated. Back skin of Balb/c mice was painted with 2, 6, or 10 µg of TPA in 100 µl of acetone weekly for two weeks. Twenty-four hours after the last dose 20 µg of SEA was administered retro-orbitally. Forty-eight hours after the SEA injection spleen lymphocyte mitogenic activity was increased in a dose-related manner, a 60% increase occurring at the highest (10 µg) dose. However, unlike its effects on lymphocytes in vitro, skin painting with TPA stimulated mitogenesis even when no other mitogen was present. Mitogenic activity in mice treated with TPA, but not with SEA, was approximately 50% of the levels induced in ace­tone-treated mice by injection of SEA. One application of 6 µg of TPA was sufficient to induce a two-fold enhancement of mitogenesis within 24 hours. Peak stimulation was reached between 48 and 72 hours after dosing. When mice received two weeks of TPA treatments, the maximum stimulation reached 48 hours after the last application was more than twice the maximum level achieved after one TPA application. An 8-fold enhancement of mitogenic activity was observed at that time.

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323 EFFECTS OF CHLORINATED PHENOLS ON IMMUNITY IN RATS. J.H. Exon and L.D. Koller, Veterinary Science, University of Idaho, Moscow, ID. Female Sprague-Dawley rats were exposed to 0, 5, 50 or 500 ppm 2-chlorophenol (2-CP) or penta­chlorophenol (PCP) from weaning to 3 weeks post­parturition after breeding at 90 days of age. Progeny were weaned at 3 weeks of age and con­tinued on chlorophenol treatment for 10 weeks at which time many immune functions were tested. Humoral immunity was measured by an indirect ELISA, cell-mediated immunity was monitored by delayed-type hypersensitivity (DTH) to oxazolone, and macrophage function was tested by phagocyto­sis of sheep red blood cells. Rats treated with cyclophosphamide were included as a positive immunosuppressed control. PCP-treated rats had significantly decreased antibody titers and DTH response and increased induced peritoneal macro­phage numbers which displayed hyperphagocytic activity. Immune responses in rats treated with 2-CP were not significantly different from controls. The data indicate that 1) the immune system may be a sensitive target for PCP toxicity but not for 2-CP, 2) closely related chloro­phenolic chemical isomers may exert different toxic effects on the immune system, and 3) PCP can exert depressive effects on some major immune parameters while enhancing others.

324 EFFECTS OF MONOETHYLPHTHALATE (MEHP) ON MOUSE ANTIBODY-PRODUCING CELLS IN VITRO. Daniel Wierda. Dept. Pharmacology & Toxicology, West Virginia University Medical Center, Morgantown, West Virginia 26506. Sponsor: John A. Thomas.

MEHP is a metabolite of diethylhexyl phthalate,
Methods for Inducing and Eliciting Delayed Contact Hypersensitivity by Vaginal or Skin Exposures in Female Guinea Pigs. Newmann, E. A., Buchler, E. V., The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45247

A technique is described that permits vaginal-only exposure to test materials without incidental skin (perianal) contamination. We have used it to determine the efficacy of the vaginal route for the induction and elicitation of delayed hypersensitivity.

2,4-dinitrochlorobenzene (DNFB) or 2,4-dinitrofluorobenzene (DNFB) will induce and elicit delayed contact hypersensitivity in female guinea pigs after vaginal or skin exposures. Female guinea pigs sensitized by skin exposures to DNFB cross-reacted in the vagina and on the skin to DNFB. Moreover, animals sensitized to DNFB cross-reacted in the vagina and on the skin to 2,4-dinitrobenzensulfonic acid, sodium salt (DNBSO_3^-).

Tissue changes produced in the vagina by DNFB in animals sensitized to DNFB cross-reacted in the vagina and on the skin to 2,4-dinitrochlorobenzene sulfonic acid sodium salt. The development of pseudocystic cufing and an overall increase in the inflammatory response.

These data suggest that guinea pigs produce delayed contact hypersensitivity by vaginal exposure to compounds that have been reported to produce similar reactions in the skin of both guinea pigs and humans.


A panel of humoral and cell-mediated immune function tests were utilized to determine the immunotoxicity of mercuric chloride, since prior studies have not completely assessed the nature of the immune defects. Six week old female guinea pigs were provided with drinking water with 0.3, 15 and 75 ppm mercury (as mercuric chloride) for 7 weeks. At termination there were 4 and 22 ppm mercury in spleen from the two highest dose groups, and no mercury accumulation in bone marrow or thymus. The 75 ppm mercury dose caused general immunosuppression but also resulted in non-specific toxicity, as indicated by depressed body, thymus, and liver weights plus hematological changes. However, several cell-mediated immune responses were also depressed in the 15 ppm dose group, where no overt toxic responses occurred. Lymphoproliferative responses to T-cell mitogens were <50% of controls and decreases in the mixed leukocyte response to allogeneic leukocytes were observed. Host resistance assays also suggested these were cell-mediated immune defects in mercury-treated mice. Dose dependent depression of pentose shunt, glycolytic, and Krebs cycle metabolism in bone marrow, thymus, and spleen were seen and believed to have been associated with some of the cellular immune defects. Neither lymphoproliferative responses to B-cell mitogens nor immunoglobulin concentrations were altered in mercury-treated mice. The only B-cell defect we observed was a decrease in the spleen PFC response to the T-dependent antigen, SRBC, at the 75 ppm dose level, but there was none to the T-independent, B-cell mitogen, LPS. The primary immunotoxicity of mercuric chloride appears to be exerted on cell-mediated immunity since several defects occurred at a lower dose than that of humoral-mediated immunosuppression.

Alterations in the Natural Killer (NK) Cell Activity of Mice Following Exposure to Nickel, Cadmium or Manganese. R. J. Smialowicz, R. R. Rogers, R. J. Garner and M. M. Riddle. Health Effects Research Laboratory, Research Triangle Park, NC 27711. (Sponsored by N. Chernoff).

Natural killer (NK) cells have been implicated as being an important effector mechanism in tumor cell cytolyis in vitro. NK cells display a spontaneous cytotoxic reactivity against syngeneic and allogeneic tumor cells in the absence of immunological priming and as such have been suggested to play a significant role as a first line of defense against newly arising malignant cells. We present evidence that several metals of environmental significance alter NK cell activity in mice. NK cell activity was determined using the in vitro Cr release cytotoxicity assay and an in vivo tumor cell clearance assay. A single intramuscular injection of NiCl_2 (18.3 mg/kg) caused a significant suppression in NK activity which returned to normal within a few days. This suppression was not related to the generation of suppressor cells. A single injection of CdCl_2 (6.25 mg/kg) caused a similar suppression in NK activity. On the other hand, a single intramuscular injection of MnCl_2 (80 mg/kg) caused a significant enhancement of NK activity.
activity which persisted for several days. The injection of A/J mice, which have low NK cell activity, with MnCl₂ caused a significant increase in NK activity as measured by both the in vitro cytotoxicity assay and the in vivo tumor cell clearance assay. These results indicate that assessment of NK cell activity using these sensitive assays can be of significant utility in assessing immunotoxic effects of environmental pollutants. Whether alterations in NK cell activity play a significant role in the development of tumors following exposure to certain toxic agents remains to be determined.

328 THE ELISA: APPROACH TO ASSESS HUMORAL IMMUNITY FOR IMMUNOTOXICOLOGY. L.D. Koller and J.R. Exon, Veterinary Medicine, University of Idaho, Moscow, ID.

Immunotoxicology is a relatively new field of investigation that is becoming recognized and used by toxicologists involved in drug and chemical evaluations. The purpose of this study was to develop a highly sensitive, quantitative immune assay procedure that could assess humoral immune responses in rats and mice used in chemical testing procedures. The enzyme-linked immunosorbent assay (ELISA) proved to be not only highly sensitive and quantitative, but also simple to perform, reliable, economically feasible, extremely accurate, required few test animals and could be automated. The antigens tested were bovine sera albumin and ovalbumin and the chemicals evaluated to validate the system were lead, polychlorinated biphenyl, cyclophosphamide and dexamethasone, all of which were immunosuppressive at the dosages tested. Practical application of the procedure appropriate to immunotoxicology will be discussed.

329 A PROINFLAMMATORY EFFECT OF TCDD ON CARRAGEENAN-INDUCED INFLAMMATION IN THE RAT PAW. H. M. Theobald, R. W. Moore and R. E. Peterson, School of Pharmacy, University of Wisconsin, Madison, WI.

As previously reported (The Pharmacologist 22: 197, 1980) a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) enhanced carrageenan-induced paw edema in male rats assayed 5 days after treatment. We have now further characterized this response. The increase in paw edema volume was detectable when assayed 2 days after 10 µg TCDD/kg, maximal after 5 days and still present at 29 days. Five days after TCDD (10 µg/kg), edema volumes in TCDD and vehicle treated rats were similar during the first 60 min after hind paw injection of carrageenan, but after 90 min the edema volumes were greater in TCDD treated rats and the difference was maximal between 3 and 4.5 hr. Thus, the effect of TCDD on paw edema occurs during the delayed phase of the inflammatory response to carrageenan. Also at 5 days, the effect of TCDD on carrageenan-induced paw edema was dose related from 0.1 µg/kg (no effect) to 100 µg/kg (near maximal effect) with an approximate ED₅₀ of 6 µg/kg. Thus, the enhancement of carrageenan paw edema is a new dose related effect of TCDD that is as sensitive as the well known effect on thymus weight. 3,4,5,3',4',5'-Hexabromobiphenyl (5 mg/kg), another halogenated hydrocarbon that binds to the TCDD binding protein, also enhanced carrageenan-induced paw edema, but 2,4,5,2',4',5'-hexabromobiphenyl (90 µg/kg) which does not bind had no proinflammatory effect. (Both compounds substantially induced liver microsomal enzymes in these rats.) The results suggest that the effect of TCDD on carrageenan-induced paw edema may be mediated by the TCDD binding protein. (Supported by NIH grant ES01332.)

330 THE EFFECT OF 12-O-TETRADECANOL PHORBOL-13-ACETATE (TPA) ON THE ANTIBODY PRODUCING CAPABILITIES OF IN VITRO SENSITIZED SPLEEN CELLS. Shopp, G., White, K., and Munson, A. Dept. of Pharmacology, Medical College of Virginia, Richmond, VA.

TPA affects several elements of the immune system and the study of the mechanisms of these effects may offer insight concerning its mechanism of action as a tumor promotor. This study examines the effect of TPA on the in vitro immunization of BDF1 mouse spleen cells to sheep red blood cells, as measured by the number of IgM antibody forming cells (AFC). The ED₅₀ that suppressed AFC was between 7.7 x 10⁻⁸M and 7.7 x 10⁻⁹M; 7.7 x 10⁻⁹M resulted in complete inhibition. TPA did not significantly affect cell viability at these concentrations (trypan blue exclusion test). A time course study showed that the suppression seen was not due to a shift of the peak response day. When TPA (7.7 x 10⁻⁹M) was added on consecutive days (day 0 = immunization; day 5 = assay for AFC), it inhibited AFC when added on days 0 and 1. Addition on day 2 resulted in 50 % inhibition, whereas days 3 and 4 resulted in a 2.2 and 1.3 fold increase, respectively. These results suggest that TPA may be affecting the macrophage early in the immune response, considering the macrophage is important in the initial 48 hours. However, because of the enhanced response on day 3, other cell types such as the suppressor T-cell may also be affected. (Supported by NIHES grant U54ES07087.)


Male Sprague-Dawley rats were fed either a low (I) mixed fat (5%) diet or a high fat diet (24%). The high fats diets contained either saturated (II), unsaturated (III) or partially saturated (IV) fats. Half of each group received DMH 15 mg/kg/5x. The toxic effect of DMH, expressed as a depression in the splenic lymphocyte transformation response to Concanavalin A (Con A) was observed only in the low fat group. The response of DMH treated rats was 4,452 ± 1,025* as compared with the untreated rats 17,813 ± 2,824* (P <.01). In comparing the three high fat diets (II, III, IV) to the low fat (I) diet, only diet III, the unsaturated fats, had a significant (P <.05) effect in depressing the transformation response to Con A. Phytomitogen and Pokedexit Motogen. These results indicate that alter-
tion in the quality and quantity of dietary fat has selective and varying effects on the local and systemic immune responses. These factors must be considered in immunotoxicology studies.

Supported in part by NIH Grant CA26917.

**CPM stimulated ~ SEM**


Nickel has been implicated as a potential toxicant in a number of clinically significant pathological findings in man. There is also evidence for significant alterations in host defense mechanisms of experimental animals treated with nickel. In the present study the effects of nickel chloride on the cellular and humoral immune response of mice was examined. A single intramuscular injection of nickel chloride (18.3 mg/kg) caused a significant involution of the thymus within two days following treatment. Thymic involution was not correlated with an increase in serum corticosterone levels. Significant reductions in the in vitro mitogen stimulated response of lymphocytes from nickel chloride treated mice (single injection of 36.6 mg/kg) were observed for the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) but not the B-cell mitogen lipopoly saccharide (LPS). Significant suppression of the primary antibody response to the T-cell dependent antigen sheep red blood cells was observed following a single injection of 18.3 mg/kg NiCl₂. In all cases the immunosuppressive effects of NiCl₂ were found to be transient with responses returning to normal within a few days. No alteration in the response of mice immunized with the T-cell independent antigen polyvinylpyrrolidone was observed following treatment with nickel. Furthermore, the phagocytic capacity of resident peritoneal macrophages from nickel treated (18.3 mg/kg) mice was not significantly different from saline-injected mice. The results suggest that NiCl₂ predominantly affects T-cell mediated immune responses.

**ORGANOPHOSPHATE (OP) POISONING AND SUPPRESSION OF THE PRIMARY IgM RESPONSE TO SHEEP RED CELLS (SRC) IN C57Bl/6 MICE. G.P.Casale, S.D.Cohen and R.A. DiCapua. Sch. Pharmacy, Univ. Conn., Storrs, CT 06268**

In a previous study we demonstrated that parathion (PAR), at doses which caused 40% lethality, selectively suppressed the IgM response to SRC in surviving mice. This study was undertaken to determine if other OP insecticides produced a similar effect at doses which produce overt signs of cholinergic poisoning. Male mice were immunized with SRC and dosed, po, 2 days later with malathion (MAL), 720mg/kg, or dichlorvos (DDVP), 120mg/kg. They were killed on day 4 post-immunization for determination of the primary IgM response by a modified Jerne plaque assay. DDVP killed 17% and MAL killed 50% of the mice within 2 days. IgM plaque forming cells, from spleens of surviving mice, were suppressed (75-80% control) by MAL but not by DDVP. Brain cholinesterase (CHE) activities in the same mice were 57% and 80% control, respectively. The IgM suppression by PAR & MAL but not DDVP may be a function of the duration and intensity of OP-induced cholinergic crisis during the post-immunization period. Mice were dosed as above or with PAR (16mg/kg) and killed at selected times. Brain CHE was decreased to 20-25% control within 3hr by each OP. With DDVP, CHE returned to 70% in 5hr after dosing; yet with both PAR & MAL, CHE remained at 15-25% control for more than 12hr. The cholinomimetic, arecoline (65mg/kg) caused a short-lived cholinergic crisis but no IgM suppression. When cholinergic crisis was extended over 4hr after sustained-release arecoline, IgM PFC was suppressed (59% control). Results suggest that IgM PFC suppression by OP is secondary to cholinergic stress, rather than a result of direct OP action on the immune system. (Supported by NIHES grants 05205 and 02524)

**EFFECTS OF PARATHION ON THE IgM AND IgG RESPONSES TO SHEEP RED CELLS (SRC) IN TWO MOUSE STRAINS. G.P.Casale, R.A.DiCapua and S.D.Cohen. Sch. of Pharmacy, Univ. Conn., Storrs, CT 06268**

Organophosphates have been reported to suppress the primary IgM response. The present study was undertaken to determine the effects of parathion on both primary IgM and IgG responses, as determined by modified Jerne plaque assay, in inbred (C57Bl/6) and outbred (CD-1) male mice. For the IgM studies, mice were immunized with SRC, dosed 2 days later with parathion (16mg/kg,po) and killed on day 4 post-immunization. Brain and plasma cholinesterase activities were about 50% of control in both strains. In 3 separate experiments with B1/6 mice, splenic IgM plaque forming cells (PFC), expressed either per 10⁶ nucleated cells or per spleen, were suppressed (10-30% of control). With CD-1 mice, the PFC response was less consistently and less severely suppressed (30-75% control). In spite of the high dose of parathion used (40% mortality in less than 2 days) the results suggest a selective effect on splenic IgM PFC. For the IgG studies, PFC were enumerated 8 or 10 days post-immunization. Parathion (16mg/kg,po) was administered to B1/6 mice either 2 days post SRC or 2 days prior to splenectomy. Additional mice of both strains were given parathion (56mg/kg) on alternate days after immunization for a total of 4 doses. IgG PFC in treated and control mice were not significantly different. The lack of an effect on IgG PFC further supports the hypothesis of a selective effect of parathion on IgM PFC. The basis for this demonstrated selectivity is not known. (Supported by NIHES grants 05205 and 02524)

**IMMUNOSUPPRESSANT AND ANTIINFLAMMATORY EFFECTS OF DIETHYLSILBESTROL (DES). Holtsapple, M.P., Morahan, P., Bick, P., and Munson, A.E. Medical College of Virginia, Richmond, VA 23298**

Female adult B36CF1 mice were administered DES (s.c.) in corn oil at dosages of 0.2, 1.0, 2.0, and 4.0 mg/kg body weight per day for 14 consecutive days. Exposure to DES produced a dose-rela-
ted decrease in the delayed hypersensitivity response (DHR) and the proliferation of the popliteal lymph nodes following challenge with sheep erythrocytes, and in the acute inflammatory response to carrageenan. The effects of the DHR to challenge with keyhole limpet hemocyanin were less dramatic following exposure to DES by two schedules. In other groups of mice, body weights, organ weights, and differential cell counts were determined. DES-treated mice gained more weight than the controls. There was a dose-dependent increase in liver weight, a slight, non-dose-related increase in spleen weight, and a remarkable thymic involution with a significant reduction noted at 0.2 mg/kg DES. Exposure to only 4.0 mg/kg DES produced significant alterations in the differential counts as the lymphocytes were increased and the polymorphonuclear leukocytes were decreased.

In parallel studies, mice were treated with DES for 14 days as before, but were allowed to recover for 30 days prior to testing. In these animals, there were no effects on either the DHR to sheep erythrocytes or on the acute inflammatory response to carrageenan, and all organ weight changes were reversed. These results indicate that a suppression of cell-mediated immunity with a corresponding involution of the thymus occurs following sub-chronic exposure to DES and that both effects are reversed within 30 days. The results with the carrageenan test suggest that at least part of the immunosuppressive activity by DES is mediated by non-specific anti-inflammatory effects. (Supported by NIEHS contract ES-1-5001.)

IN VITRO INHIBITION OF HUMAN LYMPHOCYTE TRANSFORMATION BY NABILONE. G. A. Waterhouse, L. D. Brandt, S. B. Leapman, and R. B. Forney, Department of Pharmacology and Toxicology and Department of Surgery, Indiana University School of Medicine, Indianapolis, IN.

Lymphocytes obtained from normal, healthy, drug-free individuals were incubated alone or in the presence of 1.0 µg/ml to 100 µg/ml phytohemagglutinin (PHA), 1x10^-7 M to 1x10^-3 M Nabilone*, or a combination of the two compounds. The incorporation of 3H-thymidine was used as a measure of lymphocyte responsiveness. 1x10^-3 M Nabilone completely abolished the response to all concentrations of PHA, while 1x10^-4 M Nabilone inhibited 90% of the stimulation induced by 10 µg/ml to 100 µg/ml PHA. Stimulation by concentrations of PHA lower than 10 µg/ml were completely inhibited by 1x10^-4 M Nabilone. At concentrations lower than 1x10^-4 M, Nabilone had little or no effect on the PHA induced responses. Nabilone by itself had no effect on lymphocytes, while PHA alone illustrated a dose dependent increase in 3H-thymidine incorporation.

*A synthetic cannabinoid, courtesy of Eli Lilly and Company, Indianapolis, IN.

HEINZ BODY PRODUCTION AND HEMATOLOGICAL CHANGES IN THE HEN AFTER ADMINISTRATION OF A SINGLE ORAL DOSE OF N-BUTYL MERCAPTAN AND N-BUTYL DISULFIDE. K.M. Abdo, P.R. Timmons and M.B. Abou-Dania, Department of Pharmacology, Duke University Medical Center, Durham, NC 27710 and National Toxicology Program, NIEHS, Research Triangle Park, NC 27709.

N-butyl mercaptan (nBM) is a breakdown product of S,S,S-tri-n-butyl phosphorotrithioate (DEF) and S,S,S-tri-n-butyl phosphorotrithiole (merphos) in hens and in the environment. n-Butyl disulfide is an oxidation product of nBM. A single oral dose of each compound (nBM or n-butyl disulfide) was administered in gelatin capsules to groups of five 12-month old laying hens. A third group of five hens was given gelatin capsules. One day after administration, the dosed hens exhibited weakness which progressed to unsteadiness and inability to stand by the third day. In addition, their combs turned pale 18-24 hours after dosing, and then became dark at 48 hours. After these initial reactions, the condition of the hens improved. Heinz bodies and extensive erythrocyte deformation and lysis were observed in blood smears taken from hens 48 hours after treatment. Hemoglobin concentration, hematocrit, erythrocytes and glucose-6-phosphate dehydrogenase activity were significantly lower than in the control hens, while methemoglobin was significantly higher. As the clinical condition of these hens improved, these hemolologic changes became normal. nBM caused an initial increase in plasma butyrylcholinesterase activity which returned to normal by the end of the 28-day experiment. Also, brain acetylcholinesterase activity was not different from that of the control at termination. (Supported in part by NIEHS Grant No. ES02717.)

THE EFFECTS OF DELTAMETHRIN, A SYNTHETIC PYRETHROID INSECTICIDE ON LOMOMOTOR ACTIVITY IN RATS. K. M. Crofton and L. W. Reiter, Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and Neurotoxicology Division, Health Effects Research Laboratory, US EPA, Research Triangle Park, NC 27711.

Deltamethrin is a synthetic pyrethroid insecticide that is currently experiencing a vast in-

A QUANTITATIVE IN SITU OXIDATION METHOD FOR ORGANOPHOSPHONO- AND PHOSPHOROTHIOATES. S. A. Solomon and Robert D. Zehr, Neurotoxicology Division, US EPA, Research Triangle Park, NC 27711.

Most of the well known biological effects of organophosphono- and phosphorothioates are re-

THE EFFECTS OF DELTAMETHRIN, A SYNTHETIC PYRETHROID INSECTICIDE ON LOMOMOTOR ACTIVITY IN RATS. K. M. Crofton and L. W. Reiter, Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and Neurotoxicology Division, Health Effects Research Laboratory, US EPA, Research Triangle Park, NC 27711.

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THE EFFECTS OF DELTAMETHRIN, A SYNTHETIC PYRETHROID INSECTICIDE ON LOMOMOTOR ACTIVITY IN RATS. K. M. Crofton and L. W. Reiter, Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and Neurotoxicology Division, Health Effects Research Laboratory, US EPA, Research Triangle Park, NC 27711.
crease in worldwide usage. A report is made here of the effects of deltamethrin exposure on locomotor activity. Three experiments were performed to determine: (1) the dose-effect function for changes in locomotor activity, (2) the time course of effects over a 24 hr period, and (3) the effects of 5 daily exposures. Long Evans hooded rats approximately 70 days old received deltamethrin in 0.2 ml/kg corn oil. For all experiments, activity testing was conducted for 1 hr in a figure-eight maze (Reiter, L. W. et al., Environ. Hlth. Perspect. 12:119-123, 1975).

In the dose-effect study, animals received either 0, 2, 4 or 8 mg/kg p.o. 2 hr before testing. In the time course study 4 mg/kg p.o. was administered either 1, 2, 4, 8 or 24 hr before testing. In the repeated dosing and repeated testing study, animals received 4 mg/kg p.o. either 2 hr before or 2 hr after testing for five days. Significant decreases in activity were found for the 2, 4 and 8 mg/kg groups in the dose response study. The ED50 was 3.59 mg/kg and the estimated behavioral toxicity index (LD50/ED50) was 14.5. For the time course study, significant decreases in activity were found at 1 and 2 hr after dosing, with recovery by 4 hr after dosing. The repeated dosing/testing study revealed no cumulative effect of 4 mg/kg deltamethrin administered for 5 days. These results indicate that deltamethrin is an acute behavioral toxicant with rapid onset and recovery of effects on locomotor activity.

340 ALLERGIC HYPERSENSITIVITY TO THE INSECTICIDE MALATHION IN MICE. J. R. Cushman and J. C. Street. Toxicology Program, Utah State Univ., Logan, UT.

The widely used insecticide malathion [(dimethoxyphosphinothiol)thio]butanediolic acid, diethyl ester] has been reported to cause allergic responses in some exposed people. The following studies were performed to determine whether malathion would cause cell-mediated or antibody-mediated hypersensitivity in female BALB/c mice. Delayed-type hypersensitivity was determined by change in ear thickness, 5-[125I]iodo-2'-deoxyuridine incorporation in the ear, and histology of the ear following ear challenge of mice previously treated on the abdomen. Half of the mice were pretreated with cyclophosphamide to enhance any response. No differences were observed between malathion-treated and control mice.

To elicit malathion-specific antibodies of the IgE class, a conjugate of the anhydride of the metabolite [(dimethoxyphosphinothiol)thio]butanediolic acid with keyhole limpet hemocyanin was administered intraperitoneally with aluminum hydroxide as adjuvant. The degree of conjugation was 8.7 hapten per 100,000 daltons of protein. Sera were collected following three sequential sensitizations and tested for specific IgE using the passive cutaneous anaphylaxis (PCA) test in Sprague-Dawley rats. The anhydride of the malathion metabolite coupled to bovine serum albumin was used as the challenge antigen (degree of conjugation was 12.4 hapten per molecule of protein). Specific IgE was produced following the second and third sensitizations in the mice receiving 10 and 100 µg of conjugate. This IgE antibody-mediated hypersensitivity may provide the basis for hypersensitivity occasionally observed in exposed humans. (Supported by EPA Cooperative Agreement CR-807295).


Quantitative measurements of blood and tissue paraquat (PQ) burdens are necessary to establish the in vivo importance of active PQ accumulation in the production of lung disease. Subcutaneous weekly (4x) administration of PQ (72 µmole/kg) effectively and reproducibly produce lung disease in Sprague-Dawley rats. After a single sc dose, peak blood levels (ca. 55 µmole/ml) were obtained 20-30 min after 14CH3-PQ. After 7 d the blood level was ca. 0.04 mmole/ml and tissues ranked as follows (CH3-PQ/g): carcass, lung, injection site, brain, small intestine, large intestine and feces, heart, liver, kidney, adrenals. 14CH3-PQ in carcass accounted for 79% (ca. 4.0 mmole/g) of the total body burden. Rapid PQ clearance complicates the design of studies of active lung accumulation. To achieve a stable blood level Alzet® osmotic pumps were implanted in the left thigh. Blood drawn using an indwelling jugular cannula contained 0.6-1.6 mmole PQ/ml at steady state. Highest tissue burdens after 19 d were seen in intestines, kidneys, and lung (6-11 mmole). Carcass contained ca. 6.0 mmole/g which was 54-65% of the total body burden. Anorexia, weight loss, and piloerection were observed after 18 d. At virtually the same dosage, rats given PQ using the osmotic pumps had more severe lung damage than rats injected sc. PQ is rapidly absorbed using osmotic pumps which permit establishment of a lung disease model without compromising the function of other organs, particularly the kidney. (Supported in part by NIH Biomedical Research Development Grants 1 SO8 RR07170 and 2 SO7 RR09770.)

342 SUBCHRONIC TOXICITY OF ETHYLENETHIOUREA IN MICE AND RATS. P. Kurtz, A. Peters, D. Donofrio and R. Chhabra, Battelle Columbus Laboratories, Columbus OH 43201 and National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Ethlenethiourea (ETU) is a decomposition product of ethylenebisdithiocarbamate agricultural fungicides and has been previously reported to produce thyroid carcinoma in the rat. This study examined possible differences between rat and mouse in susceptibility to the effects of subchronic exposure to ETU in dosed feed. In a preliminary study groups of 10 rats and 10 mice were continuously exposed to ETU concentrations between 500 and 8000 ppm for 14 days. Exposure to the three highest concentrations produced total (8000 and 4000 ppm) or partial (2000 ppm) group mortality in rats but no mortality in mice. The greater toxicity of ETU in rats is consistent with reports that this com-
pound has a longer half-life in rats than in mice. In the 13 week subchronic study, 10 rats per sex were exposed to ETU concentrations ranging from 60 to 750 ppm and ten mice per sex were exposed to doses ranging from 125 to 2000 ppm. There were no compound-related deaths in rats or mice. Growth depression was evident in both male and female rats exposed to 750 ppm and in male rats exposed to 300 ppm. Weight gain was mildly depressed in mice exposed to 2000 ppm. Thyroid follicular hyperplasia was seen in all the rats of the 750 and 500 ppm groups and in 16/20 rats of the 250 ppm group. Thyroid adenomas occurred in 65%, 45% and 35% of the rats in these groups, respectively. In mice, thyroid follicular hyperplasia was seen in all of the 2000 ppm group and in 19/20 mice from the 1000 ppm group. Thyroid hyperplasia is considered a preneoplastic response to ETU exposure. These observations suggest that mice may also be susceptible to the carcinogenic effects of ETU. (Supported by NIEHS Contract No. NOI-ES-8-2151).

343 SUBACUTE DERMAL TOXICITY OF LANNATE® IN THE RABBIT. G. L. Kennedy, Jr. and D. F. Edwards, E. I. du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19711
Lannate® (active ingredient is methomyl; S-methyl N-[(methyl-carbamoyl)oxy]thioacetimidate) is an insecticide used for broad-spectrum control of insects. The subacute dermal toxicity of a formulation containing 30% methomyl in a mixed solvent medium was investigated by applying either 50 or 100 mg to the shaved backs of rabbits (10 per test group) on 10 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 clinical signs were monitored daily. Erythrocytes and plasma cholinesterase activity were measured and complete gross and microscopic pathology were conducted on 1/2 of each group 3 hours after the 10th dose and on 1/2 following a 14-day recovery (no treatments) period. Two identical groups, 1 treated with distilled water and the other treated with the solvent vehicle, served as controls. Rabbits treated with 100 mg/kg Lannate® showed slight to mild skin irritation only during the exposure period. Plasma cholinesterase activity was slightly reduced (approximately 20% of pre-test values) immediately post-exposure in rabbits of the 10 mg/kg group and returned to normal during the recovery period. Erythrocyte cholinesterase activity was not affected by the compound. No compound-related effects in any of the test rabbits were detected upon pathologic examination. Rabbits treated with the mixed solvent vehicle showed severe skin irritation which resulted in cessation of treatment following the fifth dose. The skin at the treatment sites of these vehicle-control rabbits returned to normal during the recovery period and no other changes were observed.

The present research was designed to assess the potential for progressive growth of hyperplastic, adenomatous and carcinomatous mouse liver lesions induced by or associated with transplanted hepatocellular carcinoma (MHC). Pure cholangiocellular carcinoma was not found. Pathologic lesions were histologically and histochemically assessed. While normal hepatocytes were GGT negative, the earliest hepatocellular lesion demonstrated homogeneous GGT activity and the later lesions were progressively more heterogeneous. Normal bile duct epithelial cells and the epithelia of both simple and cystic cholangiomas, cholangiofibrosis, and the ductal element of mixed hepatocell-cholangio-cellular carcinoma (MHC). Pure cholangiocellular carcinoma was not found. Pathologic lesions were histologically and histochemically assessed. While normal hepatocytes were GGT negative, the earliest hepatocellular lesion demonstrated homogeneous GGT activity and the later lesions were progressively more heterogeneous. Normal bile duct epithelial cells and the epithelia of both simple and cystic cholangiomas were uniformly and intensely GGT positive. The epithelium of cholangiofibrosis was GGT negative, although entrapped areas of neoplastic hepatocytes were GGT positive. The ductal element of MHC was GGT positive. Stromal elements associated with cholangiofibrosis and MHC were GGT negative. Lesions of connective tissue origin, such as fatty infiltration and early fibrosis, and a hepatocellular carcinoma with extreme fatty degeneration and infiltration were GGT negative. While acquisition of GGT activity by hepatocytes is considered a reliable marker of preneoplasia, the significance of loss

The livers of Sprague-Dawley rats chronically fed Aroclor 1260 (100 ppm for 16 months followed by 50 ppm for 8 months sequentially developed areas of hepatocellular alteration, neoplastic nodules, and hepatocellular carcinomas. Lesions of ductal morphology consisted of simple and cystic cholangiomas, cholangiofibrosis, and the ductal element of mixed hepatocell-cholangio-cellular carcinoma (MHC). Pure cholangiocellular carcinoma was not found. Pathologic lesions were histologically and histochemically assessed. While normal hepatocytes were GGT negative, the earliest hepatocellular lesion demonstrated homogeneous GGT activity and the later lesions were progressively more heterogeneous. Normal bile duct epithelial cells and the epithelia of both simple and cystic cholangiomas were uniformly and intensely GGT positive. The epithelium of cholangiofibrosis was GGT negative, although entrapped areas of neoplastic hepatocytes were GGT positive. The ductal element of MHC was GGT positive. Stromal elements associated with cholangiofibrosis and MHC were GGT negative. Lesions of connective tissue origin, such as fatty infiltration and early fibrosis, and a hepatocellular carcinoma with extreme fatty degeneration and infiltration were GGT negative. While acquisition of GGT activity by hepatocytes is considered a reliable marker of preneoplasia, the significance of loss.
of this enzyme activity by the ductal elements of cholangiofibrosis remains to be elucidated. (Supported by the VA and NIH grant CA22140.)

IRON EXCLUSION FROM AFLATOXIN B1-INDUCED HEPATOCARCINOMA IN RATS, M.A. Gallo, L. Brooks, J. McCoy, T. Clark, Dept. Environmental and Community Medicine, Dept of Pathology, Dept of Surgery, CMDNJ-Rutgers Medical School/ Rutgers Univ. Joint Graduate Program in Toxicology, Piscataway, N.J.

Previous studies have shown that partial hepatectomy followed by an hepatic carcinogen induces foci which exclude iron. The purpose of this experiment was to test the hypothesis that altered foci induced by aflatoxin B1 (AFB1) are resistant to iron accumulation without any other manipulation, i.e., partial hepatectomy or altered diet.

Male weanling Sprague-Dawley rats received 50 µg/rat/day of AFB1 in DMSO by stomach intubation to a total dose of 1.10 mg AFB1/rat. Age- and sex-matched controls received similar volumes of DMSO. Eighteen months after administration of the last dose of the toxin half the animals in each group received iron dextran intraperitoneally (125 mg/kg/day) for three days and the other half of each group received no iron treatment.

Preserved liver sections stained with hematoxylin and eosin (H&E) indicated the presence of mixed cell hepatocellular carcinomas in AFB1-treated rats. No hepatic lesions were observed in control rats. Iron staining by the Gomori method showed exclusion of iron dextran from the tumor sites. Areas of normal tissue were deeply stained for iron, both in Kupffer cells and hepatocytes.

METHOD AND ALGORITHM FOR RAPID QUANTITATION AND MASS-ArchIVAL OF HEPATIC FOCl USING A MINICOMPUTER COUPLED DIGITIZER AND PROJECTING MICROSCOPE. J.P. Berz, Health Effects Research Laboratory, U.S. EPA, Cincinnati OH. Sponsor: R.J. Bull

Quantitation of enzymatically altered liver lesions (foci) induced by chemical carcinogens requires accurate planimetry of histologic slides and microscopic areas. By today's state-of-the-art in automation, discrimination of histochimically phenotypic GGTase rich lesions from diffuse non-specific artifacts can be only done by the trained eye. It is not probable that inexpensive, fully computerized color-pattern processing will be available for this purpose. On the other hand, the vast numbers of slides generated in one study, and the tedium and inaccuracy of manual integration methods present a major obstacle to the routine use of the liver foci assay for carcinogenic assessment of chemicals. We developed an inexpensive, accurate and rapid semi-automated method for processing large numbers of liver slides. In this procedure the histologic slide is projected onto the calibrated surface of a flat bed digitizer by means of a projecting microscope. A trained operator delineates foci boundaries by tracing at any desired magnification with a stylus. The digital output is integrated and stored in a mini-computer - disk storage device. Since the planimetry must be performed in a darkened room, and the operator cannot have access to the CRT/keyboard terminal, the digitizer surface has been divided into regions of distinct command and alphanumeric data entry through which the operator controls the data flow. Processing errors are signalled to the operator by simple audio patterns generated by the computer logic. Algorithms for data storage, and retrieval by experiment, treatment, animal, and slide numbers in random access files for statistical manipulations have also been devised.

ENHANCEMENT OF AFLATOXIN B1-INDUCED ELEVATION OF BLOOD α-FETOPROTEIN AND EMERGENCE OF γ-GLUTAMYL TRANSPEPTIDASE HEPATIC FOCl BY DIETARY VEGETABLES. J.N. Boyd, N. Misslbeck, T.C. Campbell, and G.S. Stoevensand,* Div. of Nutritional Sciences and *Dept. of Food Science, Cornell University, Ithaca and *Geneva, N.Y.

This investigation was undertaken to determine if dietary vegetables would influence response of rats to aflatoxin B1. Diets containing 25% freeze-dried ground green beans, red table beets, or butternut squash were formulated to be isocaloric and isonitrogenous to the AIN-76 basal diet. Young male Fischer rats were fed one of the diets with or without 1 ppm aflatoxin B1 (AB1) for 8 weeks. Plasma α-fetoprotein (AFP) was determined weekly. Without AB1, there were no differences in AFP levels at any time. AB1-treated groups always had significantly higher (p < .05) levels than non-AB1 groups, and among the AB1-treated groups each of the vegetable diets resulted in significantly higher levels than did the AIN basal diet. Liver sections were histochemically stained for γ-glutamyl transpeptidase (GGT). Animals receiving no AB1 had no GGT-positive foci. Among the AB1-treated groups, the basal, bean, and squash diets, respectively, resulted in 17.9, 33.0, 28.5, and 30.0% of liver section area positive for GGT, and 3.6, 32.8, 16.9, and 12.8 hepatic cell foci per cm2. These data suggest that these diets enhanced AB1-induced preneoplastic transformation in young rats. (Supported in part by NIH ES 07052.)

DOSE-RELATED INDUCTION OF PLASMA α-FETOPROTEIN ELEVATION AND EMERGENCE OF γ-GLUTAMYL TRANSPEPTIDASE-POSITIVE HEPATIC FOCl IN RATS FED AFLATOXIN B1. N. Misslbeck, J.N. Boyd, T.C. Campbell, and G.S. Stoevensand,* Div. of Nutritional Sciences and *Dept. of Food Science, Cornell University, Ithaca and *Geneva, N.Y.

The purpose of this study was to develop a model system employing oncodevelopmental proteins for rapid in vivo detection of preneoplastic transformation. Young male Fischer rats were fed 0, 0.1, 0.5, 1.0, or 2.0 ppm aflatoxin B1 (AB1) in the standard AIN-76 diet beginning one week post weaning. Blood α-fetoprotein (AFP) concentrations were determined weekly from weeks four through seven. At all times plasma AFP increased linearly with increasing dose of AB1. Animals were killed by decapitation after eight weeks. Liver sections were stained for γ-glutamyl transpeptidase (GGT) activity, and
number of GGT-positive hepatic foci per sq. cm. and % of section area positive for GGT were determined. Increasing the doses of AB resulted in 0, <1, 1.9, 3.6, and 19.4 foci/cm² and 5.5, 8.2, 12.4, 17.9, and 28.5% of area positive for GGT. Both induction of plasma AFP and emergence of GGT positive foci were dependent on dose of AB, suggesting that each may be an early indicator of preneoplastic transformation. (Supported in part by NIH environmental toxicology training grant ES 07052.)


Newborn Swiss mice were dosed IP with 25 µg of DMN or saline, and at weaning placed on drinking water containing 0.05% phenobarbital (PB), a known promoter of hepatocellular carcinomas, or water alone. Preneoplastic foci (F) and hyperplastic nodules (HN) were identified histologically by two markers; resistance to exogenous iron accumulation and an increase in gamma-glutamyl transpeptidase (GGT) activity. F (0.02-0.25 mm²) were distinguished from HN (>0.25 mm²) by size and by compression of the surrounding parenchymal cells. In the DMN & PB group, livers of affected male mice at 6, 8, 12 and 16 wk exhibited 6, 10, 17 and 12 iron resistant F/cm² and 10, 13, 9 and 9 GGT-F/cm², respectively. At these same time periods iron resistant-F in affected females ranged from 5-11 F/cm², while GGT-F ranged from 9-16 F/cm². Iron resistant HN were first observed in male mice at 8 wk; however, GGT-HN were not noted until 12 wk. By 12 and 16 wk affected males had 6 and 4 iron resistant HN/cm² while affected females had 4 and 6, respectively. At these same time points, males had 6 and 3 GGT-HN/cm² while females only displayed GGT-HN at 16 wk (6 HN/cm²). The DMN alone group contained an average of 4 F/cm² (iron resistant or GGT) while no HN were noted in any group except the DMN and PB. Lung nodules were apparent as early as 6 wk in both sexes of the DMN alone group and at 12 wk in the DMN and PB group. This model could provide a practical short-term in vivo tool for the detection of early sequential cellular alterations produced by initiators and promoters of carcinogenesis. (Supported in part by BSRG funds from the College of Pharmacy and ES-82130.)


The objective of this study was to determine if 3,3',4,4',5,5'-hexabromobiphenyl (HBB) can act as a promoter of hepatocarcinogenesis. This congener and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are similar in that they both cause 3-methylcholanthrene type induction of hepatic microsomal drug-metabolizing enzymes and produce a similar and characteristic pattern of toxic and histologic responses. TCDD has been shown to be a promoter of hepatic cancer in rats. To test the tumor promoting capacity of HBB, female Sprague-Dawley rats weighing 200-250 g were injected ip with diethylnitrosamine (DEN) at a dose of 10 mg/kg 24 hours after a 2/3 partial hepatectomy (PH). Thirty days after PH, groups of 6 rats were fed control diets or diets containing 1 ppm HBB or 500 ppm phenobarbital (PB). Groups of 3 rats without prior hepatecotomy or DEN were fed the same diets. At 6 months, rats were killed and samples were collected for histologic and histochemical evaluation. Gamma glutamyl transpeptidase (GGT) activity was used to identify enzyme altered foci. The average number of GGT positive foci/cm² were: control, 0; PB, 58; HBB, 79. Values for rats not hepatoeatomized or given DEN were: control, 0; HBB, 11. Rats fed HBB had decreased weight gain and thymic atrophy. Histologically, the liver had severe hepatocellular swelling and vacuolation and focal areas of necrosis. The promoting ability of HBB may be due to properties of a known congener or may be related to compensatory hyperplasia associated with chronic hepatotoxicity. At 1 ppm in the diet, 3,3',4,4',5,5'-HBB can act as a promoter of hepatocarcinogenesis in the two-stage model system.


Large doses of Firemaster (FM), a mixture of polybrominated biphenyls (PBB), cause hepatic carcino genesis in rats. At noncytotoxic concentrations, FM and its major congener, 2,2',4,4',5,5'-hexabromobiphenyl (HBB) inhibit intercellular communication in vitro, a property associated with known tumor promoters. The purposes of this study were to assess the in vivo capacity of PBB to promote hepatic carcinogenesis and to evaluate the reliability of the in vitro assays. FM BP-6 and HBB were compared with phenobarbital (PB), in a two-stage model for hepatocarcinogenesis devised by Pitot and associates. Female Sprague-Dawley rats weighing 200-250 g were injected ip with 10 mg/kg of diethylnitrosamine (DEN) 24 hours after a 2/3 partial hepatectomy (PH). Thirty days after PH, groups of 6 rats were fed a control diet or diets containing Pb (500 ppm), FM BP-6 (10 or 100 ppm), or HBB (10 or 100 ppm). Rats not partially hepatoeatomized or given DEN were fed Pb, FM BP-6 or HBB at the same levels. At 6 months rats were killed and tissue samples were collected for histologic and histochemical studies. Enzyme altered foci were identified by using γ-glutamyl transpeptidase. The average number of enzyme altered foci per cm² were: control, 4; PB, 58; FM BP-6 (10 ppm), 201; FM BP-6 (100 ppm), 86; HBB (10 ppm), 34; HBB (100 ppm), 84. Values in rats not hepatoeatomized or given DEN were: control, 0; FM BP-6 (10 ppm), 7; FM BP-6 (100 ppm), 14; HBB (10 ppm), 0; HBB (100 ppm), 7. FM BP-6 caused decreased weight gain, thymic atrophy and enlarged and vacuolated hepatocytes. These results indicate that FM BP-6 and HBB act as promoters of hepatocarcinogenesis.

Chronic administration of coumarin to rats results in proliferation of bile ducts considered to be either cholangiofibrosis or cholangiocarcinoma. The development of the lesion obtained in rats fed coumarin 5,000 ppm may be divided into four phases: 1) fine ductal proliferation extending between portal tracts and central veins; 2) proliferation of regular ducts around branches of the hepatic vein; 3) proliferation of irregular ducts containing mucus, polymorphs, cellular debris and embedded in a fibrous matrix; 4) the development of extensive intestinal metaplasia. These changes were preceded by hepatocyte damage, in particular damage to the canalicular membrane. Once established, ducts in centre of the lesion degenerate and were replaced by fibrous tissue. This process was associated with the accumulation of mucus, cellular debris within the duct, distension and finally rupture of the duct wall. In general, reversal to control diets, leads to almost complete atrophy of the lesion and replacement by scar tissue. However, in one animal fed coumarin for nine months and killed at eighteen months an active lesion was still present. Concurrent histochemical and biochemical studies showed an initial reduction in the activity of certain enzymes. The activity of γ-glutamyl transpeptidase was enhanced and hepatic glutathione was raised. Cholangiocarcinoma was not seen in any animal and no foci of altered hepatocyte was observed. It is concluded that the lesion induced in rats is best considered to be cholangiofibrosis with no evidence of progress to neoplasia. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food).


The histogenesis of carcinoma of liver having an adenomatous or ductal pattern is uncertain. Many consider that such tumours arise from bile ducts and is preceded by cholangiofibrosis; others consider that they are histological variants of hepatocellular carcinoma. The changes produced in the liver of rats by 3'Methyl-4-diaminoazobenzene (3'MeDAB) and by coumarin are compared. 3'MeDAB administration led to extensive oval cell proliferation and then to cholangiofibrosis. This was followed by hepatic nodules with variable enzyme patterns and later, trabecular and adenomatous or duct carcinoma. Coumarin induced cholangiofibrosis in which four distinct stages could be recognised. No altered foci, areas or nodules were seen, and no hepatocellular tumours developed. The ultrastructure appearance of cholangiofibrosis was compared with that of adenomatous areas of carcinoma. Cells forming the lining epithelium in both were similar. Carcinomas with ductal or adenomatous pattern were deep within unequivocal hepatocellular components and is considered that they represent variants of such tumours. Occasionally ductal tumours appeared to arise within cholangiofibrosis. Possibly proliferating ducts exposed to a carcinogen may be susceptible to neoplastic change. It is concluded that most of tumours (with mucus production and an adenomatous pattern), induced by 3'MeDAB, even those are variants of hepatocellular carcinoma. Cholangiofibrosis is not a prerequisite in the development of such tumours and this lesion should not be taken as indicative of a carcinogenic process. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food).


Simple cholangioma (C), cystic C, cholangiofibrosis (CF), and mixed hepatocolloidal carcinoma (MHC) were observed in the livers of 93 Sprague-Dawley rats fed Aroclor (100ppm for 16 months followed by 50ppm for 8 months) at an incidence of 30, 7, 9, and 26%, respectively. CF and MHC occurred in 81 rats fed a control diet. The cholangiocyte, a pale-staining epithelial cell in the bile duct of the portal triad, possessed the following subcellular morphology: A cleaved nucleus; few cisternae of rough endoplasmic reticulum (ER), tubules of smooth ER, Golgi complexes, and mitochondria; many ribosomes, cytoplasmic filaments, and apical surface microvilli; lateral junctions and membrane undulations; and a smooth basal surface resting on a basal lamina. A cholangiocyte subcellular morphology was observed in C cells. CF possessed cuboidal to columnar simple and pseudostratified epithelium, numerous mitoses, and few PAS-positive goblet cells. Cells of CF demonstrated features of cholangiocytes; differences included a prominent nucleus, increased organelles, and numerous desmosomes. In the MHC, the ductal cell was PAS negative and the features varied. Some cells possessed a cholangiocyte morphology. Other cells contained subcellular features typical of hepatocytes: A round nucleus, prominent nucleolus, abundant rough ER, lamellar smooth ER, lysosomes, smooth intercellular surfaces, and basal lamina, and canalicular between ductal cells and hepatocytes. Aroclor 1260 induces lesions of both hepatocellular (Toxicologist 1, 65, 1981) and cholangiocyte differentiation.

356 DINITROTOLUENE PROMOTION OF DIETHYLNITROSAMINE (DEN) INITIATED HEPATOCYTES IN VIVO. T.B. Leonard and J.A. Popp, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709. Sponsor: J.A. Swenberg

Technical grade DNT (tDNT) is a potent hepatocarcinogen when fed to F-344 male rats for one year (100% incidence of hepatocellular carcinomas). However, tDNT is only a weak initiator when evaluated in hepatic initiation-promotion systems suggesting that the promoting activity of tDNT may be a major determinant of its carcinogenic potency. The present studies were designed to ascertain whether tDNT had promot-

The hepatocarcinogenicity of dimethylnitrosamine (DMN) is sex-dependent in C3H mice following chronic oral exposure. DMN causes predominantly hepatomas in males and capillary endotheliomas in females (Den Engelse et al.; Europ. J. Cancer. 10:129, 1974). The purpose of this study was to assess the role of the promutagenic DNA lesion, O'-methylguanine (O6MG), in the cellular specificity of DMN's carcinogenicity in C3H mice. Male and female mice were fed 0 or 10 ppm DMN ad libitum in the drinking H2O for 2, 4 or 16 days. The DMN dosage varied from 1.74-2.14 mg/kg/day among the treatment groups. Livers were perfused in situ with collagenase and hepatocytes and nonparenchymal cells (NPCs) separated by differential centrifugation. DNA was isolated by hydroxyapatite chromatography and pure bases separated by HPLC and quantitated from UV-absorbing and fluorescing peaks. Concentrations of 7-methylguanine (7-MG) in the 2 liver cell populations of both sexes increased between 2 and 4 days of exposure and remained relatively constant thereafter. NPCs of both sexes accumulated O6MG over the time course of DMN administration, such that increasing O6MG/7MG ratios were observed. Male and female hepatocytes demonstrated increased removal of O6MG after exposure to DMN. Concentrations of hepatocellular O6MG in both sexes were 5 to 7 fold less at 16 days than at 4 days, resulting in decreased O6/7MG ratios. While this represents the first demonstration of cell specific alkylation in the mouse liver following chronic exposure to DMN, no correlation between cell specificity of O6MG accumulation and specificity for carcinogenesis was observed. Factors such as cell replication and differential repair of other promutagenic DNA adducts must also be considered.

2-ACETYLAMINOFLUORENE (AAF) DEPENDENT INCREASES IN EPOXIDE HYDROLASE: SPECIES, STRAIN AND SEX DIFFERENCES. M.E. Graichen and J.G. Dent, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

Exposure of rats to hepatocarcinogens causes substantial increases in hepatic microsomal epoxide hydrolase (EH) activity. The potency of compounds in increasing EH is related to their carcinogenic potency. The objective of this study was to investigate the relationship between hepatocarcinogenicity and increases in epoxide hydrolase using the known species, strain and sex differences in susceptibility to AAF carcinogenicity. Following administration of AAF in the diet or via ip injections, liver microsomes or 10,000 g supernatant was prepared. EH activity was measured with benzo(a)pyrene-4,5-oxide as substrate. Three daily ip injections of AAF, at doses between 5 and 80 mg/kg/day, caused dose-related increases in EH in male F-344 and CD rats. The log-linear dose response obtained had the same slope in both strains and the ED50 was 13 mg/kg/day. The maximum increase observed was 350% of control activity in both strains. Doses of 2-AAF up to 160 mg/kg/day failed to elicit significant increases in EH activity in CD female rats and caused only a 40% increase in female F-344 rats. No increase in EH activity was detected in guinea pigs treated with AAF at doses between 40 and 240 mg/kg/day for 3 days. In DBA2 and C57BL6 mice fed 0.02% AAF for 2 weeks, decreases (45 and 55%, respectively) in EH were observed. By comparison, F-344 male rats fed this diet exhibited a 460% increase in EH activity. Female rats, mice and guinea pigs are poor N-hydroxylators of AAF and are resistant to the carcinogenic action of AAF. These results support the hypothesis that increases in EH activity are related to the carcinogenic action of AAF and to the metabolic activation of AAF.
duced the greatest increases (7,000% relative to controls). AAF and 3'-MeDAB caused intermediate increases (600 to 1000%) and DEN and LA the smallest (< 300%). CH and BIL concentrations and activities of GGT and DTT were only increased by treatment with ANIT (< 300 to 2000%). EH was increased (150 to 600%) by all treatments except LA. At 2 and 6 weeks animals exposed to AAF, DEN and 3'-MeDAB had a direct correlation (p = 0.001) between serum BA and EH activities. In conclusion, selected hepatocarcinogens are cholestatic as determined by increased BA. An association was also noted between BA concentrations and EH activities.

360 VARIATION IN GENOTOXIC RESPONSE TO 3,2'-DIMETHYL-4-AMINOBIPHENYL (DMAB) IN HEPATOCYTE PRIMARY CULTURES (HPC) DERIVED FROM FOUR MAMMALIAN SPECIES. C.J. Maslansky and G.M. Williams, American Health Foundation and New York Medical College, Valhalla, N.Y.

The rat HPC/DNA repair assay detects the genotoxic effects of chemicals. The development of HPCs from other species facilitates study of patterns of xenobiotic metabolism and genome interaction as they may relate to differences in in vivo susceptibility to carcinogenesis. Variations in the induction of DNA repair in HPCs derived from different species have been detected in response to the carcinogen DMAB. HPCs were initiated from the rat, mouse, hamster and rabbit. Hepatocytes were exposed to DMAB and 3H-thymidine for 18 hrs. DNA repair was determined autoradiographically. DNA repair induced by DMAB varied considerably between the species investigated. Although DNA repair was maximal in hamster hepatocytes, rat hepatocytes also displayed appreciable levels. A dose dependent response was elicited from both species. Rabbit and mouse hepatocytes displayed lesser levels of repair. These differences in repair induction were reflected in variations in DMAB metabolism in HPCs derived from each species. These findings demonstrate the utility of HPCs for detecting the genotoxic potential of chemicals. Since species differences in susceptibility to chemical carcinogens exist, the test was extended to other species. Hepatocytes were isolated from Syrian golden hamsters or B6C3F1 mice by in situ perfusion of the liver with collagenase. After cell attachment, the primary cultures were washed and exposed to the test chemical and 3H-thymidine. DNA repair was determined by autoradiography. Carcinogens and their noncarcinogenic analogues from the structural classes of alkylating agents, aromatic amines, mycotoxins, polycyclic aromatic hydrocarbons, nitrosoamines, and aminoazo dyes were tested. In both species, DNA repair was elicited by all the carcinogens. No noncarcinogens were positive in the mouse HPC/DNA repair test. However, DNA repair was seen in hamster hepatocytes exposed to pyrene or aflatoxin G2, which are not carcinogenic in rats or mice, but have not been tested in hamsters. The mouse, which is not sensitive to the effects of aflatoxin B1, required a dose of 10^{-4}M to elicit maximum repair while only 10^{-6}M was required for hamster hepatocytes. These findings demonstrate that genotoxic effects of carcinogens identified using mouse or hamster hepatocytes and that species differences in sensitivity exist.

362 INHIBITION OF AFLATOXIN B1 CARCINOGENESIS IN RAINBOW TROUT BY DIETARY s-NAPHTHOFLAVONE (s-NF), AND INDOLE-3-CARBINOL (I-3-C). J.D. Hendricks, J.E. Nixon, G.S. Bailey, and R.O. Sinnhuber, Dept. of Food Sci. & Tech., Oregon State University, Corvallis, OR 97331.

Several compounds such as flavonoids, selenium, antioxidants and certain vitamins reportedly reduce the induction of cancer in experimental animals, and many function by affecting the mixed-function oxidase (MFO) system. The following compounds: 50 and 500 ppm s-NF, 1000 ppm flavone, 1000 ppm of a tangeretin-nobilitin mixture, 1000 ppm s-ionone, 1000 ppm I-3-C and 2000 ppm quercetin were examined for protection against aflatoxin B1 (AFB1) hepatocarcinogenesis and induction of the MFO system in rainbow trout. These compounds were fed to fingerling rainbow trout for 2 months. At that time the activity of several MFO enzymes and cytochrome P450 content were measured and the trout were exposed to 20 ppb AFB1 for 10 days in the same diets. After feeding the test diets without AFB1 for another month and basal diet for 11 months, the tumor incidence was determined. s-NF induced the trout MFO system in a dose-dependent manner while tangeretin-nobilitin was less effective. Flavone, quercetin, s-ionone and I-3-C did not induce the MFO system. I-3-C and s-NF provided marked protection against AFB1-induced hepatocarcinogenesis, while the other compounds were ineffective. Tumor incidences were 5.1% for 1000 ppm I-3-C, 7.5% for 500 ppm s-NF and 19.6% for 50 ppm s-NF, compared to 44.9% for the AFB1-positive control. These data are interesting because induction of the MFO system was not required for protection against AFB1 carcinogenesis. Both s-NF and I-3-C provided marked protection but only s-NF induced the MFO system. (Supported in part by U.S.P.H.S. Contract No1 CP85660 and Grant ES00541)

363 DAMAGE TO RAT LIVER DNA FROM IN VIVO EXPOSURE TO MONOCROTALINE, A PYRROLIZIDINE ALKALOID. T.W. Petry, G.T. Bowden, R.J. Huxtable, and I.G. Sipes.
Pyrrolizidine alkaloids (PAs) are known to damage DNA in vitro, to be hepatocarcinogenic in animal models, and have been linked to an increased incidence of hepatic carcinoma among South African Bantus. We have employed alkaline elution to detect and characterize DNA damage resulting from in vivo exposure of male Sprague-Dawley rats (150-280g) to single intraperitoneal doses of the PA, monocrotaline. Hepatic nuclei were isolated, and served as the source of DNA in all experiments. Histological evaluation of liver sections taken at the time of nuclei preparation revealed no signs of cytotoxicity. No evidence of single-strand breaks, as would be indicated by an increased rate of DNA elution from the filter as compared to controls, was detected at either 4 or 8 hr after doses of up to 120µg/kg of monocrotaline. As monocrotaline may be bioactivated to bifunctional alkylating agents, experiments were conducted to detect DNA cross-links. In contrast to the results of the single-strand break experiments, evidence for DNA cross-links was observed at doses as low as 5mg/kg, 4 hr after administration of the PA. DNA cross-linking was detected by an apparent decrease in the number of X-ray induced single strand breaks, and consequently a decreased rate of DNA elution from the filter as compared to controls, when nuclei from both groups were X-irradiated. Preliminary experiments to differentiate between DNA-protein crosslinks, and DNA-DNA interstrand crosslinks suggested that the two types is induced. (Supported by an AFPE Fellowship and NIH Grants ES-8-2130 and HL-25258.)


AAF-induced hepatocarcinogenesis appears to be targeted to parenchymal cells (PC). Our objective was to determine whether or not there is preferential binding of carcinogen (C) residues to the DNA of these target cells and, if so, the possible basis for the selectivity. AAF and its N-hydroxy derivative (N-OH-AAF), the first product in the metabolic activation of AAF, were employed for these studies. Male Sprague-Dawley rats weighing 200-250 g were injected, i.p., with (ring-H)AAF (64 µCi/1.8 µmoles/100 g) or (ring-H)-N-OH-AAF (100 µCi/1.8 µmoles/100 g). Animals were sacrificed at either the time of peak binding of C to DNA, 2 hr or 18 hr post-injection, when N-OH-AAF or AAF, respectively, were used, and at 3 days after maximum binding. PC and non-parenchymal (NPC) cells were isolated by centrifugal elutriation. C-modified DNA was purified by hydroxyapatite column chromatography. Following AAF administration, the µmoles of adducts formed/mg DNA of PC was 2.7 times that of the NPC. When N-OH-AAF was employed, equal amounts of adducts were found associated with DNA of PC and NPC. By 3 days after the period of maximum DNA binding 50-59% of the adducts initially present remained in the DNA of both cell types following administration of either AAF or N-OH-AAF. These results suggest that AAF preferentially damages DNA of PC due to an increased capacity of these cells to carry out the N-hydroxylation of AAF. The ability of PC and NPC to metabolize N-OH-AAF to a reactive species appears similar. The binding of C to specific regions of the genome of PC and NPC is under investigation. (Supp. by the MSU Agric. Exp. Station.)


Acrylamide is a common monomeric unit of a variety of commercial products. It has been recognized for sometime as a neurotoxin. However, its structural resemblance to ethyl carbamate (urethane), an established tumor initiator, led us to examine the carcinogetic properties of this chemical in the mouse skin. A small scale experiment was carried out to test the hypothesis that acrylamide may act as a tumor initiator. The study involved administration of a single dose of 30 mg/kg by each of 3 routes of exposure (oral, intraperitoneal and topical) to female SENCAR mice. These initiating doses were followed in 2 weeks by repeated application of 1 µg 12-o-tetradecanoyl phorbol-13-acetate (TPA) 3 times weekly for 20 weeks. At 32 weeks following the start of promotion the following cumulative tumor counts (papillomas) were noted: Oral gavage, acrylamide 8 animals in 40 (8/40) with tumors and average of 0.25 tumors per animal; distilled deionized Hz0 (DHz0) 6/20 and 0.35; I.P., acrylamide 18/40 and 0.55, DHz0 3/20 and 0.20; topical acrylamide 6/40 and 0.15 and acetone 3/20 and 0.25.

It appears from this data that acrylamide via the I.P. route is capable of initiating tumors. As in previous studies with urethane, the potency of acrylamide is greater by a systemic route than the topical route of exposure. Experiments designed to develop the dose-response relationships involved in acrylamide-induced tumorigenesis are underway.

DERMAL CARCINOGENESIS BIOASSAYS OF PYROLYSIS FUEL OIL (PFO) AND PYROLYSIS GASOLINES (PG) IN C3H MICE. L.R. DePass, L.G. Peterson, C.S. Weil, Bushy Run Research Center, Export, PA.

Pyrolysis fuel oils result from high temperature cracking of hydrocarbon components of naphtha or liquefied petroleum gas (LPG) feedstocks. These hydrocarbons are pyrolyzed at temperatures up to 950°C after which temperatures are reduced to 400°C. Further temperature reductions are accomplished by either injecting water into the process (water quench) or using a gasoline fractionator (oil quench) system. In this study 3 samples of PFO, 3 samples of pyrolysis gasoline (a C5+ fraction condensed during compression and cooling of the cracked gas) and a C5+ fraction of the pyrolysis gasoline were painted on the clipped skin of groups of 40 male C3H/HeJ mice 3 times weekly for the lifetime of the animals. Methylcholanthrene, distilled water and acetone were painted as the positive and 2 negative control substances respectively. All 3 PFO samples were...
highly oncogenic to mouse skin resulting in tumor incidences of greater than 50%. The PFO sample derived from a naphtha feedstock by an oil quench process showed significantly greater oncogenic activity than both samples from LPG feedstocks by water quench processes. Among the groups receiving PG samples, 4 skin tumors occurred in the group that received the naphtha-derived oil quench sample compared to 1 and 0 tumors respectively in the groups that received the LPG-derived, water quench samples. No skin tumors were observed in the solvent controls. These results suggest a relationship between oncogenic activity and either the feedstock (and related reaction conditions) or the cooling process used in the production of PFO and PG. Published data suggest that the feedstock may be the more important determinant of oncogenic activity. (Well and Condra, Am. Ind. Hyg. J., 38:730, 1977).

367 INITIATION-PROMOTION STUDIES WITH COAL LIQUEFACTION MATERIALS. D. Dennis Mahlum. Pacific Northwest Laboratory, Richland, WA 99352.

High boiling (>550°F) coal liquids such as heavy distillate (HD) from the solvent refined coal-II (SRC-II) process are mutagenic in the Ames assay and carcinogenic for mouse skin. Chemical characterization studies have identified a number of mutagenic constituents in HD but similar efforts to identify carcinogenic components have been hampered by the long times and high costs required for standard skin-painting assays. An initiation-promotion assay was therefore used to study the carcinogenicity of coal-derived liquids. Basic (BF), basic tar (BTF), neutral tar (NTF), and polynuclear aromatic (PNA) fractions were prepared from HD by solvent extraction. These fractions were tested for their initiating activities by applying a single dose to the shaved back of Charles River male CD-1 mice. Positive control groups were initiated with either dimethylbenzanthracene or benz(a)pyrene. Beginning two weeks after initiation, all mice received twice weekly applications of 5 µg of the promoter, phorbol myristate acetate (PMA). Papillomas were noted and recorded for each animal at time of PMA application. Heavy distillate and its fractions all showed initiating activity. However, the incidence of mice with tumors, the rate of tumor appearance, and the total number of tumors varied with the test material. Consideration of these parameters suggests the following order of activity: DMBA > HD = NTF > BaP > BTF > BF > PNA. These data are in good agreement with the results of long-term skin painting assays and suggest that initiation-promotion may provide a relatively rapid and inexpensive method for studying the tumorigenicity of coal-derived materials. (Work supported by the U.S. Dept. of Energy Contract No. DE-AC06-76RLO-1830 and National Science Foundation Grant No. SPI-8160230)


The induction of the enzyme ornithine decarboxylase (ODC) has been proposed as an early essential phenotypic change observable in mouse epidermal cells exposed to skin tumor promoters. Heavy distillate (HD), the high boiling coal liquid from the solvent refined coal process (SRC-II), is a complete carcinogen for mouse skin and therefore should elevate ODC activity in a manner similar to other promoters. To test this hypothesis, ODC induction by HD was compared with the activation of this enzyme by the potent skin tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA). A single application of 23 mg of HD or 17 nmoles of TPA to the dorsal skin of female Charles River CD-1 mice resulted in 75-fold and 270-fold increases respectively, in epidermal ODC activity. Twice-weekly applications for 4 weeks of the same doses elevated ODC levels 20-fold in those animals receiving HD and 1350-fold in the mice treated with TPA. The kinetics of induction with both HD and TPA were similar, exhibiting peak activity 3-5 hours after application and returning to near-basal levels within 12 hours; however, with multiple applications HD did not exhibit the amplification of induction in ODC activity seen with TPA. Hepatic ODC activity was also found to be elevated following dermal application of both HD and TPA. The similarity in the pattern of ODC induction after a single application of HD and TPA suggests that this assay may be useful for screening SRC materials as potential promoters. (Work supported by the U.S. Dept. of Energy Contract No. DE-AC06-76RLO-1830 and National Science Foundation Grant No. SPI-8160230)
Zinc deficiency causes hyperplasia and para-keratosis in the esophageal epithelium of many animal species, and increases susceptibility of this tissue to chemical carcinogens. Male Sprague-Dawley rats were given either a control diet (60 ppm) or a diet deficient in Zn (3 ppm). After one month on these diets, some rats received 2.5 mg/kg body weight methyl-benzyl nitrosamine (MBN), twice a week for three weeks. At the end of the dosing period, one group of control ad libitum animals was switched to zinc deficient diet and one group of zinc deficient was switched to control diet. Zinc deficiency throughout the experiment significantly enhanced tumor incidence (88% vs. 31% control diet ad lib and 14.7% control pair fed, p < .0005). Switching diets gave equivocal results. After one month on diets the zinc deficient diet only, led to much greater DNA synthetic activity than the control diet. Following the first dose of MBN, DNA synthetic activity dropped to background levels for both groups but exceeded background at end of dosing. Ten days after the last dose of MBN, DNA synthesis was significantly greater in the control groups. These results suggest that zinc deficiency acts during both the initiation and promotion stages of the carcinogenic process. (Supported in part by USPHS Grant CA 25382).


The incidence of testicular mesotheliomas due to the carcinogen FAA was studied in four-week-old male Fischer 344 rats. The experimental animals were fed the carcinogenic diet (0.06% FAA) during 3 weeks and then the control diet for 1 week. This schedule was carried out for 3 complete cycles (12 weeks). A smaller group of rats was treated for 1 complete cycle only (4 weeks) of FAA. One group of untreated controls was also available. The surviving rats were sacrificed at 59 weeks of age. The administration of FAA for 3 complete cycles resulted in a high incidence of liver, testis and Zymbal gland tumours. The testicular tumours were mesotheliomas and occurred in 9/25 rats. No such tumours were observed in animals treated for 1 cycle only or in untreated controls. Macroscopically, these tumours appeared as flat, nodular or papillary, greyish or pinkish growths on the surface of the testis and/or epididymis. Microscopically, the tumours were seen to arise from the surface of the peritesticular mesothelium. The first testicular mesothelioma was observed 30 weeks after end of treatment. No metastases from these tumours were found. The high incidence of testicular mesotheliomas, a rare type of tumour in this and other rat strains, suggests an association with treatment. The present experimental model may be useful in elucidating the mechanisms involved in the production of mesothelial tumours of the testis by chemical carcinogens.
tween binding and biological function, the competition for binding sites only between active phorbol diesters, and the low concentrations employed indicate that the binding sites for phorbol diesters on H-60 cells are receptors.

(Supported in part by EPA Grant (R808861010) and NIEHS Training Grant TT 32E507087)

374 CARCINOGENICITY OF FLUOROCARBONS: ASSESSMENT BY MG-63 and SaOS-2 with apparent Ks of 8-20 nM. and inflammatory agents in many tissues. Phorbol and ED50s were 10-20 nM, in agreement with the observed 3-fold increase in tumor promotion, was 6 times more potent than PDBu in increasing production of PGE2 and in competing for [3H]PDBu binding, while the biologically inactive parent alcohol phorbol had no effect on either response. The PGE2 response therefore appears to be mediated via the PDBu receptor. Ability of cells to increase PGE2 production in response to PE correlated with increased phospholipase activity which liberates arachidonate, the fatty acid precursor of PGE2. In responsive cells, PE treatment increased by 50% [3H]arachidonate release from biosynthetically labeled cell membranes. Increased lipase activity appeared to be substrate specific, since there was no increase in the release of either [14C]oleate or [3H]palmitate. All 4 cell lines, however, responded to PE as shown by decreased binding of epidermal growth factor, with ED50s of 5-15 nM. Thus, the inability of SaOS cells to respond to PE with increased production of PGE2 does not result from defective PE binding and may reflect an abnormality in the generation of a secondary signal which mediates this response.


Phorbol esters (PE) are potent tumor-promoting and inflammatory agents in many tissues. 20-[3H]-phorbol 12,13-dibutyrate (PDBu) binds to specific high affinity sites on several clonal lines of human osteosarcoma cells (G-392, TX-4, MG-63 and SaOS) with apparent Ks of 8-20 nM. Treatment with PDBu increased prostaglandin E2 (PGE2) production in 3 of the 4 cell lines; SaOS cells were unresponsive. Maximal increase in PGE2 production was 3- to 5-fold above control values, and ED50s were 10-20 nM, in agreement with the Ks for binding to the PDBu receptor. Phorbol 12-O-myristate-13-acetate (PMA) the most potent phorbol ester in terms of tumor promotion, was 6 times more potent than PDBu in increasing production of PGE2 and in competing for [3H]PDBu binding, while the biologically inactive parent alcohol phorbol had no effect on either response. The PGE2 response therefore appears to be mediated via the PDBu receptor. Ability of cells to increase PGE2 production in response to PE correlated with increased phospholipase activity which liberates arachidonate, the fatty acid precursor of PGE2. In responsive cells, PE treatment increased by 50% [3H]arachidonate release from biosynthetically labeled cell membranes. Increased lipase activity appeared to be substrate specific, since there was no increase in the release of either [14C]oleate or [3H]palmitate. All 4 cell lines, however, responded to PE as shown by decreased binding of epidermal growth factor, with ED50s of 5-15 nM. Thus, the inability of SaOS cells to respond to PE with increased production of PGE2 does not result from defective PE binding and may reflect an abnormality in the generation of a secondary signal which mediates this response.


Male and female Fischer 344 rats and B6C3F1 mice were administered methyl carbamate in water by gavage in a 90 day subchronic toxicity study. The animals were necropsied and given a complete microscopic examination. In rats, compound-related histopathologic lesions were found in the liver, bone marrow, spleen, and salivary gland of both sexes and in the testes of the males. The most significant lesions occurred in the liver and were characterized by proliferative changes of hepatocytes consisting of basophilic and other atypical forms, hepatocellular necrosis of a focal nature, and pigmentation of Kupffer's cells. Other toxic changes observed in rats were diffuse atrophy of the bone marrow, and acinar atrophy, duct hyperplasia and inflammation of salivary glands of both sexes, and excessive pigmentation of the spleen of males. In male but not female mice, minimal to mild hepatocellular necrosis and/or increased mitotic rates of hepatocytes were observed in animals receiving 375 to 1500 mg/kg. An hepatocellular adenoma was also observed in a high dose male mouse. Because of the patchy nature of the former lesion, it is not known whether it is directly attributable to the test substance.

The proliferative nature of the hepatic lesions suggest that methyl carbamate may be biologically active in rodents. Chronic studies to determine the carcinogenicity of this compound are in progress.

This study was performed under the Carcinogenesis Bioassay Program of the National Toxicology Program through a contract with Tracor Jitco, Inc.

377 A THREE MONTH SUBCHRONIC DIETARY STUDY WITH TERT-BUTYLPHENYL DIPHENYL PHOSPHATE (TBP) IN IN RATS. S.E. Hastings, R.W. Thomassen, M.W. Sauerhoff and G.M. Zwicker.

Stauffer Chemical Company, Environmental Health Center, Farmington, CT
SB-3018, A NEW NON-QUATERNARY THIOHYDROXIMATE

Mice showed a SC LD₅₀ of about 460 mg/kg and, at non-quaternary nitrogen of SB-3018 it appeared likely to
AChE might make SB-3018 or another of our series
brain AChE at 125 mg/kg, but reactivation was less
effect and a shorter duration of action than a
250 or 300 mg/kg of SB-3018 had a less protective
high dose of SB-3018, suggesting penetration into
brain tissue AChE from rat, chicken, and catfish to inhibition by methyl paraoxon,
paraoxon, Gutoxon, and ethyl Gutoxon were studied. The most striking differences were: 1) Catfish brain AChE was 25X less sensitive to methyl paraoxon than chicken brain AChE, and 29X less sensitive to paraoxon. 2) Chicken brain AChE was 28X less sensitive to Gutoxon than rat brain AChE, and 11X less sensitive to ethyl Gutoxon. The kinetic parameters, affinity constant (Kₐ) and phosphorylation constant (Kᵢ), were determined by the method of Hain and Iverson (Biochem. J., 100:525, 1966). For methyl paraoxon, Kᵢ was 242 µM and Kᵢ was 14.7 min⁻¹ for catfish brain AChE; for chicken brain AChE, Kᵢ was 25 µM and Kᵢ was 37.9 min⁻¹. Catfish brain AChE had 9.7X less affinity for methyl paraoxon and 2.6X slower rate of phosphorylation than chicken brain AChE. The overall difference is 25X, which agrees with the difference in IC₅₀'s (chicken 1.48 X 10⁻⁴ M; Catfish 3.73 X 10⁻⁷ M). For paraoxon, Kᵢ was 202.8 µM and Kᵢ was 22.2 min⁻¹ for catfish brain AChE; for chicken brain AChE, Kᵢ was 6.3 µM and Kᵢ was 25.2 min⁻¹. For Gutoxon, Kᵢ was 88.7 µM and Kᵢ was 8.3 min⁻¹ for chicken brain AChE; for rat brain AChE, Kᵢ was 31.5 µM and Kᵢ was 56.8 min⁻¹. For ethyl Gutoxon, Kᵢ was 58.4 µM and Kᵢ was 24.1 min⁻¹ for chicken brain AChE; for rat brain AChE, Kᵢ was 108.1 µM and Kᵢ was 565.3 min⁻¹. The kinetic studies suggest that the active site of AChE differs between species. (Supported by a SOT Fellowship sponsored by The Procter and Gamble Company to C.W. and by Research Grant ES01831 from NIEHS.)


In the search for improved reactivators of acetylcholinesterase (AChE) inhibited by organophosphorus compounds, we synthesized pBrC₆H₄C(O)C(NOH)S⁻(CH₃)₂M(C₆H₅)₆Cl⁻ (SR-3018), and evaluated its ability to reactivate AChE inhibited by ethyl p-nitrophenyl methylphosphonate (ENMP). In vitro, SR-3018 was about 1/30th as potent in reactivating eel AChE as 2-PAM. However, due to the non-quaternary nitrogen of SR-3018, it appeared likely to pass the blood-brain barrier, unlike 2-PAM, thus being of potential use in reactivating inhibited brain AChE. Tests of SR-3018 in male Swiss-Webster mice showed a SC LD₅₀ of about 460 mg/kg and, at 300 mg/kg, a protective ratio against ENMP of 1.8

379 KINETIC ANALYSIS OF SPECIES DIFFERENCE IN ACETYL-
CHOLINESTERASE SENSITIVITY TO ORGANOPHOSPHATE IN-
SECTICIDES. C. Wang and S.D. Murphy, Div. of Tox. Univ. of Texas Med. Sch., Houston, TX.

Previous studies showed that acetylcholinesterase (AChE) from different species had different sensitivity to inhibition by paraoxon and the oxygen analog of azinphos methyl (Gutoxon) (Murphy et al, TAP, 12:22, 1968). In this study, the sensitivity of brain tissue AChE from rat, chicken, and catfish to inhibition by methyl paraoxon, paraoxon, Gutoxon, and ethyl Gutoxon were studied. The most striking differences were: 1) Catfish brain AChE was 25X less sensitive to methyl paraoxon than chicken brain AChE, and 29X less sensitive to paraoxon. 2) Chicken brain AChE was 28X less sensitive to Gutoxon than rat brain AChE, and 11X less sensitive to ethyl Gutoxon. The kinetic parameters, affinity constant (Kₐ) and phosphorylation constant (Kᵢ), were determined by the method of Hain and Iverson (Biochem. J., 100:525, 1966). For methyl paraoxon, Kᵢ was 242 µM and Kᵢ was 14.7 min⁻¹ for catfish brain AChE; for chicken brain AChE, Kᵢ was 25 µM and Kᵢ was 37.9 min⁻¹. Catfish brain AChE had 9.7X less affinity for methyl paraoxon and 2.6X slower rate of phosphorylation than chicken brain AChE. The overall difference is 25X, which agrees with the difference in IC₅₀'s (chicken 1.48 X 10⁻⁴ M; Catfish 3.73 X 10⁻⁷ M). For paraoxon, Kᵢ was 202.8 µM and Kᵢ was 22.2 min⁻¹ for catfish brain AChE; for chicken brain AChE, Kᵢ was 6.3 µM and Kᵢ was 25.2 min⁻¹. For Gutoxon, Kᵢ was 88.7 µM and Kᵢ was 8.3 min⁻¹ for chicken brain AChE; for rat brain AChE, Kᵢ was 31.5 µM and Kᵢ was 56.8 min⁻¹. For ethyl Gutoxon, Kᵢ was 58.4 µM and Kᵢ was 24.1 min⁻¹ for chicken brain AChE; for rat brain AChE, Kᵢ was 108.1 µM and Kᵢ was 565.3 min⁻¹. The kinetic studies suggest that the active site of AChE differs between species. (Supported by a SOT Fellowship sponsored by The Procter and Gamble Company to C.W. and by Research Grant ES01831 from NIEHS.)


Partitioning of chlordecone, chlordecone alcohol, monohydrochlordecone, and dihydrochlordecone (4 to 100 µM) into isolated rat liver mitochondria increased the permeability properties of the inner membrane as evidenced by: inhibition of valinomycin-induced swelling, induction of passive swelling, oxidation of exogenous NADH, and induction of lysis. Associated with the increase in permeability was stimulation of state 3 and inhibition of state 3 respiration for the oxidation of succinate and glutamate. Except for the inhibition of valinomycin-induced swelling, the order of potency for all assays was chlordecone alcohol > chlordecone > monohydrochlordecone > dihydrochlordecone. Mirex, which has the same carbon skeleton as the above compounds, but con-
tains no oxygen, did not affect any of the reactions at a saturating concentration of 40 µM. The hydrated ketone or hydroxyl moiety and the chlorine content appear to be responsible for lytic and inhibitory potency. Lysis was associated with leakage of water-soluble matrix enzymes, but not solubilization of integral proteins.

**381 ASCORBIC ACID AND PARAOXAT: OXYGEN DEPLETION WITH CONCURRENT OXYGEN ACTIVATION.** M. R. Montgomery, J. Purry, S. J. Gee, and R. L. Klieger. VA Hospital and Depts. of Pharm. and Comprehensive Med., Univ. of S. Florida, Tampa, FL and Dept. of Veterinary Med., Univ. of Idaho, Moscow, ID

Ascorbic acid and paroquat produce an efficient redox pair which will deplete oxygen from physiological buffer systems. The reaction rate is three fold more rapid in sucrose buffer than in Krebs-Ringer-phosphate; in distilled water the rate is very slow but measurable. This reaction is partially blocked by superoxide dismutase (38% decrease) or catalase (36% decrease) and is inhibited by the hydroxyl radical scavenger, dimethyl sulfoxide (86% increase). Mitochondria isolated after incubation of rat lung slices with 1.0mM paroquat and 10mM ascorbate were unresponsive to addition of ADP. Also, metabolism of (1-14C)- and (6-14C)-glucose was inhibited by 50% in the same lung slice preparations. These results suggest a synergistic interaction of ascorbate and paroquat which results in disruption of subcellular energy metabolism. Paroquat accumulation, an active transport process of the pulmonary cell membrane, was also inhibited 40% by the addition of ascorbate. These results suggest that the previously reported in vivo potentiation of paroquat toxicity by ascorbate may be related to either: 1) a decreased subcellular oxygen availability, or 2) the presence of activated oxygen species, or 3) both.

Supported by grants from VA Medical Research and NIH Biomedical Research Development Grant 1 S08 RR09073-01A2.

**382 THE METABOLISM OF BROTHELAND AND ITS EFFECTS ON OXIDATIVE PHOSPHORYLATION AND CEREBROSPINAL FLUID PRESSURE.** L. D. Cherry, M. D. Gunnoe and R. B. L. van Lier (D. G. Hoffman), Toxicology Division, Lilly Research Laboratories, Eli Lilly and Co., Greenfield, IN.

Bromethalin, N-methyl-2,4-dinitro-N-(2,4,6-tribromophenyl)-6-(trifluoromethyl)benzamide is being developed as a new single feeding rodenticide. Previous data from this laboratory (The Toxicologist 1:114, 1981) suggests that bromethalin is a potent uncoupler of oxidative phosphorylation in liver and brain mitochondria in vitro. Treatment of rats with SKF-525A can substantially reduce or delay acute bromethalin toxicity suggesting that the compound must be metabolized to the active toxicant. In vitro studies of demethylated bromethalin have shown this compound to be 500-1000 times more potent than bromethalin as an uncoupler. Using malate as substrate, a 50 percent decrease in the P/O ratio was observed at 5 pmol/mg protein.

The microscopically detectable toxic effect of bromethalin is characterized by intramyelinic edema. Cerebrospinal fluid pressure (CSFP) in rats 24 hours after a 1 mg/kg dose of bromethalin was 251 ± 22.7 mm of water compared to a control value of 83 ± 5.9. CSFP returned to control levels 7 days after treatment. Daily treatment of bromethalin intoxicated rats with dexamethasone s.c. reduced the time in which CSFP returned to control levels to 4 days. Intravenous injection of hypertonic urea 24 hours after bromethalin treatment reduced CSFP to near control levels after 30 minutes. However, when infusion was discontinued, CSFP rose to near starting levels at approximately the same rate. These data suggest that the cerebral edema produced by sub-lethal doses of bromethalin can be ameliorated by treatment with steroid therapy and/or an osmotic diuretic.

**383 DIFFERENTIAL TOXIC RESPONSES OF RATS AND GERBILS TO THE INSECTICIDE CARBARYL.** I. S. Silver and H. W. Dorough. Graduate Center for Toxicology, University of Kentucky, Lexington, KY.

In the present study, the toxicity, anticholinesterase activity, and ester hydrolysis rates of carbaryl (1-naphthyl N-methylcarbamate) in female rats (R) and gerbils (G) were compared. Carbaryl was more toxic to gerbils than rats. A 300 mg/kg oral dose killed 300% (20/20) and 70% (14/20), respectively, and the 7-day oral dose LD50 was approximately 80 mg/kg/day for the gerbil and 250 mg/kg/day for the rat. When carbarle naphthyl-14C was given orally (50 mg/kg), maximum 14C-carbaryl equivalents occurred in the blood after 15 min (R, 14 µg/ml; G, 9 µg/ml) causing 46% blood ChE inhibition in the rat and 25% in the gerbil. Brain ChE was similarly inhibited (R, 56%; G, 24%). In vitro assays revealed no inherent differences in the sensitivity of blood and brain ChE to carbaryl. Carbamate ester hydrolysis, determined by measuring respiratory 14CO2 from carbaryl-14C, was much greater in the gerbil. After 4 and 24 hrs, the percent of dose hydrolyzed was 48 and 65 for the gerbil, and 17 and 28 for the rat. Since ester hydrolysis destroys the antichE activity, these data might suggest the gerbil to be more tolerant to carbaryl poisoning than the rat. The more rapid hydrolysis rate may contribute to the lower levels of carbamate in gerbil blood and the corresponding lower levels of ChE inhibition in the blood and brain. Nonetheless, the toxicity data clearly show the gerbil to be the more sensitive of the two species to carbaryl, and it is assumed that the toxicological consequences of the ChE inhibition in the gerbil is far greater than that in the rat. Symptoms of poisoning were the same in both species, thus reducing the possibility that carbaryl acted other than to inhibit ChE in the gerbil. (Supported by EPA Grant No. RB05143.)

**384 THE INFLUENCE OF DIETARY FIBER ON THE ABSORPTION AND DISPOSITION OF MIREX.** J.D. deBethizy and J.C. Street, Toxicology Graduate Program, Utah State University, Logan, UT 84322.

Selected xenobiotics are being utilized as models of putative colon carcinogens in a study of the influence of major types of dietary fiber upon xenobiotic toxicokinetics. Adult, male Wistar
with the rapid biotransformation of lindane in these mice, leads to impaired conjugation of polar metabolites, elevated tissue binding of these metabolites with reduced metabolism and/or excretion.

386 CORRELATION OF THE ESTROGENIC ACTIVITY OF o,p'-
DDT WITH NUCLEAR LEVELS OF ESTROGEN RECEPTOR.
A. K. Robison, V. R. Mukku, J. S. Ireland, and
G. M. Stancel (Sponsor: S. D. Murphy), Dept. of
Pharmacology, Univ. Texas Medical School,
Houston, TX.

o,p'-DDT and related pesticides produce estrogenic effects in vivo and in vitro. To determine if these effects result from the interaction of the pesticide with the classical estrogen receptor system we have measured estrogenic responses of the rat uterus and the nuclear translocation of estrogen receptors as a function of the dose of o,p'-DDT administered both in vivo and in vitro. Nuclear levels of estrogen receptor were measured by an exchange assay using isolated nuclei. After in vivo administration of o,p'-DDT, nuclear levels of estrogen receptor reach a maximum in 3 hours, and increases in uterine weight and DNA synthesis reach a maximum in 24 hours. In the dose range of 10-1000 mg/kg o,p'-DDT there is a high degree of correlation (r=0.98) between these two growth responses and levels of nuclear estrogen receptor. Following a 1 hour incubation of uteri with o,p'-DDT in vitro we also measured nuclear levels of estrogen receptor and the induction of a specific estrogen inducible protein (IP) using a double label isotope procedure. In the concentration range of 30-1000 uM there is also a high degree of correlation (r=0.96) between these two parameters. These results provide strong support for the hypothesis that the estrogenic effects of o,p'-DDT are indeed mediated by interaction of the pesticide with the classical estrogen receptor system in target tissues for this hormone. (Supported by NIH grants HD-08615, HD-00999 and ES-07090.)

387 ORGANOPHOSPHORUS - INDUCED NEURITE DE-
GENERATION IN HUMAN NEUROBLASTOMA
CELLS. D.G. Graham and M.B. Abou-Donia, Depts.
of Pathology and Pharmacology, Duke University
Medical Center, Durham, N.C. 27710.

Human neuroblastoma cells have been developed as an in vitro model for organophosphorus-induced delayed neurotoxicity. In the concentration range of 30-1000 uM this compound induces and maintains a variety of OP's, and the reductions follow pseudo-

order kinetics. That the hydrophobic OP's enter...
the neuroblastoma cells adsorbed to agar was shown to result in reductions in [\(^{3}H\)] epinephrine uptake, pointing to this metabolite as the ultimate neurotoxin in leptophos-induced delayed neurotoxicity. Initial experiments have shown that the in vitro effect of OPs is not shared by phenyl benzylcarbamate. (Supported by EPA Grant R806400030).

The effects of a new antiviral agent, vidarabine phosphate, the magnitude of dose levels provide an adequate margin of safety.

**THE SUBACUTE ORAL TOXICITY OF A 5:1 COMBINATION OF SULFADIMETHOXINE AND ORMETHOPRIM IN THE DOG**

T. J. Hayes, R. M. McClain, and H. Westen, Department of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, N. J.

Tablets of sulfadimethoxine and ormetoprim (5:1) were given orally to beagle dogs at dosages of 0 (control), 0 (excipients), 27.5, 82.5 and 137.5 mg/kg/day. Necropsies were done after 8 weeks of treatment to assess the effects of treatment, and after a further 12 weeks without treatment to assess the reversibility of effects.

Treatment related effects were principally those of hypothyroidism. High-dose treatment was associated with: marked thyroid hyperplasia, enlarged thyroid stimulating cells in the pituitary, decreased serum T, and T, elevated serum cholesterol, decreased hemoglobin and hematocrit, reduced heart rate and R wave amplitudes (ECG). Other high-dose findings were increased liver weights and multifocal vacuolation of hepatocytes, thymic involution, marginal testicular atrophy, decreased serum folate, and minimal eye changes. After 12 weeks without treatment, the thyroid showed functional recovery but were somewhat enlarged due to abundant colloid storage. Minimal eye change continued to be present in 1 dog, but not in another. All other changes were reversible.

Mid-dose treatment produced no changes in eyes, hemoglobin, thymus, liver or testes. Other changes listed above were observed in reduced incidence and magnitude. Changes at the low-dose were limited to elevated cholesterol, increased thyroid and liver weights, enlarged thyroid stimulating cells in the pituitary, and mild follicular thyroid hyperplasia.

These data further characterize the syndrome of sulfonamide-induced hypothyroidism in dogs and demonstrate the reversibility of these effects.

**SYSTEMIC TOXICITY STUDY OF NUCLEOTIDE ANALOG ANTIVIRAL AGENTS IN NEONATAL RATS.** A. Gough, N. Barsoum, S. Gracon and J.M. Sturgess Warner-Lambert/Parke-Davis Res. Inst., Sheridan Park, Miss., Canada (Sponsor: F. de la Iglesia)

The effects of a new antiviral agent, vidarabine phosphate on postnatal growth and development of the rat were evaluated and compared to 1- (S)-arabinofuranosylcytosine (Ara-C), an agent with established antiviral activity and teratogenic potential. The studies provided animal safety data for drug evaluation in neonatal herpes, a life-threatening condition. Groups of 2-day-old rats were given 5 consecutive daily ip doses of 400, 200, 100 and 25 mg of Ara AMP/kg or 4 mg of Ara-C/kg. Observations were made daily for clinical toxicity and animals from each group were necropsied at 7, 14, 21 and 35 days of age. Clinical, pathologic or developmental abnormalities were absent in vidarabine phosphate-treated groups and organogenesis was comparable to controls. Ara-C caused significant delay in full body growth with impairment in organogenesis. These phenomena were characterized by cerebellar cortical hypoplasia and dysplasia of the retina and renal cortex. Delayed cerebellar development was evident with Ara-C by a marked reduction in size of cerebellar folia and hypo-cellularity of the internal and external granular layers with delayed involution of the latter. Postnatal dysplasia was bilateral and extensive and characterized by rosette formation of photoreceptor cells. Immature renal tubular and glomerular structures were indicative of impaired nephrogenesis. In the absence of reference histopathological changes with vidarabine phosphate, the magnitude of dose levels provide an adequate margin of safety.

**389 SYSTEMIC TOXICITY STUDY OF NUCLEOTIDE ANALOG ANTIVIRAL AGENTS IN NEONATAL RATS.** A. Gough, N. Barsoum, S. Gracon and J.M. Sturgess Warner-Lambert/Parke-Davis Res. Inst., Sheridan Park, Miss., Canada (Sponsor: F. de la Iglesia)

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**390 INTRAVENOUS TOXICITY OF WR-2721 (NSC-296961)**


WR-2721 ((Ethanethiol,2-[3-aminopropyl]-lymno); dihydrogen phosphate ester)) is a radioprotector drug that enhances survival of mice against radiation while not protecting tumor cells. It is the first radioprotective drug approved by FDA for use in humans.

The lethality of single intravenous doses of WR-2721 was determined in mice. The defined LD, LD, and LD in mice were: males-539.8, 604.6, and 677.3 mg/kg; females-471.5, 557.1, and 658.2 mg/kg, respectively. Mice were given the 0.5 LD, LD, and LD doses and evaluated for toxicity. Dogs were given 0.1 mouse LD to 1.3 mouse LD doses adjusted to the body surface area of dogs. Clinical signs seen in the mice included hypactivity, ataxia, decreased limb tone, impaired righting reflex, dyspnea, bradypnea, and prostration. Surviving mice at termination (29 days) appeared normal and had normal weight gains. Clinical signs seen in dogs included emesis, hypothermia, hypopactivity, and ataxia. High BUN and SGOT values in treated mice at Day 2 indicated possible nephrotoxicity. Treatment related hematology and clinical chemistry changes were not observed in dogs. Pathological changes caused by WR-2721 in mice were lymphoid necrosis of lymphoid organs and renal tubular epithelial degeneration at interim sacrifice, but no drug-induced lesions were seen at termination. Drug-induced lesions seen in dogs included renal tubular epithelial necrosis; congestion, hemorrhage and edema of lungs, liver, kidney, gastrointestinal
tract and lymph nodes. The 0.1 mouse LD₅₀ dose (males-8.0 mg/kg, 160 mg/M²; females-7.0 mg/kg, 140 mg/M²) was nontoxic in dogs.

Supported by NCI Contract No. NO1-CM-17365.

391 DIFFERENTIAL TOXICITY OF N-(2-CARBOXYPHENYL) RETINAMIDE AND N-(4-CARBOXYPHENYL) RETINAMIDE IN RATS. D. Emmerling and P. Kurtz. Battelle Memorial Institute, Columbus, OH. Sponsor: G. Fisher

The toxicity of two synthetic retinoid analogs was examined and compared in 91-day gavage studies with Sprague-Dawley rats. Ten male and ten female rats were given either N-(2-carboxyphenyl) retinamide (2CPR) or N-(4-carboxyphenyl) retinamide (4CPR) at dosages of 4, 14, 50, or 150 mg/kg. Treatment with 4CPR produced typical manifestations of retinoid intoxication (bone fracture, dermal lesions, and anemia) but the required dose levels were at least three times higher than those required in positive control studies with all-trans retinoic acid. Furthermore, the two carboxyphenyl retinamides were not equally toxic. Administration of 4CPR produced a statistically significant growth depression in male rats at the 50 and 150 mg/kg dose levels while no effect on growth was observed for either sex receiving 2CPR. Significant reductions in HGB, HCT, and RBC values were seen in both sexes receiving the same two high doses of 4CPR but the hemograms of the 2CPR animals showed no significant effects. Radiographic examination of the long bones indicated fractures in the 4CPR-treated females at 50 and 150 mg/kg but no fractures in either sex given the 2CPR analog. The microscopic evaluation of tissue from high dose animals in each study did not indicate treatment-related changes. It therefore appears that N-(2-carboxyphenyl) retinamide is less toxic than the close structural analog, N-(4-carboxyphenyl) retinamide. (Supported by NCI Contract No. NO1-CP-85650.)


Tiamulin is a semisynthetic derivative of the antibiotic, pleuromutilin. It is active against mycoplasma organisms and Treponema hyodysenteriae, infectious agents in food-producing animals. Tiamulin is a semisynthetic derivative of the antibiotic possessing antitumor activity, was isolated from Actinosporangium Sp. The toxic effects of marcellomycin observed in single-dose toxicologic studies in mice iv (16.35-22.55 mg/kg) and dogs iv (2.05-4.51 mg/kg), and in multiple-dose studies in dogs iv (12 doses 0.49-1.48 mg/kg/2X/week) and rats sc (9 weekly doses 4.4-13.2 mg/kg), were dose-related and primarily manifested by suppressive action on myeloid, especially the erythrocytic and thrombocytopenic series, and on lymphoid tissues. However, initially a neutrophilic leukocytosis was observed in all species, which was considered to be due to mobilization of the marginal and bone marrow neutrophil pools. Subsequently, suppression of leukocytes was seen in dogs and in rats prior to death. Other toxicities observed included enteropathy, thyroid follicular proliferation, atrophy of the prostate and seminal vesicles, uterine hypoplasia, testicular and pancreatic degeneration, inflammation and necrosis at injection sites, hemorrhage in various organs, and serofibrinous edema in the rat study. In general, these toxicities were reversible in surviving animals during 4 to 6.5 week recovery periods. Significant cardiotoxicity was not demonstrated with marcellomycin.

393 SPECIES DIFFERENCES IN ANEMIA CAUSED BY PHTHALAZINE DERIVATIVE ANTIHYPERTENSIVE DRUGS. S. Takeyama, Y. Akiyama, T. Onoda, and T. Akimoto, Res. Inst. of Daiichi Seiyaku, Tokyo, Japan.

This experiment was performed to clarify the mechanism of species difference between rats and beagles in anemia caused by hydralazine, ecarnarine, and budralazine. These drugs produced anemia in rats receiving oral doses of 120 mg/kg and more, and in beagles given oral doses of 10 mg/kg and more. Hematological examination revealed normochromia, activation of erythropoiesis, and shortening of erythrocyte life span in both rats and beagles. The 0.1 mouse LD₅₀ dose (males-8.0 mg/kg, 160 mg/M²; females-7.0 mg/kg, 140 mg/M²) was nontoxic in dogs.
FURTHER STUDIES ON MUSCULAR DEGENERATION IN RATS AFTER POSTNATAL TREATMENT WITH 6-MERCAPTOPURINE. F.R. Alleva, T. Balazs, and L.J. Slaughter, Food and Drug Administration and Howard University, Washington, D.C.

Daily subcutaneous injections with 6-mercaptopurine monohydrate (6-MP) at 2 mg base/kg from 2 to 22 days of age has been reported to induce atrophic muscular degeneration with fat replacement in thigh and subcutaneous muscles of rats beginning at four months of age (Alleva, et al., The Toxicologist, p. 115, 1981). The present study was done to determine in one experiment whether offspring of male and female rats treated with 6-MP as above display fatty atrophy of their muscles while a second experiment was aimed at determining whether treatment of rats with 6-MP, 2 mg/kg sc, daily from 25 to 45 days of age also results in such muscular changes. In the first experiment 40 (20 females, 20 males) randomly selected rat offspring of treated parents and 14 (seven females, seven males) offspring (controls) of saline treated parents displayed normal muscles when killed at seven months of age. Eight (four females, four males) of the treated parents when killed at seven months of age displayed, as expected, fatty atrophy of their muscles while eight controls had normal muscles. Blood vessels and sciatic nerves taken from the hind legs of these treated parents were normal, adding further evidence (Alleva, et al., Drug and Chemical Toxicology, in press) that the fatty atrophy results from a primary myopathy. In the second experiment all 13 (eight females, five males) treated as well as 13 saline-control rats had normal muscles when killed at nine months of age. Mechanistic studies involving biochemical and electron microscopic examination of muscle taken from rats treated daily with 6-MP, 2 mg/kg sc, from 2 to 22 days of age are in progress.

THE EFFECT OF ADRIAMYCIN CARDIOTOXICITY ON ESSENTIAL TRACE METAL METABOLISM IN THE RAT HEART - Brian C. Lee & Klaus L. Stemmer. Univ. Cincinnati Medical Ctr., Kettering Lab. Cincinnati, OH 45267

Adriamycin (ADR, doxorubicin HCl) is a cardiotoxic anthracycline cancer chemotherapy agent that is suspected of interfering with essential trace metal metabolism in the heart. ADR cardiotoxicity is theorized to be a result of radical/peroxide damage. ADR generates O_2^-, which can be detoxified to H_2O_2 by superoxide dismutases (SODs). The essential trace metals, copper, zinc, and manganese, are associated with SODs and were examined for relationships to ADR cardiotoxicity.

Weanling male Sprague-Dawley CD rats were given ADR i.p. 2 mg/kg b.w. x 9 days. Diet and environment were trace metal controlled. Cardiac CuZn-SOD, total SOD, Cu, Zn, and Mn were assayed. EKGs and histopathology were scored. SOD was assayed in solution and in heart homogenate, with or without ADR.

Whole heart Mn was significantly decreased. There were significant non-specific EKG changes, but not QRS widening. Significant histopathological changes were seen in heart, kidney, and liver. Trends indicate CuZn-SOD was increased in the heart, but total SOD was decreased. 188 x 10^-6M ADR gave 50% inhibition of CuZn-SOD standard in aqueous solution. 3.33 x 10^-6M ADR completely inhibited total SOD in heart homogenate.

The results suggest that changes in Mn and SODs may be related to ADR cardiotoxicity.

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Pulmonary Responsiveness to Chlorcyclizine: Comparison Between Newborn and Adult Rats. S. Kacew, M.R. Parulekar and R. Narbaitz, Departments of Pharmacology and Anatomy, Univ. of Ottawa, Ottawa, Ontario.

Chronic ingestion of dietary chlorcyclizine from 3 to 6 weeks has been reported to produce an accumulation of foam cells in pulmonary alveoli of adult rats. Recent studies have shown that in newborn rats treated with chlorphenterrmine less accumulation of foam cells is apparent in pulmonary alveoli as compared to that seen in adults. Experiments were thus undertaken to (a) determine the effects of chlorcyclizine on newborn rat lung and (b) to compare pulmonary responsiveness of newborn with adult. Daily, oral administration of 30 mg/kg chlorcyclizine for 1 week produced 1-2 foam cells in a few peripheral alveoli of newborn lungs. Treatment with 10 mg/kg/day drug for 7 days produced no apparent change in newborns. Surprisingly, in adult lungs of 10 or 30 mg/kg/day chlorcyclizine-treated rats no foam cells were present. The 30 mg/kg chlorcyclizine-induced rise in foam cells of newborn lungs was associated with an increase in incorporation of thymidine into DNA and elevation in ratio of phosphatidylcholine to sphingomyelin of newborn lung. Treatment with 10 mg/kg/day drug for 7 days produced no apparent change in newborns. Surprisingly, in adult lungs of 10 or 30 mg/kg/day chlorcyclizine-treated rats no foam cells were present. The 30 mg/kg chlorcyclizine-induced rise in foam cells of newborn lungs was associated with an increase in incorporation of thymidine into DNA and elevation in ratio of phosphatidylcholine to sphingomyelin of newborn lung. In contrast, the absence of apparent foam cells in adults was accompanied by either no change or decreased incorporation of thymidine into DNA. The concentration of lung phosphatidylcholine to sphingomyelin ratio was significantly decreased from control. Our data show that the pulmonary responsiveness of newborns to chlorcyclizine differs from adults. The decrease in the ratio of phosphatidylcholine to sphingomyelin is indicative of respiratory distress to adults suggesting that newborns may adapt more readily to chlorcyclizine, a finding previously reported for chlorphenterrmine. (Supported by the Medical Research Council of Canada).
THE EFFECTS OF DRUGS AND CHEMICALS UPON THE COMPARATIVE RESPONSE TO NABILONE AND DELTA-9-THETRAYHYDROCANNABINOL IN THE ASSESSMENT OF ABUSE LIABILITY.  P. J. Pence, L. Lemberger, B. J. Cerimele, and R. B. Forney, Department of Pharmacology and Toxicology, Indiana University School of Medicine and the Lilly Laboratory for Clinical Research, Indianapolis, IN.

Nabilone is a synthetic compound similar in chemical structure to delta-9-tetrahydrocannabinol (THC), the main active ingredient in marihuana. It is a centrally acting cannabinoid which has been studied clinically because it reduces the nausea and vomiting associated with cancer chemotherapy, even in those patients who are refractory to conventional antiemetics. Since no direct quantitation of nabilone's effects compared with THC had been made, a study was designed to make qualitative and quantitative comparisons of certain physiological, psychometric, and psychomotor parameters of these drugs: to evaluate (1) abuse liability, (2) effects on psychomotor performance of such skills as driving, and (3) effects on heart rate and blood pressure. Treatments were administered both orally (po) and intravenously (iv) to 6 subjects in a double-blind manner according to a repeated measures design. Subjects were monitored for 8 hours following treatment administration. Subjects rated the 5 treatments on a preference scale from 1 (least liked) to 5 (best liked). The mean scores ± S.E. were (1) 1.17 ± 0.17 for placebo, (2) 2.92 ± 0.38 for THC po, (3) 3.17 ± 0.60 for nabilone po, (4) 3.42 ± 0.46 for nabilone iv, and (5) 4.33 ± 0.42 for THC iv. All 6 subjects stated they would use THC again either iv or po for its euphorogenic effect; 5 out of 6 subjects stated they would use nabilone either iv or po for its euphorogenic effect. No subject would use placebo again. Pharmacological effects seen and psychomotor data will be presented.


Surveys of the relevant literature indicate that the susceptibility of the endocrine tissues to compound-induced lesions may be ranked in the following decreasing order of frequency: adrenal, testis, thyroid, ovary, pancreatic islets, parathyroid and pituitary. The first two are by far the most frequently affected. Pathologists not studying endocrine effects are often unaware of the number of compounds producing specific lesions in the adrenal. Most frequently affected is the zona fasciculata and reticularis. Compounds producing either degenerative or proliferative lesions there are acrylonitrile, aminoglutethimide, aniline, chenoedoxycholic acid, cysteamine, dibromochloropropane, DDD, dilantin, dimethylbenzanthracene, ethanol, fluorohexine, hexadimethrine bromide, nitrogen oxides, polyglylutamic acids, polyanetholsulfonate plus aminocaproic acid, poly(1.5-dimethyl-1,5-dibromomethylhydram), salurin, thioacetamide and thioguanine. Lesions in the zone glomerulosa are produced by aminoglutethimide, aniline, ethanol, hexadimethrine bromide and Ponceau SX (F.D. and C. Red 84). Proliferative or hypertrophic changes in the medulla are produced by o-chlorobenzylidene malononitrile, growth hormone, nicotine, reserpine, thiouracil and degenerative lesions by cortisone. A number of compounds, especially amphiphilic ones, produce whorls of smooth endoplasmic reticulum detectable microscopically as cytoplasmic inclusions without other cellular changes.

INHIBITION OF RETROGRADE AXOPLASMIC TRANSPORT BY ACYRAMIDE. M. S. Miller, T. F. Burks and I. G. Sipes, Program in Pharmacology and Toxicology, Arizona Health Sciences Center, Tucson, AZ 85724.

Monomeric acrylamide (ACMD) produces central-peripheral distal axonopathy in laboratory animals and humans following repeated exposure. The mechanisms by which ACMD induces axonopathy are currently unknown. However, alterations in axonal transport have been suggested to be involved in the production of axonopathy. Fast and slow anterograde axonal transport have been reported to be largely unchanged in ACMD intoxicated animals. This study addresses the effects of ACMD on retrograde axonal transport mechanisms. Retrograde axonal transport was quantitated using purified nerve growth factor (NGF) iodinated by the lactoperoxidase method (80-100 µCi/mole). Male Sprague-Dawley rats (250 g) received 10 consecutive daily doses of ACMD (50 mg/kg/day ip) or saline. One day after the last dose each animal received a single unilateral injection (20 µl) of a solution containing 125I-NGF (1 µCi) in the right front footpad. At various times thereafter (4, 8, 12, 24 hours) animals were killed and accumulation of 125I was determination in dorsal root ganglia (DRG) C4-C7 was determined by gamma counting. Retrograde axonal transport of 125I-NGF was also measured rats immediately following single doses of ACMD (0.5-100 mg/kg ip). Repeated ACMD administration (10 x 50 mg/kg) significantly delayed the appearance of 125I in DRG C4-C7. Single doses of ACMD inhibited the accumulation of 125I in DRG C4-C7 by up to 90% in a dose-dependent manner (ED50 ~ 25 mg/kg ip) at times corresponding with maximum accumulation in saline treated control animals. These data suggest that alterations in retrograde axonal transport systems may be involved in the etiology of ACMD-induced axonopathy. (Supported by a Graduate Student Development Award to M.S.M. and USPHS Grants DA02163 and ES82130.)

VISUAL INDICES OF ACYRAMIDE NEUROTOXICITY IN PRIMATES. W. H. Merigan, E. Barkdoll, and J. P. J. Maurissen, Environmental Health Sciences Center, Division of Toxicology, Department of Radiation Biology and Biophysics, University of Rochester Medical Center, Rochester, NY. Sponsor: R. Wood

While peripheral neuropathy is the most marked sign of acrylamide exposure, recent reports have demonstrated concurrent central nervous system damage. We explored central nervous system effects by measuring visual thresholds and visual evoked cortical responses in macaque monkeys during acrylamide administration. Three monkeys were given 10 mg/kg/day acrylamide orally 5 days/week until they were markedly ataxic (330 to 470 mg/kg total dose). One monkey was then sacrificed and the remaining two monkeys were studied during a 140-day recovery period. A fourth
monkey, a time matched control, received vehicle.

The three experimental monkeys showed altered visual thresholds and evoked potentials well before overt signs of toxicity. Visual acuity and the acuity-fusion thresholds were reduced and the latency of pattern-reversal evoked potentials increased. After dosing was terminated in two monkeys, the latency of evoked potentials and the flicker-fusion thresholds returned to control values within a few weeks. Visual acuity of both monkeys, on the other hand, showed some recovery and then stabilized below control values.

These results indicate that visual measures are appropriate for assessing effects of acrylamide on the central nervous system. (Supported by grants ES-01885, ES-01248, ES-01247 and in part under Contract No. DE-AC02-76EV03490 with the U.S. Department of Energy.)

403 COMPARATIVE EFFECTS OF PHENYLPROPANOLAMINE WITH EphEDRINE AND DEXTROAMPHETAMINE ON SPONTANEOUS MOTOR ACTIVITY IN HAMSTERS. L.R. Weiss and S. Joyanes, Division of Drug Biology, Food and Drug Administration, Washington, D.C.

Phenylpropanolamine (PPA), an appetite suppressant, decreased spontaneous motor activity (SMA) in adult and aged hamsters (L.R. Weiss, Toxicologist 1, 20, 1981). This report compares the effects of ephedrine (EPH), a chemically related sympathomimetic, and d-amphetamine (DAP), a CNS stimulant-anorexiant with abusive potential, with PPA on SMA measured in BRS activity chambers. The drugs were administered by gavage in a volume of 10 ml of water/kg to adult hamsters 30 min prior to testing. Activity was recorded for 15, 30, 45, and 60 min and analyzed as cumulative and noncumulative counts for each time period. PPA doses were 25, 50, 100, and 200 mg/kg compared to 12.5, 25, 50, and 100 mg/kg for EPH and 1.6, 3.1, 6.3, 12.5, and 25 mg/kg for DAP. These were run with water-treated controls. A depression of SMA was noted at all doses of PPA being significant over the entire 60 min period for cumulative activity and for noncumulative counts at 15, 30, and 45 min. In contrast, EPH caused pronounced depression of SMA at 12.5 and 25 mg/kg doses of both types of activity but no significant changes at 50 and 100 mg/kg. DAP showed stimulation of SMA in doses above 3.1 and these were significant at 45 and noncumulative counts. At the 6.3, 12.5, and 25 mg/kg doses, all hamsters showed stereotyped amphetamine behavior. The results of this comparative study indicate that PPA seems to act on the brain in a manner that is pharmacologically different from DAP but similar to EPH and suggests that the possible appetite suppressant effects of PPA may not be associated with stimulation or an amphetamine-like action in the CNS.

404 TIME-COURSE AND DOSE-RESPONSE ASSESSMENT OF CHLORDECONE (C)-INDUCED TREMOR IN RATS. H.A. Tilson and J.M. Gerhart, Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. (SPON: R.B. Mailman).

A procedure based on spectral analysis of whole body movements was developed to quantify C-induced tremor in rats. Male, Fischer-344 rats were given single or repeated i.p. doses of C and the frequency (cps) over 2.5-10 Hz bands at 2.5 Hz intervals and intensity (-dbv) at each Hz interval were measured. The most prevalent Hz (Pcups) and intensity (Pdv) were also recorded. Spectral analysis of the tremor produced by single doses of C could be differentiated from the spectral analysis of movement produced by various doses of harmine (10-20 mg/kg, i.p.) and apomorphine (0.5-2 mg/kg, i.p.). The administration of 10-100 mg/kg C produced measurable tremor at 1.5, and 12 hrs postdosing. The peak effect of C on tremor occurred between 5-12 hrs postdosing. The Pcups was approximately 12-13 cps following 50-100 mg/kg C 5-12 hr postdosing. Tremor was also quantified in rats receiving 5-10 mg/kg C for 10 consecutive days. Twenty-four hrs after the last dose of C, spectral analysis indicated a Pcups that was approximately the same as that obtained following acute administration of higher doses (-12 cps). Subsequent experiments with trihexphenidyl (Artane) and pizotifen (BC-105) suggest that the tremor produced by C may have a cholinergic and serotonergic component.

Acidic amino acids cause hypothalamic lysosomal damage and neuronal necrosis in neonatal rodents when given by non-dietary means. The capacity of Monosodium Glutamate (MSG) to induce similar neuronal damage was investigated under free feeding and after a period of water deprivation. Weanling ICR strain mice (120) of both sexes were used 10 M and 10 F per group: Group I-Control; Group II-free ingestion of 5.0% Ag; MSG; Group III-14 hr. water deprivation; Group IV-water deprivation and 2.0% MSG; Group V-water deprivation and 5.0% MSG; and Group VI-5g MSG/kg given by gavage (positive control). Water deprivation caused depressed body weights. Food consumption was decreased in male mice of Groups III-V but test solution consumption was unchanged by water deprivation (Groups III-V). Mice of Group VI exhibited prostration, lethargy and 7 animals died (26%) after dosing. Histologic examination of hypothalami from all mice revealed no abnormalities in structure in Groups I-III. In hypothalami of Group IV mice, minimal histologic changes were observed in 62% of neuronal cells and in 75% from Group V. The hypothalamic lesion involving neuronal necrosis was observed in the Arcuate-Median Eminence region of all mice from Group VI. The data presented suggest that the toxicity of MSG is potentiated by water deprivation in a dose-related manner and the method of intake influences the localization and severity of the lesion. Supported in part by NIEHS Training Grant ST32ES07058-03.


There are few interaction studies in animals that are chronically exposed, and potentially tolerant to the effects of a chemical. Pentobarbital interaction with ethanol using performance on a Roto-Rod, was studied in male Sprague-Dawley albino rats weighing from 200-310 g. All rats were pre-trained to remain on a 3 inch diameter rod rotating at 10 r.p.m. for periods of 15 minutes. Experimental animals were injected i.p. daily with 1.5, 1.75, 2.0, 2.25, or 2.5 g/kg of ethanol (15.1% v/v in normal saline). After injection the animals were tested at 10 minute intervals for 90 minutes on the Roto-Rod. Tolerance to ethanol's effects were observed to develop in animals injected with doses up to 2.0 g/kg, generally by day 14. No tolerance was observed in rats at higher doses, even at the end of testing at 21 days. Dose-response curves were prepared for pentobarbital in both control and experimental rats. In control rats doses of 6 mg/kg or greater gave positive trials (falling off), on the Roto-Rod. In experimental animals doses of 10 mg/kg or greater were required to produce positive trials. Injection of 3 mg/kg (% E.D. for controls) of pentobarbital in experimental rats dosed with 2.5 g/kg of ethanol produced a significant prolongation of ataxia measured on the Roto-Rod. These observations suggest a potential method for studying other acute interactions. (Supported by NIEHS Training Grant TT32-ES-07058.)

NEUROTOXIC EVALUATION OF TETRAKIS HYDROXYMETHYL PHOSPHONIUM CHLORIDE IN FISCHER 344 RATS. D. Thake, P. Kurtz, and S. Carter, Battelle Columbus Labs., 505 King Ave., Columbus, OH 43201. Sponsor: C.L. Fisher

A previous study had indicated that the flame retardant chemical tetrakis hydroxymethyl phosphonium chloride is capable of inducing peripheral neuropathy in rodents. This study was designed to verify and elaborate upon those results. Female Fischer 344 rats were exposed daily via oral gavage to 40 or 80 mg/kg/day of tetrakis hydroxymethyl phosphonium chloride for 45 or 90 days. Neurobehavioral evaluations were conducted after 45 and again after 90 days of exposure. Rats were terminated on days 45 and 90 following initial exposures and were perfused with five percent glutaraldehyde through the ascending aorta. Brains and spinal cords were evaluated histologically by light microscopy and peripheral nerves were evaluated histologically through the use of plastic embedded sections and electron microscopy. Clinical signs associated with TPCH administration occurred only at the higher dosage level and included decreased weight gain, lack of response to external stimuli, stiffness, and paresis. Mild to severe degenerative lesions were observed in peripheral nerves (sciatic, tibial, muscle branches, and plantar) in rats given 80 mg/kg and terminated at 90 days. Mild degenerative lesions were observed in peripheral nerves of rats given 80 mg/kg and terminated at 45 days. Spontaneous motor activity, forelimb grip strength, and hindlimb grip strength, were significantly reduced at both 45 and 90 days in the rats exposed to 80 mg/kg but not in those exposed to 40 mg/kg. No significant chemical effects were seen on rectal temperature or landing-foot spread at either test interval. These results indicate that neurotoxicity results from repeated exposure to this chemical in Fischer 344 Rats.

MODIFICATION OF CENTRAL NERVOUS SYSTEM ENDOGENOUS PHOSPHORYLATION IN ORGANOPHOSPHORUS DELAYED NEUROTOXICITY. D. J. Huggins and R. J. Richardson, Toxicology Research Lab; Dept. of Environ. & Indus. Hlth., Sch. of Pub. Hlth. The Univ. of Michigan, Ann Arbor, MI.

Some organophosphorus compounds (OPs) can produce a characteristic peripheral axonopathy approximately two weeks after a single dose. To evaluate whether a disruption in endogenous phosphorylation is involved in the toxic mechanism, protein kinase-mediated phosphorylation was studied in subcellular fractions from brainstem of control and OP-treated White Leghorn hens. Proteins phosphorylated after incubation with γ-[32p] ATP were separated and visualized with SDS-polyacrylamide gel electrophoresis and autoradiography.
SIX MONTH DAILY TREATMENTS OF SHEEP WITH NEUROTOXIC ORGANOPHOSPHORUS COMPOUNDS.


The delayed neurotoxic effects of TCOP, lepto- phos, EPN and DEF on male sheep were studied during 6 months of daily treatment under field conditions. Sheep were given daily oral doses of the tested compounds at 5, 5, 1 and 5 mg/kg/day, respectively, for 180 days. The neurotoxic effects were studied on clinical, histological and biochemical bases. The results were compared with a vehicle-control group of sheep given corn oil (0.1 ml/kg/day) only. All the DEF-treated sheep died during the experiment following 77 to 92 successive daily doses. All other sheep were sacrificed 24 hrs after the 180th daily treatment. Blood, brain, spinal cord and sciatic nerve tissues were taken for histopathological and/or biochemical examinations. The results indicated that the lepto phosphorus induced a delayed neurotoxicity and paralysis in sheep following about 4 months of treatment. TCOP produced only mild ataxia in one out of 4 sheep during the last week of experiment. On the other hand some of the EPN-treated sheep showed clinical signs of neurotoxicity during the course of experiment at the tested dose level. These clinical results were supported by histopathological findings and also by biochemical results using neurotoxic esterase measurements.

IDENTIFICATION OF NEUROTOXIC ESTERASE IN HUMAN PLACENTA, P. E. Gurbia and R. J. Richardson, Toxicology Research Lab., Dept. of Environmental & Industrial Health, Sch. of Pub. Hlth., The Univ. of Michigan, Ann Arbor, MI

Neurotoxic esterase (NTE) has been implicated as the primary event in the development of delayed organophosphorus (OP) neurotoxicity. NTE has been found in the nervous tissue of all vertebrate species examined, and it has been found in non-neural tissue such as lymphocytes and ileum. We recently found that human placenta also contains NTE activity. The activity in the crude tissue is 16.1 nmoles/min/mg protein (units) when phenyvalerate is used as the substrate. Subcellular fractionation yielded mitochondria, microsomes, and post-microsomal supernatant with activities of 38.1, 36.6 and 0.6 units respectively indicating that the activity is membrane bound as in nervous tissue. Since the specific activities of the mitochondria and microsomes were approximately equal, they were pooled for other studies (referred to as P2P3). The P2P3 activity could be solubilized with Triton X-100 when the ratio of detergent to protein was adjusted to 0.30. Triton-derivatized material with paraoxon (P0) and mipafox gave mipafox pi-50 values of 4.7, 5.0 and 5.0 respectively. The activity of the solubilized enzyme is also inhibited by low levels of mercuric acetate (P1-50 of 5.8) suggesting that a sulfhydryl group as well as a serine residue is required for the ester hydrolysis. The discovery of NTE in human placenta is valuable because it is an easily obtainable tissue source of human origin. It is also present in sufficient quantities to allow complete purification and characterization studies.


Methylmercury (MeHg) is known to interfere with CNS development in humans and in several animal models. The effect of MeHg on developing mouse cerebellar cortex is being investigated. MeHg (8 mg/kg body weight) was orally administered to 2 day old BALB/c mice which were sacrificed 24 hours later. At this time the cells of the external granule layer (EGL) are rapidly dividing. Morphometric analysis of the EGL from MeHg and buffer-treated animals showed differences in several parameters. The number of cells in the EGL/unit area was counted in 5 regions of the cortex. The total number of cells was significantly reduced by MeHg treatment (Analysis of Variance <0.025). The mitotic activity of the EGL was evaluated as a possible mechanism for the decrease in cell number, but the total numbers of mitotic figures/section and the mitotic indices in the 5 regions counted were not significantly altered by MeHg. Rather, cells appeared to be injured or dying in the treated animals, as indicated by condensed nuclei. The number of cells with nuclei measuring <3 μm in diameter was increased after MeHg treatment (p<0.002). The thickness of the EGL in the same area was not significantly decreased. The percentage of late mitotic figures (anaphase/metotic figures) was reduced in the MeHg animals (p<0.002). The incomplete mitosis may contribute to the decreased cell number by causing cell death. This metaphase arrest, presumably due to loss of spindle microtubules, may be an important mechanism in MeHg-caused neurotoxicity in developing animals.
SOLVENT TOXICITY AND WATER INSOLUBLE COMPOUND
DELIVERY SYSTEM FOR IN VITRO WHOLE EMBRYO CULTURE
K.T. Kitchin and H.T. Ebron, ETD, USEPA, Research Triangle Park, NC (Sponsor: William F. Durham)

In order to study the embryotoxicity and teratogenicity of water insoluble chemicals, solvents or chemical delivery systems of low toxicity and teratogenicity to the developing embryo must be found. Therefore, day 11 rat embryos were cultured for 2 days in whole rat serum containing 0.1, 0.5 and 2.5 volume percent of ethyl alcohol, dimethylsulfoxide, acetone, tween 80, corn oil/10% acetone/90% corn oil. No adverse effects occurred at 0.1% of any solvent. At 0.5% ethyl alcohol and tween 80 significantly reduced embryonic growth and increased the incidence of embryonic abnormalities. With the exception of corn oil and acetone/corn oil, embryos cultured in media containing 2.5% of various solvents failed to grow (only 10-20% of final control embryonic DNA and protein) did not differentiate, and did not have a beating heart. Corn oil suspended in rat serum by use of ultrasound was extremely non-toxic even at concentrations of 2.5 and 10%. Growth parameters of embryos cultured in serum containing corn oil were indistinguishable from controls and overall morphogenesis was good (particularly at 2.5%). Ethyl alcohol, acetone and tween 80 showed dysmorphic effects at doses below those found to be embryolethal. The order of increasing embryotoxicity and teratogenicity of the studied liquids was corn oil<acetone/corn oil<dimethylsulfoxide<ethyl alcohol, acetone< tween 80. Any of the four water miscible solvents (at 0.1%) or a sonicated suspension of corn oil in serum (up to 2.5%) met the criteria of a non-toxic and teratogenic water insoluble compound delivery system for in vitro embryo culture.

INHALATION TERATOLOGY STUDY ON MONOCHLOROBENZENE

An inhalation teratology study on monochlorobenzene (MCB) was conducted using Fischer 344 rats and New Zealand white rabbits. Groups of bred rats and artificially inseminated rabbits were exposed to 0, 75, 210, or 590 ppm of MCB, for 6 hr/day during days 6 through 15 (rats) or 6 through 18 (rabbits) of gestation. Pregnant rats exposed to 590 ppm MCB showed evidence of toxicity, but no evidence of embryolethality or teratogenicity was apparent at any of the exposure levels tested. Exposure of pregnant rabbits to the same concentrations produced elevated liver weights in the 210 and 590 ppm groups and a slightly increased incidence of some external and soft-tissue fetal malformations in all 3 exposure groups. The incidences of fetal malformations did not increase in a dose-related manner, however; the group exposed to the highest concentration of MCB (590 ppm) exhibited the lowest incidence of malformed fetuses. To further evaluate the teratogenic potential of MCB in the rabbit, additional groups were exposed to 0, 10, 30, 75, or 590 ppm by inhalation. Fetal malformations were observed among all treatment groups, including controls; no significant dose-related pattern or increase in malformations was apparent. Based on these findings, exposure to MCB vapor during gestation was not considered to be teratogenic in either species. (Sponsored by the Chemical Manufacturer’s Association).

THERATOGENIC EFFECTS OF AZASERINE IN THE SYRIAN GOLDEN HAMSTER. B.D. Roebuck and S.J. Carpenter, Dept. of Pharmacol. and Toxicol., Dept. of Anatomy, Dartmouth Med. Sch., Hanover, NH.

Azaserine is a potent pancreatic carcinogen in the rat. The Syrian golden hamster, however, has been shown to be resistant to the carcinogenic effects of this drug. The present study was undertaken to determine if the hamster is also refractory to the teratogenic properties of azaserine. Pregnant hamsters were injected i.v. or i.p. with single doses of azaserine (1.5-3.0 mg/kg) on either day 7, 8 or 9 of gestation. Signs of
toxicity in the azaserine treated dams were not observed. Control hamsters received injections of saline. The hamsters were sacrificed on day 12 of the 16 day gestation period and a count made in each hamster of viable and resorbing (dead) fetuses. Live fetuses were examined for growth retardation and gross external malformations. Azaserine was found to have both embryolethal and teratogenic effects in the hamster.

Fetal mortality in azaserine treated dams ranged from 17 to 100% varying with the dosage, route of administration and day of gestation. For fetal mortality, a dose-response relationship was shown. Fetal mortality among the controls did not exceed 5%. The incidence of fetal abnormalities ranged from 9 to 100% in treated hamsters, versus 3% in controls. The most common fetal abnormalities were runting (9% incidence in treated versus 3% in controls) and limb defects (10% incidence in treated versus 0% in controls). These results, compared with previous studies on rats, suggest that azaserine is equally embryolethal in the hamster and the rat. The hamster is also more resistant to the teratogenic actions of azaserine than the rat. Therefore, azaserine is both less teratogenic and carcinogenic in the hamster than in the rat. Supported by NIH Grants RR-05392 and CA-26594.


The acridinylamino derivative amsacrine, an antineoplastic agent for refractory leukemias, was studied for embryotoxic or teratogenic potential in pregnant rats. The compound was given ip on Days 6 to 9 of gestation to groups of 20 female CD rats at levels of 0, 1, and 0.5 mg/kg/day. Sham and untreated control groups were used also. During the treatment period, high dose (2.0 mg/kg) dams lost weight, whereas other groups were unaffected. Weight gain suppression continued in the post-dosing period. Food consumption during treatment was comparable with control groups. Litter sizes, post-implantation losses, and fetal weights were adversely affected at 2.0 mg/kg/day; 1.0 and 0.5 mg/kg/day had no significant effects on litter and fetal parameters although there was reduced body weight of fetuses from dams given 0.5 mg/kg day and stunting at 1.0 mg/kg/day. Four fetuses from the treated groups were grossly abnormal: two at 2.0 mg/kg, one at 1.0 mg/kg, and one at 0.5 mg/kg. Two vehicle control fetuses were also abnormal. No malformations occurred in the untreated control group. Osteogenesis inhibition and minor skeletal abnormalities were manifestations of fetotoxicity among treated groups. The results indicate that amsacrine in rats induces marked embryotoxicity (2.0 mg/kg) and fetotoxicity (1.0 and 0.5 mg/kg) but frank teratogenicity was not evident even when a few malformations were seen at 1.0 and 0.5 mg/kg/day.


The teratogenic potential of acetonitrile (I), propionitrile (II) and adiponitrile (III) were evaluated in rats. Aqueous solutions of I or II or of a corn oil solution of III were administered by gavage to separate groups of 25 mated Charles River rats on gestation days 6 to 19 inclusive. Daily dosage levels (mg/kg body weight) were: (I) 125, 190 and 275; (II) 20, 40 and 80 and (III) 30, 50 and 80. Control groups of 25 mated rats received each of the vehicles alone. On gestation day 20, all females were sacrificed and the number and location of viable and non-viable fetuses and early and late resorptions and the number of total implantations and corpora lutea were recorded. All fetuses were individually weighed, and examined for external and visceral or skeletal malformations and variations.

There were some maternal deaths in each of the high dose groups with all three compounds and one death at the mid-dose level of those dosed with III. Reduced maternal body-weights occurred in the high dose groups with I and II but not III. There were no other distinct adverse maternal clinical observations in any treated groups. Embryotoxicity was observed in the high dose group with I and II as evidenced by increases in early resorptions and post-implantation losses. No teratogenic responses were observed at any dose level with any of these three nitriles.


Despite wide recognition of the teratogenic and genotoxic effects of ethanol (ETOH) consumption, few studies have examined the effects of paternal ethanol exposure on fetal development. 20 male Long Evans rats were divided into two equal groups and given either tap water (group I) or 20% ETOH (group II) as the sole source of drinking water for 60 days. Five of 30 matings (group II) as compared to Group I proved infertile. Implantation sites, live birth rates, total litter weights and pup weights were decreased while fetal deaths were increased two-fold over controls. Malformations (Microcephalus, Microphthalmia, Cranial fissure, and Hydronephrosis) were noted in 55% of the offspring of Group I compared to 12% in controls. At necropsy, one rat of Group II had gross testicular atrophy. Absolute and relative testicular weights were significantly decreased in the alcohol treated group compared to Group I. These results suggest that paternal ethanol consumption causes dominant lethal mutations and testicular atrophy, and therefore, should be considered a significant risk factor in reproduction.

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COMBINED ORAL ADMINISTRATION OF ETHANOL AND ACETAMINOPHEN IN PREGNANT MICE: POSSIBLE ANTAGONISM IN TERATOGENESIS? A. Loeb\textsuperscript{1}, R. Manke, R. LePevre, and H. Bates (Sponsor R. Abraham). Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, N.Y.

Ethanol is a teratogen in human and laboratory rodents, frequently ingested in combination with other agents. Acetaminophen is a widely used analgesic, whose effects on the developing embryo remain unclear. Pregnant female ICR strain mice were used to evaluate the possible interaction between ethanol (ETOH) and Acetaminophen (APAP); Group 1- control, group 2- 0.4ml ETOH/kg, group 3- 4.0ml ETOH/kg, group 4- 25mg APAP/kg, group 5- 250mg APAP/kg, group 6- 0.4ml ETOH and 25mg APAP/kg, and group 7- 4.0ml ETOH and 250mg APAP/kg. Equal volume doses were given by gavage from day 6 to 15 of gestation.

Fetal death rate and visceral malformation were increased 160 to 180% in mice given ETOH (group 2) as compared to controls (group 1). APAP (group 4) caused both visceral and skeletal anomalies without embryolethality. Combinations of ETOH and APAP (groups 6 and 7), caused lower fetal death rates and fewer malformations than either ethanol or APAP alone.

The data suggest that combinations of ETOH and APAP, rather than interacting synergistically, may act as antagonists, possibly through altered microsomal metabolism. \textsuperscript{1}B.S.-M.D. Program, Union College, Schenectady, N.Y. Supported in part by NEHS Training Grant STZEB07058-03 and a Basic Medical Research Grant from Royal Dutch Shell, Ltd.

ADRENAL FUNCTION AND HEPATIC METABOLISM IN ADULT MICE EXPOSED IN UTERO TO CARBOFURAN OR DIAZINON: A LONGITUDINAL EVALUATION. J. Spyker Cranmer, D.L. Avery and M.F. Cranmer, Div. of Interdisciplinary Toxicology, University of Arkansas for Medical Sciences and Jefferson Professional Services, Little Rock, AR.

The functional status of the endocrine system of mice prenatally exposed to Carbofuran or Diazinon was evaluated by assessing adrenal function and hepatic metabolism at three stages in the lifespan. Gravid dihybrid mice were fed 0, 0.01 or 0.50 mg/kg Carbofuran or 0, 0.18 or 9.00 mg/kg Diazinon daily throughout gestation. Mothers of all pesticide and vehicle-control groups gave birth to approximately equal numbers (48/group) of viable, overtly normal offspring. Adrenal production and liver reduction capacity for corticosterone were measured in these offspring at 101 days of age; plasma concentrations of corticosterone were assayed at 101, 400 and 800 days of age. Exposure to the lower doses of both anticholinesterase compounds resulted in impairment of hepatic metabolism of corticosterone in vitro due to a loss in reductive capacity per unit liver weight. Plasma levels of corticosterone were correspondingly elevated in these offspring but without a concomitant increase in adrenal steroidogenesis in vitro. No evidence for persistence of these effects was observed among older animals; plasma corticosterone levels were essentially normal when measured at 400 or 800 days of age. No differences were found between control offspring and offspring exposed to the higher dose of either insecticide. This non-linear dose response is consistent with other reports. Results suggest that prenatal exposure to these low levels of Carbofuran or Diazinon has only a minimal effect on endocrine function and that this effect is transitory.

IMMUNOLOGICAL EFFECTS OF COMPLEX CHEMICALS ON THE DEVELOPING IMMUNE SYSTEM OF RATS EXPOSED IN UTERO. J.E. Morris and T.M. Graham, Battelle, Biology Department, Pacific Northwest Laboratory, Richland, WA and F.D. Andrew, Syntex Research, Palo Alto, CA.

Responses of the developing immune system to Aroclor 1254 and Solvent Refined Coal I (SRC-I) and II (SRC-II) materials were measured in rats. Rats were exposed in utero by daily gavage of the dam on days 12-16 of pregnancy, and were killed at 40 d of age. The dose groups included: 0.7 ml/kg/day process solvent (PS, SRC-I), 0.3 ml/kg/day heavy distillate (HD, SRC-II), 0.8 ml/kg/day 2.5% Aroclor 1254 (Ar, positive control), corn oil (vehicle control), and untreated rats (shelf control). Body, spleen, thymus, liver and brain weights were recorded. Lymphocyte function was measured by mitogen-induced lymphocyte activation assays (in vivo correlate of cell-mediated immunity) using cells from the peripheral blood, spleen and thymus. The distribution of T-lymphocytes in spleen cell preparations was based on \textsuperscript{3}H-Uridine uptake. PS-exposed offspring had reduced body (10%) and thymus (10%) weights while HD-exposed rats had weight reductions of the body (10%), thymus (22%) and liver (14%). Ar-exposed rats showed weight decreases in body (50%), thymus (50%), liver (28%) and brain (7%). HD- and Ar-exposed animals had significantly decreased thymus cell responses to phytohemagglutinin (35% and 11% respectively); AR-exposed rats also had decreased peripheral blood response to the mitogens Concanavalin-A (50%) and pokeweed mitogen (50%). T-lymphocyte populations in spleen cell preparations are not altered in exposed rats.


The functional status of the immune system of mice prenatally exposed to a chlorinated hydrocarbon pesticide was evaluated by assessing cell-mediated and humoral immune responses. Gravid Balb/c mice fed 0, 0.16 or 8.0 mg/kg chlordane daily throughout gestation produced viable, overtly normal offspring. Cell-mediated immune response (CM1) responses were measured in 101-day-old offspring by contact hypersensitivity responses to oxazolone. Mice were sensitized by application of 25 \textmu l of 8.0% solution of oxazolone to the de
nuded flank. Five days later, offspring were challenged by percutaneous application of 10 µl of an 0.1% oxazolone solution to the dorsal surface of the ear. Degree of induration was determined by daily micrometer measurement of ear thickness for 3 days after challenge. Subjects exposed to 8.0 mg/kg/day chlordane had a significantly (p < 0.01) depressed CMI response when compared to offspring of the control or lower dose groups. The primary humoral response to sheep red blood cell (SRBC) inoculation was determined at 101 days of age using the hemolytic plaque assay. Subjects were immunized with 0.1 ml of 1% washed SRBC. IgM antibody released from spleen cells was detected by addition of complement which caused lysis of the SRBCs. There were no differences between treated and control groups in the number of plaque-forming cells (PFC) produced by spleen cells. Results of this study revealed two significant findings: (1) a severe depression of the functional CMI response in apparently normal offspring, and (2) no effect on the T-cell dependent humoral immune response.


Diazinon, an extensively used organophosphate insecticide, was administered to pregnant New Zealand rabbits to evaluate its prenatal toxicity. The compound was orally administered by gavage at O (control), 7, 25, and 100 mg/kg from days 6-18 of gestation. All doses were weighed throughout the study and toxic signs evaluated. Fetuses were weighed, sexed, and evaluated for external, visceral, and skeletal abnormalities and variations. Significant doe mortality (40.9 percent) with concomitant pharmacologic signs were observed at 100 mg/kg. Neither embryotoxicity (decreased fetal weight and increased embryo-lethality) nor frank teratogenicity (external, visceral or skeletal anomalies) were observed at 100 mg/kg. Furthermore, lower dose levels of 7 and 25 mg/kg produced no significant maternal or fetal toxicity. The findings suggest that maternal toxicity was achieved at dose levels below those which cause fetal weight reduction and increases in either embryo-lethality or malformations. There was no evidence of a teratogenic effect at any of the dose levels tested.


It was shown earlier that progestosterone metabolites elicited contrasting action on hepatic drug metabolism (Dhami et al., Toxicology, 14, 99, 1979). This raised the question whether this action is connected with membrane-bound phospholipids. To test this, the effects of 16a-hydroxyprogesterone and 5α-pregnan-3α-ol-20-one on fatty acid content and composition of total liver and microsomal phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions were studied. 16α-Hydroxyprogesterone significantly increased the fatty acid content of both PC and PE fractions of total liver and microsomes. Both saturated and unsaturated components were raised, unsaturated to greater extent, so sat/unsat ratio was decreased. In contrast, pregnanolone significantly decreased total fatty acid, saturated and unsaturated fractions both in PC and PE components. The reduction of unsaturated fatty acid was greater resulting in an increased sat/unsat ratio. The contrasting action of these test compounds was observed in 16:0, 16:1, 18:0, 18:1, 18:2, 20:3, 20:4, 22:5 and 22:6 in PE. 16α-Hydroxyprogesterone significantly increased, while pregnanolone decreased mono- and polyunsaturated fatty acids. Drug metabolism is also increased or decreased by these test compounds respectively. Changes in unsaturation of fatty acids are connected with phase equilibrium changes of lipid membranes. Thus, it may be that the regulatory action of progesterone metabolites on the function of the endoplasmic reticulum is associated with intrinsic structural changes connected with modifications of the phospholipid moiety.


Chemically reactive metabolites have been implicated as mediators of toxicity, including carcinogenicity. Glutathione is conjugated with reactive metabolites resulting in detoxification and excretion. Numerous studies have shown that thiols are important in the degradation of Captan. The objective of this study was to determine the effect of Captan Technical in vivo on duodenal sulfhydrlys. Male and female B6C3Fl mice were administered Captan as a single oral gavage in corn oil at doses of 0,20,200 or 2000 mg/kg. Mice were sacrificed at 24, 72 hrs. Captan administration resulted in an increase in duodenal sulfhydrlys. Significant increases were observed as early as 2 hrs. following administration of Captan and persisted for at least 72 hrs. The mean value of reduced duodenal sulfhydrlys (+s.d.) for control males and females was 1595±170 µg/g and 1524±152 µg/g, respectively. In a separate study to evaluate the effects of subchronic ingestion on duodenal sulfhydrlys, Simonsen mice ingested diet containing Captan Technical at levels of 500,1000,2000,4000,8000 or 16000 ppm for up to 30 weeks. Mice were sacrificed on days 1,2,5,9,14,21,35,49,84 and 213 days of treatment. Duodenal sulfhydrlys were elevated at the 500 ppm level and above at all sacrifice intervals. Maximal increases were about 160% of control values. Contrary to a proposed genetic mechanism for captan-induced small intestinal tumors, the results of this study do not show a depression of sulfhydrlys. Perhaps captan-induced tumors are produced through an epigenetic mechanism. Recent observations lend credibility to this hypothesis.

To evaluate the possible contribution of covalent binding to the Ames positive responses reported for many 9,10-anthraquinone (AQ) derivatives, studies with \(^{14}C\)-labelled 1,4-dihydroxy, 1-amino-2-methyl and 1-nitro-2-methyl AQ have been performed to examine their binding to DNA and protein in vitro. The chemicals were incubated with calf-thymus DNA in Tris buffer pH 7.0 at 37°C for varying times in the presence or absence of a rat liver S9 fraction. DNA was isolated by phenol/cresol extraction and precipitation, and subjected to ribonuclease and protease treatment before assessment of bound radiolabel. All three AQs bound to DNA, with 1,4-dihydroxy and 1-nitro-2-methyl AQ requiring S9 (0.5 and 0.7 pmol/mg DNA respectively after 60 min. incubation) while 1-amino-2-methyl AQ bound in the presence and absence of S9 (3.2 and 1.7 pmol/mg DNA respectively). Binding to protein was measured with S9 fraction as above, precipitating the protein with TCA, and exhaustively extracting the unbound AQ with solvents. 1,4-dihydroxy, 1-amino-2-methyl and 1-nitro-2-methyl AQ all bound to protein with values after 45 min. incubation of 1050, 790 and 270 pmol/mg protein respectively. Such binding was not seen when albumin was used, suggesting a requirement for enzymic activation of the compounds to enable binding to protein. These data suggest that covalent interactions with DNA may be responsible for the Ames test results with certain nitro and amino AQs. The role of covalent binding in the activity of 1,4-dihydroxy AQ and other AQs which were more active in the absence of S9 in the Ames test, is not however clear.


Benzene and cyclophosphamide both cause a wide variety of disorders of the haemopoietic system. Both compounds can affect the proliferating and non-proliferating cells of the bone marrow, and thus have the potential to alter erythropoiesis. Rats have been treated with benzene or cyclophosphamide and the erythroid system in vivo compared with the production of erythrocytes in vitro. Benzene (two s.c. doses of 2.2 g/kg) caused a marked reduction (70% at day 3) in the amount of 2-\(^{14}\)C glycine incorporation (over 24 hrs) into newly formed erythrocytes. This reduction in erythrocyte production was confirmed by a reduction (80% at day 3) in the number of reticulocytes present in the peripheral blood, and was coincident with a depletion of nucleated cells (50% at day 3) and haem synthesis (70% at day 3) in the bone marrow. Cyclophosphamide, (50 mg/kg) caused a greater reduction in erythrocyte production (96%), reticulocytes (90%), bone marrow nucleated cells (90%) and haem synthesis (90%). The recovery of these parameters to control levels was complete by day 7. With both these compounds there was a rebound increase in the incorporation of 2-\(^{14}\)C glycine into newly formed erythrocytes. This occurred at day 10 for benzene (40% increased) and day 13 for cyclophosphamide (100% increased). These data suggest that the initial reduction in erythrocyte production is caused by the depletion of nucleated cells in the bone marrow, whilst the delayed recovery and rebound increase in erythrocyte production must be the result of prolonged effects on more immature bone marrow cells.


The report (Sammet et al., Toxicol. Environ. Health. 5, 785, 1979) that partial hepatectomy protected rats against benzene toxicity and our recent observation that mice were similarly protected suggested that a toxic metabolite of benzene travels from liver to bone marrow. We attempted to characterize the metabolite by administering each of a series of known and suspected benzene metabolites to Swiss Albino mice. We measured their effect on red cell production using the \(^{59}\)Fe uptake technique of Lee et al. (Toxicol. Appl. Pharmacol. 27, 431, 1974). Mice were treated twice per day for three days with saline, a negative control, benzene (1760 mg/kg, sc) in corn oil, a positive control, or the test compound. On day 4 \(^{59}\)Fe was injected via tail vein and 72 hr later the blood was sampled for \(^{59}\)Fe uptake. The following compounds were administered sc at the designated doses expressed in mg/kg: phenol, 172; hydroquinone, 47.5; resorcinol, 250; catechol, 31 and 62; p-benzoquinone, 17; a combination of p-benzoquinone, 17, with hydroquinone, 47.5. The ip route was used for hydroquinone, 50; 1,2,3-benzenetriol, 150; 1,2,4-benzenetriol, 20; 1,3,5-benzenetriol, 350; dibenzo-p-dioxin, 100; muconic dialdehyde, 2; 2,5-dihydroxy-1,4-benzoquinone, 8.5 and 17. Unlike benzene, none of the compounds tested markedly reduced \(^{59}\)Fe uptake. The reactive metabolite of benzene has yet to be identified.

Supported by NIH grant 5PS00322.


Benzene metabolites (bm) are responsible for benzene-induced bone marrow depression (Snyder et al., Rev. Biochem. Tox. 3, 123, 1981). To determine relative toxicity of the metabolite(s), mouse granulocyte-macrophage colony-forming units (CFU-GM) were exposed to five bm. CFU-GM cultures were grown in agar suspension (Metcalf, Hemopoietic Colonies, New York; Springer Verlag, 1977). Medium (4=MEM) was supplemented with 20% FBS and a 1:2 dilution of Salmonella typhimurium endotoxin-induced murine colony stimulating factor (ECSM).
factor. Phenol (P), catechol (C), p-benzoquinone (PBO), hydroquinone (HO), or 1,2,4-benzenetriol (BT) was added. Concentrations of bm ranged from 10^{-8} to 10^{-3} M; zero-concentrations were verified by HPIC analysis. Granulocyte and monocyte colonies per plate were determined on day 7 and expressed as % of control. P was the least toxic bt, producing no suppression of colony formation below a concentration of 10^{-3} M. PBO and HQ began to suppress colony formation at 10^{-3} M and were the most toxic bm tested. C was less toxic than PBO and HQ. BT was intermediate between C and P. The combined concentrations of bm in bone marrow of benzene-treated mice (Longacre et al., J. Toxicol. Environ. Health 7, 223, 1981) were similar to those which inhibited growth in CFU-GM. Although the data from the CFU-GM studies suggest that these compounds are potentially hemotoxic, it has yet to be demonstrated that they are active in vivo.

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The reactivity of trans,trans-muconaldehyde, a possible toxic metabolite of benzene towards platelets after 14-day retinoic acid treatment in rats. S. D. Harrison, Jr. and C. J. Viau, Graduate Center for Toxicology, University of Kentucky, Lexington, KY.

430 The reactivity of trans,trans-muconaldehyde, a possible toxic metabolite of benzene towards glutathione. G.S. Rao, G. Khz, and B.D. Goldstein, Dept. of Environmental and Community Medicine, CMMNJ-Rutgers Medical School/Rutgers University Joint Graduate Program in Toxicology, Piscataway, NJ.

At present the metabolite(s) responsible for the leukemogenic action of benzene is not known. We have hypothesized that muconaldehyde, an alpha, beta-unsaturated six-carbon diene dialdehyde, may play a role in benzene hematotoxicity. Alpha,beta-unsaturated aldehydes are known to react with SH-containing compounds. In the present studies, we investigated the kinetics for the reaction of trans,trans-muconaldehyde with glutathione. The rate of the reaction was followed by measuring the decrease in optical density at 272 nm, the major absorption band of muconaldehyde which disappears after 1,2-addition of glutathione. First order reaction rates with respect to muconaldehyde were observed in borate, pH 8.1, and tris, pH 8.1 and pH 7.4. The apparent second order rate constants were 79.3, 43.2, and 29.1 M\(^{-1}\) s\(^{-1}\) in borate, pH 8.1, tris, pH 8.1 and tris pH 7.4 respectively. These data show that the reaction is pH-dependent and varies with the type of buffer used. The stoichiometry of the reaction obtained by measuring the amount of muconaldehyde and glutathione consumed at t=30 min was 1:1 on a molar basis in tris buffer. It has been suggested that the rate of reaction between alpha, beta-unsaturated aldehydes with sulfhydryl compounds is directly proportional to their toxicity. In comparison with literature data on toxic alpha beta-unsaturated aldehydes, our studies show that muconaldehyde is less reactive than acrolein but far more reactive than presumed toxic lipid peroxide decomposition products of this nature.

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432 Evaluation of exposure levels to phthalate acid esters during blood transfusion. R. Kardish, M. Tocchi, G. Posen, R.K. Smiley, J.M. Bowman, and C. Rock, Canadian Red Cross, B.T.S., Ottawa Ontario, *Renal Dialysis Unit, Ottawa Civic Hospital, +Department of Medicine, Ottawa General Hospital and +Canadian Red Cross, B.T.S., Winnipeg, Manitoba. Sponsor: J.S. Woods

The risk of exposure to plasticizers such as di-2-ethylhexyl phthalate (DEHP) which leach from plastic medical devices is currently being reassessed in view of the recent documentation from N.I.H. of the carcinogenicity of DEHP. This subject is of concern not only in the multi-transfused patient whose plasticizer intake is relatively large but also to the blood donor population, who may be exposed to plasticizer during routine pheresis procedures. To assess the exposure level in these various groups, blood samples were analyzed for DEHP and its more toxic metabolite mono-2-ethylhexyl phthalate (MEHP) using HPIC. Of the 28 hemodialysis patients, DEHP was detected in 9 pre-dialysis blood samples (1.0 to 14.0 µg/ml) and in 9 post-dialysis blood samples (1.0 to 14.5 µg/ml). MEHP was detected in 7 of the pre- and 14 of the post-dialysis samples. However, no phthalate acid esters were detected in the blood of 7 severe Factor VIII deficient hemophiliacs either before or after cryoprecipitate administration, although the total intake of DEHP and MEHP per hemophiliac was as much as 5.0 mg and 0.3 mg per week respectively. No detectable levels of DEHP or MEHP were observed in the pre- or post-blood samples of donors undergoing plasmapheresis or cytapheresis. Patients who have been multiply plasmapheresed for many year did not show detectable levels of metabolism in rats, we have explored whether retinoic acid (RA) affects the ability of platelets to metabolize AA. Young, adult male Sprague-Dawley rats were treated once per day for 14 days by oral gavage with RA (20 mg/kg/day) or RA (20 mg/kg/day) + indomethacin (IND, 5 mg/kg/day).

Control rats received diluent (0.2 ml/100 gm of body wt) consisting of aqueous propylene glycol (10%) and Cremophor EL (8%). On Day 12 (first treatment = Day 1) all rats received 0.5-1.0 µCi of 14C- AA sc. Pooled 24-hr urine samples were collected for Days 12-14. Rats were killed 24 hr after last treatment, and blood was collected for preparation of platelet-rich plasma (PRP). Mixtures of PRP with 14C- AA, CaCl\(_2\), and thrombin (2.5 U) were incubated 10 min at 37° and then frozen. The following AA metabolites were separated by TLC and quantitated by liquid scintillation: prostaglandin (PG)E\(_2\), 6-keto-PGF\(_2\)\(_a\), and thromboxane (TX)B\(_2\). The data, expressed as fmoles of 14C- AA converted to each metabolite, indicate that the ability of rat platelets to metabolize 14C- AA is not affected by 14 days of oral RA. RA + INDO produced a significant decrease in the production of PGF\(_2\)\(_a\) (144 ± 12 fmoles/10 min, \(\pm\) SEM) vs control (201 ± 12 fmoles/10 min) in this assay. The data suggest that repeated doses of retinoids may not alter the capacity of platelets to support oxygenation processes critical for the regulation of aggregation, but the possibility of effects on endogenous AA pools warrants further study. ([Supported by McDowell Cancer Network and Amer. Cancer Soc. grant IN108F.])
The data suggests that while there is exposure to phthalate esters during blood transfusion the accumulative dosage and therefore the risk is relatively small.

**PROMUTAGEN ACTIVATION BY SCHISTOSOMA MANSONI INFECTED MICE.** S.A. Lacey, J.G. Babish, and J.R. Georgi. Dept. of Preventive Medicine, N.Y. S. College of Veterinary Medicine, Cornell University, Ithaca, N.Y.

Hepatic cytochrome P-450 mediated promutagen activation was examined in *Schistosoma mansoni* infected, female, CD-1 mice. Animals were exposed by tail immersion to either 108, 254, or 438 cercariae/well; the duration of infection was 30, 60, or 90 days prior to killing. Liver homogenates (25% w/v) from control and parasite infected animals were centrifuged at 9000 x g. Supernatant fractions from the 9000 x g centrifugation (S-9) were used to examine the metabolic activation of 2-acetoxyantracene (AA), 2-acetamidofluorene (AAF), aflatoxin B1 (AFB1) and benzo (a)pyrene (BP). *Salmonella typhimurium* TA100 was used to detect mutagenic metabolites. Fractional log doses of S-9 protein (S) were assayed using a promutagen concentration giving 75% maximal mutagenic response of control S-9.

S-9 from *S. mansoni* infected mice, in general, produced significantly fewer revertants per mg S-9 protein for all compounds. Cercarial dose and duration of infection showed no correlation with depression of mutagenic activity. However, at 30 days post infection, BP mutagenicity was greater in infected animals than controls. Plots of revertants/m mole P-450 vs mmole P-450 showed (1) decreasing mutagenicity of all compounds with increasing P-450 concentration and (2) a decrease in revertants/m mole P-450 in infected animals at all concentrations of P-450 for AA, AAF, and AFB1.

The decreased production of mutagenic metabolites per mmole P-450 indicates a parasite mediated effect on a specific form(s) of P-450 or a change in ratio of other enzymatic processes involved in deactivation of reactive metabolites of the P-450 system.

**COMPARATIVE METABOLIC ACTIVATION OF PROMUTAGENS BY FERRET AND RAT HEPATIC S-9 FRACTIONS.** K.A. Frederick, J.G. Babish, and B.E. Johnson, Department of Preventive Medicine, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York.

Investigation of biotransformation reactions in the ferret is requisite for its development as a toxicologic research model. Several chemical promutagens subject to metabolic activation by the hepatic microsomal mixed-function oxidase system were assayed by *Salmonella typhimurium* reversion to histidine prototrophy, using the 9,000 x g supernatant fraction (S-9) of constitutive ferret and rat hepatic tissue homogenates. Amounts of S-9 were varied in an effort to determine the optimal activating concentration in each species for those xenobiotic compounds tested. The slope of the linear portion of the dose-response curve, the potency (revertants per ug), and the lowest significant doses of all test compounds were calculated. For both species, the most favorable dose-responses were observed at S-9 levels of 25 to 100 ul per plate (500 ul) for 2-acetamidofluorene, cyclophosphamide, and aflatoxin B1, 7,12-Dimethylbenz(a)anthracene and hydrazine effected similar responses regardless of the S-9 level, especially at their higher doses. Response to benzo(a)pyrene was equivocal. Mutagenic activity was determined per ul S-9 as well as per nmole Cytochrome P450. Overall responses were qualitatively similar between the species, although some quantitative differences were shown to exist.


L-azaserine is a potent carcinogen that, in bacteria, can cause mutation and bacteriophage induction without prior activation by mammalian enzymes. However, we found that rat tissue extracts markedly enhanced azaserine phage inducing activity. In the Inductest of Moreau, et al, prophage induction in Escherichia coli (λ) by azaserine alone began only after a 40-60 minute lag. Addition of rat hepatic post mitochondrial supernatant (S9) enhanced induction by reducing the lag; more than 50% of the population was induced by 40 minutes. Preparations from rats fed a diet deficient in methyl groups were about twice as effective in the enhancement of induction at a given time as S9 fractions from control animals. (The methyl group deficient diet greatly enhances hepatocarcinogenesis by azaserine). Enhancing activity was found also in pancreatic extracts, but in lesser amount than in liver and was unaffected by diet. Most of the S9 activity was found to be in the cytosol; it was destroyed by trypsin. Hepatic cytosol enhanced also azaserine mutagenesis in *Salmonella typhimurium*, at short times of exposure. Under Inductest conditions cytosol promoted incorporation of label from 14C-azaserine into the bacteria, most of it into acid insoluble material, usually after a 15-20 minute lag. This pattern differed from the rapid active transport and incorporation of the amino acids tryptophan and proline into bacteria that was not affected by cytosol. The results suggest that cytosolic factor(s) that are influenced by diet can act on azaserine so that it enters bacteria more readily and reacts with macromolecules.


Previous investigators have employed the formation of metabolic-intermediate complexes from nor-benzphetamine in conjunction with other ligand interactions to characterize 4 different subpopulations of cytochrome P-450 in microsomes from phenobarbital induced rat livers. The use of piperonyl
butoxide, which can also produce a metabolic-intermediate complex, enables 2 of those 4 subpopulations to be further differentiated. A subpopulation of cytochrome P-450 capable of forming a metabolic-intermediate from norbenzphetamine but not interacting with metyrapone can be separated into 2 different types, one that can and one that cannot form a metabolic-intermediate complex from piperonyl butoxide. Another subpopulation that forms a metabolic-intermediate complex with norbenzphetamine and interacts with metyrapone can be similarly subdivided into 2 types; one that also forms a metabolic-intermediate complex from piperonyl butoxide and one that cannot. Of the 6 types present in induced microsomes, 3 appear novel to phenobarbital induction. They comprise 2 types that can form metabolic-intermediate complexes from norbenzphetamine and not piperonyl butoxide, but only one interacts with metyrapone, and a third type which interacts with metyrapone but is unable to form metabolic-intermediate complexes from either substrate. Of the 3 types existing in both induced and non-induced microsomes, only 1 (which interacts with metyrapone and forms metabolic-intermediate complexes from both substrates) occurs at a higher concentration in induced microsomes.

The use of metabolic-intermediate complexes in conjunction with conventional heme ligands has enabled the characterization of 3 types of cytochrome P-450 in non-induced microsomes and 6 types in phenobarbital-induced rat liver microsomes. Supported by USPHS Grant #CA 15760.

**437 THE DISPOSITION AND METABOLISM OF DIPHENYLHYDANTOIN IN MATERNAL, FETAL AND NEONATAL TISSUES AFTER PERINATAL EXPOSURE OF RAT DAMS.** A.E. Chin, P.J. Kurtz, B.D. Carlton, A.C. Peters, and R.S. Chhabra, Battelle Memorial Institute, Columbus, OH, and National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Female Fischer 344 rats were fed 800, 240, 80 and 0 ppm diphenylhydantoin (DPH) from two weeks prior to breeding until treatment to evaluate the effect of perinatal exposure to DPH on the disposition and metabolism of a subsequent single i.v. dose of DPH (50 mg/kg) given during gestation or postpartum. In rats administered radiolabeled DPH on Day 18 of gestation the levels of radioactivity retained in maternal and fetal tissues four hours after injection of radiolabeled DPH were dependent upon the level of prior maternal exposure indicating a dose-dependent difference in clearance rates. These results can be explained in terms of a balance between an induction of DPH metabolizing enzymes resulting in an increased DPH elimination and a saturation of these enzymes due to an accumulation of DPH during the perinatal exposure period. In lactating females administered radiolabeled DPH on Day 12 postpartum, dam blood levels of DPH equivalents correlated with milk levels and blood levels in the pups, indicative of neonate exposure via the milk. Accumulation of DPH equivalents was observed to occur in maternal and neonatal livers consistent with the liver as a primary organ of DPH metabolism. The capacity of the fetus and neonate to metabolize DPH was less than that of the dam (Supported by NIEHS Contract No. N01-ES-8-2151).

**438 EFFECT OF DOSE ON CAFFEINE METABOLISM BY THE ISOLATED PERFUSED RAT LIVER.** Satu M. Somani, Dept. Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62708, U.S.A.

Metabolism of caffeine has been extensively studied in animal and man. However, there is paucity of data on the effect of dose on its metabolism and its uptake by the isolated perfused rat liver. The perfusion studies were carried out at four different doses of caffeine; 25.6(5), 77.3(15) 154(30) and 309(60) umole (mg/kg) rat body weight. 14C-caffeine solution (1 ml) for each dose was added to the perfusate in the reservoir after equilibration of liver for about 30 min. At least four livers were used at each dose level. 0.2 to 0.5 ml of perfusate sample was taken at different time intervals. Radioactivity was measured in each perfusate sample and the amount of radioactivity retained in the liver was calculated by mass balance equation at each time interval. Caffeine and its metabolites were determined in 1 ml perfusate by HPLC. The uptake of caffeine by the liver appeared to be dependent on the dose. The amount of drug taken up ranged from 8 to 35 percent. A linear increase in the uptake of caffeine by the liver on a per gm basis was seen with the increase in dose, at the end of the experiment. The uptake was not a saturable process at the doses studied. Major metabolites (M) of caffeine viz. theophylline, theobromine, 3-methylxanthine, 1-methylxanthine and 3-methyluric acid were determined. The metabolites (M) in the perfusate were 15.4% at 5 mg/kg which consistently decreased to 4.6% at 60 mg/kg dose, whereas the percent of metabolites (M) remained almost the same (~5%) in the liver at the end of the experiment irrespective of dose.

**439 DRUG METABOLISM IN RODENTS INFECTED WITH TRYPANOSOMES OR TREATED WITH TRYPANOCIDES.** H.G. Sheretz, Kettering Laboratory, Univ. of Cincinnati, Cincinnati, OH 45267, and J. Ed. Hall and J.R. Seed, Dept. Parasit., Sch. Pub. Hlth., Univ. NC, Chapel Hill, NC 27514. SPONSOR: P.B. Hammond

We used two animal models to evaluate the acute and chronic effects of infection with Trypanosoma brucei gambiense on hepatic drug metabolism. The mouse was used as a model for the acute infection, since it exhibits fulminating parasitemia about 3 days post inoculation. The field vole Microtus montanus was used to evaluate the chronic infection, since after inoculation it undergoes waves of parasitemia for over 4 weeks. Three days after inoculation in mice, there was hepatic edema and decreases in tissue DNA, cytochrome P-450 and capacity for metabolizing several foreign compounds. In Microtus, after 28 days there was tissue edema and a large decrease in hepatic cytochrome P-450 content and related enzyme activities. However, there was little
change in other endoplasmic reticulum enzyme markers not relating to cytochrome P-450. These markers include glucose-6-phosphatase, cytochrome b5 and NAD(P)H-cytochrome c reductases. The data indicate that there is selective toxicity for hepatic cytochrome P-450 during trypanosome infection in Microtus. In both the mouse and Microtus, common trypanocides such as Suramin, Melarsospol B, ethidium bromide and Stilbam, were themselves found to be potent inhibitors of drug metabolism in vitro. Furthermore, after chronic treatment of mice with Suramin or Melarsospol B, there was a significant increase in pentobarbital sleeping times, indicative of an impaired capacity for pentobarbital metabolism and detoxification in vivo. We suggest that during trypanosomiasis or with associated chemotherapy, there is a reduced capacity to metabolize foreign compounds. (Supported by UNDG/World Bank/WHO and NIH-GM 27928).

### 440 THE EFFECT OF PREGNANCY AND ESTRADIOL-17ß TREATMENT ON THE BILARY TRANSSPORT MAXIMUM OF ORGANIC ANIONS IN THE ISOLATED PERFUSED RAT LIVER. A.C. Auunnakul and M. Vore, Grad. Center for Toxicology and Dept. of Pharmacology, Univ. of Kentucky, Lexington, KY.

The purpose of this study was to investigate the effect of pregnancy and estrogen on the biliary transport maximum (Tm) of three organic anions in the isolated perfused liver system. Livers from untreated female (control), estradiol-17ß (E2) treated female (1 mg/kg/day, s.c. for 14 days) and pregnant (19-21 days of gestation) rats were perfused with 20% male rat blood in a Krebs-Ringer bicarbonate buffer equilibrated with 95% O2/5% CO2 at 37°C. Dibromosulfophthalein (DBSP), [14C]-phenyl-5-parahydroxyphenylhydantoin (HPPH) and [14C]morphine were infused for 90 min into the perfusate for a total dose of 41.2, 18 or 40.5 µmol respectively. The concentration of [14C]HPPH and [14C]morphine declined in the perfusate whereas the concentrations of [14C]HPPH-glucuronide (HPPH-G) and [14C]morphine-glucuronide (MG) increased during the 90 min experiment indicating that the rate of formation of the glucuronide exceeded its rate of excretion in bile. The Tm (nmol/min/g liver) of DBSP was 26.8±1.4, 30.1±2.7, 18.6±0.8; of HPPH-G was 25.0±1.4, 15.6±1.8, 18.0±0.8; and of MG was 20.9±2.2, 9.4±1.2, 10.6±1.8 for control, Ez-treated and pregnant rats respectively. This indicated that Ez treatment significantly decreased the Tm for HPPH and MG but not for DBSP whereas pregnancy decreased (P<0.5) the Tm for all three organic anions. Bile flow (µl/min/g liver) was significantly decreased in E2-treated and pregnant rats only in the experiments using morphine, thus the effects of pregnancy and estrogen on bile flow do not appear to be responsible for the decrease in biliary excretion. These data suggest the presence of multiple carriers for organic anions which are differentially affected by estrogen treatment and pregnancy. (HD13250).

### 441 INDUCTION OF HEPATIC PHASE II BIOTRANSFORMATION IN THE RAT. T.N. Thompson, J.B. Watkins, 7. Gregus, and C.D. Klaassen, Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS.

Numerous xenobiotics and endogenous chemicals induce phase I biotransformations by enhancing synthesis of cytochrome P-450. Since induction of phase II metabolism has not been thoroughly studied, effect of the following xenobiotics on phase I and phase II biotransformation were evaluated: phenobarbital (PB), 3-methylcholanthrene (3-MC), progynonolone-16α-carbonitrile (PCN), 2, 3, 7, 8,-tetrachlorodibeno-p-dioxin, isosafrole (1SF), trans-stilbene oxide (TSO), butylated hydroxyanisole (BHA). These inducers produced the expected effects on hepatic cytochrome P-450, ethylmorphine and benzphetamine N-demethylation, benzo-[a]pyrene hydroxylation, ethoxyresorufin O-deethylation, and styrene oxide hydration. PCN and 3-MC were the only chemicals that increased N-acetylation of p-naphthylamine (60 and 40%, respectively). Glutathione conjugation of dichloronitrobenzene, dinitrochlorobenzene and sulfobromophthalein was induced from 50 to 170% by TSO, BHA, ISF, and PB. Glucuronidation was also enhanced by treatment with these inducers. For example, PB increased glucuronidation of chloramphenicol (250%) and morphine (115%); 3-MC increased conjugation of 1-naphthol and p-nitrophenol (190% and 80%, respectively); PCN enhanced chloramphenicol (15%) and morphine (50%) conjugation but had no effect on that of 1-naphthol or p-nitrophenol. Most dramatic effect was the 1100% increase in glucuronidation of di-galactosylin monodigitoxoside by PCN. PCN was the only inducer to enhance sulfation of 2-naphthol (28%), taurocholate (111%), and dehydroepiandrosterone (140%). In conclusion, PCN induces a wider spectrum of phase II reactions than the other microsomal enzyme inducers. (Supported by USPHS Grants AM 14513 and ES 07079).

### 442 ALCOHOL-STRESS INHIBITION OF MIXED FUNCTION OXIDASE METABOLISM IN THE RAT. D. Brown, J. Ning, M. Bogdanoff, I. Krull, S. Kuttab, and L. Shargel. Toxicology Program, Col. Pharm. & Allied Hlth Prof Northeastern Univ, Boston, MA.

Stress and Alcohol are environmental factors which alter the metabolism of xenobiotics. Both acute stress and acute alcohol inhibited in vitro and in vivo metabolism of Hexobarbital in the rat. (Chung and Brown, 1976 Life Sci. 18 123). This investigation gives further evidence that acute alcohol and/or acute stress inhibit other hepatic MFO pathways.

Rats were given 1 to 4 g/kg Alcohol (ETOH) and/or stressed by hind leg ligature (HLL). The rats were sacrificed (1 hr) by decapitation, livers excised and homogenized in cold 1.15% KCL. The 9000 x g fraction was used to determine Aminopyrine N-demethylase (AP) and Aniline hydroxylase (AH) activities.

AP activity was inhibited 26% by 3 g/kg ETOH and 44% by 4 g/kg. HLL (30 min) inhibits AP activity 12 to 22% by ETOH (1, 2 & 4 g/kg). HLL (30 min) inhibits AP activity 20 and 28% respectively (P<0.05).

AH activity was inhibited 12 to 22% by ETOH (1, 1.5, 2 & 4 g/kg). HLL (30 min) inhibits AH 17%. HLL and ETOH inhibits AH 26 to 37% (P<0.05).

Benzpyrene (BP) metabolism was measured by disappearance using 10,000 x g fractions from rats pre-treated with 3-methylcholanthrene (30 mg/kg). Acute ETOH 5 g/kg 1 hr prior to sacrifice inhibited BP metabolism 16.7% and corticosterone (12.7mg/ kg ip) 15 minutes before sacrifice inhibits 31.2%. In drenalactomized rats neither treatment inhibi-

Fenitrothion[0,0-dimethyl-O-(3-methyl-4-nitrophenyl)] phosphorothioate is a widely used organophosphate insecticide. In addition to the expected inhibition of tissue esterases, inhibition of hepatic mixed function oxidases (MFO) and depletion of hepatic glutathione (GSH) have also been demonstrated after Fenitrothion (FEN) exposure. This study was undertaken to investigate the dose and time response relationships of these FEN effects. At 2 hr after ip injection of 50, 200 or 500 mg FEN/kg, in mice, brain cholinesterase (CHE) was reduced to 52, 52 and 36% of control activity respectively. Within 12 hr, activity had returned to 75% of control after 500 mg/kg. Liver carboxylesterase (CE) activity was reduced to 91, 78 and 56% of control, respectively, at 2 hr after FEN. It declined further at 5 hr and by 12 hr liver CE was at 4% of control after 500 mg/kg. Hepatic GSH was decreased maximally at 5 hr after each dose (32% control after 500 mg/kg), and some recovery occurred by 12 hr (45% control after 500 mg/kg). In contrast, hepatic MFO (aniline hydroxylation and p-nitroanisole O-demethylation) activities were reduced to 15-28% of control as early as 2 hr after 500 mg/kg and remained at that level through 12 hr. MFO activity 2, 5 or 12 hr after 50 mg/kg was 45, 63 and 75% of control. Results indicate that the MFO and brain CHE responses to FEN are similar, with maximal inhibition occurring by 2 hr. In contrast, maximal inhibition of liver CE does not occur until 12 hr after FEN. The long-lasting biochemical effects of FEN, in the absence of cholinergic symptoms, may be of toxicologic importance.
cell surface morphology and the development of massive centrilobular congestion due to entrapment of red blood cells (RBC) before the appearance of necrosis. These phenomena have been investigated by scanning electron microscopy (SEM). Within 1.5 hr of acetaminophen administration (750 mg/Kg by gavage), SEM of centrilobular areas reveals endocytic vacuoles at the sinusoidal poles of hepatocytes, reduced numbers of microvilli in the Disse space, loss of the study-like projections from the lateral surfaces of hepatocytes, dilation of bile canaliculi, and enlargement of the sinusoidal lining cell fenestrations. By 3 hr after acetaminophen RBC's are in the Disse space, between hepatocytes, and within the endocytic vacuoles of many hepatocytes. The number of RBC's within the Disse space is sufficient to collapse the original sinusoidal lumen by 6 hr. Sinusoidal lining cells are not lost, but apparently held in position by cytoplasmic projections from hepatocytes, preservation of intercellular junctions, and anchorage by fat storing cells within the Disse space. We suggest that hepatotoxic congestion develops as a result of alterations in the relationship between hepatocytes and sinusoidal lining cells which then separate and thus produce an expanded Disse space. RBC's enter the Disse space through the enlarged fenestrations in the sinusoidal lining cells. (Supported by the Canadian Liver Foundation).

447 THE NEPHROTOXICITY OF 1,2-DICHLOROETHANE AND 1,1-DICHLOOROETHYLENE IN MALE SPRAGUE-DAWLEY RATS. N.M. Jackson, W.J. Al-Hassan, and R.B. Conolly, Toxicology Program, Sch. of Pub. Hlth, The University of Michigan, Ann Arbor, MI. 
Sponsor: H.H. Cornish

1,2-Dichloroethane and 1,1-dichloroethylene are important industrial chemicals. Male Charles River Sprague-Dawley rats were exposed by inhalation to various concentrations of these chemicals for four hours and killed 24 hours after the start of exposure. In normally fed rats exposed to 2000 ppm 1,2-dichloroethane, there was a significant (p <0.05) increase in kidney to body weight ratios (gm kidney/100 gm body weight; exposed: 1.73 ± 0.12 vs. control: 0.97 ± 0.08), BUN (mg urea nitrogen/100 ml; exposed: 50.61 ± 4.35 vs. control: 8.37 ± 0.61) and serum creatinine (mg creatinine/100 ml; exposed: 3.46 ± 0.46 vs. control: 0.83 ± 0.02). Rats fasted 18 hours prior to exposure to 2000 ppm 1,2-dichloroethane had BUN and serum creatinine values significantly higher than fed exposed rats. These values were: BUN (fasted: 65.37 ± 9.73 vs. fed: 50.61 ± 4.35) and serum creatinine (fasted: 4.51 ± 0.35 vs. fed: 3.46 ± 0.46). Rats exposed to 1000 ppm 1,2-dichloroethane for four hours showed slight but significant increases in serum creatinine values at both 24 and 48 hours post-exposure. Rats exposed to 5000 ppm for four hours all died between 2 and 18 hours after the end of the exposure. The acute nephrotoxicity of a structurally related chemical, 1,1-dichloroethylene was also investigated. Since in addition to their structural similarities, 1,2-dichloroethane and 1,1-dichloroethylene share common metabolic pathways, the interactive effects of exposure to mixtures of these two chemicals were evaluated. Supported, in part, by training grants T32 GM07767 NHL, and T32 ES07062, NIEHS.

448 THE EFFECT OF UBIQUINONE-10 ANTAGONISM UPON THE ACUTE TOXICITY OF DOXORUBICIN. A.B. Combs, E. Lewandowski, and *K. Polkers, Dept. of Pharmacology of the College of Pharmacy and *Inst. for Biomedical Research, Univ. of Texas, Austin, TX. 
Sponsor: D. Acosta

Previous work in this laboratory has shown that ubiquinone-10 (CoQ) can reduce the acute toxicity of doxorubicin (DOX) in rats and mice. It is not clear whether that protection is due to the non-specific antioxidant actions of CoQ, or to its very specific coenzyme functions. The present study was designed to help differentiate between these mechanisms. The in vitro CoQ-antagonist, 2-hydroxy-3-n-dodecylmercapto-1,4-naphthoquinone (I), or its vehicle, 10% Tween-20, was injected ip at a dose of 50 mg/kg in female CD-1 mice. It was injected two hours following a 10 mg/kg ip dose of phytonadione. One hour following I, single acute doses of 15 or 20 mg/kg of DOX or its vehicle, water, were given ip. Animal survival was determined on a daily basis. After 12 days, the survival frequencies were: 1) untouched controls and vehicle controls, 100% each (10/10 each), 2) DOX, 15 mg/kg, + vehicle for I, 92% (23/25), 3) DOX, 20 mg/kg, + vehicle for I, 65% (17/26), 4) water, 10 ml/kg, + I, 96% (25/26), 5) DOX, 15 mg/kg, + I, 36% (9/25), and 6) DOX, 20 mg/kg, + I, 12% (3/26). The data indicate that an antagonist to CoQ can enhance the toxicity of DOX. This, in turn, provides evidence that a more specific action than antioxidation may be involved in the reduction of DOX toxicity by CoQ. 
Sponsored by Daniel Acosta.

449 EFFECT OF A LOW-PROTEIN DIET ON CONTRACEPTIVE STEROID-INDUCED CHOLESTASIS IN FEMALE RATS. A. Idris, U.A. Boelsterli, H. Kotik, and T. Balagg, Division of Drug Biology, Food and Drug Administration, Washington, D.C.

Due to the wide use of oral contraceptives (OC) in human populations suffering from malnutrition, an increasing number of women might be exposed to the hepatotoxic effect of OC when the liver is compromised due to protein deficiency. To study such a possible interaction in the rat model, groups of female Sprague-Dawley rats (6 to 10 per group) were given daily oral doses of a combination of OC, 17α-ethinyl estradiol (EE) and 19-norethisterone (NE), for a maximum of 8 weeks. Due to the wide use of oral contraceptives (OC) in human populations suffering from malnutrition, an increasing number of women might be exposed to the hepatotoxic effect of OC when the liver is compromised due to protein deficiency. To study such a possible interaction in the rat model, groups of female Sprague-Dawley rats (6 to 10 per group) were given daily oral doses of a combination of OC, 17α-ethinyl estradiol (EE) and 19-norethisterone (NE), for a maximum of 8 weeks. A low-dose group received 0.25 mg/kg EE and 2.5 mg/kg NE. A high-dose group was given weekly doubled doses until 4 mg/kg EE and 40 mg/kg NE were reached. The rats were fed a low-protein (LP) diet (8%), starting 8 weeks before treatment with OC, and continuing throughout the study. Cholestatic liver injury, as measured by basal bile flow, bile acid secretion, and BSP excretion, could be induced in control rats receiving the LP diet alone. In control animals on a normal diet, OC administration alone also produced a marked dose-dependent cholestatic effect.
However, the combination of a chronic LP diet with OC resulted in an ameliorating effect on the pathophysiology of bile secretion and hepatic excretory function. Thus, in the rat, a mild protein malnutrition does not enhance the cholestatic effect of the steroids but rather protects the liver from cholestatic liver damage caused by an LP diet or OC administration alone.


A potential prescreen cytotoxicity assay is being evaluated. Isolated hepatocytes (1.5 x 10⁸ cells per ml) were incubated for up to 6 hr at concentrations of 10, 1 or 0.1 mM in 3 ml of hormonet-supplemented Waymouth's medium. Using glutamate-oxaloacetate transaminase (GOT) release as the test criterion, the lowest concentrations eliciting GOT release were: 0.1 mM_Ncadmium acetate, chlorohexidine diacetate, 2-chlorobiphenyl, 4-chlorobiphenyl, mercuric acetate; 1 mM_Nfor 3-amino-1,2,4-triazole, sodium selenate, 2,4-dinitrophenol, iodooacetate, sodium arsenite, 2,4,2',4'-tetrachlorobiphenyl, tris(2,3-dibromopropyl)phosphate; and 10 mM for diethanolamine, hydrazine hydrate, parquat, phosphonoacetic acid, styrene, and styrene oxide. CBrCl₃, CCl₄, 1,1,1- and 1,1,2-trichloroethane and CHCl₃ were active in the 1-10 mM range, with CBrCl₃ and CCl₄ being the most cytotoxic of these haloalkanes in the prescreen. Dimethylthrosamine did not release GOT even at a concentration of 10 mM. To determine if cytotoxicity is occurring at lower concentrations than indicated by GOT release, other viability indices such as ATP level and urea synthetic capacity of hepatocytes are being assessed. This type of assay may be useful as a rapid, economical prescreen to identify chemicals warranting further animal studies. This work was supported by EPA Contract 68-01-5079.


Ethylene diamine (EDA) was evaluated for potential genotoxic activity using a battery of in vitro and an in vivo mammalian tests: Chinese Hamster Ovary (CHO) gene mutation, Sister Chromatid Exchange (SCE) with CHO cells, Unscheduled DNA Synthesis (UDS) in primary rat hepatocytes and a Dominant Lethal study using Fischer 344 rats. In vitro tests evaluated a minimum of five doses spanning a wide range of concentrations. EDA did not produce a dose-related mutagenic effect in either the CHO mutation test or in the SCE test with or without a rat liver S9 activation system. With hepatocytes, no dose-related effect was produced by EDA in UDS tests at two different labora-

MUTAGENIC ACTIVITY OF COCAINE NITROXIDE RADICAL IN THE Ames SALMONELLA TEST SYSTEM. M. A. Evans and Joseph Jankauskis. Interdisciplinary Toxicology Program, Dept. Pharmacology, University of Illinois Medical Center, Chicago, IL 60680.

Cocaine (C) produces an acute hepatic necrosis in mice pretreated with agents which enhance microsomal metabolism. The necrosis is species specific for the mouse and is associated with decreased glutathione concentration and increased covalent binding of radio-labelled cocaine to microsomal protein. Incubation of cocaine or norcocaine with mouse liver microsomal suspensions and a NADPH generating system produces a nitroxide radical based on the appearance of the characteristic electron spin resonance spectra for nitrooxides. The half life for the nitroxide in the microsomal suspension at 4°C is less than four minutes. Norcocaine (10µg) incubated with mouse hepatic S-9 fraction showed weak mutagenicity (31 revertants/plate) with TA 98 and strong mutagenicity (836 revertants/plate) with TA 1538. No mutagenicity was observed with hepatic S-9 fraction from rat or rabbit or with heat-treated mouse hepatic S-9 fraction.

Further studies demonstrated that chemically synthesized cocaine nitroxide was directly mutagenic with TA 1538 but other known metabolites of cocaine were inactive without activation. The apparent greater mutagenicity of cocaine nitroxide in TA 1538 vs. TA 98 suggests that the nitroxide directly alters DNA structure and is not dependant on errors during DNA repair. This is consistent with results from in vitro studies which demonstrated that the nitroxide reacts irreversibly with guanine, cysteine and reduced glutathione to form a covalently-bound adduct.


Benzo(a)pyrene [B(a)P] can be activated by cytochrome P450 containing monoxygenase system to potent mutagens and highly reactive DNA binding species. Since the microsomal drug-metabolizing system requires the presence of both reducing agent and molecular oxygen, the role of activated oxygen species in the activation of B(a)P to an active mutagen was investigated. In these studies we have examined the antimutagenic effects of
several known scavengers of different activated oxygen species in the Ames Salmonella typhimurium test system in the presence of B(a)P with and without liver microsomal (S-9) fraction. In the presence of S-9, the polycyclic aromatic hydrocarbon B(a)P, was confirmed mutagenic using tester strains TA 98 and TA 100. Superoxide dismutase, which removes O_2^- radicals, at 10 \( \rightarrow \) 100 \( \mu \)g/ml on agar plates had no significant effect on the number of revertants produced by activated B(a)P. Catalase, which removes H_2O_2, at 10 \( \rightarrow \) 100 \( \mu \)g/ml or mannitol, which removes OH\(^*\), at 3 \( \rightarrow \) 12 \( \mu \)M had trivial effect. Dimethylfuran (DMF), an effective singlet oxygen scavenger, however, inhibited the mutagenic response of activated B(a)P in a dose-dependent manner. Thus, in TA 100 strain using 0.5 \( \mu \)g/plate B(a)P, the inhibition of number of revertants by 3, 6 and 9 \( \mu \)M DMF was 40, 60 and 75%, respectively. In TA 98 strain, using 4 \( \mu \)g/plate B(a)P, 6 \( \mu \)M DMF inhibited 50% of the number of revertants. These levels of DMF were neither toxic to the cells nor were they found to have any mutagenic activity in the absence of B(a)P. The antimutagenic activity of DMF was dependent upon the activation of B(a)P by S-9 system. These data indicate a major role for singlet oxygen in promoting mutagenesis (Supported by U.S. Dept. of Energy).

454 IN VIVO GENOTOXIC EFFECT OF 1,2-DICHLOROETHANE IN MALE B6C3Fl MICE. R.D. Storer, P.A. Bank, and R.B. Conolly, Toxicology Program, Sch. of Pub. Hlth, The University of Michigan, Ann Arbor, MI Sponsor: R.H. Cornish

Results from the N.C.I. Bioassay Program have shown that 1,2-dichloroethane (1,2-DCE) is carcinogenic to the liver of male B6C3Fl mice when administered chronically by gavage. Our objective is 1) to characterize the extent of DNA damage produced in vivo in the liver of male B6C3Fl mice following single oral doses of 1,2-DCE and 2) to identify what, if any, role glutathione conjugation has in the in vivo activation of 1,2-DCE to a DNA-damaging agent. Male B6C3Fl mice were gavaged with a single 200 mg/kg dose of 1,2-DCE dissolved in corn oil and sacrificed 4 hours later. Suspensions of liver nuclei were prepared and analyzed for evidence of DNA damage by alkaline sucrose density gradient centrifugation. Animals dosed orally with corn oil or by I.P. injection with 50 mg/kg dimethyl nitrosamine (DMN) served as negative and positive controls respectively. In a series of three experiments, gradient profiles from 1,2-DCE treated animals consistently showed slower sedimentation relative to controls. The percent of the total DNA recovered in the first five fractions of the gradients was significantly decreased; from 68.1 \( \pm \) 9.3% (control) to 52.4 \( \pm \) 12.3% (treated) (mean of three experiments \( \pm \) std. dev., P < 0.02). By contrast, DMN (10 mg/kg) reduced the percent of the DNA recovered in the first five fractions from 68.1 \( \pm \) 9.3% to 7.0 \( \pm \) 3.0%. Liver/blood weight ratios, serum sorbitol dehydrogenase levels, and light microscopic evaluation at 24 hours after 1,2-DCE treatment (200 mg/kg) did not reveal any evidence of liver necrosis. Liver glutathione levels (as non-protein sulfhydryl, 1 hour post dose) were significantly depleted (59.1%) in 1,2-DCE (200 mg/kg) treated animals relative to controls.

455 PHENOBARBITAL ALTERATION OF 1-NITRONAPHTHALENE LEVELS IN TARGET ORGANS OF TOXICITY. D. Dankovic and H.H. Cornish. Toxicology Program, Sch. of Pub. Hlth, The University of Michigan, Ann Arbor, MI

1-Nitronaphthalene (1-NN) is acutely toxic to both lung and liver; pretreatment with phenobarbital prevents lung toxicity, but potentiates liver damage. It was previously shown that phenobarbital lowers the total level of 1-NN + metabolites by more than 50% in liver, and 80% in lung. The levels of unchanged 1-NN in lung, liver and blood have now been measured, using a chloroform/methanol/water system for extraction, and reverse isotope dilution for quantification.

Following phenobarbital pretreatment 1-NN levels in lung, liver, and blood were 7.9, 5.5, and 6.7%, respectively, of the levels in the 1-NN treated control animals. These data suggest that an increased rate of metabolism and excretion prevents 1-NN from reaching the lung in sufficient quantities to produce lung damage. The excretory rate and metabolic profile of 1-NN in control and phenobarbital pretreated animals is now being investigated.

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Following injections of aqueous solutions of the B group metals zinc, cadmium and copper, a low molecular weight, sulfur-rich protein named metallothionein containing these metals at various mole ratios can be isolated. The absorption, circular dichroism (CD) and magnetic circular dichroism (MCD) recorded for Cd, Zn-MT isolated from rat livers, from crab and guinea pig livers, are all very similar. Comparison of the absorption and MCD spectra measured for polypeptide model compounds confirms that the signal in the 250 nm region arise from S->-Cd charge transfer. The effect of changing the pH, loading the protein with Cd\(^{2+}\) and extensive dialysis were followed using all three techniques. Analysis of the spectral changes suggests that while both the Zn\(^{2+}\) and Cd\(^{2+}\) may be displaced at low pH values, only the Cd\(^{2+}\) is rebound when the pH is returned to 7. In addition, when excess Cd\(^{2+}\) is added into the energies of the charge transfer transitions change significantly, which implies that the effect of added Cd\(^{2+}\) is to alter the protein conformation, which in turn results in a change in the stereochemistry around the original, protein-bound Cd\(^{2+}\) ions. The metal-free protein prepared by exhaustive dialysis at low pH does not rebind Cd\(^{2+}\).
the hypothesis that tetraploid and octaploid hepatocytes, but not diploids, are predisposed to tumor formation. Unlike the adult rodent liver, neonatal rats have a single class of diploid hepatocytes, and it was of interest to investigate whether the liver, which is carcinogenic, would induce premature development of polyploidy. To investigate this hypothesis further, neonatal rats (5 or 15 day old) were given the chlorocarbon, mirex (4.5 mg/kg) daily for 7 days.

Hepatic nuclei were isolated and analyzed on the Coulter Counter for alterations in volumes and frequencies. No significant differences were found between the nuclei of experimental and control groups. These observations lead us to believe that diploid hepatocytes are resistant to transformation by carcinogenic agents, and may explain why primates and humans, whose livers are preponderantly diploid, are not susceptible to these agents.

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DEPLETION OF GLUTATHIONE BY METHYL CHLORIDE AND RELATIONSHIP TO LIPID PEROXIDATION. D.J. Kornbrust and J.S. Bus, Chemical Industry Inst. of Toxicology, Research Triangle Park, NC, 27709.

Inhalation of methyl chloride by male B.C.B. mice results in a concentration-dependent depletion of glutathione (GSH) in liver, kidney and brain. Exposure for 6 hr to 100 ppm CHCl₃ (the current TLV) lowered liver GSH by over 40%, while exposure to 2500 ppm for 6 hr reduced liver GSH to 2% of control levels. For those exposures which decreased liver GSH to less than 20% of control levels, the extent of liver GSH depletion was closely correlated with the capacity of a 9000 x g supernatant fraction from the liver to undergo lipid peroxidation (LP), quantitated by measuring the amount of thiobarbituric acid-reactive material formed during a 20 min. incubation. Addition of GSH to the incubation prevented the LP. Also, surviving mice exhibited a rapid recovery of liver GSH such that by 4 hr post-exposure LP was no longer demonstrable. GSH was depleted to a lesser extent in mouse brain and kidney, compared to liver, and no relationship to peroxidation was observed for single exposures to CHCl₃. A dose-dependent decrease in liver GSH was also produced by diethylmaleate, although a nearly lethal amount (2 ml/kg) was required to lower liver GSH to less than 10% of control levels under which conditions the amount of LP was 3.5-fold less than in mice exposed to 2000 ppm CHCl₃. GSH was depleted to a lesser extent in CHCl₃ in the liver, kidney and brain of Fischer-344 rats, a species less sensitive to the toxic effects of CHCl₃. Exposure of rats to 2000 ppm resulted in a 4-fold higher level of GSH remaining in the liver and a 30-fold lower amount of LP compared to mice similarly exposed. These findings suggest that depletion of GSH by CHCl₃ to levels insufficient to prevent the occurrence of lipid peroxidation may underlie the hepatotoxicity of the compound.

TRIHALOMETHANES AND RAT RENAL AND HEPATIC ORNITHINE DECARBOXYLASE ACTIVITY. R.E. Savage, Jr., P. Wiehl, C. Guion and M.A. Pereira, Health Effects Research Laboratory, United States Environmental Protection Agency, Cincinnati, OH 45268 and Ohio University College of Osteopathic Medicine, Athens, OH 45710. Sponsor: R.J. Bull

We have previously reported that CHCl₃ is a very potent stimulator of rat hepatic Ornithine Decarboxylase (ODC) activity. The ability of several trihalomethanes to effect either hepatic or renal ODC activity in male and female Fischer 344 rats was investigated. Chlorodibromomethane was the most potent stimulator of hepatic ODC in both males and females while having a unique inhibitory effect on male renal ODC. The other compounds tested were all stimulatory in male and female livers, slightly stimulatory in male rat kidney but all were virtually without any effect in female kidney.

To further investigate the mechanism of trihalomethane stimulation of hepatic ODC, animals were pretreated with Diethyl Maleate (DEM, 325 mg/Kg) 2 hrs. prior to treatment with saline (control) or CHCl₃. The level of hepatic ODC in DEM treated rats was not different from control. However, the effect of DEM on CHCl₃ induced ODC stimulation was a 3-fold enhancement of the stimulation observed by CHCl₃ itself. Although, the effect of CCl₄ was not statistically different from CHCl₃, the effect of DEM on CCl₄ induced ODC stimulation was not nearly as dramatic as its effect on CHCl₃. Furthermore, the direct addition of CHCl₃ to in vitro ODC incubations from control animals had no effect on ODC activity. These findings suggest that the induction of ODC by CHCl₃ is related to its metabolism.

EFFECT OF TCDD ON THE RESPONSE OF RAT LIVER TO PARTIAL HEPATECTOMY. B.J. Christian and R.E. Peterson, Univ. of Wisconsin, Madison, WI.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) enhanced ³H-thymidine (Tdr) incorporation into rat hepatic DNA after 1/3 hepatectomy (TAP 58: 398, 1981). Our aim was to further describe the response. It was found that this response to a 1/3 hepatectomy performed 5 days after TCDD, was dose related from 1 µg/kg (no effect) to 30 µg/kg (maximum) with an apparent ED₅₀ of 4-6 µg/kg. The effect was greatest if rats were 1/3 heptactomized 5-10 days after 5 µg/kg of TCDD. If rats treated with TCDD (5 µg/kg, 5 days) were not heptactomized, or were given a 2/3 hepatectomy, ³H Tdr incorporation was similar to control. However, if rats were laparotomized ³H Tdr incorporation was 2-3x higher than control. Hepatic enzyme activities and ³H Tdr incorporation into liver DNA were also compared at designated times from 2 to 32 hr after 1/3 hepatectomy. Activities of ornithine decarboxylase (a marker of G₂ progression), tyrosine aminotransferase, and ß-glutamyl transpeptidase were altered by 1/3 hepatectomy. However, all changes in enzyme activities were similar in the two groups. In contrast, ³H Tdr incorporation was 2-3x higher in the TCDD group 24-28 hr after the operation. To determine if the enhanced incorporation into DNA was caused by more hepatocytes entering S phase, autoradiography was done. Labeling indices for periportal, midzonal and centrilobular hepatocytes...
in control and TCDD-treated rats were similar. Thus, TCDD enhances 3H Tdr incorporation into hepatic DNA of 1/3 hepatetomized rats by a mechanism that does not involve more hepatocytes entering S phase. (Supported by NIH grant ES01332.)

**461 COMPARISON OF BODY WEIGHT LOSS AND OXYGEN CONSUMPTION IN RATS TREATED WITH 2,3,7,8- TETRACHLORODIBENZO-P-DIOXIN (TCDD) AND PAIR-FED CONTROL RATS.** M. D. Seefeld, S. W. Corbett, R. E. Keesey and R. E. Peterson, Univ. of Wisconsin, Madison, WI.

Male rats were given a single oral dose of TCDD (15, 25 or 50 µg/kg) and paired mates, matched on a body weight basis, received an equivalent volume of acetone/corn oil. Daily food intakes were corrected for spilled food since TCDD treatment caused a progressive dose-related increase in spillage. Failure to account for spillage results in overestimating the amount of food consumed by TCDD-treated rats with consequent overfeeding of pair-fed controls. Young rats (80-100 g) given 25 µg TCDD/kg displayed an immediate reduction in food intake and weight gain which persisted over the 35 day post-treatment period, and 65% of the rats in this group died. Pair-fed controls exhibited an identical weight response but only 13% of the rats died. Experiments using adult rats (275-300 g) demonstrated that pair-fed controls display identical weight loss during the first 10 days after treatment as rats given 25 and 50 µg TCDD/kg. After day 10, pair-fed rats exhibited a greater ability to maintain their body weight on the reduced food intake than TCDD-treated rats. In the 25 and 50 µg/kg dosage groups, 33 and 75% of the rats died respectively, whereas in the respective pair-fed groups 0 and 15% of the rats died. In rats housed in a thermoneutral environment, doses of 15 and 50 µg TCDD/Kg depressed total and resting oxygen consumption, however, oxygen consumption was similarly reduced in pair-fed controls. These studies demonstrate that reduced food intake is the primary cause of body weight loss and depressed oxygen consumption in TCDD-treated rats. (Supported by NIH Grant ES01332.)

**462 EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON FOOD INTAKE, BODY WEIGHT AND ENERGY EXPENDITURE IN THE RAT.** M. D. Seefeld, S. W. Corbett, R. E. Keesey and R. E. Peterson, Univ. of Wisconsin, Madison, WI.

Food intake, body weight and energy expenditure were studied in male rats (275-300 g) treated with a single oral dose of TCDD (5, 15, 25 or 50 µg/kg) or acetone/corn oil. Immediately after TCDD treatment a progressive dose-related decrease in food intake and body weight was observed. At 5 and 15 µg TCDD/kg the effect was reversible in that these groups were consuming appropriate amounts of food for their reduced weights by days 15 and 25 respectively, whereas the reductions in intake at the 25 and 50 µg/kg doses were more persistent. Energy expenditure was assessed in rats housed in a thermoneutral environment by monitoring motor activity and total and resting oxygen consumption before and after 0, 15 and 50 µg TCDD/kg. Motor activity was depressed by both doses of TCDD. Total oxygen consumption was reduced 13 and 22% below control levels in the 15 and 50 µg/kg groups respectively, one week after TCDD. This effect was reversible at the low dose but not at the high dose. Similar depressions in resting oxygen consumption were also found. Rats whose weight had been lowered before treatment (by food restriction) exhibited hyperphagia immediately after dosing (when food was provided ad lib) demonstrating that the ability to eat is retained after treatment. These studies indicate that TCDD-induced weight loss is not due to an increase in metabolic rate. Rather, we suggest that TCDD may lower a "set-point" for regulated body weight in a dose-dependent manner. If true, the reduced food intake would be secondary to the rat's effort to reduce its body weight to a lower maintenance level. (Supported by NIH Grant ES01332.)


2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent inducer of cytochrome P450-mediated monooxygenase activities, including aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin O-deethylase (ECOD). In certain inbred strains of mice, this response is regulated by a single genetic locus coding for a cytosolic receptor protein. In the present study the induction of ECOD activity by TCDD and several halogenated analogues was examined in 5 human cell lines derived from squamous cell carcinomas (SCC) of epidermal and oral epithelia. The cells were grown in the presence of a lethally irradiated feeder layer of mouse 3T3 cells in Dulbecco-Vogt modified Eagle's medium supplemented with 5% fetal calf serum. Addition of varying concentrations of TCDD (10-11 - 10-7 M) to confluent cultures of SCC 4, 9, 13 and 15 cells resulted in a dose-dependent increase in ECOD activity with an ED50 ranging from 1 to 3 nM. Near maximal induction was observed within 24 h. Under the same culture conditions, addition of TCDD to SCC 12P2 cells at concentrations up to 1 µM produced no significant increase in ECOD activity. The structure-activity relationship for the induction of ECOD activity in the four responsive lines (SCC 4, 9, 13 and 15) showed the same stereospecificity required for binding to the TCDD receptor in murine liver. Using sucrose density gradient analysis, specific binding of radiolabeled TCDD was detected in the cytosol fractions from the responsive cell lines, whereas no specific binding could be detected in the SCC 12P2 cells. These data indicate the presence of a putative receptor for TCDD in cultured human epithelial cells that appears to regulate at least one inducible enzyme activity.

The effects of chlordecone, mirex, and four photo derivatives of mirex on K+-stimulated p-nitrophenyl phosphatase in rat brain synaptosomes were determined. Additionally, the effect of chlordecone on the substrate and K+ activation kinetics was also determined. Rat brain synaptosomes were prepared by Ficol-Sucrose gradient centrifugation and the enzyme activity was determined by colorimetric method. Dose-response curves were determined by assaying the enzyme activity in the absence and presence of several concentrations of the test chemicals. The sensitivity of the enzyme to the various chemicals was highly variable. Chlordecone was the most effective inhibitor with an IC50 of 7 µM. All other chemicals showed a maximum inhibition of 20% at 20 µM concentration. The order of inhibition at 20 µM concentration was chlordecone (100%) > 8-mono hydro mirex (20%) > 2, 8-dihydromirex (10%) > mirex (10%) > 10-monohydro mirex (9%) > 5,10-dihydro mirex (2%). Double reciprocal plots of the data obtained on the effect of chlordecone on the substrate and K+ activation kinetics showed that the Vmax and Km were decreased with respect to paranitrophenyl phosphate, while it was classical non-competitive inhibition with respect to K+ activation. These results suggest that chlordecone is the most effective inhibitor among its structural analogs tested on paranitrophenyl phosphatase in rat brain synaptosomes. (Supported by PHS Grant ES-02443 of the National Institute of Environmental Health Sciences).

465 RELATIONSHIP OF TISSUE NON-PROTEIN SULFHYDRYL GROUPS (NPS) TO THE TOXIC EFFECTS OF 1,2-DIBROMO-3-CHLOROPROPANE (DBCP). W. M. Kluwe, A. Greenwell and F. Harrington (J. A. Moore, sponsor), National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC.

DBCP is a nematocide causally associated with infertility in male pesticide formulators and shown to produce testicular, epididymal, renal and hepatic injury in rats. Subcutaneous administration of DBCP to adult, male rats transiently depleted hepatic and caput (head) epididymal, but not renal or testicular NPS contents. The liver, kidney and testis exhibited increases in tissue NPS within 48 hr of treatment. The glutathione-depleted diethyl maleate (DM) transiently lowered hepatic, renal and caput epididymal NPS in a dose- and time-dependent manner. Single injections of DBCP produced dose-dependent lesions in the kidney, testis, caput epididymis and liver. DM prior to DBCP enhanced the nephrotoxic potency of DBCP (greater elevation of BUN and more severe renal tubular necrosis in DM pretreated animals). Serum glutamic pyruvic transaminase activity was greater in DM pretreated than in non-pretreated animals after DBCP. However, DM alone appeared to be hepatotoxic, suggesting additive effects on the liver. Seminiferous tubular degeneration was greater in rats pretreated with DM than in non-pretreated controls.

These results indicate that DBCP is a depleter of hepatic and caput epididymal NPS in the acutely-toxic dose range. Since NPS concentrations were not lowered in two of the major target organs, kidney and testis, acute DBCP injury would not appear to be dependent on local glutathione depletion. However, the greater susceptibility of kidney and testis to DBCP injury following DM pretreatment suggests that hepatic NPS may protect against DBCP toxicity in a general sense.


We have suggested that chlordecone (CD) enhances the hepatotoxicity of CCl4 by altering the metabolism of CCl4. If altered metabolism of CCl4 is indeed the mechanism of CD potentiation, then administration of sublethal doses of CCl4 would be expected to lower parameters associated with the hepatic MFO system to a proportionately greater extent in CD-treated rats than in control rats. Male Sprague-Dawley rats (250-350 g) were treated with CD, po (10 mg/kg) in corn oil (c.o.) and control received c.o. vehicle alone. Twenty-four hr later, rats received an ip injection of CCl4 or c.o. vehicle. Rats fed c.o. initially received 250 µl CCl4/kg, while rats fed CD initially, received 25 µl CCl4/kg. Twenty-four hr later, the animals were used to measure hepatic microsomal protein, cytochrome P-450, microsomal glucose-6-phosphatase, and aminopyrine-N-demethylase activity. Serum enzymes (SGPT, SGOT, TCD) were quantitated to serve as indices of hepatic damage. Our findings show a significant increase in cytochrome P-450 content, G-6-Pase, and aminopyrine-N-demethylase activity in rats fed CD than in the control group and a parallel decrease in microsomal protein, cytochrome P-450, G-6-Pase, and aminopyrine-N-demethylase activity in both CD and control animals which received CCl4. These results are supportive of the primary working hypothesis that CD potentiates CCl4 hepatotoxicity by altering the metabolism of CCl4, and suggest that the mechanism of this alteration in metabolism involves induction of a P-450 species responsible for the bioactivation of CCl4. (Supported by ES-01369 and ES-07045).

467 EFFECT OF CHLOROFORM AND DRINKING WATER CONCENTRATES ON MOUSE RENAL CORtical SLICE UPTAKE OF P-AMINOHIPPURATE. L.W. Condie and C.L. Smallwood, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. Sponsor: R.J. Bull

A recent research thrust of our laboratory has been the evaluation of target organ toxicities from exposure to environmental chemicals. The effect of environmental chemicals on active uptake of p-aminohippurate (PAH) by mouse renal cortical slices was utilized as a potential screen for nephrotoxicity. Other renal function tests included blood urea nitrogen (BUN) and serum creatinine while serum glutamate pyruvate transaminase (SGPT) activity was measured as an indicator of liver function. Male CD-1 mice were administered chloroform by daily oral gavage for 14 days prior to sacrifice at levels of 150 mg/kg, 75 mg/kg and 38 mg/kg. PAH uptake was inhibited by 64% and 17% in kidney slices taken from the 150 mg/kg and 75 mg/kg dose groups, respectively. BUN and SGPT values were only elevated in the high
dose group. Organic chemicals were concentrated from drinking water of two cities by reverse osmosis. Reverse osmosis extracts were daily injected intraperitoneally (200 mg/kg) for three days prior to sacrifice. PAH uptake into kidney slices was inhibited 25% by this treatment while BUN and serum creatinine levels were not altered. These studies suggest that the measurement of PAH uptake in mouse renal cortical slices may serve as a useful tool for screening nephrotoxins that are present in complex environmental mixtures.

**468 PROTECTIVE EFFECT OF AUCUBA JAPONICA AGAINST CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE.** K. H. Yang, T. J. Kwon, and I. M. Chang (Spon: R. E. Peterson). Dept. of Biological Science and Engineering, Korea Advanced Institute of Science and Technology, and Natural Products Research Institute, Seoul National University, Seoul, Korea

Many plants have been used as herbs in oriental medicine for thousand years, but in most cases their effectiveness has never been proved. Among them Aucuba japonica has been known to have protective effect against liver diseases. Our object was to determine if extracts from Aucuba japonica protects CCl₄-induced liver damage and to isolate active compound for possible therapeutic use. Leaves of the Aucuba japonica were extracted with methanol (90%, v/v) for 6 hrs. The methanol extract was dried under reduced pressure and then dissolved in saline. Rats were pretreated with the extract for two days (600 mg/kg/day, p.o) and CCl₄ (0.5 ml/kg, ip) was administered 30 min after the second dose. Pretreatment of the extract effectively protected CCl₄-induced depression in plasma disappearance and biliary excretion of bromosulfophthalein (BSP) determined 24 hr after the CCl₄ challenge. Percent recovery of BSP in bile in 60 min for control, CCl₄, extract + CCl₄ treated rats was 66.8 ± 1.9, 56.2 ± 1.4, and 71.2 ± 1.8, respectively. Pretreatment of the extract also protected CCl₄-induced increased SGPT activity and liver triglyceride content. Further purification of the extract suggested that an iridoid glycoside, aucubin is the most probable active compound. (Supported by 1982 Korea Science and Engineering Foundation Research Grant)

**469 THE EFFECT OF CCl₄ EXPOSURE ON MITOCHONDRIAL MEMBRANE PHOSPHOLIPIDS.** A. Wisner-Gebhart, R.A. Head, and M.J. Brabec. Toxicology Program, Sch. of Pub. Hlth, The University of Michigan, Ann Arbor, MI

Over a period of 10 to 66 hours following CCl₄ exposure, alterations in the phospholipid (PL) composition of rat hepatocyte mitochondrial membranes parallel the loss of function of this organelle. The percentage of phosphatidylcholine (PC) decreases and that of phosphatidylethanolamine (PE) increases in the microsomal and mitochondrial outer membranes; while the inner membrane shows changes in the opposite direction for each of these PL's. Many authors have demonstrated the direct free radical effect of CCl₄ on the PL's of the microsomes but never of the mitochondrial membranes. Alterations to the mitochondrial PL's may therefore occur as a secondary effect of PL synthesis on and transfer from the microsomes. The major route of synthesis of PC in rat liver is the sequential methylation of PE; therefore the in vivo incorporation of label from [³H]-ethanolamine occurs in both PC and PE. The PC:PE ratio of counts decreases in the microsomal and mitochondrial outer membranes and increases in the inner membrane 44 hours after CCl₄ exposure - the same time that mitochondrial function and PL composition are most greatly altered. Two microsomal methyltransferases are responsible for the conversion of PE to PC. The in vitro activities of both enzymes decrease in the 10 to 40 hour period after CCl₄ exposure. A soluble liver protein that functions in vivo to transfer PL's between membranes has been described. The activity of this protein increases after CCl₄ exposure, suggesting that in vivo it is part of the repair mechanism working to restore membrane PL composition. (Supported by PHS grant ES01919 and NIEHS training grant ES07062.)

**470 THE EFFECT OF 1,3-BUTANEDIOL ON THE HEPATOTOXIC ACTION OF CCl₄ IN RATS.** L.A. Hewitt, W.R. Hewitt and G.L. Plaa, Dept. de pharmacologie, Université de Montréal, Montréal, Québec, Canada.

1,3-Butanediol (BD) has several potential uses in the processing of human and animal diets, including use as a source of calories. BD can increase circulating ketone bodies. Evidence suggests that ketosis can potentiate the hepatic action of halogenated anethesia (Hewitt et al.; Fed. Proc. 33: 3118, 1980). BD given po potentiates CCl₄ hepatotoxicity (Hewitt, Plaa; TAP 47: 177, 1979). The present study characterizes the hepatotoxic response of male Sprague-Dawley rats to CCl₄ following pretreatment with BD. Animals were given free access to drinking solutions containing 0, 0.1, 1, 5, and 10% (w/v) BD. During the 7-day pretreatment period, body weight, ketone excretion and solution consumption were monitored daily. Collateral experiments determined plasma ketones and liver GSH on Days 1, 3 and 7. On Day 7 one-half of the rats in each group received CCl₄ (0.1 ml/kg, ip) in corn oil; the balance received corn oil alone. On Day 8 the liver damage was assessed by histological and biochemical parameters (plasma bilirubin, GPT and OCT). BD reduced GSH and increased plasma ketones on Days 3 and 7. CCl₄ toxicity was enhanced in a dose-related manner, with a BD threshold between 0.1 and 1.0%. Possible mechanisms include induction of MFO enzymes. BD was a relatively weak inducer of the usual MFO parameters: 10% BD increased cyt. P-450 (~ 57%) and aniline hydroxylase activity (~ 85%); aminopyrine N-demethylase was not altered. However BD greatly increased the in vivo binding of [³H]-CCl₄ to microsomal protein (aerobic ~ 9-fold, anaerobic ~ 3-fold). These results show that BD enhances the hepatotoxicity of CCl₄ and that this is, in part, the result of increased biotransformation. (Supported by grants from NSERC, NRMPD, and IRSSTQ).

**471 EFFECT OF CALCIUM ANTAGONISTS ON CARBON TETRACHLORIDE-INDUCED LIVER NECROSIS.** L.D. Schuman and M.A. Evans, Interdisciplinary Toxicology Program, Environmental and Occupational Health
The present studies were designed to evaluate the effects of calcium antagonists on carbon tetrachloride-induced necrosis. Mice received a single intraperitoneal dose of CCl₄ (0.2 ml/kg) and then administered at selected time schedules the calcium chelator ethylenediamine tetraacetate (EDTA) (200 mg/kg) or the organic calcium antagonist verapamil (10 mg/kg). Both EDTA and verapamil significantly decreased CCl₄-induced hepatic necrosis at 24 hrs. as determined by histopathological examination. Treatment with EDTA or verapamil alone produced no significant changes from control in liver histology. Animals receiving EDTA and CCl₄ also showed significantly lower serum glutamic pyruvic transaminase (SGPT) levels, but only if administration began 30 minutes prior to injection of CCl₄. Administration of verapamil to animals receiving CCl₄ failed to lower elevated SGPT levels. Hence, SGPT levels in this study do not appear to be a sensitive index of hepatoprotection by the calcium antagonist. This may be due to the presence of different intracellular pools of calcium. Both calcium antagonists failed to significantly block the increased liver calcium at 24 hours. These results may reflect the massive influx of calcium subsequent to removal of the antagonists at 12 hours and/or the presence of different intracellular pools of calcium. These studies indicate that co-administration of calcium antagonists significantly reduces CCl₄-induced hepatic necrosis in mice. However, the serum transaminase values and liver calcium content were not consistent with the observed reduction in cellular necrosis as evidenced by histopathology.

**ROLE OF HEPATIC MICROTUBULE SYSTEM IN CHLORINATED HYDROCARBON INDUCED HEPATIC STEATOSIS.** F.M. Selan and M.A. Evans, Interdisciplinary Toxicology Program, Dept. Pharmacology, University of Illinois Med. Ctr., P.O. Box 6998, Chicago, IL 60680.

Recent studies have demonstrated that inhibitors of the tubulin assembly-disassembly process can also produce a decreased lipoprotein secretion and intracellular accumulation of triglycerides in the liver. The purpose of this investigation was to examine the role of the hepatic microtubular (MT) system in chlorinated hydrocarbon-induced hepatic steatosis. Microtubules were separated from free tubulin by differential centrifugation and measured by a modified radiolabelled colchicine binding assay which was shown to be selective and quantitative for hepatic tubulin protein. Production of steatosis was assessed by measurement of total liver lipids recovered by the method of Folch. The release of triglycerides (TG) from the liver was estimated in vivo by the method of Byers and Friedman (Amer. J. Physiol. 198: 629-631, 1960) using Triton WR 1339. At doses consistent with the production of fatty liver, carbon tetrachloride (CCl₄) produced a significant decrease in hepatic MT in male mice. The decrease in MT was time-dependent and restoration of hepatic MT at 72 hrs. was associated with a decrease in total liver lipids to control values. Disassembly of MT and hepatic steatosis was achieved with doses of CCl₄ as low as 0.025 ml/kg and maximal disassembly was observed at 0.1 ml/kg. These results were consistent with observed CCl₄-induced inhibition of hepatic triglyceride secretion. Further studies conducted with methylene chloride (CH₂Cl₂) (2 ml/kg, P.O.) also demonstrated a significant decrease in hepatic secretion of TG at 2 hrs with an increased hepatic TG content observed at 6 hrs. The increased accumulation of hepatic lipids was consistent with a 25% decrease in liver MT produced by methylene chloride. These results indicate that disruption of the tubulin assembly-disassembly process is associated with the development of acute hepatic steatosis produced by the chlorinated hydrocarbons.

**PCBs: EFFECTS OF STRUCTURE ON THE INDUCTION OF CYTOCHROME P-448 DEPENDENT MONOOXYGENASES IN HEPATOMA CELLS. T. Sawyer and S. Safe, Guelph Waterloo Centre for Graduate Work in Chemistry, Chemistry Department, University of Guelph, Guelph, Ontario, and Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX. Sponsor: L. W. Robertson**

The relative activities of polychlorinated biphenyls as inducers of cytochrome P-448 dependent monooxygenases were determined by measuring the dose effecting half-maximal (EC₅₀) induction of benzo[a]pyrene hydroxylase and ethoxyresorufin O-deethylase activities in rat hepatoma H-4-II-E cells in culture. There was an excellent correlation between the relative potencies of the individual polychlorinated biphenyls using both enzyme assays and the most active congener, 3,3', 4,4',5-pentachlorobiphenyl, was only 3-4 times less active than 2,3,7,8-tetrachlorodibenzo-p-dioxin. In addition there was an excellent correlation between the in vivo and in vitro activities of the polychlorinated biphenyls and their relative affinities for the cytosolic Ah receptor protein. (Sponsor: National Cancer Institute of Canada).

**EFFECTS OF STRUCTURE ON THE ACTIVITY OF POLYBROMINATED BIPHENYLS (PBBs) AS MICROSOMAL ENZYME INDUCERS. L. W. Robertson, A. Parkinson and S. Safe, Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX. and Department of Chemistry, University of Guelph, Guelph, Ontario, N1G 2W1 Canada.**

The effects of structure on the activity of synthetic polychlorinated biphenyl (PBB) congeners as microsomal enzyme inducers has been studied in the immature male Wistar rat. Using enzymic assays and ligand-binding measurements, PBB congeners were classified as phenobarbital (PB)-or 3-methylcholanthrene (MC)-type inducers of cytochrome P-450 dependent monooxygenases. Important qualitative differences in the modes of induction exist between PBBs and their chloro analogs. Where analogy exists, PBBs are in general the more potent inducers. The structure-activity rules for PBBs as PB-type inducers are as yet unresolved. PBBs with at least 1 meta and 2 para substituents are MC-type inducers of cytochrome P-450, whereas PCBs require at least 2 meta and 2 para substituents for this activity. PBBs which are mono or di ortho derivatives of the MC-type inducers may be mixed (PB + MC)-type inducers. (Sponsored in part by the Natural Sciences and Engineering Research Council of Canada).
HEPTA- AND OCTACHLORONAPHTHALENE CONGENERS ARE POTENT INDUCERS OF RAT HEPATIC MICROSOMAL BENZO[a]PYRENE HYDROXYLASE. M. A. Campbell, L. Safe, S. Bandiera, L. W. Robertson and S. Safe, Department of Chemistry, University of Guelph, Guelph, Ontario, N1G 2W1 Canada and Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Induction of rat liver microsomal enzymes by three polychlorinated naphthalene congeners octachloronaphthalene, 1,2,3,4,5,6,7- and 1,2,3,4,5,6,8-heptachloronaphthalene was investigated. The congeners were prepared by repeated recrystallization from Halowax 1051 in chloroform/petroleum spirits (octachloronaphthalene) and lithium aluminum hydride reduction of octachloronaphthalene (1,2,3,4,5,6,7-heptachloronaphthalene). The compounds were administered to immature male Wistar rats in doses ranging from 12 to 1200 µmol/kg-1. On the basis of enzymic activities as well as ligand binding (CO and EIC) measurements and SDS polyacrylamide gel electrophoresis, the results indicated that the heptachloronaphthalenes were potent MC-type inducers (maximum activity < 60 µmol/kg-1). Octachloronaphthalene, also an MC-type inducer was much less potent (maximum activity at 300 µmol/kg-1) and a number of isomeric dichloronaphthalenes did not induce microsomal benzo[a]pyrene hydroxylase.

INHIBITION OF RAT LIVER ALDEHYDE DEHYDROGENASE BY CARBON TETRACHLORIDE. J.J. Hjelle, J.H. Grubbs, D.G. Beer and D.R. Petersen, Dept. of Pharmacology, School of Pharmacy, Univ. of Colorado, Boulder, CO 80309. Sponsor: C.D. Kalassen.

Current data suggests that aldehydic products of lipid peroxidation possess substantial cytotoxic properties. Carbon tetrachloride, a potent stimulator of hepatic microsomal lipid peroxidation was tested for possible effects on hepatic cellular aldehyde metabolism. Carbon tetrachloride administered intragastrically at a dose of 1 ml/kg 24 hr before sacrifice produced an elevation in serum alanine amino transferase activity, hepatic fatty infiltration, centrilobular necrosis and significant decreases in the content of hepatic microsomal cytochrome P-450. Concurrently, the aldehyde dehydrogenase (ALDH) activity (E.C. 1.2.1.3) of mitochondrial and cytosolic fractions was significantly depressed. The lower Km ALDH located in the mitochondria showed a 43% lower specific activity compared to control values. Cytosolic ALDH specific activity was decreased 33% by carbon tetrachloride treatment. In addition to these in vivo findings the low Km ALDH of mitochondria was shown to be inhibited during in vitro lipid peroxidation under a variety of conditions. These data suggest that aldehyde metabolism is impaired following CC14 exposure, an effect that may prolong the intracellular half life of aldehydic products of lipid peroxidation. Supported by NIAAA grant - 03527.

ELEVATION OF HEPATIC CONJUGATED DIENES IN THE ABSENCE OF CELLULAR INJURY IN 1 DAY-OLD RATS AFTER CARBON TETRACHLORIDE (CC14) TREATMENT. A.T. Shehata and M. Hitchcock, Yale University and John B. Pierce Foundation, 290 Congress Ave., New Haven, CT. 06519.

We investigated the time course of onset of CC14-induced hepatotoxicity in 1- to 3-day-old rats. Activities of hepatic aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in control animals were identical in all age groups and were, respectively, 218±25 and 20±2.7 Sigma units per g liver. Twenty-four hrs. following CC14 treatment (1 ml/kg, i.p.) plasma activity of AST in rats injected at age 1, 2 and 3 days were increased, respectively, by 1.2±0.3, 810±20 and 2380±45%: plasma activity of ALT in the same groups were, respectively, 0.5±2, 0.5±2, 0.5±2, 2380±45% and 613±20%. These data suggest age difference in susceptibility to CC14 rather than lack of hepatic enzymes to be released. Gas chromatographic analysis of heptane-extracts of liver, kidney, lung and fat homogenates from rats of all age groups did not detect the presence of CC14 metabolites. In 1-day-old rats, conjugated dienes, a measure of lipid peroxidation, were found to be significantly elevated only in liver homogenates 90 mins following CC14 treatment (1 ml/kg, i.p.) (absorbance at 243 nm of lipid per g liver: 0.12±0.0147 for CC14-treated rats and 0.08±0.0066 for corn oil-treated rats; p<0.05). Under these conditions, hepatic activities of AST and ALT were unchanged. The results suggest that lipid peroxidation can occur without hepatic injury in CC14-treated newborn rats. Supported by Grant 5ST32ES-07086.
THE TRANSFER OF 3,4,5,3',4',5'-HEXACHLOROBIPHENYL (6-CB) FROM MOTHERS TO FETUSES AND NURSING OFFSPRING. M.J. Vodcnik and J.J. Lech, Dept. of Pharm. & Tox., Med. Col. Wisconsin, Milwaukee, WI

The pharmacokinetic behavior of 14C-6-CB in virgin and pregnant/lactating Sprague-Dawley mice was assessed. Mice were injected IP with 3mg/kg 6-CB on Day 2 of pregnancy and sacrificed at various times along with matched virgin controls. Tissues were prepared for liquid scintillation counting and hepatic microsomal ethoxyresorufin-O-deethyla-
tion (EROD) determined. Suckling offspring from 6-CB-treated mothers were sacrificed at selected times, tissues analyzed for 14C activity and hepatic microsomal EROD assessed. No significant differences were noted in the tissue distribution of 6-CB between virgin and pregnant mice on Days 6, 12, or 19 of gestation. EROD activity between the two groups did not differ at Days 6 or 12 of pregnancy but was significantly depressed in pregnant

maternal adipose tissue. During lactation, 6-CB was rapidly eliminated in milk. While no detectable elimination of 6-CB from the adipose tissue of virgins occurred, the t 1/2 of elimination of 6-CB from the fat of lactating mothers was 2.5 days. These greatly enhanced rates of elimination were observed in all maternal tissues examined. Nursing offspring accumulated the PCB in liver and both coplanar and non-coplanar isomers. Supported by HD 13591 and MOD 15-9.

CHOLESTYRAMINE ENHANCES FECAL EXCRETION OF PENTACHLOROPHENOL. K. Rozman, L. Ballhorn, T. Rozman, C. Klaassen, and H. Greim. Gesellschaft fur Strahlen-und Umweltforschung mbH Muenchen, 8042-Neuherberg, FRG and Dept. of Pharmacology, University of Kansas Medical Center, Kansas City, KS.

Three male rhesus monkeys were provided with a bile duct bypass and were dosed twice with 14C-pentachlorophenol (50 mg/kg, po) four weeks apart. During the seven days following the first dose, the monkeys excreted 60 and 3% of the dose into urine and feces, respectively. Up to 30% of the dose was excreted into bile in one day indicating a considerable enterohepatic circulation of pentachlorophenol. Since cholestyramine binds acidic compounds such as carboxylic acids and phenols, it was of interest to determine if this resin would enhance the elimination of pentachlorophenol. Therefore, twenty-four hr after the second dose of 14C-pentachlorophenol, the monkeys were placed on a diet containing 4% cholestyramine for six consecutive days. During this time 28 and 55% of the dose was excreted into urine and feces, respectively. The body burden of pentachlorophenol was markedly reduced by cholestyramine treatment as its total excretion (urinary plus fecal) was enhanced by 40%. While cholestyramine increased the plasma disappearance of pentachlorophenol, it produced a more marked decrease in its urinary excretion. This effect suggests that a considerable amount of pentachlorophenol in urine probably originates from the enterohepatic cycle. Cholestyramine enhanced the fecal excretion of pentachlorophenol from 8 to 163 mg. Since only about 104 mg was excreted into bile, cholestyramine probably also increases elimination of pentachlorophenol directly across the intestinal wall.


An in vitro toxicity test is being developed to compare cytotoxicity of chemical substances toward cultured corneal endothelial cells with ocular irritancy in vivo. Confluent monolayers of corneal endothelial cells are established in 24-well dishes from primary cultures, incubated with various concentrations of test substances and positive and negative control substances,
then evaluated for a biochemical and morphological indices of cytotoxicity. Preliminary results indicate that benzalkonium chloride, silver nitrate, hydrogen peroxide, and lauryl sulfate, mediate half-maximal $^{51}	ext{Cr}$ release at $4 \times 10^{-4}$M, $8 \times 10^{-4}$M, $2 \times 10^{-5}$M, and $4 \times 10^{-6}$M doses, respectively, during 1 hour incubations. Relatively non-toxic substances, propylene glycol, dimethylsulfoxide, and ethanol, were not cytotoxic at concentrations less than 1 M. $^{51}	ext{Cr}$ release was demonstrated to be dependent on duration of the exposure to a test substance as well as concentration. At 10, 30, 60, and 90 minutes, benzalkonium chloride at $10^{-4}$ M elicited 48%, 49%, 56%, and 60% of the maximum $^{51}	ext{Cr}$ release. Other assays will quantify TUN release, morphometric analysis of endothelial cells, lymphocyte activation, and vital dye or dye exclusion methods of measuring cytotoxicity in vitro. These studies will be extended to include a variety of known ocular irritants and non-irritant substances, to afford a broad data base with which to confirm or invalidate the in vitro assays as a model system for screening potential ocular irritants.

This work was supported by a grant from the New England Anti-Vivisection Society.


The ocular safety of materials is determined primarily by observing the irritation produced by test agents instilled directly into the rabbit eye. The object of this study was to review the scientific basis for published guidelines, particularly those recently developed by the Organisation for Economic Cooperation and Development (OECD) and the Interagency Regulatory Liaison Group (IRLC). These guidelines are essentially the same and recommend instillation of 0.1 ml material into the rabbit eye with observation for at least 72 hr. To increase cost-effectiveness and optimize use of animals, the guidelines reduce the number of animals and permit exclusions based on very high ($\geq 11.5$) or low ($\leq 2$) pH, and demonstrated dermal irritation. This is based on the high probability that agents meeting these criteria will be severe eye irritants. Data reviewed support excluding strong alkalies (pH $\geq 11.5$). Exclusions of acids below pH 2 is not as well supported. Data indicate that the majority of severe dermal irritants would also be eye irritants. The monkey appears the most predictive animal model for human response; however, the rabbit still remains the species of choice due to practical considerations and the large data base which is useful for comparative purposes. Generally greater sensitivity of the rabbit eye compared to the human allows a conservative extrapolation to human risk. The development of alternative methodologies (e.g. in vitro tests) and the use of topical anesthetics is discussed. A tier strategy to eye irritation testing is proposed on the basis of pH, dermal irritation and results from other tests. (Supported by the EPA, Contract No. 68-01-6176, C. Auer, Project Officer. Contents do not reflect agency policy.)


The use of monomeric and polymeric isocyanates in a wide variety of industries has been increasing. Little is known about the toxicity of polymeric isocyanates. The pulmonary and sensory irritation of an aliphatic polyisocyanate (DES-N, Mobay Chemical Corporation), based on hexamethylene diisocyanate (HDI) were studied in Swiss-Webster male mice during aerosol exposures in the range 25-130 mg/m$^3$. The aerodynamic equivalent diameter (AED) and geometric standard deviation for the aerosol were 0.58 $\mu$m and 2.35, respectively. High speed liquid chromatography was used to determine both free HDI and DES-N in the exposure chamber. Each exposure was for 4 hours during which the tidal volume pattern and respiratory rate of groups of 4 mice were recorded. Unlike the monomeric isocyanates, DES-N acted predominantly as a pulmonary irritant, evoking little sensory irritation. The concentration required to reduce the respiratory rate 50% due to pulmonary irritation was found to be 57.1 mg/m$^3$. The LC50, determined by counting the number of deaths within the 24 hour period following the 4 hour exposure was found to be 91.2 mg/m$^3$. On groups of animals sacrificed 2 hours after the 4 hour exposure, the concentration to increase lung weight by 50% was found to be 45 mg/m$^3$. Based on comparisons with other pulmonary irritants, the maximum concentration permitted in industry should be 1 mg/m$^3$ with a time weighted average for an 8 hour period of 0.6 mg/m$^3$.
Many organic industrial chemicals, including toluene diisocyanate (TDI) are known to cause asthmatic reactions in sensitized workers. In order to prevent sensitization, it is necessary to understand the relationship between exposure concentration and development of pulmonary sensitivity. An animal model was used to evaluate this relationship. Groups of guinea pigs were exposed via inhalation to concentrations of TDI ranging from 0.1 to 0.5 ppm. Animals were exposed to the TDI vapors for 3 hours per day on 5 consecutive days. On day 21 blood was drawn from animals for evaluation of TDI-specific antibodies. TDI-specific respiratory sensitivity was determined by bronchial provocation challenge (BPC) using TDI antigens. No antibodies were detected in animals exposed to 0.1 ppm TDI. However, animals exposed to 0.25 ppm or greater displayed TDI-specific antibodies in their sera and antibody titers increased in a logarithmic relationship with TDI exposure concentration. Similarly, bronchial sensitivity was not apparent in animals exposed to 0.1 ppm TDI but was evident in animals exposed to higher TDI concentrations. However, exposure of animals to concentrations of 2 ppm or greater resulted in pulmonary damage and animals were less able to respond upon BPC with TDI antigens. Recognition of the concentration-response and threshold concentration relationship governing inhalation sensitization to TDI should permit establishment of safe airborne exposure levels for industrial workers to prevent sensitization. Supported by NIEHS Grant #ES01532 and NIOSH #OH00865.

EVALUATION OF BENOXAPROFEN PHOTOTOXICITY IN AN ANIMAL MODEL. G. T. Brophy (SPON: D. G. Hoffman). Toxicology Division, Lilly Research Laboratories, Greenfield, IN 46140

A wide variety of drugs induce phototoxic effects clinically, viz. phenothiazines, sulfonamides, psoralens, tetracyclines. Phototoxicity has been associated with the clinical use of benoxaprofen in some cases. Benoxaprofen phototoxicity was investigated using an in vitro mouse tail model (Acta Dermatovener (Stockholm) 56:373, 1976). Briefly, test compounds were administered to ICR mice, the intact tails were irradiated with UVA for 5 hours and at 24 hours the evaporable water content of a tail segment was determined to quantitate edema formation resulting from phototoxic inflammation in the tail. The classical phototoxic agent chlorpromazine had a minimum phototoxic dose (MPD) of 2.5 mg/kg (i.p.) whereas the MPD of benoxaprofen was 10 mg/kg (p.o.); these doses are comparable to those used clinically. Benoxaprofen is not significantly metabolized in the mouse, rat, monkey or man and metabolic induction or inhibition with phenobarbital or SKF 525A did not alter benoxaprofen phototoxicity. The two major benoxaprofen UV degradation products were comparatively less phototoxic than the parent compound (MPD = 900 mg/kg). These derivatives do not appear to be responsible for benoxaprofen phototoxicity.

DERMAL SENSITIZATION BY TOLUENE DIISOCYANATE (TDI). E. J. Kirchner, E. J. Burden, C. S. Brunkhorst, and M. A. Friedman. American Cyanamid, Wayne, NJ.

TDI is known to cause pulmonary sensitization and has recently been shown to cause allergic skin reactions. The present study was designed to determine the threshold dose for dermal sensitization. Young adult guinea pigs received an open epicutaneous induction dose (50 mcg) of 8% TDI in n-butyl ether on day 0. On day 5, animals were challenged with 0.025, 0.05, 0.1, 0.2 and 0.4% TDI (25 mcg). Twenty-four hours after the day 5 application, the mean Draize irritation scores for the challenge doses of the 8% group were 2.4 and 3.4 for 0.025 and 0.4% TDI, respectively, and from the 40% group, 2.4 and 4.2, respectively. The vehicle control and challenge doses produced scores of 0.6-1.0, and since all the challenge doses produced scores greater than 2.0, no threshold was apparent at these induction and challenge levels. An additional experiment was performed at lower doses where animals received an induction dose (50 mcg) of either 4 or 8% of TDI and were challenged with 0.0, 0.006, 0.012, 0.025, 0.05, and 0.1% (25 mcg). Twenty-four hours after the challenge applications, the mean Draize irritation scores for the challenge doses of the 4% groups were 1.0, 1.0, 0.9, 1.6, 1.4 and 1.8, and the 8% groups were 1.0, 1.1, 1.1, 2.1, 2.4 and 3.0, respectively. The data from the second experiment suggest that TDI has an apparent no effect challenge level of 0.012% (3 mcg) when 4% (2,000 mcg) and 8% (4,000 mcg) induction doses were used.


The chronic toxicity and carcinogenicity of a process-derived recycle oil (RO) from a high-BTU coal gasification pilot plant is being determined. RO collected during several experimental runs was pooled to form a representative sample. Both the RO and its nonvolatile organic components (NVO) are being tested. Treatment groups consist of 50 SKH hairless mice either untreated...
or painted 3 times/week with RO, NVO, benzo(a)-pyrene (+ control), or acetone (solvent control). Mice are examined for gross evidence of inflammatory, hyperplastic, or neoplastic dermal effects and for systemic changes through analysis of blood and urine. Dermatitis, scaling, and scab formation were seen following oil treatment, being most severe in the NVO group. Urine and serum were collected during weeks 22, 26, 35, and 39. Urine was normal in all groups except that with NVO, which displayed a transient reduction in volume. The NVO-treated mice had elevated serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), decreased urine GOT and increased urine and serum urea nitrogen (UN) levels, and creatinine (C) excretion. The RO-treated mice were normal except for increased serum GPT and urine C levels. Although results are incomplete, they indicate pathological changes taking place in the liver (GPT), kidneys (UN), and muscle (GOT & C). Benzo(a)pyrene and both oil-treated groups developed skin tumors starting 10-12 weeks into the experiment. The NVO-treated mice displayed a more severe tumor response with a shorter induction time. Histologic examination of the tumors is in progress. (Work supported by US DOE under contract No. W-31-109-ENG-38.)


Toxic axonal peripheral neuropathies are accompanied by early degeneration of the distal ends of long ascending sensory axons in the dorsal columns and gracile nuclei (central-peripheral distal axonopathy). This study sought to determine whether detectable electrophysiological changes preceded axonal degeneration in this system, and whether such changes are reversible. Monkeys were daily injected with levels of either 10, 3, 2 or 1 mg/kg of acrylamide monomer. Brainstem somatosensory evoked potentials (SEPs) and peripheral nerve conduction velocities were recorded weekly from surface electrodes prior to and during intoxication. The earliest abnormality detected (1-2 weeks in the 10 mg/kg animals) was a latency shift of approximately 200 usec in the onset of near-field SEP activity overlying the gracile nucleus following unilateral electrical stimulation of the peroneal nerve. This CNS effect preceded observed morphological or behavioral abnormality, or alteration in the conduction velocity recorded overlying distal sural nerve, cauda equina, or spinal cord below C5. The SEP profile recorded overlying the cuneate nucleus remained unchanged following median nerve stimulation. When intoxication was stopped at this stage, the configuration of the SEP gradually returned to normal. Progressively longer delay before onset of latency shift occurred in animals intoxicated at lower levels. Animals intoxicated with 1 mg/kg first displayed physiological abnormalities only after 18 months of daily dosage. This study suggests that the non-invasive brainstem SEP is a sensitive tool for the early detection of peripheral neuropathies of the distal-axonopathy type, that these conditions are potentially reversible and that extremely prolonged delay of onset can occur with low-level intoxication.

491 EVIDENCE FOR PYRROLE FORMATION IN THE PATHOGENESIS OF HEXANE NEUROPATHY. D.C. Anthony and D.G. Graham, Dept. of Pathology, Duke University Medical Center, Durham, N.C. 27710.

The hypothesis that covalent binding of 2, 5-hexanedione (HD) to neurofilament proteins is the initial injury in the molecular pathogenesis of hexane neuropathy was studied using the structural analogue 3, 4-dimethyl-2, 5-hexanedione (DMHD). Deaths of steric properties, DMHD should form a pyrrole ring more rapidly than HD after imine formation and be less likely to form dimesines, two possible mechanisms of neurofilament aggregation. Twenty-four Sprague-Dawley rats (170 gm) were divided into four equal groups and injected i.p. five times a week with HD (6 mmoles/kg), HD (0.25 mmoles/kg), DMHD (0.25 mmoles/kg) or water. When hind limb paralysis occurred, the animals were perfused with 4% glutaraldehyde. The DMHD-treated animals showed a more rapid clinical course characterized by weight loss and progressive fore-and-hind-limb weakness, reaching end-point after 19.6±1.2 days. Rats receiving HD at 16 times the dose given the DMHD rats reached end-point after 35.8±2.6 days, with no appreciable fore-limb weakness. After 8 weeks of treatment, the low-dose HD-treated animals showed no signs of toxicity. Morphologic studies revealed the characteristic giant axonal swellings with proximal paranodal neurofilament aggregations in the high-dose HD-treated and DMHD-treated animals, although in the latter group the changes occurred much more proximally with severe Wallerian-like degeneration distally. In vitro formation of pyrrole was more rapid when ethanolamine was reacted at pH6 with DMHD than with HD, occurring approximately seven-fold more rapidly at 37°C. These findings suggest that pyrrole derivatization of neurofilament lysyl residues follows imine formation and that more rapid irreversible alterations lead to more proximal aggregations of neurofilaments. (Supported by NIEHS grant ES 02611-01.)


Sponsor: G.L. Plaa

In March 1979, an endemic disease due to chronic poisoning from polychlorinated biphenyl (PCB) contamination of rice oil, widely consumed in central Taiwan. Exposure to PCB in human subjects and experimental animals cause various metabolic and physiologic abnormalities including immune suppression. We have studied cellular and humoral immunity among 30 PCB-poisoned patients, and 23 normal human subjects. The results and conclusions are summarized as follows: 1) PCB poisoning caused decreased concentrations of IgA and IgM but not that of IgG. We also found that the percentages of total T-cells, active T-cells, and Th-cells were decreased, while the percentages of B-cells and Tr-cells were not affected. 2) Monocytes and polymorphonuclear leukocytes obtained from patients have lower percentages of cells bearing immunoglobulin and complement re-

Dimethylnitrosamine (DMN) has been reported to induce BHK-21 Cl 13 cell growth in soft agar, in the absence of an added activation system. To determine if DMN is mutagenic to BHK cells, an ouabain resistance mutation (Or+) assay was established. BHK cell growth was inhibited by 0.5 mM ouabain in the growth medium; this inhibition was overcome by the addition of K+. DMN was then compared to the mutagenic methylating agents MNNG and nitrosocimetidine (NC). MNNG and NC yielded dose dependent induction of Or+ clones. DMN was not mutagenic. Optimal phenotypic expression for 3 weeks growth in nonselective medium. The ability of the test compounds to methylate DNA in BHK cells was determined; MNNG and NC yielded detectable levels of 7-methylguanine, DMN did not. The compounds were tested for the ability to induce BHK cell growth in soft agar, and MNNG, NC, and DMN did at 1µM, 20µM, and 338µM respectively. The results suggest that BHK cells are not able to activate DMN to a mutagenic intermediate. Therefore, DMN appears to induce BHK cell growth in soft agar by a non-genotoxic mechanism. NC was found to be as effective a mutagen and inducer of BHK cell growth in soft agar as MNNG at equitoxic concentrations, approximately 4 fold less effective at equimolar concentrations. This work was supported by the NIEHS and NIH grants P32-ES0519401, CA-09214, CA-12227, and CA-23451.


The pharmacokinetics of styrene was defined in B6C3Fl mice exposed by inhalation to either 80, 200, 600 or 1200 ppm vapor for up to 12 hr. Continuous 12 hr exposure to styrene resulted in plateau blood levels of approximately 0.71, 1.69, 39.3 and 84 µmols/ml for the 80, 200, 600 and 1200 ppm exposures respectively. The plateau blood concentrations increased proportionately with exposure between 80 and 200 ppm, but disproportionately between 200 and 1200 ppm. The 3-fold increase from 200 to 600 ppm caused a 23-fold increase in the plateau concentration and the 6-fold increase from 200 to 1200 ppm caused a 50-fold increase in the plateau concentration. After a 6 hr exposure to 80 or 200 ppm, styrene was eliminated from the blood according to a 2-compartment linear model; however, after a 6 hr exposure to 600 or 1200 ppm, styrene was eliminated according to a non-linear saturable model. In addition to the blood data, tissue levels, non-protein sulfurhydryl depletion and liver histopathology supported that the transition from linear to non-linear kinetics occurs between 200 and 600 ppm.

These studies demonstrate that the fate of styrene in the B6C3Fl mouse, as in the rat, is dependent on exposure concentration. Therefore, at high saturating doses, toxicological information will be of limited value for hazard assessment purposes. (Supported by the Chemical Manufacturers Association).
absorbed from the GI tract and lung. Radioactivity from the compound was distributed in a fairly uniform manner throughout the tissues of the rat. Urine and feces were the primary routes of excretion with less than 2% of the administered dose being excreted as $^{14}$CO$_2$.

More than 96% of the administered dose was eliminated within 48 hours after dosing. These data indicate that the route of administration, oral or endotracheal, had little effect on the distribution of radioactivity within the body or on the pattern of elimination. In comparing results from animals receiving DETA at 500 mg/kg with those receiving the compound at 50 mg/kg there was a significant increase in urinary excretion and a significant decrease in $^{14}$CO$_2$ elimination at the higher dosage level. There was also a shift in the percentage of DETA and metabolites recovered under the peaks of urinary chromatographic profiles for the two dosage levels. These observations suggest that saturation processes may be involved at the high dosage level. Three pharmacokinetic parameters (bioavailability, total clearance and terminal half-life) of DETA in the rat were compared at the dosage level of 50 mg/kg following oral, endotracheal and iv dosing. Irrespective of the route of administration, the pharmacokinetic parameters are comparable.


Meperidine carboxylesterase activity can be measured in liver subcellular fractions by coupling the release of ethanol to the reduction of a tetrazolium dye. This particular study was undertaken to compare and contrast the activities present in Swiss mouse liver and in human liver. The latter samples were obtained from seven patients undergoing abdominal surgery and were normal in histologic appearance. In mouse liver, the meperidine carboxylesterase activity was distributed between the mitochondrial and microsomal fractions, with approximately 2/3 occurring in the mitochondrial fraction. In contrast, human liver meperidine carboxylesterase activity was detected only in the microsomal fraction. The meperidine carboxylesterase activities in mouse liver mitochondria, mouse liver microsomes, and human liver microsomes were characterized by determining Michaelis–constants and organophosphate sensitivities. The K$_m$ and Vmax values (T=37º) for the mouse liver mitochondrial activity, the mouse liver microsomal activity, and the human liver microsomal activity were estimated, respectively, as 87 uM, 1,62 mmol/min/mg protein; 460 uM, 1.4 mmol/min/mg protein; and 830 uM, 0.8 mmol/min/mg protein. With paroxon as the inactivator, the corresponding IC$_{50}$ values (T=37º, pH=8.0, incubation time=20 min) were 0.3 uM, 1.2 uM, and 4 uM, and with DFP, 0.15 uM, 0.5 uM, and 0.5 uM. These results suggest a similarity between the liver microsomal meperidine carboxylesterases of mice and humans, but also show that the mouse has at least one liver mitochondrial esterase with a relatively high affinity for meperidine and a relatively high sensitivity to organophosphate inactivators. Supported by NIEHS, Grant #E702616.

**PHARMACOKINETIC STUDIES OF 3,3',4,4'-TETRACHLOROAZOBENZENE AND 3,3',4,4'-TETRACHLOROAZOXYBENZENE IN RAT.** M. T. Stephen Haia and Charles F. Burant. Environmental Toxicology Center and Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.

3,3',4,4'-tetrachloroazobenzene (TCAB) and 3,3',4,4'-tetrachloroazoxybenzene (TCAOB) were recently found to pose occupational health hazards among chemical workers handling contaminated chloroanilide herbicides. Both compounds are genotoxic and can cause dramatic atrophic changes in the lymphoid organs, particularly in the thymus of laboratory rodents. The pharmacokinetic profiles of TCAB and TCAOB were investigated in male Sprague-Dawley rats. TCAOB was found to be cleared from the body more rapidly than TCAOB when a single oral dose of [14C]-labeled TCAB or TCAOB was administered to rats. While 66% of the administered TCAB dose was excreted via the urine and feces within the first 24 hr, TCAOB-treated animals were only able to clear 37% of the administered dose by the same elimination route. Examination of the tissue distribution of the remaining radioactivity 5 days after dosing indicated that, for both compounds, the adipose tissue as represented by the epididymal fat pad contained the highest levels of radioactivity. High concentrations were also found in various excretory organs. The rapid clearance of TCAB and TCAOB by rat as observed in the present study is significant since these agents are isosteric to 2,3,7,8-tetrachlorodibenz-p-dioxin, and all three molecules bind reversibly with high affinity to the same cytosolic receptor. These pharmacokinetic data may explain the differences between binding affinity and biological potency seen with these compounds in previous studies. (Supported in part by USPHS Grants, R01-ES01737 and R23-ES01737 from NIEHS & Grant #110066, from the Graduate School, Univ. of Wisconsin-Madison.

**SPECIES DIFFERENCES IN THE METABOLISM AND DISPOSITION OF MORPHOLINE.** Sohn, O.S., Fiala, E.S., Conaway*, C.C. and Weisburger, J.H. Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, N.Y. 10595 and *Texaco Inc., Beacon, N.Y. 12508.

Morpholine (tetrahydro-1,4-oxazine) is an important industrial chemical with a wide variety of applications. Thus, it is essential that its metabolism and disposition be thoroughly understood. We examined the blood plasma levels and urinary metabolites of morpholine in three rodent species: the Sprague Dawley rat, the Syrian golden hamster and the strain II guinea pig. Marked differences were found between the guinea pig and the other two species with respect to plasma levels as well as the metabolism of morpholine. After the i.p. administration of 125 mg/kg of morpholine-$^{14}$C (50 μCi) per animal, the blood plasma half-lives in the rat, hamster and guinea pig were: 115 min., 120 min. and 300 min. respectively. In all three species, approx. 80% of the radioactivity was excreted in the urine in 24 hrs. However, while non-metabolized morpholine-$^{14}$C constituted up to 99% of the urinary radioactivity in the rat and hamster, a signifi-
501 METABOLISM OF ACRYLONITRILE BY ISOLATED FISCHER 344 RAT HEPATOCYTES. L.E. Geiger, L.L. Hogy, F.P. Guengerich, and R.A. Neal. Center in Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232 and Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709.

Acrylonitrile (AN), with 1980 U.S. production of 1.8 billion pounds, is used in the production of plastics and elastomers. Among its toxic effects, AN has been found to produce brain and mammary tumors in the Fischer 344 rat. The liver is not a target organ for this compound, but it is likely to be a major site of metabolism of the compound and we have investigated the ability of isolated Fischer 344 hepatocytes to metabolize the compound. We have found that these cells transform AN by both conjugative and oxidative pathways. The major metabolite of AN is a conjugate of reduced glutathione (GSH), S-(2-cyanoeethyl)-GSH. However, we have found that hepatocytes will oxidize the double bond of AN to form the epoxide of AN (2-cyanomethylene oxide). This material has been identified by co-chromatography with an authentic standard on an HPLC system. After 2 hr of incubation with 400 µM AN, the ratio of epoxide to GSH conjugate was found to be 0.074. SCN, a known urinary metabolite of AN, has also been established as a hepatocyte metabolite of AN through the use of mass spectrometry. The ratio of SCN to GSH adducts was found to be 0.169 after 4 hr of incubation with 250 µM AN. It has been hypothesized by numerous investigators that SCN arises from an unstable cyanohydrin which is formed by AN epoxide. The demonstration of epoxide production by hepatocytes provides experimental support for this hypothesis.


PCP concentrations in casual blood and urine specimens from occupationally-exposed men have been reported, but previous reports have not specified the temporal relation between specimen collection and exposure. We collected serial blood and urine specimens from each of 5 volunteers, occupationally-exposed for at least 3 weeks. Specimens were collected before, during and after work on the last of 7 consecutive workdays, and thereafter for about 72 hours away from work. Pre and post work plasma and urine concentrations were similar for each of the individuals. The averages ranged from 0.8 to 12.0 µg/mg in plasma, while casual urines obtained during the same day ranged from 0.25 to 1.76 mg PCP/mg creatinine. These values are comparable to those reported by others. Since Braun et al., (1979, in Toxicology and Occupational Medicine, Elsevier, N.Y.) have shown that 86% of ingested PCP was eliminated in volunteers' urine within a week of dosing, 24 hr urinary excretion of PCP should be a good index of occupational exposure. We found that individuals excreted 0.8 to 5.9 mg/day in the urine with an apparent elimination half-life of about 175 hrs; the amounts excreted in urine were consistent with estimated pulmonary uptake at or below the current ACGIH-recommended TLV®. Concurrent measurements of PCP in the breathing zone showed considerable variability, but provided the same relative estimate of individual exposure with greater convenience.

503 COMPARISON OF THE ACUTE TOXICITY OF TETRAMETHYL-SUCINONITRILE (TMSN) AND SUCCINONITRILE (SN). F.A. Doherty, R.P. Smith and V.H. Ferm, Dept. of...
Chemical alteration of rat liver UDP-glucuronic acid (UDPGA) content. J.B. Watkins and C.D. Klaassen, Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103.

Glucuronidation, a major phase II reaction, is involved in metabolic conversion of xenobiotics to more water soluble compounds which are then excreted into urine and/or bile. This process is dependent upon enzymatic activity of UDP-glucuronyltransferase and intracellular concentrations of UDPGA. Many xenobiotics alter hepatic glucuronyltransferase activity, but their effect on UDPGA content is unknown. Male Sprague-Dawley rats were pretreated with: 1) microsomal enzyme inducers (7,8-benzoflavone, benzo(a)pyrene, butylated hydroxyanisole, isosafrole (ISF), 3-methylcholanthrene, phenobarbital, pregnenolone and 13) which contains trace quantities of TCDD. Because of possible maternal hepatic or reproductive effects of this uncharged, low molecular weight, lipophilic compound 0, 30, 100, 300 and 1,000 mg/kg/day of unpurified TCB was orally administered to pregnant rats on days 9, 10, 11, 12 and 13 and the animals were sacrificed on day 14 of pregnancy. No maternal deaths were recorded and body weight gain was significantly decreased only in the 1,000 mg/kg/day group. Maternal liver weight, liver to body weight ratio and hepatic microsomal protein content were observed after exposure to diethylether, while the other anesthetics reduced UDPGA about 25%. Borneol and galactosamine decreased UDPGA by 85-90%. Thus, numerous xenobiotics alter the concentration of UDPGA in rat liver which may influence the rate of glucuronidation. (Supported by USPHS grant AM 14513 and ES 07079).

Presystemic elimination of diethylstilbestrol by rat liver. T.N. Thompson and C.D. Klaassen, Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS.

Some chemicals do not effectively enter the general systemic circulation even though they are readily absorbed by the gastrointestinal tract. This phenomenon is known as the first-pass effect or presystemic elimination. Hepatic and/or intestinal biotransformation and hepatic extraction are primarily responsible for this phenomenon. Therefore, any xenobiotic which is efficiently excreted by the liver into bile might be expected to exhibit presystemic elimination. Accordingly, the synthetic estrogen diethylstilbestrol (DES) was examined to determine if the liver decreases its systemic availability. Male Sprague-Dawley rats were administered \(^{14}C\)-labeled DES (0.005, 0.05 and 0.5 mg/kg) into either the ileocolic (portal administration) or femoral (systemic administration) vein. Plasma and bile samples were collected for 90 min and the concentration of total DES, unchanged DES and its glucuronide were determined. Both total and unchanged DES disappeared from the plasma more rapidly after portal than systemic administration. In contrast, DES glucuronide constituted a greater percentage of total plasma DES after portal than systemic administration. Comparison of the area under the plasma concentration vs time curve (AUC) indicated that the systemic availability of DES after portal administration was less than 40% of that after systemic administration. The rate of biliary excretion of total DES during the first 15 min was higher following portal administration than by the systemic route. These results indicate that the liver can reduce the systemic availability of DES by 60-70%. (Supported by USPHS Grant AM 14513).

Hepatic effects of 1,2,4,5-tetrachlorobenzene in the pregnant rat. K.T. Kitchin, ETD, USEPA, Research Triangle Park, NC (Sponsor: William F. Durham)

1,2,4,5-Tetrachlorobenzene (TCB) is an industrial intermediate used in the production of 2,4,5-trichlorophenoxyacetic acid, a herbicide which contains trace quantities of TCDD. Because of possible maternal hepatic or reproductive effects of this uncharged, low molecular weight, lipophilic compound 0, 30, 100, 300 and 1,000 mg/kg/day of unpurified TCB was orally administered to pregnant rats on days 9, 10, 11, 12 and 13 and the animals were sacrificed on day 14 of pregnancy. No maternal deaths were recorded and body weight gain was significantly decreased only in the 1,000 mg/kg/day group. Maternal liver weight, liver to body weight ratio and hepatic microsomal protein content were
unaffected by TCB treatment. Although day 14 NADPH-cytochrome c reductase activity was not affected, the maternal hepatic microsomal cytochrome P-450 content was significantly increased by administration of 1,000 mg/kg/day of TCB. Microsomal N-demethylation of aminopyrine was slightly increased from 2.4 to 3.4 and 3.5 mmoles/mg protein/min at doses of 300 and 1,000 mg/kg TCB. However, maternal hepatic microsomal ethoxyresorufin O-deethylase activity was greatly increased from 14 to 30, 40, 50 and 49 pmole/mg protein/min in pregnant rats administered 0, 30, 100, 300 and 1,000 mg/kg/day TCB. The microsomal rates of p-nitrophenol and phenolphthalein glucuronidation were not increased by TCB administration. The maternal hepatic microsomal enzyme induction observed after TCB administration to pregnant rats suggests the presence of both cytochrome P-450 and P-448 inducers in the sample of 1,2,4,5-tetrachlorobenzene used.

**507 FREE RADICAL ACTIVITY INCREASE IN SUBCELLULAR FRACTIONS FROM PCB INDUCED RAT LIVER. D. Havkin-Frenkel, J.D. Rosen and M.A. Gallo, Dept. of Food Science, Cook College, Rutgers Univ.; Dept. of Environmental and Community Medicine, CMDNJ-Rutgers Medical School, Rutgers Univ./Rutgers Medical School Joint Toxicology Graduate Program, Piscataway, NJ Chlorinated hydrocarbons have been shown to be tumorigens in rodent species. Several of these compounds, including PCBs, do not induce mutations in in vitro systems and must be considered as possible epigenetic tumorigens. Aroclor 1254 induces mixed function oxidases in rodents and this induction may lead to the increased production of oxygen radical species. Studies were conducted in subcellular fractions to determine the ability Aroclor 1254 induced livers to form hydroxy radical ex vivo. Male Sprague-Dawley rats (100-125g) were administered Aroclor 1254, I.P., in corn oil at 0, 10,50 and 100 mg/kg/day for four (4) days. Livers were homogenized in 5 volumes of 0.15M KCl and fractions separated by differential centrifugation. Relative liver weight was increased (p < 0.01) at 100mg/kg/day as were aniline hydroxylase and o-demethylase in the post-lysosome/peroxisome supernatant fraction (approximately 7-fold and 2-fold respectively). Hydroxy radical measured by ethylene production per gram liver was increased 270% in the supernatant fraction of the high dose group. There was a dose dependent increase in lipid peroxidation in the whole homogenate (p<0.07). These data suggest that the increase in oxygen radical species from PCB is the result of the induction of mixed function oxidases.

(Sponsored in part by N.J. Agricultural Experiment Station)

**508 THE EFFECTS OF PRENATAL EXPOSURE OF HEXACHLOROBENZENE ON MIXED FUNCTION OXIDASES AND RENAL FUNCTION IN THE MINK. G.F. Rush, V. Adler, M. Bienavies, R. Aulerich and J.B. Hook, Department of Pharmacology and Toxicology, Center for Environmental Toxicology and Department of Animal Science, Michigan State University, East Lansing, MI 48824.

Hexachlorobenzene (HCB) is a ubiquitous environmental contaminant that has been shown to induce cytochrome P-450 and a variety of microsomal mixed function oxidases (MFO). Minks were administered either 0 ppm, 1 ppm or 5 ppm HCB in the diet 21 weeks prior to mating and were continued on HCB supplemented diet throughout gestation. Kits were weaned and placed on a regular diet containing no HCB and at 16 weeks of age were killed. Prenatal HCB treatment caused a dose dependent increase in hepatic cytochrome P-450 (0.09±0.01, 0.12±0.01, and 0.17±0.01 mmole/mg protein in 0 ppm, 1 ppm and 5 ppm treatment groups respectively). In contrast, microsomal ethoxyresorufin-O-deethylase (EROD) ethoxycoumarin-o-deethylase (ECOD) and benzphetamine-N-demethylation (BND) were not significantly different in any treatment groups. Renal EROD, ECOD, BND and cytochrome P-450 were not altered in any treatment groups. There were no pathological changes evident in either kidney or liver. HCB treatment had no effect on renal function as determined by blood urea nitrogen and accumulation of p-aminohippurate (PAH) and tetaethylammonium (TEA) by renal cortical slices. It is interesting to note however, that kidney slices from mink demonstrated very little ability to accumulate PAH (slice to medium S/M ratio=2.46 ± 0.12) while TEA transport was much greater (S/M TEA ratio=13.13 ± 0.96). These data indicate that prenatal exposure of minks to HCB will have residual effects on components of the hepatic MFO system. (Supported by USPHS Grant ES00560.)

**509 DOSE-DEPENDENT BIOCHEMICAL RESPONSE TO AND METABOLIC EXCRETION OF BROMOBENZENE IN RATS. S. Chakrabarti and J. Brodeur. Dép. méd. trav. et hyg. mil., Fac. méd., Univ. Montréal, Montréal, Que., Canada. Adult male rats were treated (i.p.) with different doses of bromobenzene (BB) (0.1, 0.5, 1.0, 2.5 and 5 mmole/kg) in corn oil and sacrificed 24 h after each dose. While there was no significant increase in such biochemical response at 0.1 and 0.5 mmole/kg BB dose, the activities of serum transaminases (SGOT and SGPT) attained their maximum values at 1 mmole/kg BB dose and remained fairly at the same value at higher doses of BB. In metabolic excretion studies, various urinary metabolites of BB were measured at 0-7½ h, 7½-24 h and 0-24 h periods. At each of these time intervals, neither the volume of urine nor the concentration of creatinine was affected by different doses of BB. Total nonprotein sulfhydryl contents started to increase from 0.5 mmole/kg BB and reached a fairly constant value at higher doses of BB at each time interval. Among four hydroxymetabolites of BB, the percent of o-bromophenol increased with increasing doses of BB and reached a maximum of ~37% at 5 mmole/kg dose at 0-7½ h but decreased to ~24% during 0-24 h. The m-bromophenol initially increased to ~31% at 2.5 mmole/kg BB but decreased to ~15% during 0-24 h. However, the percent of p-bromophenol remained fairly constant (~40%) even with increasing doses of BB during different time periods. The percent of p-bromocatechol decreased from 22% at 0.1 mmole BB to nearly zero at 2.5 or 5 mmole BB during 0-7½ h and then increased to a fairly constant 25% level at higher doses. The amount of total hydroxymetabolites excreted per dose of BB injected decreased
with increasing doses of BB. Thus, as the biochemical response to BB hepatotoxicity increased and attained a maximum, the % dose excreted with regard to total urinary metabolites decreased with increasing doses of BB. (Supported by MRC Grant, MA-6159).


The binding of TCDD and its congeners to a cytosolic receptor protein appears to correlate with their ability to elicit toxic effects in mammals. Differences in species susceptibility to TCDD may be related to alterations in the binding properties of these receptors. Utilizing a hydroxylapatite assay system, we determined the number of receptors, apparent equilibrium dissociation constants (Kd), and the ability of TCDD congeners to bind to hepatic and thymic cytosolic receptors from male guinea pigs, rats, hamsters, C57BL/6J, and B6D2F1/J mice. Hepatic receptor concentrations ranged from 23 to 74 fmol/mg cytosolic protein. There appears to be no relation between hepatic receptor number and the acute LD50 values for these species. However, those species (guinea pig, rat) which have been found to be most sensitive to TCDD-induced thymic atrophy showed the highest levels (47-140 fmol/mg) of thymic receptors as compared to B6D2F1/J mice which showed the lowest levels (6-10 fmol/mg). In the liver there appeared to be a general relation between receptor affinity towards TCDD and the acute LD50 values in these species. Hepatic receptors from the guinea pig showed the highest affinity (Kd = 0.6nM) while those from B6D2F1/J mice and hamsters demonstrated lower affinities (0.32-0.42 nM). A similar relationship was observed in the thymus. Also, the ability of various TCDD congeners to bind to hepatic receptors generally decreased with increasing acute LD50 values. These data suggest differences in properties of the receptor molecules may contribute to species differences in TCDD-induced toxicity. (Supported by NIH Grant ES02515 and a Grant from the Pharmaceutical Manufacturers Association.)

511 EFFECT OF ZINC ON BROMOBENZENE-INDUCED ACUTE HEPATOTOXICITY IN RATS. S. Chakrabarti and J. Brodeur, Dép. méd. du travail et hyg. du milieu, Fac. Méd., Univ. de Montréal, Montréal, Qué., Canada H3C 3J7

When adult male rats were treated with 0.5, 2, and 10 mg/kg (i.p.) of zinc chloride (ZnCl2) 24 h prior to the i.p. injection of 2.5 mmole/kg of bromobenzene (BB) and sacrificed 48 h after the BB dose, significant increases in the levels of activities of the serum transaminases (SGOT and SGPT) from those of bromobenzene-alone-treated rats were observed at 0.5 mg/kg of ZnCl2 whereas an increase (but not significant) in such activities were noticed at 2 mg/kg of ZnCl2. Pretreatments of rats for 48 h with the above doses of ZnCl2 however produced no such effect. Treatment of rats with 2 mg/kg ZnCl2 6 h prior to the administration of 2.5 mmole/kg BB resulted in significant elevation of SGPT activity only, but no such effect was observed when these were administered simultaneously. But treatment with 2 mg/kg ZnCl2 6 h before the administration of 1 mmole/kg BB or simultaneously with BB produced a reduction in the activities of the transaminases (although not significant) from those of BB-alone-treated rats. When rats were fed 50, 250 and 500 ppm of ZnCl2 in drinking water daily for four weeks prior to the injection of 2.5 mmole/kg BB and sacrificed 48 h after the BB dose, a reduction in the SGOT activity and a tendency towards reduction in the SGPT activity were noticed in 250 ppm treated rats only. Liver microsomal system showed an increase in the concentration of microsomal proteins and the activity of aminopyrine N-demethylase in such chronically treated rats. Thus changes in the hepatotoxicity of BB depend not only on the dose of zinc but also on the time of its exposition. (Supported by MRC Grant, MA-6159).

512 HEXACHLOROBUTADIENE NEPHROTOXICITY IN PIPERONYL BUTOXIDE TREATED RATS. M.E. Davis, Dept. of Pharmacol. and Toxicol., West Virginia Univ. Medical Center, Morgantown, WV 26506 (Sponsor: M.J. Reesor)

Hexachlorobutadiene (HCBD) is a by-product of chlorinated ethylene synthesis. Treatment with HCBD causes acute renal failure and degeneration of the straight portion of the proximal tubule. HCBD is metabolized by rats, possibly by oxidation and probably by conjugation with glutathione (GSH). The present studies examined the effects of blocking mixed function oxidase (MFO) activity on the development of nephrotoxicity. Male, Sprague-Dawley derived rats were maintained in stainless steel metabolism cages for daily urine collection. Piperonyl butoxide (PIP; 50 mg/kg) was administered i.p. 60 min before HCBD (300 mg/kg). Renal function was assessed after 5 hr, 1 and 2 d. Renal blood flow (RBF) was decreased in both groups at 5 hr and then returned to control values by 2 d. At each time RBF was decreased slightly, but not significantly, more in the HCBD group compared to PIP+HCBD. Glomerular filtration rate was concomitantly reduced and was not affected by pretreatment with PIP. Blood urea nitrogen was significantly elevated on both days and was not influenced by PIP pretreatment. HCBD treatment decreased urine osmolality and caused glucosuria equally in control and PIP pretreated rats. Hepatic GSH concentrations was not decreased by PIP and PIP pretreatment did not block the HCBD-induced decrease of GSH concentration. These data suggest that activation of HCBD by MFO is not necessary for development of nephrotoxicity. (Supported by WVU Medical Corp. and NHI Biomedical Research Grant 5 S07-RR05433-18)

513 HEXACHLOROCYCLOPENTADIENE (HEX) DISPOSITION AND METABOLISM IN RATS. C. C. Yu, and Y, H. Atallah, Research and Development Department, Velsicol Chemical Corporation, Chicago, IL 60611

Hex is an intermediate for the synthesis of flame retardants and cyclodiene insecticides. 14C-Hex was administered to both male and female Sprague-Dawley rats by oral intubation (po, 10-25 mg/kg) and to female rats by intravenous injection.
EFFECTS OF CHRONIC TREATMENTS WITH MERCURY OR SELLNIIUM ON BROMOBENZENE-INDUCED ACUTE HEPATOTOXICITY IN RATS. S. Chakrabarti and J. Brodeur. Dép. Méd. du travail et hyg. du milieu, Fac. Méd. Univ. de Montréal, Montréal, Qué., Canada H3C 3J7

Our previous studies have shown that acute treatment with mercury or selenium could protect the bromobenzene (BB)-induced acute hepatotoxicity in rats (Fed. Proc. 40: 738, 1981). In this study, adult male rats were fed 10, 50 and 100 ppm of mercuric chloride in drinking water daily for four weeks prior to the administration of BB (2.5 mmole/kg, i.p.) in corn oil and sacrificed 48 h after the BB dose. Increased levels of the activities of serum transaminases (SGOT and SGPT) as induced in the BB-alone-treated rats were neither reduced nor further increased due to such mercury pretreatments. No changes in the liver microsomal enzyme activities were noticed in such mercury-treated rats. Thus the different action of mercury on the hepatotoxicity of BB depends on the nature of exposition of the metal among other factors. On the other hand, when rats were given 2.5, 10 and 20 ppm of sodium selenite in drinking water daily for four weeks prior to the injection of 2.5 mmole/kg BB and were sacrificed 48 h after the BB dose, increased level of SGOT activity in BB-alone-treated rats was significantly reduced in 2.5, 10 and 20 ppm selenite pretreated groups, whereas the activity of SGPT was reduced in 20 ppm selenium pretreated group, although tendency towards such a reduction was noticed in other two pretreated groups. Liver microsomal enzyme activities did not change except an increase in the aminopyrine N-demethylase at 2.5 ppm of selenium. Thus both acute and chronic pretreatments with selenium could reduce the hepatotoxicity of BB. Based on existing information, such protection against BB hepatotoxicity may be attributed to an induction of increased hepatic glutathione levels by selenium. (Supported by MRC Grant, MA-6159).

EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON RNA POLYMERASE I INDUCTION IN RAT LIVER. C.L. Potter, L.G. Sipes and D.H. Russell, Toxicol. Prog. and Depts. of Pharmacol. and Anesthesiol., Univ. of Arizona, Tucson, AZ 85724.

Previously, we have reported that pretreatment of rats with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits the extent of hepatic induction of ornithine decarboxylase, the rate limiting enzyme in biosynthesis of the polyamines (putrescine, spermidine and spermine). Since increased hepatic ornithine decarboxylase activity has been positively correlated to a subsequent increase in RNA polymerase I activity, we examined the effects of TCDD on RNA polymerase I induction. Male Sprague-Dawley rats (100-150 g) received a single i.p. injection of 5 µg/kg TCDD in acetone:corn oil (1:17). Controls received vehicle only. Hepatic RNA polymerase I activity was subsequently stimulated 25-50% by partial hepatectomy or by administration of xemethasone (0.5 mg/kg) in normal saline. At 5 hr or 16 hr after partial hepatectomy, or 4 hr after dexamethasone, times at which TCDD has been shown to strongly inhibit ornithine decarboxylase induction, animals pre­treated one week earlier with TCDD exhibited RNA polymerase I activity 20-50% lower than controls; i.e. TCDD-treated rats exhibited 0-50% as much stimulation of RNA polymerase I activity as did the controls. In unstimulated liver, RNA polymerase I activity, as well as protein DNA, RNA and spermine levels, remained essentially the same in control and TCDD-treated rats one week after treatment. However, TCDD-treated rats had significantly lower putrescine and spermidine concentrations. These results indicate that TCDD pretreatment may prevent a normal growth­stimulated expression of ornithine decarboxylase and RNA polymerase I activities. (Supported by CA-14783 and 5-T32-ES07091.)


2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an extraordinarily potent toxin, teratogen and carcinogen. In exposed humans, chloracne, a skin disorder characterized by altered patterns of growth and differentiation in the epidermis and its appendages, is one of the most common clinical findings. In initial studies of the molecular mechanisms of toxicity, several human cell lines derived from squamous cell carcinomas (SCC) of epidermal and oral epithelia have been surveyed for their response to TCDD. The cells were grown in the presence of a lethally irradiated feeder layer of mouse 3T3 cells in Dulbecco-Vogt modified Eagle's medium supplemented with 5% fetal calf serum. Under conditions of normal growth with continuous exposure to various concentrations of TCDD (10-11 to 10-7 M) for 3 wks, colony expansion

Ototoxic interaction of CHL and noise was first observed by D.W. Glenn (M.S.thesis,'79) in audio-
indicate a difference in bioavailability in the preps. Thus, low dose oral CHL following brief noise exposure results in transient or permanent ototoxicity. Bioavailability of CHL preps may account for the observed difference in duration of ototoxicity.

A MATHEMATICAL MODEL TO STUDY THE TIME COURSE AND DOSE-DEPENDENT IN VIVO EFFECT OF TOCP ON CHICKEN BRAIN NEUROTOXIC ESTERASE ACTIVITY. S.A. Soliman, D. Svendsgaard, and L.W. Reiter, Neurotoxicology and Biometry Divisions, Health Effects Research Laboratory, US EPA, Research Triangle Park, NC 27711.

The time course and dose-dependent in vivo effect of TOCP on hen brain neurotoxic esterase (NTE) were studied. Hens were given a single oral dose of 0 (corn oil only), 50, 100, 250, 500 or 750 mg/kg of TOCP. On days 1, 2, 3, 7, 10, 14, 21 and 28 after treatment 3 hens per group were sacrificed and brain NTE activity levels were determined. Hens were also observed daily for abnormalities in gait. Results indicated that dose levels of 250 mg/kg or greater induced signs of delayed neuropathy. For each dose of TOCP peak inhibition of brain NTE activity, computed as percentage of each corresponding control mean, occurred on day 2 and the effect was dose-dependent up to 10 days after treatment. By 21 days post-treatment all treatment groups had returned to greater than 90% of control. Mathematical analysis of these data indicate that the level of NTE activity following TOCP administration can be described by the following equation:

$$E = 100 \left( 1 - \frac{k_a}{k_e} - \frac{k_a}{k_e} \left( \exp \frac{k_a - \exp k_e}{C_2} \right) \frac{D C_1}{C_0 + C_1} \right)$$

where E is the percent NTE activity, t is the time (in days) following exposure, D is the dose in mg/kg and $k_a$, $k_e$, $C_1$, and $C_2$ are constants determined from the data. Johnson (J. Environ. Sci. Hlth B15:823-841, 1980) has reported that OP-induced delayed neuropathy develops only after 80% inhibition of NTE has occurred; accordingly, the minimum ataxia dose of TOCP in the hen is estimated to be 143 mg/kg.

NEUROBEHAVIORAL EFFECTS OF REPEATED EXPOSURE OF MICE TO ORGANOPHOSPHATE INSECTICIDES. R.C. MacPhail and S.A. Soliman, Neurotoxicology Division, US EPA, Research Triangle Park, NC 27711.

These studies evaluated the effect of 30-day exposures to TOCP (T), parathion (P) and cyanophos (C) on the motor activity of mice and on brain levels of acetylcholinesterase (AChE) and neurotoxic esterase (NTE). Mice were tested at five-day intervals and received all treatments p.o. after the test session. In different experiments, mice (N=5-10) received OP dosages that were 4%, 10%, 25%, 37.5% or 50% of the acute LD50s previously determined in our laboratory. Each experiment also included mice that received either no treatment or the corn-oil vehicle.

None of the OPs produced an appreciable effect on motor activity at dosages that were 25% or less of the appropriate LD50, whereas at 50% of the appropriate LD50 all mice died within a few days of exposure. At dosages that were 37.5% of the appropriate LD50 both T and P substantially reduced motor activity; these effects were maximal on the 10th day of dosing and did not change over the next 20 days of dosing. The effects of T and P were also proportionately comparable on both ambulation and rearing. C produced no noticeable effects on motor activity throughout the dosing regimen. Moreover at 37.5% of the LD50, T, P and C inhibited brain AChE by 22, 87 and 65%, and NTE by 56, 0 and 49%, respectively.

These results suggest that certain OP insecticides produce enduring effects on the motor activity of mice which appear to be unrelated to AChE or NTE levels in brain. In addition, the proportionate constancy of effects on ambulation and rearing suggests that motor impairment produced by OPs in mice is not related to a hindlimb weakness similar to that observed in many other species.

PLASMA ACETYLCHOLINESTERASE ACTIVITY—A POTENTIAL MARKER OF PERIPHERAL NEUROTOXICITY. B.F. Bass and A.M. Goldberg, Division of Toxicology, The Johns Hopkins University, Baltimore, Md.

Increases in plasma acetylcholinesterase (AChE) activity have been associated with various naturally occurring neuromuscular disorders. The following studies were undertaken to examine whether chemically induced disorders would result in a similar increase and, if so, would this increase precede overt toxicity. Two well known neurotoxic compounds, 2,5-hexanediol and acrylamide, were chosen.

Sprague-Dawley rats were exposed via their drinking water to 0.5% 2,5-hexanediol for up to nine weeks. By week 6, 12% of the animals exhibited signs of the neuropathy and by week 8, all treated animals had developed peripheral motor disturbances. Plasma AChE activity was found to differ significantly between control and treated groups (p<.01). At week 4, plasma AChE activity in the treated group was elevated. This upward trend continued, reaching statistical significance at week 10 (p<.01). In the second study Sprague-Dawley rats were initially administered 3 daily doses of acrylamide (50mg/kg). Two days later, daily injections were resumed for six days, but at half the dose (25mg/kg). By day 6 serum AChE activity was found to be significantly higher in the treated animals than in controls (p<.01), at a time when only 29% of the treated animals exhibited overt signs of peripheral nerve toxicity. Not until day 12 did all of the treated group show motor dysfunction.

In conclusion, the above studies are further evidence that plasma AChE activity is a marker of neuromuscular disorders, whether chemically induced or naturally occurring. The data further suggest that plasma AChE activity is a potential early indicator of peripheral neurotoxicity and would be a useful tool in toxicity testing. Supported in part by NIEHS 07067 & 00454.


Inhibition and aging of neurotoxic esterases (NTE), a phenyl valerate (PV) hydrolase, by organophosphorus compounds results in a delayed neuropathy in sensitive species. Rats and hens differ in their sensitivity to the neurotoxic actions of these compounds. The purpose of the present study was to compare the distribution of NTE in discrete homologous areas of the rat and hen nervous system. Male, Sprague-Dawley rats and adult, white Leghorn hens were decapitated and homogenized. NTE activity was determined by the differential titration of PV esterases using paraoxon and mipafox. In the rat, highest activity (in mmol/mg protein) was in the hypothalamus (9.1 ± 0.9) and lowest in the hippocampus (5.0 ± 0.5). Intermediate values were found for the brainstem (8.0 ± 0.6), lumbar cord (7.1 ± 1.1), amygdala (7.0 ± 0.5), thoracic cord (6.9 ± 0.2), cervical cord (6.3 ± 0.3), cerebral cortex (5.8 ± 0.6), caudate nucleus (5.5 ± 0.3) and cerebellum (5.2 ± 0.7). In the hen highest activity was in the cerebral cortex (19.6 ± 1.1) and the area homologous to the rat caudate nucleus (19.5 ± 1.5) whereas lowest activity was in the lumbar (4.2 ± 0.4) thoracic (2.8 ± 0.2) and cervical (2.2 ± 0.1) spinal cord. Intermediate values were found in the area homologous to the rat amygdala (15.1 ± 0.6), hippocampus (15.0 ± 1.3), cerebellum (13.9 ± 1.3), hypothalamus (9.7 ± 0.5) and brainstem (8.6 ± 0.4). These differences in distribution of NTE activity in the nervous system of the two species may be related to their differential sensitivity to the neurotoxic actions of organophosphorus compounds. (Supported by NIH grant ES01611-03)

525 ACUTE AND CHRONIC TOLERANCE TO ORGANOPHOSPHORUS INSECTICIDES. L. G. Costa, B. W. Schwab and S. D. Murphy, Div. of Toxicology, Univ. of Texas Med. School, P.O. Box 20708, Houston, TX, 77025.

Male mice, treated for two weeks with the insecticide disulfoton (0,0-diethyl 2-ethylthioethyl phosphorodithioate; 10 mg/kg/day) became tolerant to the hypothermic and antinociceptive effects of a single injection of disulfoton and the muscarinic agonist oxotremorine. Tolerant animals presented a reduced binding of the specific muscarinic antagonist (3H)-quintuxidyl benzilate (3H-QNB) in central and peripheral tissues. In forebrain, the number of receptors (Bmax) was decreased 40% with no change in the affinity constant. Acetylcholinesterase (AChE) activity was 15% of control. 48h after a single injection of disulfoton (10 mg/kg) mice were more resistant than control to the hypothermic and antinociceptive effect of a second administration of the same insecticide and to oxotremorine. Tolerance was not present 96h after a single administration of disulfoton. AChE activity was 65 and 27% inhibited after 48 and 96h, respectively. The first injection of disulfoton gave a 74% inhibition of AChE activity after 4h. Similar degrees of inhibition (73 and 72%) were found after a second injection at 48 or 96h. (3H)-QNB binding did not differ from control at either time point while high affinity binding of the muscarinic agonist (3H)-oxotremorine was significantly decreased (-3%) 48h after disulfoton. These results show that in chronic tolerance there is a decrease of (3H)-QNB binding, possibly indicating a loss of central muscarinic receptors. In acute tolerance antagonist binding is unaffected and only a decrease in agonist binding was found, indicating a differential regulation of muscarinic receptors depending on duration of exposure. (Supported by NIH grant # ES 01831 and Training Grant GM 07405).

526 EVIDENCE FOR LEPTOPHOS-INDUCED NEUROPATHY IN CHICKS EXPOSED ON INCUBATION DAY 14. L. P. Sheets and S. Norton, Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS 66103.

It has been reported that the young of all sensitive species are resistant to organophosphate-induced delayed neuropathy (OPIDN) and that chicks become sensitive around 60 to 70 days of age. OPIDN is characterized as a distal axonopathy with symptoms first becoming apparent 7 to 14 days following treatment. A single 250 mg/kg oral dose of leptophos (an organophosphate insecticide) injections at 48 or 96h. (3H)-QNB binding did not differ from control at either time point while high affinity binding of the muscarinic agonist (3H)-oxotremorine was significantly decreased (-3%) 48h after disulfoton. These results show that in chronic tolerance there is a decrease of (3H)-QNB binding, possibly indicating a loss of central muscarinic receptors. In acute tolerance antagonist binding is unaffected and only a decrease in agonist binding was found, indicating a differential regulation of muscarinic receptors depending on duration of exposure. (Supported by NIH grant # ES 01831 and Training Grant GM 07405).
ALTERATION OF TISSUE COENZYME-A LEVELS DURING ACRYLAMIDE INTOXICATION.  M.J. Miller, M.S. Miller, I.G. Sipes and D.E. Carter.  Toxicology Program and Dept. of Pharmacology, University of Arizona, Tucson, AZ 85721.

Chronic low-level exposure to the neurotoxin acrylamide (ACMD) produces central-peripheral distal axonopathy. The mechanism of ACMD-induced neuropathy is currently unknown, but has been postulated to occur through inhibition of the glycolytic-TCA pathway. Schoental and Cavanagh (1977) suggested that ACMD may bind directly to coenzyme-A (CoA) and thus decrease the available CoA content required for neuronal metabolism and maintenance of axon integrity. To test this hypothesis, CoA content of neural and non-neural tissues during ACMD intoxication has been measured. Sprague-Dawley rats (300 g) received daily doses of ACMD (50 mg/kg/day ip) or saline. Rats were sacrificed after cumulative ACMD doses of 50, 150, 250 and 350 mg/kg. Cerebral cortex, cerebellum, spinal cord, heart and liver were removed immediately and assayed for CoA content by the radioenzymatic assay of Chan and Ebadi (1981). Neuropathy was assessed by measuring the duration of response to a 1% zingerone solution applied to the eye. Prolonged response to zingerone was detected following cumulative ACMD doses of 150 mg/kg or greater. No depletion of tissue CoA content was observed at times corresponding with the development of neuropathy. Tissues demonstrated a significant (p < .05) increase in CoA content after a cumulative ACMD dose of 250 mg/kg. These data demonstrate that the mechanism by which ACMD induces axonopathy does not involve depletion of CoA. Elevated levels of CoA detected suggest impaired utilization of CoA during the development of axonopathy. (Supported by a Graduate Student Development Award to M.J.M. and NIEHS ES-82130.)

Three phenylphosphonate derivatives have been synthesized and tested for toxicity in hens. Two of the compounds, O-ethyl S-benzyl phenylphosphonothioate (Inezin) and O-methyl O-benzylidene-phenylhydrazone phenylphosphonothioate, which are used as fungicides, had low acute toxic effects in hens. LD50 values were greater than 2000 and 1000 mg/kg, respectively. No signs of delayed neuropathy were detected in hens that received Inezin or the hydrazone compound after single oral doses of 2000 or 1000 mg/kg were administered. On the other hand, O-n-propyl O-(2-propynyl) phenylphosphonate (also known as NIA-16388) which has been recommended for use as an insecticide synergist, was found to be relatively toxic in hens; its 24-hr LD50 value was approximately 340 mg/kg. This compound produced delayed neuropathy in hens given an oral dose of 400 mg/kg and pretreated with atropine sulfate in order to minimize cholinergic effects. The clinical signs of neurotoxicity were apparent 12-17 days after treatment and were similar to those produced by tri-o-cresylphosphate (TOCP). Degenerative lesions of axons were observed in the sciatic nerve and spinal cord of hens sacrificed 30 days after treatment with the NIA-16388 and resembled those produced by TOCP and similar compounds. In addition, administration of NIA-16388 inhibited hen brain neurotoxic esterase by 90% at 24 hrs after administration. These results indicate that the organophosphorus insecticide synergists should also be tested for their ability to produce delayed neuropathy in hens.
ever, concentration-dependent NTE inhibition occurred (0.12-12 uM) when metabolic activation was used (35% inhibition at 12 uM leptophos). The procedure described has usefulness for (1) screening delayed neurotoxicity of small quantities of research organophosphorus esters and (2) studying metabolic mechanisms for delayed neurotoxicity. Preliminary results suggest leptohiphosphorothioate requires metabolic activation to produce delayed neurotoxicity.

530 ASSESSMENT OF FENITROTHION NEUROTOXICITY IN THE HBN. H.D. Durham and D.J. Ecochelchon, Dept. of Pharmacol. Ther., McGill U., Montreal, CANADA

A report of human poisoning by fenitrothion, (O-C-dimethyl O-(4-nitro-m-toly1)phosphorothioate) suggested that this agent caused a persistent neuropathy. To test this hypothesis, groups of fasted adult white Leghorn hens were treated with single, toxic doses of fenitrothion (500 mg/kg), TOTP (500 mg/kg) or trichlorfon (100 mg/kg) in peanut oil or aqueous solution by oral gavage. At post-treatment intervals of 1, 7, 14, 28, 42 and 56 days, birds were anesthetized with chloralose (100 mg/kg). Maximal conduction velocity of the sciatic nerve and post-tetanic potentiation (PTP) of the soleus muscle contraction to nerve stimulation at 0.4Hz were measured as indices of peripheral nerve and muscle function. To elicit tetani, the sciatic nerve was stimulated at frequencies of 50-200Hz. The birds were killed by exsanguination and brain and spinal cord AChE and neurotoxic esterase (NTE) were assayed as well as hepatic and renal carboxylesterase (CB) and plasma pseudocholinesterase (PChE). No change in maximal conduction velocity was observed in any treatment group although TOTP-treated birds were clinically neuropathic. No inhibition of PTP was observed after treatment with fenitrothion or trichlorfon but results with TOTP were variable. TOTP caused marked reductions in NTE, little effect on AChE but inhibited PChE and tissue CE at days 1 and 7 of post-treatment. Fenitrothion and trichlorfon had no effect on NTE at any post-treatment interval but markedly reduced brain and spinal cord AChE, plasma PChE and hepatic and renal CE at days 1 and 7. The results demonstrate that, unlike TOTP, fenitrothion (and trichlorfon) administered as single toxic doses did not cause a persistent peripheral or central neuropathy detectable by the parameters measured.


Clinical and epidemiological evidence has implicated toluene as a potential neurotoxicant associated with solvent abuse by humans, especially adolescents, but controlled laboratory verification in animals is lacking. Therefore, we assessed the neurotoxic potential of toluene compared with that of the known neurotoxicant hexane, which is also present in abused solvent mixtures. Male weanling rats (Fisher-344) were exposed by inhalation to toluene (900 or 1400 ppm) or hexane (2000 ppm) 14 hr/day, 7 days/week for 14 weeks. Both solvents inhibited weight gain. Hexane caused a neurotoxic syndrome characterized by reduced grip strength (esp. hindlimb), motor activity, and startle responses. Initial acquisition of a conditioned avoidance response (CAR) was also impaired, but subsequent performance was intact. Toluene did not cause the peripheral motor symptoms associated with exposure to hexane. However, CAR acquisition and performance was impaired along with a tone intensity discrimination test. The latter effect was still evident several weeks after the last exposure to toluene. These results can be interpreted to suggest that toluene may cause a persistent cognitive dysfunction without the peripheral symptoms caused by hexane. (Supported by NIDA Contract No. 271-80-3712)


Although the most apparent deficit accompanying alcohol exposure is in motor control, sensory disturbances have been reported. For example, methyl alcohol affects persistence in the auditory system, disrupting a rat's ability to detect gaps in white noise, as measured by reflex modification. The present experiments examined gap detection following ethyl alcohol poisoning, and, further, its interaction with organic mercury poisoning. Rats (n=8) were given progressive doses of 0.00, 0.25, 1.00, and 2.00 g/kg (IP, 16% w/v solution) at one hour intervals. Thirty minutes after each injection, gap detection was assessed by presenting gaps of 0, 2, 4, 6, 8, 12, and 20 msec in white noise, 190 msec before a startle eliciting tone burst. As with methyl alcohol, the effect of the gap progressively declined with dose level to methyl alcohol. The size of the control startle reflex was significantly decreased by 85% (twice the effect of methanol). To examine the interaction between methyl alcohol and methyl mercury poisoning, 15 rats (6 control, 6 with 13 mg/kg, and 3 with 40 mg/kg, methyl mercury chloride) received the dosing regimen. All animals showed a similar decline in gap detection with increasing dose, and no interactions were found. Differential behavioral responding under these two compounds may reflect differences in metabolism and compartmentalization resulting from the differences in molecular structure. These results are part of an ongoing effort to characterize behavioral performance following exposure to alcohols of different carbon length. Supported by NEIHS grant ES01248.


This study was undertaken to assess the role of esterase activity on the action of heroin in the
mouse and on the binding of heroin to opiate receptors in rat brain homogenate. Prior inhibition of peripheral esterase activity by triorthotolylphosphate (TOTP), 125mg/kg, ip 18 hr before test, in male mice, increased the analgetic potency of heroin but not of morphine. The potency of 6-mono-acetyl morphine (6-MAM) was only marginally increased. The ED50 of heroin, by the acetic acid-induced writhing test, without esterase inhibition was 0.24mg/kg sc (0.20-0.28, 95%CI) and following peripheral (but not central) esterase inhibition was 0.042mg/kg (0.024-0.074). Peripheral plasma cholinesterase (CHE) and carboxylesterase activities were inhibited 80 and 93% respectively, while brain CHE was unaffected by TOTP pretreatment. This suggests that peripheral esterases hydrolyze the acetyl group at position #3 of heroin while prevention of this hydrolysis by TOTP increased the lipophilicity, CNS-penetrability and potency of heroin. In opiate receptor binding studies, heroin displaced 3H-ethorphine (IC50 = 3x10^-7 M). The addition of paraoxon to the incubation media resulted in a lower potency (IC50 = 1.6x10^-6 M). Morphine or 6-MAM without paraoxon yielded IC50 values vs ethorphine, of 2x10^-7 M and 9.2x10^-8 M respectively; the addition of paraoxon failed to significantly alter these binding constants. Results suggest that esterase activity in the binding preparation deacetylates heroin at position #3 and that this hydrolysis is necessary for optimal opiate binding. (Supported by NIDA Grant #DA-016112).

534 CONDUCTION VELOCITIES OF SENSORY AND MOTOR NERVES IN GALACTOSE NEUROTOXICITY. D.A. Delio and H.E. Lowndes, Dept. Pharmacology, CMDNJ, NJ Medical School, Newark, NJ.

Abnormalities of sensory nerve conduction are an early feature of diabetic neuropathy; such changes are consistent indicators of subclinical neuropathy. Galactosemic diabetic neuropathy is characterized by an accumulation of galactitol, accompanied by osmotic swelling in sciatic nerves. Although impaired motor nerve conduction velocity (MNCV) has been detected in this experimental diabetic neuropathy, a corresponding defect in sensory nerve conduction velocity (SNCV) has not been investigated. Sprague-Dawley rats (250-300 gms) were fed a 40% galactose diet for 3 weeks. Conduction velocities of single sensory and motor axons between sciatic or tibial nerves and dorsal or ventral roots were recorded and compared to age and weight matched controls. No significant differences between age matched control and galactosemic animals were observed. However, when compared to weight matched controls, both sensory and motor conduction velocities in whole nerve trunks and single fibers were decreased (p<0.05); the decrease in whole nerve SNCV was more pronounced for both nerves (p<0.05). After terminating the galactose diet for one week, sensory and motor conduction velocities recovered in sciatic and tibial nerves, with values approaching or exceeding those of controls. These data suggest that galactose feeding induces a reversible peripheral neuropathy which encompasses sensory and motor fibers, both proximally and distally.


Adult exposure to triethyltin (TET) produces degeneration of white matter, edema, vacuolation of myelin and histotoxic hypoxia (Magee et al., 1957) and visual evoked response (VER) disturbances (Dyer et al., 1981). Perinatal TET exposure also produces changes in the adult VER (Dyer et al., 1981). Acute adult trimethyltin (TMT) exposure produces selective lesions within the limbic system as well as VER changes (Dyer et al., 1981). In this study, the VER was used to study visual system development following perinatal exposure to either TET or TMT. On postnatal day 5, Long-Evans hooded rat pups were injected ip with either 0, 3 or 6 mg/kg TET bromide in saline or intubated with 0, 5, 6 or 7 mg/kg TMT chloride in saline. On postnatal days 12 and 21, pups were anesthetized (Chloropent) and tested using the VER. Averaged responses to single and paired-flashes (500 msec flash separation) were derived. In the single-flash paradigm, TET exposure increased day 12 P1 latency and day 21 P1N1 peak-to-peak amplitude. TMT exposure on the other hand increased day 12 N3 latency and day 21 P1N1 and N1P2 amplitudes. No significant TET or TMT differences were observed for responses recorded during the paired-flash paradigm, suggesting that visual system recovery function was not impaired. The VER changes observed on days 12 and 21 in this study are different from those seen in adult animals following day 5 TET exposure (Dyer et al., 1981), suggesting a progression in the nature of the TET visual system toxicity during postnatal development. The qualitative differences in the VER changes following day 5 TET and TMT exposure suggest different sites and mechanisms of action for these structurally similar organotin compounds.


Comparative effects of aldrin, dieldrin, telodrin, endrin and isodrin on different ATPase activities in beef heart mitochondrial (BHM) and rat brain synaptosomal (RBS) fractions were determined in vitro. BHM fractions were prepared by the conventional centrifugation method and the RBS were prepared by Ficol-Sucrose gradient centrifugation method. Na+K+ ATPase, oligomycin-sensitive (0.5) and insensitive (0.1) Mg2+ ATPases, and K+-paranitrophenyl phosphatase (KPNPase) were determined in RBS and only the Mg2+ ATPase activities were determined in BHM. Dose-response curves were determined by assaying the enzyme activities in the absence and presence of 10-120 µM concentrations of each test chemical. BHM (0.5) Mg2+ ATPase activity was inhibited by all 5 chemicals tested. Aldrin and telodrin were the most potent inhibitors with an ID50 of 40 and 80 µM respectively. About 30% inhibition was observed with dieldrin, endrin and isodrin and the inhibition
was not dose-dependent. 0.1 Mg\(^{2+}\) ATPase was not significantly inhibited by any chemical except aldrin. RBS ATPases were also sensitive to these compounds. Aldrin and telodrin were more effective than others. A 50% inhibition of 0.5 Mg\(^{2+}\) ATPase activity was obtained at 80 µM of aldrin and telodrin. Na\(^{+}\)K\(^{+}\) ATPase and O.I Mg\(^{2+}\) ATPase activities showed a maximum inhibition of 40% at the highest concentration tested for aldrin and telodrin. KPNPase was not inhibited significantly by any compound tested. These results suggest that ATPase system in heart and CNS may be selectively inhibited by aldrin and telodrin but not by their structural analogs. (Supported by PHS Grants ES-02443 awarded to D.D. and RR-00810 awarded to B.D.M.)


The clinical effectiveness of nitroimidazole hypoxic cell radiosensitizers such as misondazole (MIS) and metronidazole (METRO) is considerably reduced by the dose limitation imposed by neurotoxic side effects. The purpose of this investigation was (i) to determine if data on the relative neurotoxicity of nitroimidazole sensitizers in mice using different quantitative functional endpoints were similar, (ii) to compare misondazole (MIS) with desmethylnicotidazole (DESMIS) in relation to central nervous system (CNS) versus peripheral nervous system (PNS) effects and, (iii) to determine potential mechanism(s) of neurotoxicity. The data indicate good agreement between the functional endpoints used to determine relative neurotoxicity (rotorod performance and high frequency hearing loss) and the incidence of CNS and PNS pathology in mice. In general, CNS and PNS lesion development coincided suggesting these effects are separate. MIS is 2 times more toxic per administered dose and equitoxic per brain exposure dose compared with DESMIS in BALB/cka mice. DESMIS is slightly more toxic than MIS to the CNS of C3H mice. DESMIS given i.v. resulted in more severe PNS lesions than either i.p. or oral DESMIS or i.p. or oral MIS. Significant decreases in the lactate/pyruvate ratio of the brain stem of MIS treated mice indicates that inhibition of glycolysis is a potential mechanism of neurotoxicity. Results are evaluated in relation to their predictive significance to clinical testing of sensitizers. (Supported by National Institute of Health Grants NS-16147 and CA-11051 and the United Cancer Council, Rochester, New York.)
Groups of 5 adult hens were exposed to vapors of n-hexane, methyl iso-butyl ketone (MiBK) or mixtures of n-hexane and MiBK. Inhalation exposure of either 1,000 ppm n-hexane, 2,000 ppm n-hexane or 1,000 ppm MiBK caused leg weakness during the 90-day exposure. The clinical condition of these birds improved during the 30-day observation period after their exposure to the vapors. These hens lost weight during treatment, but regained most of the lost weight after cessation of exposure. By contrast, simultaneous exposure to mixtures of 1,000 ppm n-hexane and 1,000 ppm or 500 ppm MiBK caused leg weakness and ataxia which progressed to paralysis. Although the clinical condition of these hens slightly improved during the 30-day observation period, more of these hens remained paralyzed at the end of the experiment. The neurological dysfunction was accompanied by swollen axons and by degeneration of axons and myelin of the spinal cord and peripheral nerves. These hens lost weight during exposure but regained some of the lost weight during the observation period. Hens simultaneously exposed to 1,000 ppm n-hexane and 100 ppm MiBK were not different from those exposed only to 1,000 ppm n-hexane. The mechanism of the joint neurotoxic action of n-hexane and MiBK seems to be related to liver microsomal induction in treated animals. (Supported in part by NIOSH Grant No. OH00823).

Four groups of hens (5 hens/group) were treated for 90 days with a daily dermal dose of 1.0 mg/kg of EPN (85%) in 0.1 ml of acetone on the unprotected back of the neck. Three of these groups were exposed simultaneously to 100, 50, or 10 ppm of MBK (methyl n-butyl ketone : methyl iso-butyl ketone 7:3) - in inhalation chambers. One group was treated only with the dose of EPN. Three other groups of hens were exposed continually in inhalation chambers to the same concentrations of MBK without EPN treatment. The treatment period was followed by a 30-day observation period. Combined exposure to both EPN and MBK caused more severe signs of neurotoxicity than exposure to either individual compound. The severity of histopathological lesions in hens given daily dermal doses of 1 mg/kg EPN in combination with inhaled MBK depended on the MBK concentration. There clearly were more lesions in the 100 ppm MBK : 1 mg/kg EPN group than in the others. In most hens in this group, Wallerian degeneration was seen along with large paranodal axonal swellings. The morphology and distribution of histopathologic lesions were characteristic of MBK-induced lesions. In the 50 ppm MBK : 1 mg/kg EPN group axonal swelling was observed but was not clearly identifiable as paranodal. Hens treated with 10 ppm MBK : 1 mg/kg EPN had minimal unequivocal lesions with rare axonal swellings. These were not as large as those seen in MBK neurotoxicity, but instead resembled the histopathologic lesions caused by EPN. (Supported in part by NIOSH Grant No. OH00832).

Hexane inhalation can cause neurotoxicity, apparently through formation of the metabolite 2,5-hexanediol (HD). In previous work, we had shown (J. Neurotox. Text., in press) that in adult rats, sub-chronic hexane exposure led to increasing HD blood levels over several days, apparently due to induction of liver microsomal enzymes. When blood HD levels were maintained at 50 µg/ml or more by chronic hexane exposure, detectable neuropathological changes occurred within 4-8 weeks. A further question remained as to the neurotoxic potential of hexane in young, prepuberal animals, since liver metabolic enzyme levels often increase during development (and adolescents are a primary human population of solvent abusers). We exposed weanling male Fischer 344 rats to 1000-2000 ppm of hexane on various schedules, in acute and chronic experiments, including a direct comparison to adult males. HD was synthesized well in weaning rats, and its levels increased in blood for several days of constant exposure to hexane at 1000 or 2000 ppm, as in adult males. However, HD levels in the blood of weanlings were about 50 to 80% of those in adults with the same exposure. The subacute hexane toxicity appeared correspondingly less in the weanlings than in the adults. In contrast, in a chronic exposure, rats exposed to hexane from weaning to adulthood developed neuropathology at a rate consistent with that in rats exposed first as adults, with an increase in liver and decrease in testicular weights, and decrease in weight gain similar to those observed in young adults. (Supported by NIDA Contract No. 271-80-3712.)

Clinical signs of neuropathology associated with the abuse of volatile solvents containing toluene, hexane and other compounds indicates a need to independently determine the neurotoxic effects of components of solvent mixtures. Hexane has been extensively studied in the laboratory but rigorous, experimental verification of the neurotoxicity of toluene is lacking. We compared the effects of toluene and hexane on several electrophysiologic indicators of peripheral and central nervous system activity. Male weanling rats (Fischer 344) were exposed by inhalation to toluene (900 or 1400 ppm) or hexane (2000 ppm) 14 hr/day, 7 days/week for 14 weeks. Hexane caused...
a mild neuropathy indicated by increased latencies of the ventral caudal nerve action potential and components of the somato sensory, visual and auditory cortical evoked responses. Hexane affected only the amplitude of the 5th component of the brainstem auditory evoked response. This finding was in contrast to previous results showing an effect on latency of that component with exposure of rats to 1000 p.p.m. hexane for 24 hrs/day. Only the effects on the ventral caudal nerve, and decreased amplitude of cortical and brainstem auditory components persisted for one week after termination of exposures. Toluene had an effect only on the amplitude of the fifth component of the brainstem response. These findings are consistent with the results of behavioral measures indicating a general lack of neurotoxicity of toluene, except for tests of cognitive function which indicate deficits of learning and discrimination. (Supported by NIDA Contract No. 271-80-3712)

544 THE EFFECT OF TRACE HALOTHANE ON 2',3' CYCLIC NUCLEOTIDE 3' PHOSPHODIESTERASE (CNPASE) IN THE DEVELOPING RAT BRAIN. J.J. Nagelhout, F.C. Beutchin, R.T. Louis-Ferdinand, Coll. Phcy. & All. Hlth. Prof., Wayne State University, Det. MI 48202

On subcellular fractionation, CNPase activity is primarily localized in the myelin fraction of rat brain and has been used as an enzyme marker of myelination. Chronic exposure to subanesthetic concentrations of the volatile anesthetic Halothane inhibits leucine incorporation into myelin in rats exposed during the prenatal and early postnatal period (Wiggins et al. 1979).

In order to determine the effect of trace concentrations of Halothane on the developmental profile of CNPase, 2 groups of pregnant Sprague Dawley rats were exposed to 500 p.p.m. or 250 p.p.m. Halothane in air, 8 hours per day, 5 days per week from the third day of conception through postnatal day 10. Control animals received air alone. The specific activity of CNPase was decreased by 500 p.p.m. Halothane 34% (p<.05) and 15% at postnatal days 17 and 20 respectively. Body weight was decreased in the treated group 15%, 20% and 12% at days 17, 20 and 25. In the 250 p.p.m. group, CNPase activity was decreased at 17 days (p<.01), and body weight decreased at 14, 17 and 20 days. Brain/body weight ratios were unaffected at either dose.

The data indicate that pre & postnatal Halothane exposure delays the development of the myelin marker enzyme, CNPase. This suggests that exposure to trace concentrations of Halothane during neurodevelopment inhibits myelination in the rat. (Supported by University Anesthesiologists of Detroit)

545 BEHAVIORAL EVALUATION OF SENSORY IRRITATION BY OZONE, J. L. Tepper, R. Wood, B. Weiss. Depts. of Pharmacology and Radiation Biology & Biophysics, Div. of Toxicology, Environmental Health Sciences Center, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642

Ozone causes changes in behavior at concentrations as low as 0.12 ppm. To determine if its irritant properties are of behavioral significance, we used a behavioral test in which mice respond to turn off, or escape an irritant. Initially, mice were trained to turn off 1000 ppm ammonia. Each mouse was required to poke its nose five times into one of two conical recesses. Completion of this requirement terminated delivery of the irritant for one minute, and produced a facial shower of clean humidified air from the conical recesses. Irritant delivery was also terminated after 60 seconds if the animal did not respond. Control procedures demonstrated that this behavior is not due to a general increase in activity resulting from irritant exposure, nor is it maintained by the presence of the facial shower alone. After the concentration-effect curves for ammonia were determined, each mouse was exposed to ozone in an ascending and descending sequence of concentrations (0.25-24.0 ppm).

Ozone exposures alternated every other day with exposures to 1000 ppm ammonia as a control for changes in baseline behavior. As the concentration of ozone increased, the number of escape responses increased and the latency decreased. Thus ozone, a lower airway irritant, will maintain escape behavior. Supported by DAO0623, ES01247, and in part under contract with DOE DE-AC02-76EV05490.

546 MACROCYCLIC (CROWN) ETHERS: THEIR TOXICITY NEURO-MUSCULAR PHARMACOLOGY AND STRUCTURE ACTIVITY RELATIONSHIPS. S. C. Gadd, P. A. Cavigan, W. D. Adams and G. Schieman, Allied Corporation, Morristown, NJ; University of Cincinnati, Cincinnati, OH and CMDNJ - Rutgers Medical School, Piscataway, NJ.

The six compounds currently comprising the commercially available Crown Ethers and Crown Ether derivatives (12-Crown-4, 15-Crown-5, 18-Crown-6, Dibenzo-18-Crown-6, Dicyclohexano-18-Crown-6, and Hexacyclen trisulfate) were evaluated for acute lethality in mice (i.p. and oral), rabbits (dermal and oral) and rats (dermal and oral).

In all species, the LD50 by any single route were bound to be 18-6-15 C-5 < 12-14-4 with the relative lethality of the substituted compounds depending on route.

Associated with the three parent Crown Ethers have been reports of marked acute and intermediate term neuromuscular, cardiovascular, and behavioral effects, which has been alternately hypothesized to be due to either ion trapping/chelating (in the ring structure) or to a neurologically active metabolite. The neural effects were instigated in rats via the i.p. route. The neurologic effects of the unsubstituted Crown Ethers were found to parallel the lethal effects, but this was not the case for the substituted derivatives. From this and other observations, it is established that the unusual neurologic and cardiovascular effects are due to a metabolite and not due to an ion trapping mechanism.
Intracellular recordings from giant axon formations in IDPN neuropathy. H.E. Lowndes and B.G. Gold, Dept. Pharmacology, CMDNJ, NJ Medical School, Newark, NJ.

Giant axon formations (GAF) are large swellings resulting from accumulation of neurofilaments in the first proximal internodes of motor axons (Griffin et al, Science, 202, 663, 1978). The purpose of this study was to determine the effect of the GAF on action potentials (AP) conducted along individual axons. Cats were given 2,5-iminodipropionitrile (IDPN) 50 mg/kg/week and investigated 5 weeks after the first injection. Action potentials evoked by antidromic stimulation of motor axons or orthodromically by dorsal root stimulation were recorded in GAF with microelectrodes. Impalements in GAF were discriminated from those in motoneurons by the absence of EPSPs and IPSPs. APs indistinguishable from those in normal axons were occasionally seen. Others displayed double hump potentials which occurred randomly during the course of the AP. Hump potentials occurring on the rising phase of the AP on antidromic stimulation shifted to the falling phase upon orthodromic stimulation during the same impalement. The characteristics of these action potentials are strikingly similar to the reflection potentials predicted to result from sudden increases in diameter of axons (Parnas et al, J. Neurophysiol. 39, 909, 1976). Since motoneurons in IDPN treated cats often generate repetitive APs upon single stimulation, it is conjectured that the repetition might arise in part from reflection of APs from GAF and subsequent re-activation of the motoneuron. GAF occur in some cases of amyotrophic lateral sclerosis and this phenomenon may contribute to the electromyographic observations in this disorder.

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up to 20 weeks or 200 mg/kg 2,5-HD for up to 7 weeks. Electrophoretic analysis and protein staining using a modified Ehrlich's reagent revealed conversion of CNS protein amino groups to substituted pyrrole adducts in 70 and 200 mg/kg exposure groups, but not at 20 mg/kg. Although binding was detected in many CNS proteins, the myelin basic protein exhibited particularly strong affinity for 2,5-HD. Hydrolysis and amino acid analysis of myelin basic protein from treated rats revealed conversion of lysine ε-amino groups to pyrrole derivatives. Cessation of dosing resulted in eventual clearance of the adduct from spinal cord myelin protein. Electrophoretic studies provided no evidence for simple neurofilament protein cross-linking as the mechanism of action of 2,5-HD. Hens at the two upper dose levels displayed neurotoxic signs and pathologic changes similar to those seen in rodents and man, demonstrating the susceptibility of this species. These results provide evidence for pyrrole formation as the mode of in vivo binding of 2,5-HD, and suggest a possible direct action of the compound upon myelin protein. (Supported by a Society of Toxicology Predoctoral Fellowship sponsored by the Procter and Gamble Co.).

551 EPHAPTIC TRANSMISSION BETWEEN MOTONEURONS IN CATS WITH θ,θ′-IMINODIPROPIONITRILE NEUROPATHY. H.E. Lowndes and B.G. Gold, Dept. Pharmacology, CMDNJ, NJ Medical School, Newark, NJ.

Cats given θ,θ′-iminodipropionitrile (IDPN) develop giant axon formations (GAF) in the first proximal internodes of motor axons (Gold et al, Neuropathol Appl. Neurobiol., 1981). GAF become demyelinated as they enlarge, giving rise to possible electrical 'short-circuiting' or ephaptic transmission to adjacent motoneurons, dendrites or GAF. Cats were given IDPN, 50 mg/kg once weekly for 5 weeks. Action potentials were recorded in lumbar motoneurons using micropipettes and evoked by antidromic stimulation of ventral root filaments. In normal cats, an action potential can be evoked only by a single motor axon. Ventral roots were divided into two or more filaments which were stimulated sequentially after successful impalement of a motoneuron. Of those cells tested in the IDPN-treated cats, action potentials could be evoked in 12% of the motoneurons by stimulation of more than one filament. Thus ephaptic transmission occurs in IDPN neuropathy and most likely results from the presence of the GAF. ‘GAF are also seen in some cases of amyotrophic lateral sclerosis (ALS) (Carpenter, Neurol. 18,841,1968). Ephaptic transmission may thus underlie the doublet and synchronous action potentials seen electromyographically in this disorder.

Supported by the ALS Society of America.


Parenteral administration of monosodium glutamate (MSG) to rats produces convulsions as an acute response to the neuroexcitant properties of MSG. MSG presumably excerts its neurotoxicity via excessive stimulation of excitatory receptors in select brain regions that lack blood brain barriers. Recently, it has been reported that neonatal administration of MSG results in increased susceptibility to pentylenetetrazol-induced seizures in adult mice, however this finding has not gone unchallenged. The aim of the present study was to ascertain if neonatal administration of MSG produces permanent alterations in CNS excitability. Male CF-1 mice were injected (s.c.) on post-natal day 4 with either 4 mg/g MSG (n=6), 1 mg/g MSG (n=6) or saline (n=12). In addition, a fourth group (n=4) received 4 mg/g MSG (i.p.) on post-natal day 30. Seizures were chemically-induced with flurothyl ether on post-natal days 60, 90 and 120 and time (sec. ± SEM) to full clonic-tonic seizure was recorded. The seizure threshold for the saline-treated mice was 305 ± 12.7 and did not vary over time. Mice treated on post-natal day 30 had elevated seizure thresholds (451 ± 12.7) on all doses of flurothyl ether. Mice that received MSG neonatally exhibited a significant increase in time to seizure on the day 60 measure (4 mg/g 408 ± 54.4; 1 mg/g 442 ± 41.8) which reverted to control thresholds on days 90 and 120. In conclusion, MSG treatment increases flurothyl ether seizure threshold and the permanence of this effect appears to be dependent on the age at which MSG-induced neuronal damage occurs. (Supported by NIEHS grants ES07094 and ES02277).


Decalin (decahydrobenzene) is a saturated cyclic hydrocarbon which has a wide variety of industrial uses. Published data indicate this chemical produces a specific type of nephrotoxicity in male rats after 90 days in a continuous inhalation exposure regimen. The purposes of our study were 1) to determine whether or not a similar type of renal toxicity could be induced in a shorter time period in male rats and 2) to determine if male mice were also sensitive to the nephrotoxic effects of decalin inhalation exposure. Fischer 344 male rats and C57Bl/6 male mice were exposed continuously (22 hours/day, 7 days/week) for 20, 28 or 35 days to decalin vapors at 0, 25, 52.5 and 125 ppm. Kidney lesions were observed in all 3 test groups of rats after either 20, 28, or 35 days exposure. The nephrotoxicity was characterized by 1) hyaline droplet formation in the cytoplasm of the proximal convoluted tubule epithelium, and 2) the presence of granular casts at the corticomedullary junction. These changes were time and dose dependent and are similar to the renal toxicity reported in male rats after 90 days of continuous inhalation. No pathologic effects were observed in other tissues of rats nor in the kidneys of mice exposed to the high dose of decalin. Our results show that the nephrotoxicity can be produced by decalin in
less than 90 days, a situation which will make it more feasible to conduct studies designed to evaluate the functional significance of the kidney damage observed in the male rat.

554 A SIX MONTH MULTI-SPECIES INHALATION STUDY WITH MALEIC ANHYDRIDE. R. D. Short, F. R. Johannsen, C. E. Ulrich*, Monsanto Co., St. Louis, MO 63166 and *T.R.D.C., Mattawan, MI 49071

This study was initiated to assess the safety of atmospheres that contained maleic anhydride. Accordingly, rats (15/sex/group), hamsters (15/sex/group), and monkeys (3/sex/group) were treated 6 hours a day 5 days a week for 6 months. Atmospheres were generated by subliming maleic anhydride and were monitored using Tenax collection columns and gas chromatography to detect total maleic; i.e. maleic anhydride plus maleic acid. Hematology, clinical chemistry and urinalysis were performed in all species at 3 and 6 months. Pulmonary function tests (respiratory rate, tidal volume, resistance and compliance) were performed in monkeys at 0, 3 and 6 months. In addition, a histopathological examination was performed in 30 tissues from both sexes of animals in control and high dose groups. The mean analytical concentrations were 0, 1.1, 3.3 and 9.8 mg/m³ of total maleic. Dose related signs of nasal and ocular irritation were observed. These signs included discharge, sneezing, gasping and coughing. No significant treatment-related mortality was observed in any species. Reduced weight gains were observed only in mid and high dose rats, which had terminal body weights greater than 90% of control values. No treatment-related effects were observed in hematology, clinical chemistry, urinalysis, and pulmonary function. Microscopic evaluation of tissues revealed no histopathologic changes related to treatment. Therefore, no effect levels, which disregard overt signs of irritation, were considered to be 1.1 mg/m³ of total maleic for rats and 9.8 mg/m³ of total maleic for hamsters and monkeys.


Formaldehyde (CH₂O) induced squamous cell carcinomas in the nasal turbinates of F-344 rats exposed to 6 or 15 ppm of CH₂O (6 hr/day, 5 days/week, 2 years). Disposition studies using ¹⁴CH₂O showed that a principal site of absorption was the nasal cavity. The concentration of radioactivity in the nasal mucosa was approximately proportional to both the airborne concentration and the time of exposure. An equivalent concentration of 0.7 µmol of¹⁴CH₂O/g of tissue is attained after 6 hr of exposure to 6 ppm, and 1.7 µmol of¹⁴CH₂O/g of tissue after 6 hr of exposure to 15 ppm. Equivalent concentrations of¹⁴CH₂O in other tissues (liver, lung, heart, kidney, spleen, small intestine, brain, testes) were typically one or two orders of magnitude less than in the nasal mucosa. As the radioactivity measurements represent upper limits to attainable CH₂O concentrations, which may be much greater than the actual concentrations, a method was developed to assay CH₂O directly using gas chromatography-mass spectrometry. A free and (acid-labile) bound CH₂O, but not irreversibly-bound CH₂O, are detectable. Rats were exposed to CH₂O (6 ppm, 6 hr/day, 10 days) and sacrificed within 10 minutes of the last exposure. Concentrations of CH₂O in the nasal mucosa were apparently unchanged from control (endogenous) values (0.5 µmole/g), suggesting that absorbed CH₂O in the mucosa must be rapidly metabolized and/or bound irreversibly. Rapid metabolism is possible since homogenates of the nasal mucosa were as effective as liver homogenates in catalyzing the NAD⁺-dependent oxidation of CH₂O to formate.

556 PATHOLOGICAL EFFECTS OF CHRONIC FORMALDEHYDE INHALATION IN RATS AND MICE. W. D. Kerns, K. L. Pavkov, and D. J. Domofrio, Battelle Columbus Laboratories, Columbus, OH 43201 and R. J. Orulla, CIT, RTP, NC. 27709.

Male and female Fischer 344 rats and B6C3F1 mice were exposed to formaldehyde vapor concentrations of 2, 6, or 15 ppm for 24 months. Selected animals from the study were maintained for an additional six months without formaldehyde exposure. Nasal carcinomas were observed in rats exposed to 6 and 15 ppm and in mice exposed to 15 ppm. Neoplasia was preceded by dysplastic and metaplastic alterations of the respiratory epithelium in the anterior portion of the nasal cavity in rats and mice. In the 2-ppm exposure group (rats), squamous metaplasia was the only change present. In the 6-ppm (rats) and 15-ppm (rats and mice) exposure groups, there was continued progression of the metaplastic epithelium to squamous epithelial hyperplasia with increased keratin production and then to squamous papillary hyperplasia with foci of cellular atypia. These lesions then progressed to carcinomas "in situ" and finally to invasive squamous cell carcinomas of the nasal cavity. At 27 months (3 months post-exposure), there was a decrease in the frequency of squamous metaplasia in the rat (2 and 6 ppm) and mice (6 and 15 ppm). In the 15-ppm exposure group (rats), there was no progression of squamous metaplasia. Bone marrow hyperplasia was also associated with formaldehyde inhalation in male and female rats (15 ppm). In summary, formaldehyde vapor was shown to be a nasal carcinogen in rats (6 and 15 ppm) and mice (15 ppm). Supported by Chemical Industry Institute of Toxicology.

557 NASAL MUCOSAL RESPONSE INDUCED BY A RESPIRATORY IRRITANT (METHYL CHLOROACETATE). D. G. Keyes, R. J. Kociba, J. W. Henck. Toxicology Research Laboratory, Health and Environmental Sciences, USA, Dow Chemical U.S.A., Midland, MI 48640.

Groups of male and female Fischer 344 rats were exposed to 0, 10, 25 or 75 ppm methyl chloroacetate vapor 6 hours/day for 11 consecutive days. Parameters monitored included appearance and demeanor, body weight, organ weights, clinical
558 INHALATION TOXICITY OF A FLUOROTELOMER - ZONYL®-TELAL. B. A. Burgess, S. D. Nash, and G. L. Kennedy, Jr., B. I. du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware 19711 Zonyl® TELA (CAS #25398-32-7) is a mixture of perfluoroalkyl iodides consisting mainly of C-6 to C-12 telomers and is a raw material used in the manufacture of a number of functional fluorochrome materials such as surfactants and repellents. The material is a paste or liquid at room temperature and boils around 116°C. Male rats were exposed for single 4-hour periods to vapor/aerosol evolved from spraying the material onto a heated surface and boils around 116°C. Male rats were exposed for single 4-hour periods to vapor/aerosol evolved from spraying the material onto a heated surface. Rats exposed to 10 ppm MCAC exhibited a lesser degree of toxicity noted as minimal eye irritation and epithelial hyperplastic changes in the nasal turbinates. Thus, this study indicates that morphologic changes in the upper respiratory tract are the most sensitive index of exposure of the rat to vapors of methyl chloroacetate. As with all inhalation studies in rats, these data must be interpreted in view of the fact that the rat is an obligatory nasal breather.

559 GUINEA PIG RESPIRATORY RESPONSES TO ISOCYANATOETHYL METHACRYLATE- AND ISOCYANATOETHYL PROPIONATE- PROTEIN CONJUGATES. L. S. Mullin, C. K. Wood and N. D. Krivanek, E. I. du Pont de Nemours and Company, Inc., Haskell Laboratory, Newark, DE Exposure to certain isocyanates has been associated with development of respiratory sensitization in some people. In this study, guinea pig respiratory response to protein conjugates of isocyanatoethyl methacrylate (IEM) and isocyanatoethyl propionate (IEP) was evaluated by the method of Karol (AIHA J. 39:546-556, 1978). Guinea pigs were exposed daily up to two weeks to an aerosol of bovine serum albumin (BSA) or BSA conjugated with IEM or IEP. After the initial exposures, the guinea pigs were challenged with BSA, isocyanate conjugated to guinea pig serum albumin (GSA), or isocyanate monomer or polymer. Cross response to IEM and IEP conjugates was also tested. After a two week induction, guinea pigs exposed to BSA-IEM conjugated from 24% to 98% exhibited a marked increase in respiratory rate and in some cases bronchospasms. Response was related to degree of conjugation. Guinea pigs exhibiting a response to BSA-IEM reacted to BSA-IEP. No responses to unconjugated BSA were observed. In guinea pigs responding to BSA-IEM, >0.1 ppm IEM monomer vapor elicited similar but delayed responses; 0.01 ppm IEM did not. An IEM polymer aerosol with <0.0004% monomer did not elicit a response. These data suggest a response threshold. Guinea pigs exposed to BSA-IEM (52% conjugated) had sensitization-type responses and cross reacted to BSA-IEM and GSA-IEP. Application of BSA-IEP or IEP monomer to the scratched skin of guinea pigs that responded by inhalation to BSA-IEP resulted in immediate wheal and flare response not seen in unexposed animals. All of these responses suggest immediate hypersensitivity.

560 ETHYLENE GLYCOL MONOBUTYL ETHER (EGBE) VAPOR 9-DAY AND 90-DAY INHALATION STUDIES IN FISCHER-344 RATS. D.E. Dodd, W.M. Snellings and B. Ballantyne, Union Carbide Corporation, Bushy Run Research Center, Export, PA.

The toxicity of EGBE vapor was studied in male and female rats in order to assess possible hazards from short-term repeated exposures to high concentrations and longer-term repeated exposures to lower concentrations. In the shorter-term study rats were exposed for 9 days (6 hrs/day) to EGBE concentrations of 245, 86, 20 or 0 (control) ppm. The most noteworthy finding was in blood, where a statistically significant depression of red blood cell (RBC) count (approximately 20% below control), hemoglobin (Hgb), mean corpuscular Hgb concentration with increased nucleated RBC's, reticulocytes and mean corpuscular volume were observed in males and females at 245 ppm. Less marked, but statistically significant, effects on erythrocyte parameters were found in rats exposed to 86 ppm EGBE. An additional group of rats allowed 14-days postexposure recovery had a substantial, but not complete, reversal of the affected blood parameters. In the subsequent longer-term study, rats were exposed to EGBE at concentrations of 77, 25, 5 or 0 ppm for 13 weeks (6 hrs/day, 5 days/wk). Slightly, but statistically significant, decreases in RBC
count (13% below control) and Hgb, accompanied by an increase in mean corpuscular Hgb, were observed in the 77-ppm-treated female rats after 6 weeks of exposures. Females of the 77 ppm group also had a transient decrease in body weight gain. However, at the conclusion of the 90-day study, there were no significant effects at any of the EGBE concentrations on body weight, organ weights, hematology, and urine or clinical chemistries. Furthermore, no gross or microscopic lesions related to EGBE exposure were found. These findings confirm the known RBC target toxicity of EGBE and demonstrate the occurrence of this effect above the current TLV of 25 ppm.

561 NITROETHANE: A 13-WEEK INHALATION TOXICITY STUDY IN RATS AND MICE. Cushing, T. S., Bell, T. J., Burek, J. D., Potts, W. J., and McKenna, M. J., Tox. Res. Lab., Dow Chemical USA, Midland, MI 48640

A 13-week inhalation study was conducted to assess the toxicity of nitroethane (NE) in F-344 rats and B6C3Fl mice. In the 13-week study each species was exposed to 0, 100, 350, or 1000 ppm (0.0, 0.3, 1.0 or 3.0 mg/l) of NE for 6 hr/d, 5 d/wk for a total of 64-65 exposures with an interim sacrifice of rats and mice after 20-21 exposures.

Exposure of rats to 1000 ppm NE resulted in decreased body weight gain, elevated methemoglobin levels (MetHb) with cyanosis, increased reticulocytes and Heinz bodies in peripheral blood and associated splenic extramedullary hematopoiesis. Other major target organ effects in these animals included degenerative and inflammatory changes in the olfactory nasal epithelium, hepatocellular vacuolization, decreased cytoplasmic granularity of renal cortical tubular epithelium and ductal epithelial cells of the salivary glands. Rats exposed to 350 ppm NE showed similar but less severe changes in MetHb, spleen, nasal turbinates and salivary glands. Minimal changes in MetHb, spleen and salivary glands were observed in rats exposed to 100 ppm NE.

Mice exposed to 1000 ppm NE showed increased MetHb, evidence of toxicity in the salivary glands, liver, olfactory nasal epithelium and multinucleated spermatids in the testes. Overall these changes were less severe than those observed in rats. Less extensive toxicity was observed in mice exposed to 350 ppm and only MetHb, liver, salivary glands and nasal turbinates were affected. Mice exposed to 100 ppm NE showed minimal changes in the nasal turbinates and transient (even during continued exposure) effects on salivary gland epithelium only. The 100 ppm NE exposure concentration was judged to be a minimal effect level under the conditions of this study.


Carbonochloridic acids (chloroformates) comprise a class of chemical reactants widely used as industrial intermediates. They are corrosive by skin or eye contact and may be irritating and toxic when inhaled. Fischer 344 rats (5/sex/exposure level) were exposed for 1-hour to the following chloroformates (CF): methyl-CF, ethyl-CF, sec-butyl-CF, isobutyl-CF and phenyl-CF. CF vapors were generated using a Battelle-developed generator and concentrations were monitored by IR spectrophotometry. Toxic signs for all CF's included ocular and nasal discharge, edema, lachrymation and weight loss; most deaths occurred within 24 hours of exposure. Rats that died generally had lung hemorrhage and edema. In the methyl-CF study additional rats were sacrificed for histopathology. Rats at 0.36 and 0.47 mg/l dose levels survived within 24 hours had alveolar hemorrhage and mucus epithelial necrosis and degeneration in the nasal turbinates, trachea, bronchi and bronchioles. Regenerative changes indicated resolution of previously observed lesions by days 9-10. Calculated LC50's (mg/l) for both sexes combined were: methyl-CF, 0.45; ethyl-CF, 0.87; sec-butyl-CF, 1.82; isobutyl-CF, 1.81; phenyl-CF, >1.24. Correlation of these LC50's with molecular weight (MW) fit a linear model; correlation coefficient 0.9996. Slopes from regression analyses of male and female data are 0.025 and 0.041, respectively, supporting increased male sensitivity. Phenyl-CF did not cause death at the highest achievable exposure level (1.24 mg/l) in agreement with the linear model prediction (2.5 mg/l). This work indicates that the toxicity of chloroformates may be predicted from their molecular weights. Supported by PPG Industries, Inc.

563 DISPOSITION OF METHYL ETHYL KETONE IN F-344 RATS AFTER INHALATION EXPOSURE. J. S. Bus, D. Deyo, M. Cox and E. L. White, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709

The purpose of this study was to characterize the disposition of methyl ethyl ketone (MEK) in Fischer-344 rats after a single inhalation exposure to 500, 1500 and 5000 ppm MEK. Male rats were exposed for 6 hr to (1,2-3H)MEK, after which total excreta (urine, feces, expired CO2 and MEK) were collected for 72 hr. In other experiments various tissues were analyzed for MEK and the metabolites 2-butanol, 3-hydroxy-2-butanol (3H2B) and 2,3-butanediol (2,3BD) by gas chromatography-mass spectrometry at selected times after exposure to nonradioactive MEK. Radioactivity collected in the various excreta fractions plus tissues and carcass (expressed as percent of total radioactivity collected) was as follows: at 500 ppm, exhaled MEK 16%, 12CO2, 46%, urine 32%, feces 12% and tissues and carcass 5%; at 1500 ppm the distribution of the respective fractions was 31%, 26%, 38%, 1% and 3%; and at 5000 ppm the distribution was 50%, 14%, 33%, 1% and 2%, respectively. Blood MEK concentrations 0.5 hr after exposure were 40, 189 and 739 µg/ml for the respective groups and had declined to lower concentrations in all tissues (blood, brain, liver, kidney, lung, and sciatic nerve) by 12 hr after exposure. Both 2-butanol and 3H2B were detected in all tissues examined, but at concentrations generally less than 50% of MEK. 2,3BD was observed only in blood. These results indicate that MEK is extensively metabolized after inhalation exposure and that saturation of MEK metabolic pathways occurred over the exposure range studied.
564 EFFECT OF CHLORPHENTERMINE (CP) ON THE PULMONARY CLEARANCE OF (14C)-5-HYDOXYTRYPTAMINE (5-HT) IN RABBIT IN VIVO. T. Morita, L. S. Angeline and H. M. Mehendive, Dept. Pharmacol. & Toxicol., Univ. Miss. Med. Ctr., Jackson, MS 39216, USA.

Previous studies from this laboratory have demonstrated that the pulmonary clearance of 5-HT is perturbed by CP, an observation possibly mechanistically related to drug-induced pulmonary hypertension. The objective of these experiments was to determine if the effects of CP could be demonstrated in vitro. Pulmonary uptake and metabolism of (14C)-5-HT were examined in single-pass pulmonary perfusion using reference indicator-radiosotope dilution technique (Catravas and Gillis, JPET 213: 120, 1980). Male New Zealand albino rabbits (1.9 ± 0.6 kg) were surgically prepared under pentobarbital anesthesia (NembutalR, 40 mg/kg). The right jugular vein and left carotid artery were then cannulated to the level of the right atrium and ascending aorta, respectively. Arterial BP, blood pH, rectal temperature, blood hematocrit were monitored during the experiment. After 25 min stabilization, controls received injection of saline vehicle and CP treated animals received CP iv (jugular vein, 0.5, 1 or 3 mg/kg). Ten min later, 1 ml fresh saline solution containing 500 µg cardigreen (CG) and 0.5 µC (14C)-5-HT was injected into jugular vein and simultaneously blood samples from the artery catheter were collected at 1 sec intervals for 31 sec (dead space 6 sec) and analyzed for 5-HT and 5-HIAA. Extraction of 5-HT for controls was 79.2 ± 2.3 (n = 6), which was reduced significantly at higher doses of CP. Portion of metabolite in each fraction in controls was increased from 0 to 49% during 25 sec. These results indicate that 5-HT is metabolized by the lung during single-pass and that prior administration of CP results in inhibition of uptake and metabolism of 5-HT. (Supported by HL-20622).

565 AN IN VITRO MODEL FOR THE EXPOSURE OF LUNG ALVEOLAR EPITHELIAL CELLS TO TOXIC GASES. P. O. Zamora, R. E. Gregory, A. P. Li and A. L. Brooks (Sponsor: R. O. McClellan), Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185

An in vitro model of lung alveolar tissue has been developed by culturing lung cells of Type II cell origin on collagen gels and subsequently, incubating the cultures at an air/water interface. The cells can be maintained for up to 2 weeks at the interface and the cultures provide a system to expose lung cells directly to toxic fumes and gases. Nitrogen dioxide (NO2) was used to test the responsiveness of the cultures to toxic gases. Exposure to NO2 resulted in cytotoxicity, decreases in protein synthesis, and morphological alterations similar to those found in vivo, but at lower doses. Dose-response relationships were determined for one-hour exposures at room temperature to 0.5-6.0 ppm NO2 in 95% air - 5% CO2 (flow rate = 2.6 liter/minutes; chamber volume 5.4 liters). Time-response was determined at 6 ppm NO2 for 15-60 minutes. The cultures were analyzed for trypan blue dye-exclusion, clonogenicity, and 3H-Lysine incorporation. Increasing concentrations of NO2 caused linear increases in cytotoxicity, decreased protein synthesis, and morphological damage. Increasing time of exposure also decreased cell viability. The morphological damage after 24 hours included cell lysis, detachment of cells from the basement membrane, and the appearance of cytoplasmic vacuoles, distended nuclei, and cells with degraded nuclear membranes. This exposure model provides quick, reproducible information on several cellular endpoints and may be useful in short-term toxicological testing of toxic gases. The system is currently being tested with a variety of toxic gases and will be expanded to include assays of genotoxicity. (Supported under DOE Contract No. DE-AC04-76EV01013.)

566 MAJOR FINDINGS IN A TWENTY-FOUR-MONTH INHALATION TOXICITY STUDY OF METHYL CHLORIDE IN MICE AND RATS. K. L. Pavkov, W. D. Kerns, C. E. Chrisp, D. C. Thake, R. L. Persing, H. H. Harroff, Battelle Columbus Laboratories, Columbus, OH; E. J. Gralla, CIIT, RTP, NC.

Male and female B6C3Fl mice and Fischer 344 rats were exposed to 0, 51, 224 or 997 ppm methyl chloride (MeCl) 6hr/day, 5 da/wk, for 24 mo. Sacrifices in randomly selected animals occurred after 6, 12, 18, and 24 mo. Parameters evaluated included: body weight, organ weights, pathology, hematology and urinalyses. Survival was adversely affected in both species and sexes at the 997-ppm exposure concentration. Growth rate was significantly (p<0.05) impaired (997 ppm) in both species. Nearly all mice (997 ppm) had signs of neurofunctional impairment (18, 21, and 22 mo). Histopathologic lesions related to MeCl exposure included: in male mice, renal cortical tubular cysts and adenocarcinomas (997 ppm), renal cortical adenomas (997 and 224 ppm) and cortical microcysts (51 ppm); splenic lymphoid depletion and atrophy (997 ppm); seminiferous tubule degeneration and atrophy (997 ppm). In both male and female mice, lesions included granular cell layer (cerebellum) degeneration and atrophy (997 ppm) and hepatic cellular degeneration and necrosis (997 ppm). In male rats, decreases in the relative and absolute weights of testicles (997 ppm; 18 and 24 mo) were seen. Histopathology showed (6, 12 and 18 mo) significant (p<0.05) incidence of testicular tubular degeneration and atrophy (997 ppm) when compared with controls. It did not become more prevalent (p>0.05) with increased exposure time. Methyl chloride induced pathologic lesions were not observed in female rats. Supported by CIIT.

567 INTERACTIVE EFFECTS OF JP-5 VAPOR EXPOSURE AND ELEVATED TEMPERATURE ON RENAL LESION INDUCTION. L. L. Pitts II, A. F. D'Addario, M. J. Cowan, Jr., and R. H. Bruner*. Naval Medical Research Institute/Toxicology Detachment and †Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB OH 45433. Sponsor: M. E. Andersen. Interactions between chemical toxicity and elevated temperatures are incompletely understood. Exposures to fuel vapors or chemicals often occur at temperatures greater than 25°C (eg. ship engine rooms). We have studied the effects of
568 THE EFFECT OF BENZENE INHALATION ON MURINE HEMATOPOIETIC PRECURSOR CELLS (CFU-e, BFU-e, CFU-gm).


The inhalation of benzene has been shown to produce severe blood dyscrasias which may ultimately include pancytopenia or leukemia. To investigate these effects assays reflecting committed stem cell functions [erythroid colony forming units (CFU-e), erythroid burst forming units (BFU-e), and granulocytic/macrophagic colony forming units (CFU-gm)] were used. Male B6D2F1 mice were exposed by inhalation to 4000 ppm of benzene for 8 hrs a day for either 0, 1, 2, 3, 5, 7 or 14 days. Groups of mice were sacrificed at 0900 hrs on the morning following exposure for the given period. Femoral marrow was sterilely collected from 6 femurs and pooled. Nucleated cells (2 x 10^6) as a single cell suspension were plated in 1 ml of α-medium with methylcellulose in quadruplicate. Following one day of exposure, femoral cellularity, BFU-e, and CFU-gm/plate were significantly depressed. The cellularity remained depressed for the duration of the exposure. On the other hand, BFU-e and BFU-e/plate were depressed for 3 or 2 days, respectively, and then rebounded to significantly higher concentrations than controls. By day 7 the concentrations of BFU-e and BFU-e/plate were elevated so that the total number of BFU-e or BFU-e/femur had actually rebounded to control values. CFU-gm/femur remained depressed at all exposure periods. CFU-e, BFU-e or CFU-gm/spleen did not increase. The toxic effects of benzene on hematopoietic stem cells are immediate and unequivocal, and are not ascribable to migration of stem cells between femur and spleen.

569 CHANGES IN PHOSPHOLIPID BIOSYNTHETIC ENZYMES IN TYPE II CELLS AND MACROPHAGES (PAM) ISOLATED FROM RAT LUNGS AFTER NO EXPOSURE. E.S. Wright, M.J. Vang, J.N. Finkelstein and R.D. Mavis, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY Sponsor: P.E. Morrow

The activities of phospholipid biosynthesizing enzymes and subcellular marker enzymes were measured in Type II cells and PAM isolated from rat lungs 48 hours after exposure to air or 40 ppm NO. DNA and protein increased in cell fractions from NO-exposed lungs, but NO exposure had no effect on the percent of Type II cells in isolated cell fractions. A general increase in biosynthetic enzyme activities (units/mg DNA) was observed in Type II cells from NO-exposed rats, but no change was detected in the activity of the mitochondrial marker enzyme, NADPH cytochrome c reductase. The increases were 171 and 168% and phosphatidate phosphohydrolase increased 69%. Glycololphosphate phosphatidyltransferase increased 143% and succinate cytochrome c reductase, the mitochondrial marker enzyme, increased 111%. PAM recovered by lavage from normal rat lungs were enriched in enzymes of PC synthesis relative to Type II cells, but contained three-fold less glycerolphosphate phosphatidyltransferase activity. Exposure to NO reduced in a significant inverse relationship in NADPH cytochrome c reductase activity (46%) in PAM, but no change in any biosynthetic enzymes. The increases in activity of phospholipid biosynthesizing enzymes in Type II cells, but not PAM, is consistent with a specific stimulation of surfactant phospholipid biosynthesis in Type II cells 2 days after exposure to NO, when Type II cell proliferation is occurring.

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crease in dry lung weight, accompanied by a 1.5% increase in water content. This increased dry weight and the absence of a significant change in the amount of DNA and protein per unit dry weight indicated that the increased lung weight of this group was due to increased cellularity. Lung connective tissue content increased as a result of acrolein exposure. As a function of dry weight, elastin levels were 177% of control values in animals exposed to 4.0 ppm acrolein. Significant increases were observed in the collagen concentration in both the 1.4 and 4.0 ppm group where it was 111% and 133%, respectively, of the control group.

Stepwise discriminant analysis was used to identify those pulmonary function and biochemical parameters which best distinguished four groups of acrolein exposed rats. This technique selected and linearly combined a set of significant discriminating variables which forced the exposure groups to be as distinct as possible. When completed the effectiveness of a discriminating function, based on these variables, was checked by using it to classify each animal originally studied. Groups of male Fischer 344 rats were exposed to 0, 0.4, 1.4 or 4.0 ppm acrolein for 62 days. Pulmonary function tests and connective tissue measurements provided more than 25 variables used for discriminant analysis. Collagen, elastin and the expiratory flow rate at 25% vital capacity explained 89% of the dispersion among the 4 exposure groups. A discriminant function based on these variables correctly classified 69% of the animals. Because the 0.4 and 1.4 ppm animals were poorly classified, each exposure group was assessed individually with the control group. Analyses were conducted using either the biochemical measurements, the respiratory physiology data, or all of the parameters. Collagen most effectively discriminated the individual exposure groups (with DNA in the 1.4 ppm group) if biochemical variables were used. The most discriminating physiology variable was different for each exposure group regardless of analysis used. This variation of the most significant parameter may be the result of distinct lesions at each exposure concentration.

The Dahl selected rat lines, one susceptible to salt-induced hypertension (DS) and the other resistant to salt-induced hypertension (DR) were subchronically exposed to filtered air, 0.4, 1.4, or 4.0 ppm acrolein. All of the DS rats exposed to 4.0 ppm acrolein died within the first 11 days, while 60% of the DR animals survived. Neither dose dependent blood pressure changes nor altered behavioral characteristics were evident following acrolein exposure. Surviving DR animals exposed to 4.0 ppm acrolein had increased serum alkaline phosphatase (71%), SGOT (42%), and SGPT (59%) levels when compared to control animals. Exposure to 4.0 ppm acrolein also led to pulmonary edema and significant increases in collagen and elastin content of lung. There was a marked difference in the pulmonary pathology observed in DS and DR rats exposed to 4.0 ppm acrolein. The lungs of the DS rats exhibited severe airway epithelial necrosis with edema and hemorrhage, while surviving DR rats primarily showed a proliferative change. Following exposure to 0.4 and 1.4 ppm acrolein, both rat lines displayed similar pathologic change. Epithelial hyperplasia and/or clusters of macrophages were usually found near terminal bronchiolar areas. These findings suggest that further investigation of the physio-
pathologic sensitivity of the DS rat line may elucidate a model for investigating the underlying characteristics of stress susceptible populations.

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**Effects of Inhaled Toluene and m-Xylene on Conditioned Behavior.** G. David Gentry, Ronald W. Wood, Department of Radiation Biology and Biophysics, Environmental Health Sciences Center, Division of Toxicology, University of Rochester Medical Center, Rochester, NY 14642.

Four rats performed five days per week on a fixed interval 5 minute (FI 5) schedule of food reinforcement (i.e., the rats received sweetened condensed milk for the first lever press after five minutes had elapsed since the previous reinforcer). After behavioral indices were stable, the rats inhaled toluene or m-xylene for two hours immediately preceding the behavioral sessions on Tuesdays and Fridays; Thursdays were sham-exposure days. The toluene and m-xylene concentrations were 560, 1000, 1780, and 3000 ppm given first in ascending, then in descending order; two rats received toluene first and the other two m-xylene first. Changes in post-reinforcement pause, response rate, and index of curvature were concentration-related. Across the 100 minute behavioral session, decreasing blood levels were associated with both increases and decreases in response rate. The direction of change was a function of exposure concentration: for lower concentrations there was an initial increase in rates; for 3000 ppm there was an initial decrease followed by an increase. Index of curvature showed the largest magnitude effect for the three measures, and post-reinforcement pause showed the slowest recovery. This research was supported by grants DA00623, ES01247, ES05106 and DE-AC02-76EV03490 with the Department of Energy.

**Subacute Inhalation Toxicity of Aniline; the Relationship between Methemoglobin Formation and Toxicity.** F. O. O'Neal, E. I. du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE 07111.

Sponsor: G. L. Kennedy, Jr.

Male rats were exposed head-only to 0, 17, 45 and 87 ppm aniline 6 hrs/d, 5 d/wk for 2 wks. Methemoglobin (MetHb) levels were monitored throughout exposure and a subsequent 13-day recovery period. Rats were divided into two groups and clinical and histopathological examinations performed following the last exposure and the 13th recovery day. Similar MetHb levels were observed in rats exposed to 0 and 17 ppm aniline. During exposure to 45 and 87 ppm aniline, MetHb levels ranged from 1.7- to 6-fold and from 7.5- to 26-fold greater than controls, respectively. These values gradually decreased to control levels by the 11th day of recovery. Aniline exposures of 45 and 87 ppm also affected other hematopoietic parameters. Significant decreases in erythrocyte count, mean cell hemoglobin and hematocrit and an increase in immature erythrocytes were observed after 10 exposures. Exposure to 87 ppm aniline depressed erythrocyte, neutrophil, and platelet counts throughout the recovery. The organ to body weight ratios for the liver, spleen and thymus were also significantly altered. A 14% increase for liver (87 ppm) and 48 and 272% increases for spleen ratios were observed after 10 exposures to 45 and 87 ppm aniline, respectively. These values returned to normal during the recovery period. Thymus ratios were elevated 9 and 30% above controls in the 87 ppm group following the exposure and recovery periods, respectively. These results suggest a relationship between MetHb levels and several toxicity parameters in rats exposed to aniline.


The toxicity of morpholine, an important industrial chemical, was investigated by the inhalation route of exposure. Experimental exposures for the subchronic study were 25, 100 and 250 ppm six hours/day, five days/week for a total of 13 weeks. Rats exposed to these doses showed striated breathing and sleep patterns. Irritant effects of morpholine exposures were evident mostly in the high exposure group, and a bloody discharge was observed around the nose and mouth in the 250 ppm dose rats after the first week of exposure; salivation was also observed. Ten rats/sex/dose level were sacrificed after 7 weeks exposure. Exposure to 250 ppm morpholine resulted in focal erosion of the maxillary turbinates and focal squamous metaplasia, which was observed in 6/10 male rats and 2/10 female rats. A sporadic increase in porphyrins in the Harderian gland sections was noted. Rats sacrificed at 13 weeks confirmed results observed at week 7; the lesions were more advanced and involved the nasal septum and anterior nasal cavities. Evidence of progressive chronic murine pneumonia was observed in the 250 ppm dose groups and lower dose groups. No other compound-related histomorphologic alterations were observed in 25 or 100 ppm exposure groups. There were no compound-related effects on body weight, clinical chemistry, gross necropsy, or organ weight data.


Isocyanates are highly reactive chemicals used in the manufacture of polyurethanes. Exposure to
small amounts of isocyanates produces respiratory symptoms in immunologically sensitized persons. Exposure to higher concentrations of isocyanates produces similar respiratory symptoms in all individuals as a result of irritation. The ability of isocyanates to function as inhibitors of serine proteases (Brown and Wold, Biochem. 12, 828, 1973) prompted investigation of the effects of industrial isocyanates on cholinesterase. Hexamethylene diisocyanate (HDI), 2,6 toluene diisocyanate (2,6 TDI) and hexyl isocyanate (HI) completely inhibited purified human serum cholinesterase at molar ratios of 4:1 to 8:1 (isocyanate added: enzyme). Isocyanate inhibition was active site directed since known competitive cholinesterase inhibitors protected the enzyme. 2,4 TDI, phenyl isocyanate and o-tolyl isocyanate showed little enzyme inhibition. With these isocyanates, molar ratios of 50:1 or greater were required for 50% inhibition. Enzyme inhibition was also achieved by in vitro exposure of cholinesterase to 1 ppm atmospheres of isocyanates. Under these conditions HDI and HI were the most potent inhibitors. The inhibition of cholinesterase by isocyanates was reversible with a half time of approximately 12 hours. Maximum reversibility occurred at pH 7.5. These results indicate that isocyanates are potent inhibitors of cholinesterase. The reversibility may explain the recovery from respiratory rate depression observed in animal models of sensory irritation where animals are exposed to vapors of isocyanates. Supported by the Winters Foundation and NIOSH #OH00865.

578 CELL SPECIFIC ACTIVATION AND DETOXIFICATION OF BENZO(a)PYRENE BY TWO CLONED MURINE TESTICULAR CELL LINES. R. Filler and L. S. Woods, Biology Division, Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, TN. Sponsor: W. M. Haschek

The interaction of environmental toxins within the male reproductive system is complicated by the presence of several distinct testicular cell types. To define more clearly the potential relationship between chemically-induced toxic effects and endogenous metabolic capability of specific testicular components to activate and detoxify chemical agents, we have examined two cloned cell lines identified as of the Leydig (TM3) and Sertoli (TM4) cell types. The polycyclic aromatic hydrocarbon, benzo(a)pyrene [B(a)P], was used as a probe for defining cellular metabolic activity. TM3 cells metabolized B(a)P 160x more effectively than did TM4 cells. This difference in chemical activation was correlated with major differences in the formation of 9,10-, 4,5-, 7,8-dihydrodiols, quinones and 9-0H-B(a)P derivatives as determined by HPLC. No significant amounts of 3-0H-B(a)P were detected. Long-term (72 hrs.) incubation of TM3 cells with 5 µg/ml B(a)P did not produce cytotoxic effects. Therefore, the role of two detoxification reactions was assessed by determining the presence of glucuronic acid conjugates and sulfate ester derivatives. Treatment of water-soluble metabolites with 8-glucuronylase resulted in the hydrolysis of 4,5- and 7,8-dihydrodiols and 3 and 9 phenol metabolites. Similar treatment with arylsulfatase released only 4,5- dihydrodiol. These observations demonstrate that, for at least two cell types in the testis, there is not only a difference in biochemical capability to activate chemical agents but a structural specificity for selective conjugation of cytotoxic and transforming reactive intermediates. [Operated by UCC Corp. with the U.S. DOE]

579 IN VIVO METABOLISM OF THE ANTI-JUVENILE HORMONE PRECOCE II AND THE GENERATION OF DIHYDRODIOL METABOLITES. M. T. Stephen Haia and Scott J. Grossman, Environmental Toxicology Center and Department of Entomology, University of Wisconsin, Madison, Wisconsin, 53706.

Precoce II (6,7-dimethoxy-2,2-dimethyl-2H-benzo-[b]pyran), a potent anti-juvenile hormone, induces precocious metamorphosis and sterilization in several insect species, and has been proposed as a prototype fourth-generation insect control agent. This compound was recently found to be acutely toxic in male Sprague-Dawley rats at intraperitoneally administered doses of 300 mg/kg and above. The primary lesion was severe centrolobular necrosis of hepatic parenchymal cells. Liver damage was evidenced by the massive release of glutamicoxalacetic transaminase and glutamic-pyruvic transaminase into the serum. When a 100 mg/kg dose of [3H]-precoce II was administered to rats, it was found to be rapidly metabolized and excreted from the body. Approximately 74% of the recovered dose was cleared within the first 24 hours. Identification of the various urinary metabolites was accomplished via the use of: a) Amberlite XAD-2 column chromatography, b) high pressure liquid chromatography, and c) gas chromatography-mass spectrometry. One of the most significant findings is that precoce II is metabolized to a stereoisomeric cis/trans mixture of 3,4-dihydrodiol in vivo as well as in vitro. It is thus postulated that hepatotoxicity occurs as a result of an overload of a highly reactive epoxide intermediate formed during metabolism. (Supported in part by a USDA Hatch Grant, #2407, from the College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison, and a Biomedical Research Grant, #181532, from NIH.)


N-Vinyl-2-pyrrolidinone (NVP) is the monomer from which polyvinylpyrrolidinone preparations are made. The latter are of great pharmaceutical value and contain a small percentage of NVP whose metabolism and disposition is unknown. Elimination studies of [14C(vinyl)]-NVP showed that blood levels of the intact compound drop rapidly after i.v. administration with an elimination half life of 1.5 hours. Following administration (i.v.) of [14C(vinyl)]-NVP, total radioactivity in the urine of rats after 6 hours ranged from 40-65% of the dose, while that excreted in the bile ranged from 17-20% of the dose. Intact NVP in these cases was found to be less than 0.2% of the dose in the urine and 2.3% in the bile. Of the total

Nitroarenes have been detected in photocopy toners and diesel exhaust particulates and are potent mutagens without metabolic activation in the Ames Test. Certain nitroarenes show S-9 Mix dependent activity in mammalian cell culture assays, which indicates that S-9 contains the enzyme system capable of activating these compounds. Rat liver functions such as cytosol, microsomes and S-9 supernatant were found to reduce anerobic 1-nitropyrene (1-NP) to 1-aminopyrene (1-AP). The reaction was stopped by addition of HCl. Substrate (1-NP) was extracted with cyclohexane, the product extracted from the alkalized aqueous fraction in cyclohexane. 1-AP and 1-NP concentrations were determined by spectrofluorimetry.

The rate of reduction was much greater upon addition of NADPH and was dependent upon the amount of S-9 Mix added. Not all 1-NP added is recoverable as 1-NP plus 1-AP, indicating that some material may exist as intermediates (-NH2OH or -NO). These results imply that mammalian enzymes can reduce 1-NP to potentially more reactive species.

The excretion and tissue distribution of [14C]-2,4,7-trinitro-9-fluorenone in the rat. William F. Busby Jr., Mark E. Goldman, and Gerald N. Wogan; Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139 and Frank Aldrich and Thomas Smith; IBM Corporation, White Plains, NY 10601.

Experiments have been performed to measure urinary fecal excretion and tissue distribution following administration of the photoconducting compound [14C]-2,4,7-trinitro-9-fluorenone (TNF) in 275 g male Fischer rats. The skin absorption of 14C-activity administered, 69-75% appeared in the urine in the first 24 hours. Distribution studies of 14C-activity in vital tissues and organs of rats 6 hours after i.v. administration of 14C(vinyl)-NVP showed that the liver (4.3-6.5% of the dose) and small intestines (3.5-5.7% of the dose) are the organs with the highest accumulation of 14C-activity. Preliminary attempts to elucidate the metabolic fate of 14C-(vinyl)-NVP from urine samples showed 12% of the radioactivity was incorporated into acetate and radioactivity was recovered as 1-NP plus 1-AP, indicating that 14C(vinyl)-NVP is readily metabolized and eliminated in the rat. The liver and small intestines are the organs with the highest accumulation of 14C-activity and the urine and bile are the major routes of elimination.


Previous studies have shown 2-naphthyl glycine to be a major metabolite of 2-methylnapthalene (2MN) in rats. Ring oxidation of 2MN by hepatic microsomes from several species and formation of 2-naphthoic acid (2NA) from 2MN by rat liver microsomes have been reported. In order to gain insight into the methyl group oxidation of methylated PAHs the oxidation of 2MN to 2NA was studied in rat and rainbow trout liver homogenates (LI) and subcellular fractions via the use of 2MN, 2-hydroxymethylnapthalene (2MNHO) and 2-naphthaldehyde (2NCHO) as substrates. The homogenates (4 ml, 25M sucrose/g liver) were fractionated by centrifugation. The tissue preparations were incubated with substrate (generally 1 mM, 0.05M NAD, 0.05M NADP, 15M MgCl2, 12M G-6-P, 0.04M PO4 (pH 7.4) and 0.125M sucrose at 37°C for rat preparations and 22°C for trout preparations. After incubation, analysis for 2MN, 2NCHO and 2NA was done by HPLC using a C-18 column and elution with H2O-CH3ON-CH3COOH (65:35:0.1) Rat LI formed 2NA from all 3 substrates approx. 6 times as rapidly as did trout LI. The ability to metabolize 2NCHO to 2NA was present in the various fractions of rat and trout liver. With 2MNHO as substrate, cytosol was the most active fraction from trout liver and the activities of individual fractions accounted for total homogenate activity, while mitochondria was the most active fraction from rat liver and the activities of individual fractions did not account for total homogenate activity. Treatment of rats and trout with 100mg/kg Aroclor 1254 ip in corn oil increased the metabolism of 2MN and 2MNHO by rat LI but not by trout LI. The pattern of metabolites formed with rat LI and fractions also changed. Supported by NIH Grants ES01985 & ES01080.

Disposition of dioctyl phthalate following oral exposure in rats. D. G. Pegg, Biomedical Science Department, General Motors
The disposition of acetaldehyde oxime (AAO), a chemical intermediate used in the production of insecticides, was determined in the male Fischer 344 rat. Animals were given (1,2-14C)AAO, orally, at dose levels of 0.75, 7.5, 75, 375 and 750 mg/kg, and the excretion of 14CO2 in the exhaled air, and excretion of radioactivity in the urine and feces for up to 96 hr following dosing was determined. Radioactivity remaining in the liver, kidneys, testes, muscle, spleen, fat and blood at the end of the experiment was also determined. Fecal and urinary excretion accounted for 1-2.9% and 11.7-14.7% of the administered radioactivity, respectively, by 96 hr. No dose-related differences were noted in the amount of radioactivity excrated in the urine and feces. Reversed-phase HPLC analysis of the urine of animals given (14C)AAO indicated the presence of at least 4 metabolites. On the basis of chromatographic characteristics, one of the minor metabolites has been identified as acetic acid. The primary route of excretion of (14C)AAO-derived radioactivity was as exhaled CO2 accounting for 63-68% of the administered radioactivity by 96 hr. Elimination by this route at the lower dose levels was rapid; at a level of 7.5 mg/kg nearly 60% of the administered radioactivity had been excreted by 8 hr. With increasing dose, however, a nonlinear increase in the maximum rate of excretion of 14CO2 was observed. At the highest dose level, only 20% of the administered radioactivity had been excreted as 14CO2 by 8 hr. Pretreatment of animals with disulfiram (an aldehyde dehydrogenase inhibitor) resulted in a significant decrease in the initial rate of excretion of 14CO2, indicating that the two-carbon metabolism of AAO is via the tricarboxylic acid cycle.

As part of a two-year chronic toxicity study, the pharmacokinetics of ethylenediamine (EDA) was studied in Fischer 344 rats of both sexes at day zero (naive animals), 6 months (controls and high level animals) and 18 months (controls and high level animals). The rats, which were randomized along with the rest of the animals on the toxicity study, were taken for pharmacokinetic and metabolic studies at the specified time. A single po dose of 50 mg 14C-EDA·2HC1/kg was given to each rat and the plasma kinetics was followed for a 24-hr period. Four pharmacokinetic parameters (absorption rate constant, terminal half-life, area under the curve and apparent metabolic rate constant) were compared with respect to age, sex and dose. With absorption rate constant, there appeared to be a dose-related difference. The rats subjected to chronic dietary EDA·2HC1 at 0.35 g/kg/day (high level animals) had a slower rate of absorption. In the case of the terminal half-life, the differences, if any, were not obvious. This was probably due to the variability among the animals. With area under the curve, there was a strong indication for age-related changes. The older rats had higher values for area under the curve than the younger rats. The apparent metabolic rate constant was derived from the rate of formation of 14CO2 as a result of 14C-EDA·2HC1 dosing. The comparison of this constant under the various experimental conditions revealed sex-related differences. The female appeared to be more active metabolically.
radiochemical(s) in the following parameters was compared with respect to age, sex, and dose: excreta (urine and feces), \(^{14}\text{CO}_2\), major organs (liver, kidney, lung and brain) and the carcass. There was no apparent sex-related difference in the amount of radiochemical(s) eliminated via the excreta. However, there were indications of age-related limitation of excretory capability. The dose-related difference in excretion is apparently limited to the 18-month old animals in which the EDA-treated rats (both sexes) had a higher rate of excretion in both the first and the second 24-hr periods. The expiration of \(^{14}\text{CO}_2\), an indicator of EDA biotransformation, may be age-, sex- and dose-dependent. In general, a higher rate was observed in older, female, control rats. The relatively low \(^{14}\text{CO}_2\)-expiration rate and small differences among the EDA-treated rats are interpreted as the result of competition from non-labeled EDA in the dietary intake. A few age-, sex- and dose-related differences in the level of radiochemicals in the major organs and carcass were also observed. The probability of enzyme induction and/or capacity limited processes exists.


Acrylonitrile (VCN), a widely used monomer, is a demonstrated carcinogen. In order to investigate its biological fate a comparative pharmacokinetic and covalent binding study of 1-\(^{14}\text{C}\) and 2,3-\(^{14}\text{C}\) VCN was carried out at different time intervals in rats following a single oral dose of 46.5 mg/kg (0.5 LD50). Within 24 hours 60% of the administered radioactivity from 2,3-\(^{14}\text{C}\) VCN appeared in urine where as it was only 40% from 1-\(^{14}\text{C}\) VCN. The fecal excretion of radioactivity from 2,3-\(^{14}\text{C}\) VCN was 4-5 fold higher than that from 1-\(^{14}\text{C}\) VCN. Total exhalation of \(^{14}\text{CO}_2\) and \(^{14}\text{CN}\) from 2,3-\(^{14}\text{C}\) VCN (2% of administered dose) was considerably lower than that from 1-\(^{14}\text{C}\) VCN (12%). Highest levels of radioactivity from 1-\(^{14}\text{C}\) and 2,3-\(^{14}\text{C}\) VCN in blood were attained at 3 and 6 hr respectively. Unlike 1-\(^{14}\text{C}\) VCN, the radioactivity from 2,3-\(^{14}\text{C}\) VCN was comparatively higher in globin and membrane fractions of the red cells whereas it was negligible in the heme fraction. Highest covalent binding of 1-\(^{14}\text{C}\) and 2,3-\(^{14}\text{C}\) VCN in tissues occurred at 6 and 1 hr, respectively. The percent covalent binding of 2,3-\(^{14}\text{C}\) VCN was significantly higher in most tissues and their subcellular fractions as compared to 1-\(^{14}\text{C}\) VCN. These studies indicate differential interaction and binding of VCN, labelled at cyano moeity (1-\(^{14}\text{C}\)) versus the vinyl moeity (2,3-\(^{14}\text{C}\)). (Supported by NIH grant ES01871).


The pharmacokinetics of inhaled methyl chloride (MeCl) were studied in male F344 rats and beagle dogs. Animals were exposed via head-only chambers to 50 or 1000 ppm MeCl for 6 hr (rats) or 3 hr (dogs). Blood was sampled during and after exposure, and MeCl was measured by gas chromatography. Also, a procedure for measuring vapor uptake (in rats) was developed and used for measurements of MeCl uptake at apparent steady state.

In both species a linear 2-compartment open model accurately described parent compound pharmacokinetics of inhaled MeCl. Blood MeCl concentrations (at apparent steady-state) were proportionate to exposure concentration. These values averaged 190, 3900 ng/g (rats), and 160, 3700 ng/g (dogs) in animals exposed to 50 and 1000 ppm respectively. Post-exposure elimination of MeCl from blood was unaffected by exposure concentration in either species, but was slightly slower in dogs than rats. Uptake of MeCl at steady state (measured in rats only) was approximately proportionate to exposure concentration; the values obtained were 10 and 165 ng/g/min in 50 and 1000 ppm exposed rats. Estimates of MeCl metabolism by a 2-compartment open model suggest that MeCl metabolism (relative to body weight) is greater in rats than dogs.

Although it has been reported that the dog is more sensitive to MeCl toxicity than the rat, the pharmacokinetic data (notably steady-state blood MeCl concentration and AUC for blood concentration vs time) do not suggest that there should be profound species-dependent differences in sensitivity. Of course, factors other than blood concentration of the parent compound may influence the relative sensitivity of the two species.

590 LUNG TOXICITY DUE TO SIMPLE HETEROCYCLIC COMPOUNDS IN MICE. R. Wiley, L. Gammal, G. Traiger, H. Choo, and S. Baraban, Departments of Medicinal Chemistry and Pharmacology, The University of Kansas, Lawrence, KS 66045.

3-Methylfuran is a component of atmospheric smog, and causes significant necrosis of Clara cells in the lung when administered either parenterally or by inhalation. Although there is good evidence that a metabolite is responsible for the damage, it has not been possible to elucidate the nature of the process. In order to determine whether other simple heterocycles would also cause lung damage, and hopefully identify some toxic compounds whose metabolism it would be possible to study, we developed an efficient synthesis for 3-alkylfurans, and produced 3-methylfuran, 3-ethylfuran, and 3-pentylfuran. The toxicity of these compounds, as well as furan, 2-ethylfuran, 2-furamide, and 3-methylthiophene in mice was studied. The parameters used to determine pneumotoxicity were histological observations, lung weight to body weight ratio, and rate of DNA synthesis as measured by rate of 3H-thymidine incorporation.

It was found that 3-methylfuran, as expected, exhibited pronounced lung toxicity using all three criteria. Surprisingly, very little 3-methylfuran was found in lung. 3-Ethylfuran was also intensely toxic, and was found in very high concentration in the lung. 3-Pentylfuran, although present in high concentration, was not toxic. 2-Ethylfuran, 2-furamide, and furan gave evidence of toxicity in the thymidine incorpora-
tion test, exhibited edema on histological ob-
ervation, and were found in lesser concentra-
tion in lung. 3-Methylthiophene was not toxic
in any of the tests. The metabolic reasons for
these differences remain to be elucidated. Sup-
ported by GM-26,366.

591 EFFECT OF AMMONIA ON HEPATIC MICROSOMAL ENZYME
ACTIVITY IN THE MOUSE. J.C. Kapeghian, A.B.
Jones and I.W. Waters, Depts. of Pharmacology and
Pharmaceutics, Sch. of Pharmacy, Univ. of Missis-
pippi, University, MS 38677. Sponsor: W.M.
Davis. It has been suggested that NH3 liberated
from urine and feces could be responsible for de-
creased hepatic microsomal parameters seen in
rats housed in a dirty environment. This hypo-
thesis was tested following both NH4Cl adminis-
tration and NH3 gas exposure (dynamic) in male ICR
mice. All hepatic microsomal enzyme assays were
performed on the 12,500 g supernatant fraction.
Neither acute nor repeated (6 d) NH4Cl adminis-
tration (250 mg/kg, i.p.) had any effect on hexo-
barbital sleep time, aminopyrine N-demethylase
(type I), or aniline hydroxylase (type II) activ-
ity. Acute NH3 gas exposure at non-lethal
levels (1350 ppm; 4 hr) had no effect on hexobar-
bital hypnotosis; however, lethal concentrations
(4380 ppm; 4 hr) increased this parameter. Acute
exposure to lethal NH3 levels (4700 ppm; 4 hr)
increased total microsomal protein and type I en-
zyme while type II activity was unchanged. There
was no inhibition of either enzyme when hepatic
microsomal fractions were incubated in NH3/O2 at-
mospheres. Repeated NH3 (4 hr/d; 4 d) at 522,
350 or 115 ppm reduced microsomal protein and
type I enzyme but not type II; body weights were
depressed after 522 or 350 ppm. When mice ex-
posed to air only were pair-fed to control for
body weight reductions following repeated NH3,
the microsomal effects were indistinguishable
from NH3-treated animals. In no instance did non-
lethal NH3 exposures cause inhibition of type I
or type II enzymes (per mg microsomal protein);
therefore, our results indicate that NH3 is not a
hepatic microsomal enzyme inhibitor in mice.
(Supported in part by NIH Grant ST32 GM07099-05
and by the University of Mississippi Research In-
stitute of Pharmaceutical Sciences).

592 DISPOSITION OF CARBARYL-14c RESIDUES FOLLOWING
INHALATION AND ORAL EXPOSURE. B. L. Roberts and
H. W. Dorrough, Graduate Center for Toxicology,
University of Kentucky, Lexington, KY.

Preliminary studies in our laboratory showed
that nose-only exposure of rats to carbaryl (L-
14C-naphthyl-N-methylcarbamate) vapors for 1 hr
resulted in over 80% of the inhaled dose being
retained in the body and that the distribution and
excretion after 24 hrs did not differ from
that of an oral dose. The present investigation
was conducted to determine the distribution of
inhaled residues shortly after exposure when body
burdens are greatest, and to compare the finding
with those of orally treated animals. Female
Sprague-Dawley rats were administered 6 µg/kg
during a 1-hr exposure to the vapors and the same
dose was given orally to other animals. Analyses
were performed 1, 2, and 4 hrs after treatment. 14c-Carbaryl equivalents in the muscle, brain,
lung, trachea, fat, and liver were essentially the
same for both routes of exposure, but residues after
1 hr in the blood and kidney of animals ex-
posed by inhalation were 3X the level of those
from rats receiving an oral dose. After 4 hrs,
the level of radiocarbon in the blood was similar
for both groups, although the kidney maintained
the differential observed after 1 hr. A markedly
different pattern in disposition was evident when
the remaining carcass was homogenized and aliquots
radioassayed. The carcass of animals treated or-
ally contained 22, 20 and 5% of the dose after 1,
2 and 4 hrs, while corresponding values for ani-
mals inhaling the carbamate were 80, 55 and 50%
of the dose. Urinary elimination of radiocarbon
after 24 hrs accounted for approximately 90% of
the oral or inhaled dose and, consequently, no
difference was noted in total body burden. How-
ever, the data clearly show that inhalation of
carbaryl results in a much greater exposure of the
total body to the residues than does the same dose
consumed orally. (Supported by EPA Grant # R805143).

593 COMPARATIVE HYDROLYSIS AND DISPOSITION OF INHALED
AND ORALLY ADMINISTERED CARBOFURAN-CARBONYL-14C
IN RATS. M. Trono and H. W. Dorrough, Graduate
Center for Toxicology, University of Kentucky,
Lexington, KY.

Rats treated with a single oral dose of carbofuran-
an hydrolyzed 66% of the administered radiocarbon
in 12 hrs as determined by quantitation of ex-
pired 14CO2. Animals exposed to carbofuran va-
pors for 1 hr at approximately the same dosage
level (175 µg/kg) hydrolyzed 12 times less of the
carbamate. Approximately 18% of the single oral
dose was eliminated in the urine in 12 hrs, while
rats exposed to carbofuran vapors eliminated only
7%. Fecal elimination of radiocarbon for both
routes of exposure was less than 6% of the dose.
Tissues from both groups were assayed for radio-
carbon content at 1, 3, 12, and 24 hrs after
treatment. Most tissues contained higher levels
of residues in the inhalation treated rats, with
the difference decreasing with time. The radio-
carbon content of the GI tract was greater in the
orally treated rats at 1 and 3 hrs after ex-
posure, but at 12 hrs the GI tract of inhalation
treated rats contained a significantly larger
(p < .05) quantity of the administered dose (11%) than those treated orally (7%). Radiocarbon in
the blood of inhalation treated rats decreased
only slightly during the 24 hrs after exposure,
with 7% of the dose in the blood at 1 hr and 6% at
24 hrs. Conversely, the levels in orally
treated rats declined from an initial 6% of the
dose at 1 hr to 1% at 24 hrs. The ratio of brain
to blood radiocarbon was significantly higher in
inhalation than in orally treated rats, while the
reverse situation occurred in the liver. Since
carbofuran is a potent anticholinesterase agent,
the greater transfer of residues from the blood
to the brain suggests that an inhalation exposure
would be more significant toxicologically than an
equivalent oral dose. (Supported by EPA Grant
No. R805143).
Retention and Fate of Inhaled Aldicarb in Rats.

H. H. Lysy and H. W. Dorough, Graduate Center for Toxicology, University of Kentucky, Lexington, KY.

Aldicarb [2-methyl-2-[(1C-methylthio)-propionaldehyde-O-methylcarbamoyl oxime] vapors were administered to rats via nose-only exposure for 1 hr and the retention, distribution, and excretion of the insecticide determined. The mean retention of the inhaled vapors (0.1 and 0.2 ppm in the air) was 83.4 ± 6.6% and was independent of the ppm in the air and of the minute volume of the animals, which ranged from 118 to 290 ml/min. Urinary elimination over a 24-hr period accounted for 81% of the retained radioactive dose and was the same as that of an equivalent oral dose. With exception of the liver where residue levels were similar, the tissues of animals exposed to aldicarb vapors contained higher levels of radiocarbon during the first 12 hrs than those from rats treated orally but little difference was noted after 24 hrs. The blood of the inhalation treated animals contained 5, 4, 2 and 1% of the dose after 1, 3, 12 and 24 hrs, while for the orally dosed animals the maximum levels did not exceed 2% of the dose and were undetected after the 3-hr sampling period. Approximately 50% of the radiocarbon in the urine of inhalation treated rats was unextractable with organic solvent, whereas 80% could not be extracted from urine of those dosed orally. Although there were a number of quantitative differences among the organoextractable metabolites, the greatest difference was that 5 to 10 times more of the aldicarb sulfone derivative was present in this urine fraction from animals treated with the aldicarb vapors. The tissue and excretory data indicate that the detoxification of aldicarb is much less efficient when inhaled than when ingested. (Supported by EPA Grant No. R801543.)


The scientific validity of using inhalation data to predict risks of toxic injury upon ingestion of halocarbon contaminants in drinking water is uncertain. In the first of a series of comparative oral/inhalation studies, the pharmacokinetics of inhaled 1,1-dichloroethylene (1,1-DCE) was studied. Anesthetized male Sprague-Dawley rats inhaled 25, 75, 150, or 300 ppm 1,1-DCE for 4 hrs. from a mylar bag via a tracheostomy with a miniaturized one-way breathing valve. Periodic samples were taken adjacent to the valve from the separate influent air and exhaled breath streams and analyzed for 1,1-DCE content by gas chromatography. Pulmonary retention, or % uptake, decreased over time following initiation of exposures until equilibria were established. Percent uptake, as measured after reaching initial equilibrium, varied inversely with the 1,1-DCE vapor concentration. Monitoring of respiratory flow rates with integration to yield minute volumes enabled the measurement of cumulative body burdens of 1,1-DCE at each sampling point. Apparent saturation kinetics were reflected by significant increases, following an initial plateau, in exhaled 1,1-DCE levels after the first hour of exposure. Periodic blood sampling from the femoral vein, upon cessation of the 150 ppm exposures, yielded data consistent with a two compartment open pharmacokinetic model. The half-life during the initial, rapid redistribution phase was approximately 2 min, while that for the elimination phase was 17 min. These results indicate that 1,1-DCE is readily absorbed systemically and eliminated via the lung, and that these processes are dependent upon time and intensity of exposure. (Supported by EPA Grant No. R808282)


Our previous studies have shown that subacute treatment of rats which chlorphentermine (CP) (50 mg/kg/day, po, 7d) roughly doubles the total pulmonary phospholipids and significantly enhances the accumulation of CP in perfused lungs. It was of interest to determine if the accumulation of CP was associated with a particular fraction of the lung, and if the enhanced uptake was correlated with the increased phospholipids. Male Sprague-Dawley rats were treated with CP in saline as indicated above the controls received the vehicle only. Artificially ventilated isolated rat lungs were perfused with Krebs Ringer bicarbonate buffer containing bovine serum albumin. After perfusion, lung homogenate was subjected to standard fractionation procedures. Some lungs were examined histopathologically. No correlation was observed between increased CP uptake and increased lung phospholipids. After 60 min of perfusion, CP was distributed uniformly among the subcellular fractions and CP treatment did not alter this. However, pulmonary macrophages obtained from CP treated animals contained 8 times more CP than in controls. The increased CP uptake by the lung following treatment coincides with the increased CP in the lung lavage of treated rats. The macrophages in the lung tissue and lavage fluid from rats treated with CP were more numerous and were larger than those observed in control lungs. These results indicate that a closer stoichiometric association may be found between accumulated CP and macrophage uptake rather than with the pulmonary phospholipids as previously thought. (Supported by HL-20622 and ES-07045)
598 REPEATED INHALATION CHALLENGE OF GUINEA PIGS DIS-
PLAYING DELAYED RESPIRATORY SENSITIVITY. J. Stadler and M.H. Karol, Dept. of Ind. Env. Hlth. Sci., Grad. Sch. of Pub. Hlth., Univ. of Pittsburgh, Pittsburgh, PA 15261

Delayed-onset sensitivity reactions, characterized by symptomatology commencing several hours following exposure, have been noted to occur in industrial workers. Frequently repeated episodes result in earlier onset of symptoms. The underlying pathogenesis of this allergic response is unknown. An animal model of delayed-onset pulmonary sensitivity was employed to evaluate these responses. To induce delayed-onset sensitivity, guinea pigs were injected with Freund's Complete Adjuvant, then challenged three weeks later with an aerosol of tuberculin PPD. Upon first challenge, reactions were apparent after 6 hours and reached maximum response 9-12 hours following inhalation of aerosol. Animals were challenged at regular 2 week intervals with PPD aerosol for a total of five challenges. Pulmonary sensitivity responses were elicited by each of the challenges. However, the onset of response was earlier with repeated inhalation challenge and maximal response was frequently observed 4-5 hours following exposure. The earlier response was not a result of development of non-specific pulmonary hyperreactivity since control animals repeatedly exposed to PPD aerosol failed to demonstrate any pulmonary response. Serologic evaluation of experimental animals at the height of responsiveness failed to indicate the presence of cytoplphilic antibodies. However, histologic examination of pulmonary tissue revealed mononuclear cell infiltration consistent with delayed immunologic reaction. Evaluation of this animal model may provide insight into the immunologic mechanisms governing delayed-onset pulmonary reactions in sensitized industrial workers. Supported by NIHES #ES01532.


The genotoxicity of particulate organic matter collected from air polluted with forest fire smoke was compared to that from air collected at the same site in Lexington, KY on days without forest fire smoke. The benzene/acetone extracts from these samples were assayed using the Salmonella reversion assay and the sister chromatid exchange (SCE) assay in cultured human lymphocytes. Salmonella strains TA98 and TA100 were used with and without the addition of bromochlor-induced rat liver homogenate (S9). Each sample induced dose-related increases in mutagenicity and SCE. However, on the basis of volume of air sampled, the smoke-filled air induced 12 to 14 times more bacterial reversions in TA100 and 16 to 38 times more reversions in TA98 than did smoke-free air. Similarly, on a volume basis smoke-free air induced 1.6 times more SCE than did smoke-free air. Furthermore, on the basis of particulate weight the smoky air extract induced 5 to 16 times more bacterial reversions and 21 times more SCE than did the extract from smoke-free air. These results indicate that the higher mutagenicity of the smoky air was due not only to the heavier particulate load of the air but also to increased mutagenicity of the particles. This is probably a consequence of the higher fraction of organic-extractable matter in the smoke particles: 53% vs 5%. (Supported by contract S4529 of the Institute of Minerals Research, Comm. of Kentucky).

600 THE BEHAVIOR OF INHALED CARBON DISULFIDE (CS2) IN RAT BLOOD. C.W. Lam and V. DiStefano, Division of Toxicology, Univ. of Rochester, School of Medicine, Rochester NY. Sponsor: D. Taves.

It has been reported that the correlation between CS2 concentrations in blood and in air is poor, and that blood CS2 cannot be used as an indicator of exposure. One of the reasons for this statement may be that the CS2 is present in the blood in two forms, free and bound. Our studies were undertaken to characterize these two forms of CS2 in the blood of exposed rats. Both forms of CS2 in the blood increased linearly with inhalation concentration. The bound CS2 in the blood also increased linearly with time when rats were exposed to 2 mg/l CS2 up to 8 hours. Free CS2 was eliminated rapidly by a two exponential first order process (half-times 8.5 and 54.1 min); bound CS2 was eliminated similarly but more slowly (half-times 2.2 and 42.5 hours). The direct proportionality of blood bound CS2 concentration to the inhalation concentration and exposure time coupled with the slow elimination of blood-bound CS2 suggest that it may be used as an indicator for CS2 exposure. In all in vitro studies, CS2 bound to both rat and human blood. The majority (85%) of blood CS2 was found in the red blood cells. Plasma and hemolysate of RBC from CS2-exposed rats were dialyzed at 4°C. There was no detectable loss of bound CS2 from the plasma and only a slight loss from
602 Mutagenicity Testing of Melamine. 

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Melamine (1,3,5-triazine-2,4,6-triamine) is an important industrial monomer used in the production of high pressure laminate resins, molding compounds and resins. The only noteworthy biological responses to melamine include crystalluria and bladderstone formation in chronic studies and convulsions in acute studies. In order to further understand its biological activity, melamine was tested in a battery of short term mutagenicity assays. In the Ames Salmonella assay, strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to melamine in the presence and absence of metabolic activation at doses up to 5000ug/plate. Under conditions of the test no increase in the number of revertants/plate was observed relative to control in any treatment group. Melamine was then tested in the CHO/HGPRT forward mutation assay (O'Neill, J.P.et.al. Mut Res., 45:91, 1977) and in the in vitro CHO sister chromatid exchange assay (Perry, P.et.al. Nature, 251:156 (1974). In both of these assays, CHO cells were exposed to concentrations of melamine ranging from 600 to 1000 ug/ml with and without liver S-9 activating fraction. Results of the CHO/HGPRT assay indicate melamine failed to induce an increase in the mutation frequency above that of controls. Similarly, no increase in the number of sister chromatid exchanges were observed in the CHO cells exposed to melamine. These data clearly suggest that melamine does not represent a genetic risk under conditions of exposure and use.


Dimethylnitrosamine (DMN), a potent hepatocarcinogen and mutagen, requires metabolic activation to exert its biological effects. Although it has been suggested that hepatic DMN metabolism is catalysed by cytochrome P-450 dependent mixed function oxidase (MFO) enzymes, recent studies have provided evidence for several pathways of DMN metabolism. We have investigated the effect of a number of substrates and inhibitors of monoamine oxidase (MAO, EC 1.4.3.4) on DMN metabolism and DMN-induced mutagenicity. The metabolism of DMN to formaldehyde by rat hepatic postmitochondrial supernatant fractions was markedly inhibited by a number of MAO inhibitors (e.g. aminoacetonicitrile, benzyl cyanide, indazole and pargylene) and by MAO substrates (e.g. benzylamine, 2-phenylethylamine, tryptamine and tyramine), but not by the deaminated aldehyde, acid and alcohol products of the latter class of compounds. At the concentrations which inhibited DMN metabolism the MAO substrates and inhibitors had little effect on a range of MFO enzyme activities. Whilst the mutagenicity of DMN in the Ames test was markedly inhibited by MAO substrates and inhibitors, these compounds had little effect on the mutagenicity of either aflatoxin B1 or benzo(a)pyrene. These results suggest the participation of an enzyme(s) with MAO-like properties, unrelated to cytochrome P450, in the hepatic metabolism/bioactivation of this nitrosamine. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food.)
The present study was undertaken to evaluate the effect of timing of ethyl carbamate (EC) administration relative to 5-bromodeoxyuridine (BrdU) infusion on SCE induction and persistence of SCE inducing lesions in bone marrow (BM) and alveolar macrophage (AM) cells of BDF1 mice. When EC was given immediately prior to BrdU infusion, i.e. at the beginning of the 1st cell cycle of BrdU incorporation, SCE frequencies observed in 2nd division metaphases of AM and BM were 32.8 ± 4.8 and 28.9 ± 4.2, respectively. When BrdU infusion was allowed to progress for a third cell cycle, non-reciprocal frequencies in AM and BM were comparable to those previously described (AM, 32.3 ± 3.3; BM, 23.0 ± 2.8) and reciprocal exchanges were elevated (AM, 6.1 ± 0.7; BM, 5.8 ± 0.6) over controls (AM, 1.5 ± 0.07). Persistence of SCE inducing lesions as suggested by elevated reciprocal frequencies was confirmed by experimental data following approximately a 2 cell cycle delay prior to BrdU infusion (AM, 12.2 ± 1.0; BM, 9.7 ± 1.2). However, SCE frequencies observed in 2nd division cells following EC injection between the 1st and 2nd cycles of BrdU infusion was higher than expected (AM, 39.3 ± 3.1; BM, 42.3 ± 3.0). Unlike previously studied mutagens our observations with EC are in contrast to the general assumption that SCE in 2nd division cells are a cumulative total of those induced during both 1st and 2nd cycles of BrdU incorporation. Additional experiments are in progress in an attempt to clarify these inconsistencies. (This research was supported in part by EPA Grant CR806-815-01/2/680/10/07 and BRS 2507R005451 from Division of Research Resources, DHHS).

A multicellular in vivo assay was employed to evaluate DEB as an SCE inducer in bone marrow (BM) and alveolar macrophage (AM) cells of intact and in BM, AM and regenerating liver cells of hepatocarcinomatized SW mice. Relative to baseline SCE levels, significant increases were induced over a DEB concentration range of 9.7 µg/kg (1.5 x baseline) to 291 µg/kg (7.5 x baseline). No cellular specificities were apparent as similar dose-response relationships were obtained in all cell types.

An acute i.v. injection was approximately 1.5 times more effective in inducing SCE than a comparable i.p. dose. Regardless of the route of injection, 193.8 µg/kg DEB produced similar cellular responses in SW and BDF1 mice. The effect of acute i.v. administration of 193.8 µg/kg DEB alone was compared to the dose required to induce reciprocal exchanges in a minimum effective dose. The mammalian SCE test was designed for evaluating carcinogenesis potential of the antineoplastic agent amssacrine, an acridinylamino anisidine derivative. Previous genotoxicity assays showed frameshift mutagenesis at the hisC3076 locus in Salmonella TA-1537 but no confirmed mutagenesis in TA-98, TA-100, TA-1535, and TA-1538. In Chinese hamster ovary cell assays, amssacrine induced sister-chromatid exchanges and hyperdiploid chromosomes. There are no data available on mammalian point mutation with amssacrine and a V-79 Chinese hamster lung cells assay was conducted to detect HPRT locus mutation. Cytotoxicity was established at 2 µg/ml and one hour exposures were used with 2, 1, 0.5 and 0.25 µg drug/ml in duplicate flasks. Positive control was ethylmethane sulfonate at 800 µg/ml. Lethality occurred at 2 and 1 µg/ml. A significant number of 6-thioguanine resistant mutant colonies were induced at 0.5 and 0.25 µg/ml. The results indicate high mutagen sensitivity of this assay when compared to the dose required to induce reverse mutation in prokaryotes with a 1,000-fold separation in minimum effective dose. The evaluation of the battery of tests conducted unequivocally render a genotoxic potential. Further, the mammalian cell assays demonstrate a differential effect on various genetic endpoints and that cytogenetic lesions observed in Chinese hamster cells are capable of leading to specific locus mutation. The correlation of this mutagenic potential in somatic and germinal cells in vivo has not been established.

Chlorination of drinking water has been shown to produce mutagenic byproducts in addition to the trihalomethanes. Much of the precursor material of these products is thought to be humic and fulvic acids. Consequently, we are exploring the use of humic acids as model precursors of mutagenic chemicals formed by water chlorination.
Determination of mutagenic activity were performed by use of a bacterial mutation assay (Ames test) with five Salmonella tester strains. Mutagenic activity was detectable in human solutions after chlorination with HOCl with strains TA98 and TA100. The addition of S-9 caused a 70% decrease in activity. The formation of mutagens was linearly related to humic concentration in the range of 0.2-1.6 mg/ml TOC and to chlorine concentration in the range of 0.1-1.0 chlorine:carbon molar ratios. Using a humic TOC concentration of 1 g/l, a chlorine:carbon ratio of 1:1, and an initial reaction pH of 7.0, the level of mutagen formation was measured at 2,323 net revertants/mg TOC for TA100, and 309 net revertants/mg TOC for TA98. When different sources of the humic materials were used, the levels of mutagens formed were similar. Mutagenic activity was not detected when chlorination was carried out at alkaline pH (11.5). Little effect on activity was observed after heating the chlorinated solutions to 37°C or after purging the solutions to remove volatile compounds. Raising the pH of the chlorinated solutions to pH 10 resulted in a 35% loss of activity. These results indicate that primarily non-volatile mutagens are formed from the chlorination of humic substances which are similar in several respects to the mutagens found in drinking water concentrates.


Halogenated dibenzo-p-dioxins are very toxic compounds which are produced in side reactions in the preparation of herbicides and also by incomplete combustion. The 2,3,7,8-tetrachloro-dioxin isomer has been extensively studied and found to induce certain mixed function oxidases. Reports of its mutagenicity conflict, but the best evidence today indicates that it is not mutagenic. 2-Nitrodibenzo-p-dioxin was prepared by heating the dipotassium salt of catachol with 2,3 dichlorobenzene in DMSO. Likewise, 2,3 dichloro-7-nitrodibenzo-p-dioxin was prepared by heating the dipotassium salt of 4,5 dichlorocatachol with 3,4 dichloronitrobenezene. This compound was identical to that prepared by nitration of 2,3 dichlorodibenzo-p-dioxin.

Both nitrodioxins were mutagenic for Ames tester strain TA1538. No S-9 fraction was required because the bacterial nitroreductase can reduce the nitrodioxins to the active species. Neither dioxin was mutagenic for strains TA1535 nor TA1537.

These results indicate that the dibenzo-p-dioxin ring system can bind to DNA or other critical targets and suggests that the lack of mutagenicity of 2,3,7,8 TCDD and other halogenated compounds may result from lack of metabolic activation and not from improper binding.


Yamasaki and Ames(PNAS 74:3555,1977) reported the mutagenic activity of XAD-2 urine concentrates (U.C.) from cigarette smokers in Salmonella typhiurium. The same techniques may be used to monitor human exposure to various mutagenic/carcinogenic substances. In this connection we felt that further studies on the following would be useful: 1. Dose-response relationship (number of cigarettes smoked versus mutagenic activity), 2. Persistence of mutagenic activity in smokers' U.C. after cessation of smoking, 3. Possible interaction(s) of smoke components with other substances, 4. Possible geographical variations in urine mutagenicity. We studied the above using cigarette smokers and nonsmokers from Lexington, Ky. and nonsmokers from Ashland, Ky. A standardized protocol was used to assay the mutagenic activity of cigarette smokers' and nonsmokers' XAD-2 U.C.s in Salmonella typhiurium TA98. We found that there was no dose-response relationship in a population of 16 different smokers while a clearly positive dose-response was observed in an individual smoker studied. Secondly, we found that mutagenicity though gradually decreasing can be detected in a smoker's urine even a week after the person stopped smoking. In combination with the aromatic amin, 2-aminoanthracene, cigarette smokers' U.C. showed a synergistic effect on mutagenicity. In addition, we observed a significant difference in the mutagenicity of non-smokers' U.C.s (control) from the two different areas. The significance of these findings will be discussed in relation to the application of body fluid assays for detecting occupational exposure to mutagens/carcinogens. This work was supported by ARC contract No. 79-207, CO-7162-79-1-302-0917


Pyridoquinazolines may be useful in preventing local allergic reactions due to mast-cell mediator release. In vitro bioassays were applied to evaluate the genotoxic potential of (2-methyl-11-oxo-11H-pyrido[2,1-b]-quinazoline-8-carboxylic acid), the archetype compound in this series. Salmonella typhiurium plate incorporation assays run at 1 to 1000 µg/plate in the 5 standard tester strains and mitotic gene conversion assay in yeast strain D4 revealed no microbial mutagenicity. Mice receiving 500 mg/kg po exhibited no significant mutagenic activity of urinary metabolites. Sister-chromatid exchange (SCE) in mouse lymphoma L5178Y and in hamster eel Is was increased in the metabolic activation phase and not in the nonactivation phase, respectively. The magnitude of these effects was small (less than 1.6 fold over controls) and not consistently dose related. The consequences of this SCE inducing capacity was studied in V79 hamster lung cells for mutation at the HGPRT locus. At toxicity limited doses ranging from 18 to 75 µg/ml, which induced SCE in the previous assays, there was no induction of 6-thioguanine resistant mutants. Since antiallergy compounds exert effects on the cell membrane, the weak SCE-inducing capacity may be non-specific and attributable to var-
ious degrees of bromodeoxyuridine interaction with DNA in the presence of drug. Semi-quantitative probabilistic analyses indicate minimal intrinsic genotoxic potential complemented by the lack of systemic toxicity in experimental animals.

611 GENOTOXICITY EVALUATION OF AMSACRINE.
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Acridinylamino derivatives constitute compounds with antineoplastic properties. Comparative genotoxicity and hence carcinogenic potential of 4′-(9-acridinylamino) methane-sulphon-m-anisidide (amsacrine) was studied in several bacterial and mammalian cell in vitro assays. In a Salmonella plate incorporation assay, reverse mutation was observed only at 269 µg/plate in the nonactivation phase in frameshift tester strain TA-1537. In a preincubation modification of this assay, significantly increased revertant counts were obtained in TA-1537, TA-1535, and TA-100 at a cytotoxic dose of 672 µg/plate without S9. In the presence of S9, mutagenic activity was manifest only with TA-1537, indicating that potential base substitution activity of amsacrine was prevented more readily than its frameshift activity specific for TA-1537. At these high cytotoxic doses, a significant portion of presumed revertants did not grow in histidine-deficient medium and could have represented phenocopies. By contrast, doses as low as 0.02 µg/ml in culture medium without S9 induced a greater than fivefold increase in sister-chromatid exchange (SCE) rate and chromosomal damage frequency in Chinese hamster ovary cells in vitro. SCE response was more sensitive to S9 detoxification than the chromosomal aberration frequency. The wide differences between the effective dose of amsacrine in Salmonella and mammalian cell assays illustrated the relative insensitivity and perhaps lack of utility of bacterial mutagenesis assays in predicting the mutagenic/carcinogenic potential of acridinylamino compounds.


Metyrapone (2-methyl-1,2-di-3-pyridyl-1-pro-anone), an inhibitor of adrenal steroid hydroxylation, is used clinically in assessment of pituitary-adrenal function and in treatment of Cush- ing's syndrome. Metyrapone has been shown to inhibit several microsomal enzyme activities and to stimulate the hydroxylation of acetaminophen and aniline. The presumed mechanism of inhibition of drug oxidation is binding to one or more phenobarbital (PB)-inducible forms of cytochrome P450. Metyrapone has been used as an inhibitor in microsomal-mediated Salmonella mutagenesis assays (Hales and Jain, Biochem. Pharmacol. 29,256, 1980). Since our studies of chloroallyl compounds as Salmonella mutagens have demonstrated PB-induced microsomal activation (Loper et al, EMS 12th Meet- ing, P12, p.56, 1981; manuscript in prepn.), we tested metyrapone as an inhibitor using these and several control carcinogens. Metyrapone alone was neither toxic nor mutagenic (TA98, TA100, TA1535); it gave a dose-dependent inhibition of mutagenesis of aflatoxin B1 (up to 4-fold in TA100), triallate (6-fold, TA1535, and sulfanilic acid (7-fold, TA1535), and a dose-dependent enhancement of mutagenesis of benzo (a) pyrene (5-fold, TA98), 2-acetylaminofluorene (3-fold, TA98), and 2-aminoanthracene (6-fold, TA98). The S-chloroallyl thioethers, triallate and diallate, have been proposed to form proximate mutagens by a similar mechanism initiated by sulfonation (Schuphan et al, Science 205, 1013, 1979). We have shown both to be preferentially activated by PB-induced S9. However, while metyrapone inhibited triallate mutagenesis, diallate mutagenesis was neither inhibited nor enhanced. Supported in part by EPA grant CR806872. L. D. is supported by NIHES Toxicology Training Grant ES07071.


The time course for induction of sister chromatid exchanges (SCE) in bone marrow cells was studied in mice administered either methyl- nitrosourea (MNU) or ethylnitrosourea (ENU). Intraperitoneal injection of MNU, 50 mg/kg, at intervals up to 36 hours prior to and 19.5 hours after 5-Bromo-2′-dioxouridine (BrdU) pellet implantation resulted in an induction of 10.1 (SEM = 0.6) SCE per cell at 2 hours post BrdU implantation with a reduced response at time points before or after BrdU implantation. Baseline SCE was 3.6 (SEM = 0.4). These results indicate that the DNA damage induced by MNU as reflected by an increased incidence of SCE is repaired by bone marrow cells in approximately 2.8 cell cycles and the biologic half-life of the repair is 18 hours. Based on the repair phase data one can predict the SCE results for MNU administered post BrdU implantation.

The time course of SCE induction following ENU (50 mg/kg) administration is more complex with at least two distinct repair phases apparent. The maximum induction of SCE at 2 hours after BrdU implantation is 10.9 (SEM = 1.2). A relatively slow repair phase is evident from 4 to 0.5 cell cycles prior to BrdU implantation. A second and faster repair phase is apparent from 0.5 to 0 cell cycles prior to BrdU implantation. The biological half-life for fast repair is approximately 3 hours and that of the slow repair is approximately 39 hours. These results imply that a protocol utilizing a fixed time point for compound administration post BrdU may give inadequate and/or misleading results in SCE assays. (Supported by EPA Grant 808861010)
Talin protein is obtained by aqueous extraction of the fruit of Thaumatococcus daniellii indigenous to West Africa. It is 2500 x sweeter than sucrose, is non-cartogenic and has the property of enhancing and extending many flavours. The extract contains several related sweet proteins (Thaumatins) of known amino acid composition and sequence. They contain a minimal complement of amino-acids apart from histidine and there are no unusual peptide linkages or end-groups. The digestibility of Talin protein in rats is equal to that of egg-albumen but has a lower biological value reflecting the absence of histidine. Sub-acute studies in rats and dogs have shown the 'no-effect level' to be in excess of 2 g/kg/day. This dose was non-teratogenic when administered to pregnant rats from Days 6-15 of pregnancy, and did not induce dominant lethal mutations in male mice treated on 5 consecutive days.

A double-blind cross-over study in which human volunteers received either 100 mg Talin protein/day or lactose for 14 days showed no immunological reaction as judged by the absence of an anaphylactic antibody response. The absence of sensitisation was confirmed in a double-blind study in which 50 volunteers consumed 15 g chewing gum/day containing either 150 ppm Talin protein or placebo for 28 days. Clinical examination further revealed that the protein was non-irritant to the buccal mucosa. It may be concluded, therefore, that the use of Talin protein either as a high-density sweetener or a flavour enhancer in food and pharmaceutical products will not be hazardous.

Organophosphate (OP) insecticides are frequently involved in human poisonings. Hemoperfusion is a purported option for treatment of severe poisoning cases when conventional atropine and pralidoxime therapy fails. Rats dosed with 3 mg/kg of parathion i.v. were hemoperfused with a cartridge containing 10 g of XAD-4 resin; hemoperfusion was initiated 30 minutes after injection. Ninety minutes and five hours after dosing, blood was taken from the inlet and outlet sides of the cartridge. Five hours after dosing, rats were sacrificed and the brains and livers were excised and frozen until analyzed by gas chromatography. Tissue clearance of parathion and the total amount of parathion removed were calculated. Parathion was effectively cleared from the blood, but the level of parathion in the brain and liver was not altered by hemoperfusion. The total amount of parathion removed was less than 1% of the dose administered. The ability of hemoperfusion to protect against parathion lethality was examined. After orally administering an LD 99 dose of parathion to rats, hemoperfusion with a cartridge containing 10 g of XAD-4 was begun. Hemoperfusion neither protected against lethality nor delayed the time to death. Kinetic studies indicate that parathion is rapidly distributed to tissues (t1/2 = 14 min.) and the volume of distribution is large (Vd = 45 l/kg). Because of the kinetic properties of parathion, hemoperfusion is not an effective mode of therapy for parathion toxicity. (Supported by NIH Grant ES-07079 and U.S. Army DAMD 17-78-C-8039)

Our previous studies have established that the 12-fold greater toxicity of chlorpyrifos (0.0-diethyl 0-3,5,6-trichloro-2-pyridinol phosphorothionate) (CPS) compared to its dimethyl analog, methyl chlorpyrifos (MCPS), can only partially be explained by extensive glutathione-mediated detoxification of MCPS. The present study identifies additional factors which contribute to this difference. Recovery of brain acetylcholinesterase (AChE) and carboxylesterase (CarE) from inhibition in vivo was faster in mice that received CPS (70 mg/kg) than in mice given an equitoxic dose of MCPS (1000 mg/kg). This faster recovery following CPS probably reflects the more rapid elimination and lower tissue levels of CPS, since brain levels of CPS were 40-50 times higher than those of CPS. Furthermore, no CPS was present 48 hr following administration whereas MCPS was measurable until 7 days after administration. Neither oxon could be detected at any time point. Additionally, the t1/2 for mouse brain AChE reactivation in vitro was 30 min following inhibition by MCPS-oxon (MCPO), but was 8 hr after inhibition by CPS-oxon (CPO). Moreover, after inhibition by MCPO AChE eventually returned to control values, while CPO-inhibited AChE only returned to 50-55% of control levels. The concentration of CP required to inhibit 50% of the AChE activity of mouse brain homogenate (IC50) was 3.6 nM, while that for MCPO was 1733.5 nM. This substantial difference in IC50s and the rates of reactivation of inhibited AChE probably contribute to the marked difference in acute mammalian toxicity of CPS and MCPS. (Supported by grants ES01831 and ES05223 from NIEHS.)


The rabbit was investigated as an alternate animal model for evaluating the acute oral toxicity of intact pesticide granular formulations. Trithion® Technical (Fig 1) is an organophosphorous insecticide-acaricide which may be prepared in granular formulation for crop-field application. While the technical grade Trithion has a purity of 90.5%, the 20G formulation product contains 20.9% active ingredient. In contrast Trithion Technical, a liquid, is easily administered to animals by gavage to determine toxicity, but reliable toxicity testing of the intact granular formulation is more difficult since for many technical reasons it cannot be readily administered directly to rats. Also, if one considers administration of granular material in gelatin capsules to rats there are definite limitations on the amount of granular material that can be administered conveniently. This study was divided into three parts. A feasibility study was first conducted in which it was shown that intact granules in gelatin capsules were administered to rabbits. The second part involved a comparison of the sensitivity of both rats and rabbits to oral doses of the technical material. In the third part of the study, data were evaluated to compare the toxicity of the granular formulation and the non-granular technical material in rabbits to quantitate the reliability of predicting granular product toxicity based on technical material toxicity.


Although it has been well established that many physiological processes exhibit diurnal rhythms in man and animals, this is toxicologically considered when assessing toxicity. These studies therefore were initiated to determine whether the time of dosing significantly influences the actions of a classic hepatotoxin, carbon tetrachloride (CCL4) and a nephrotoxin, mercury chloride (HgCl2). Adult male S.-D. rats were dosed orally with CCL4 (0.05, 0.25, 0.5 ml/kg) and by ip injection with HgCl2 (1 mg/kg) during their active cycle (6 am-6 pm) and sleep cycle (6 pm-6 am) to compare the toxicity of intact pesticide granular formulations. This toxicology was measured by increases in the enzymes ornithine carbamyl transerase, arginine succinate lyase and sorbitol dehydrogenase in serum. Significant increases over controls in the levels of these enzymes were observed in animals treated with 0.05 ml CCL4/kg at 8 hr. When HgCl2 was given at 8 am, 0.25 ml/kg was required to produce significant increases in levels of the enzymes. Similarly, dosing with HgCl2 at 8 am produced maximal decreases in renal cortical transport of organic ions at 24 hr; after HgCl2 dosing at 8 pm, maximal decreases in transport were not seen until 48 hr post-dosage. Alkaline phosphatase and maltase appeared in greater quantities in the urine sooner when HgCl2 was given at.
8 pm. Rhythmic changes in toxicant tissue disposition and metabolism, as well as glutathione (GSH) levels may explain these observations. Maximal levels of GSH were found at around 6 am in the liver, and at 3 am and 3 pm in the kidney. The findings in this study indicate that animals may be more sensitive to chemicals when exposed during their active span. This is most representative of human exposure situations, since man commonly encounters chemicals during his waking hours. (Supported by EPA Grants R808282 and CR807449 and NIEHS Training Grant ES07090)

622 HEAT PRECIPITATION OF ACID SOLUBLE COLLAGEN AS A SCREEN FOR SEVERE CORNEAL OPACIFIcANTS, S. J. Williams, E. I. du Pont de Nemours & Co., Inc., Haskell Laboratory for Toxicology & Industrial Medicine, Newark, DE 19711 Sponsor: G. L. Kennedy, Jr.

An in vitro screen to detect severe eye irritants prior to in vivo testing could avoid production of irreparable damage to the rabbit eye. Since permanent corneal injury is the most insidious form of chemically induced eye damage, a model matrix of collagen and glycosaminoglycan, the most abundant biochemical entities in the cornea, was employed to detect chemical damage. Twelve water soluble chemicals, 7 with known ability to produce corneal opacity and 5 inactive in the cornea, were prepared at 10% concentrations. Zero, 0.01, 0.05 or 10.0 mL of test solution was mixed with 3 mL of collagen solution (0.1% acid-soluble calf skin collagen, 0.2% chondroitin sulfate in 0.05M tris buffer with 0.2M NaCl) in separate cuvettes. The test mixtures were warmed for 2 minutes at 37° and transferred to an isothermal spectrophotometer with % transmittance (540 nm) read every 2 min for up to 80 min. In controls an opaque, semi-solid gel forms with a T-1/2 to maximum of 20 min. With test compounds, the gelation kinetics may be altered. All 5 compounds known to be without effect in vivo had no effect on gelation. Of the remaining 7 chemicals known to be opacifiants, 5 exhibited positive effects while 2 were inactive. Thus, the results from this in vitro test concurred in 83% of the cases with the known in vivo toxicity of each compound. These preliminary results will be expanded by broadening the range of chemicals studied and by prospective studies of new compounds.


This study was designed to assess the toxicity potential of Allyl Methacrylate (AMA) following repeated dermal applications in rabbits for 28 days. Four groups of rabbits were treated with the test material at dose levels of 0, 25, 50 and 100 mg/kg/day. One satellite group of six male and six female rabbits was also treated with 100 mg/kg/day of AMA. Mortality, behavioral reactions, growth and food consumption were observed and measured along with hematology, blood biochemistry, absolute and relative organ weights and pathology parameters. No overt signs of toxicity or behavioral abnormalities were observed among the rabbits. There were no mortalities and no significant adverse effects in the low dose and control rabbits. Of the remaining 7 chemicals studied and by prospective studies results will be expanded by broadening the range of chemicals known to be opacifiants, 5 exhibited no effect on gelation. Of the remaining 5 chemicals known to be without effect in vivo had no effect in vitro. Controls consisted of five untreated hens from each age group. After dosing, the hens were observed for 21 days. The results showed that development of TOTP-induced delayed neurotoxicity was age dependent. While the youngest group (2 month old) did not differ from control hens, all other age groups developed delayed neurotoxicity. Four hens of the 3-month group developed gross ataxia, while the fifth hen progressed to paralysis. Among the 6-month hens, three showed severe ataxia and two developed paralysis. The groups of 12 month old and 18 month old hens each had 3 hens with paralysis and two with ataxia with near paralysis. All 30-month old hens developed paralysis. Histopathological examination showed degeneration of the axons and myelin in the spinal cord and peripheral nerves. Severity of histopathological changes in nervous tissues paralleled that of the clinical condition. Plasma butyrylcholinesterase (BuChE) activity in control hens varied inversely with age. Control hens in the 2-month age group had the highest plasma BuChE activity while the 18-month old hens had the lowest BuChE activity (43% of the first group). This pattern also held true for the TOTP-treated hens. In addition, the younger treated hens recovered plasma BuChE activity, while the older hens had a partial recovery. These results suggest that plasma BuChE may play, at least in part, an important role in the insensitivity of young chickens to delayed neurotoxicity. (Supported in part by NIEHS Grant No. ES02717).
625 EFFECT OF LUNG, LIVER AND KIDNEY TOXICANTS ON RESPIRATORY RATES OF MICE. P.J. Hakkinen*, R. Frankel†, M. Harden, L.A. Balogh*, and H.P. Witschi. *Univ. of Tenn-Oak Ridge Grad. Sch Biomedical Sciences and Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

Studies by Travis et al (Radiat. Res. 84: 133, 1980), and Collis et al (Br. J. Cancer 41: 901, 1980) have shown that changes in the respiratory rate (RR) of mice correlated well with histological changes for both early and late x-ray- and cyclophosphamide-induced lung damage. In the present studies, mice were exposed i.p., i.t. or by inhalation to the lung toxicants, butylated hydroxytoluene (BHT) ± 70% oxygen (O2) ± 200 rad thorax x-irradiation, bleomycin ± 80% O2, cyclophosphamide ± 80% O2, cadmium chloride aerosol, 3-methylfuran, methylcyclopentadienyl manganese tricarbonyl, beryllium sulfate; the kidney toxicant, mercuric chloride; the liver toxicant, carbon tetrachloride; or were starved. Time course studies of the RR changes measured with a total body plethysmograph found that each lung toxicant produced a distinctive change in RR with different days for onset of changes and for peak RR. Studies with BHT ± 200 rad found that the RR right before sacrifice (2 weeks after BHT) correlated well (r = 0.97) with the degree of pulmonary fibrosis as measured by changes in hydroxyproline content. Liver and kidney toxicants and starvation on the other hand produced decreases or slight increases only in RR. These studies suggest that RR measurements in mice allow non-invasive quantitation of the development and resolution of acute injury following lung toxicants. Research sponsored jointly by the Office of Health & Environ. Res., U.S. Dept. of Energy under contract W-7405-eng-26 with Union Carbide Corp. & subcontract 3322 from Biol. Div to the Univ. of Tennessee. †Oak Ridge Assoc. Univ. undergraduate student, Summer 1981 from SUNY, Stony Brook, NY.

626 CHRONIC TOXICITY OF MELAMINE IN FISCHER 344 RATS. G.N. Rao, P.J. Giesler, T.E. Palmer, R.W. Mast, M.A. Friedman, and C.B. Shaffer, Raltech, Division of Ralston Purina Company, Madison, WI. and American Cyanamid Company, Chemicals Group, Wayne, NJ.

Melamine is an intermediate in the manufacture of resins, plastics, and chemical compounds. Chronic exposure of rats to diets containing 0.1 and 1.0% melamine in the diet for two years resulted in melamine phosphate calculi, epithelial hyperplasia, and benign papillomas of the bladder at the 1.0% level. The papillomas probably resulted from irritation due to calculi. The objective of the present study is to evaluate inherent tumorigenicity of melamine, if any, in the absence of bladder calculi. Fischer 344 rats, males at 0.01, 0.05, and 0.12% melamine in the diet and females at 0.01, 0.1, and 0.2% melamine in the diet are being treated for a minimum of 24 months and a maximum of 30 months. Statistically significant differences in body weights, feed consumptions and pharmacotoxic signs were not evident between the treated and control groups during the first 24 months. Hematology, clinical chemistry and urinalysis determinations on 10 animals of each sex at each dose level and control, in urine at 6, 12, 18, and 24 months did not show any differences between the treated and control groups. Necropsy of 10 animals of each sex of each dose level and control at 18 months did not show any bladder calculi and treatment related gross lesions. Histopathological evaluation of tissues of 10 animals of each sex at the control and high dose groups necropsied at 18 months showed changes of nephrosis in kidneys of control and treated groups, and a few thyroid and pituitary tumors in the control as well as the treated animals. The incidence and severity of these lesions in the high dose males and females are not statistically different from the respective control groups. Clinical and pathological evaluation of all animals which died during the course of the study or necropsied at termination of the treatment period is in progress.


Sponsor: F. Reno.

Ribavirin (Virazole, 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a potent antiviral drug that is effective against Lassa fever. Rhesus monkeys and man develop anemia during high-dose treatment with ribavirin. The mechanism by which the anemia occurs may involve RBC destruction, suppression of hematopoiesis or both. The effect of ribavirin on blood and bone marrow was measured in monkeys injected IM with ribavirin for 10 days at a dose of 30 or 100 mg/kg/day. Both groups developed a normochromic, normocyctic anemia by day 10 that was mild in the low-dose group and severe in the high-dose group. Recovery from anemia was complete by day 42. Total and differential leukocyte counts, MCH and MCHC did not change from control levels. Reticulocyte counts and MCV increased after treatment and returned to control levels by day 65. Osmotic fragility of erythrocytes was not changed. Dose-related thrombocytopenia occurred on days 15-22; in both groups; platelet counts returned to control levels on day 42. Dose-related erythroid hypoplasia occurred in bone marrow by day 10 followed by hyperplasia on day 22 and a return to control level by day 42. Myeloid precursors were not affected. Differential counts of erythroid precursors showed a significant (p < 0.05) decrease in late erythroid forms; early forms were either unchanged or increased. Megakaryocytes were significantly increased (p < 0.05) on day 10 in both groups. Vacuolization was observed in erythroid precursors in both groups on day 10 only. Blood and bone marrow effects of ribavirin were reversible when treatment was discontinued.

628 MORPHOLOGIC CHANGES IN THE GASTRIC MUCOSA OF RATS AND DOGS TREATED WITH AN ANALOG OF PROSTAGLANDIN E. A. W. Kramer, Jr., W. J. Dougherty, A. R. Belson, B. M. Henderson, American Cyanamid Company, Medical Research Div., Wilbur G. Malcolm Toxicology Laboratories, Pearl River, N.Y. 10965

Sponsor: M.J. Iatropoulos, Pearl River, N.Y. 10965

American Cyanamid Company compound CL 115,574, an analog of prostaglandin E is active orally in inhibiting gastric acid secretion and in protecting against gastric ulcers induced by stress.
ethanol and non-steroidal anti-inflammatory drugs. Sprague Dawley rats were given doses up to 0.2, 2.0 and 20.0 mg/kg/day for 6 months. Beagle dogs were given doses up to 0.4, 0.8 and 1.6 mg/kg/day for a year. The only toxic clinical sign was diarrhea, which occurred at all dose levels in both species. Microscopic findings at necropsy were limited to the gastric mucosa. They consisted of a dose related widening of the cuticular ridge in the mid and high dose groups. Microscopically, it consisted of a simple hyperplastic of the cuticular ridges; stratified squamous epithelium. The dogs showed a multifocal hyperplasia of the foveolar epithelium in the pyloric antrum. Neither species had atypical cellular changes in their hyperplastic lesions. Furthermore, the hyperplasia in the dog was well differentiated and without pseudostratification of cells and increased mitotic activity. These hyperplastic changes are possibly adaptive reflecting a pharmacologic effect of a prostaglandin possibly related to "cytoprotection."

Sponsor: W.M. Haschek
There are at least 4 reported instances of acute renal failure in humans associated with massive skin exposure to diesel fuel. This suggests that middle distillates can penetrate the skin and, under certain conditions, are potentially nephrotoxic. In the course of a chronic dermal carcinogenesis bioassay of shale oil derived jet and diesel fuels, we have obtained clinical and histopathologic evidence which indicates that these materials, while possessing a very low skin carcinogenic potency, have the potential to induce both acute and chronic renal damage following prolonged skin exposure.
Clinical findings included elevated water consumption and urine production and low urine osmolality. Acute lesions were microscopic and were restricted to the cortex. There was dilatation of tubules in the corticomedullary zone. The distended tubules were filled with proteinaceous precipitates. There was focal necrosis and degeneration of the tubular epithelium.
After 68 weeks of three times weekly dermal exposure, severe renal pathology was evident, both grossly and microscopically. Again, the cortex was most severely involved. Lesions were focal, as well as diffuse. The overall frequency and severity of lesions was both dose and treatment related. Lesions were also more severe in female than in male mice. Histologically, the chronic lesion consisted of a loss of tubular parenchyma. Fibrosis was not present, nor were there any inflammatory infiltrates. Glomeruli did not show changes other than those related to senescence. (Research sponsored by the Office of Health and Environmental Research, US DOE, under contract W-7405-eng-26 with the Union Carbide Corporation.)

The effects of CS₂ on several physiological measures were studied to determine the beneficial effects of intraperitoneal saline on evoked potentials from peripheral nerve and brain, for assessing neurotoxicity in rats. Measurements included body weight, rectal temperature, tail temperature, forelimb and hindlimb grip strengths, latency of the compound action potential of the ventral caudal nerve, parameters of the somatosensory, auditory, and visual cortical evoked responses, and of the brainstem auditory evoked response (BAER). The rats had chronic electrode implants and were tested weekly while awake and restrained. Twelve rats each were dosed with 0.0, 171, 286, or 400 mg/kg of CS₂ in sesame oil (i.p., 2 ml/kg) once per day, five days per week for 12 weeks. Preliminary results after four weeks of injections were as follows: temperatures remained constant and equivalent among the groups; body weight increases were observed in all groups but weight gain was slowed in medium and high dose groups; forelimb and hindlimb grip strengths were reduced in the medium and high dose groups; latency of the caudal nerve action potential was unaffected but latency of the fifth component of the BAER was lengthened in proportion to the dose of CS₂ administered.
Supported by EPA Grant R807150020

631 AGE-RELATED SUSCEPTIBILITY OF RAT ENDOCRINE PANCREAS TO CYPROHEPTADINE. S.A. Chow and L.J. Fischer, Dept. of Pharmacology, Toxicology Center, University of Iowa, Iowa City, IA.
Cyproheptadine (CPH), an antiserotonin-antihistaminic drug, produces pancreatic β-cell damage after repeated oral doses to adult rats. Characteristics of this effect are a reduction in insulin content and vacuolization of the endoplasmic reticulum. The purpose of this study was to examine possible age-related changes in CPH-induced pancreotoxicity. CPH at doses of 5, 11, 22.5 or 45 mg/kg was given orally once daily for two days to 10-, 15-, 25- or 50-day-old Sprague-Dawley rats. Serum and pancreatic insulin (IRI) and serum glucose were measured 24 hrs after the last dose. In all age groups, CPH caused a dose-dependent decrease in pancreatic and serum IRI levels. These effects of CPH, however, were greater in young rats. In 50-day-old rats, a significant decrease in pancreatic and serum IRI was detected only at high doses (22.5 and 45 mg/kg). However, in 10- and 15-day-old rats, the effects were observed after the 5 mg/kg dose. No significant difference in serum glucose level was detected among various doses and age groups. Electron microscopic examination of pancreatic β-cells from young animals indicated a loss of insulin-containing granules and changes in the endoplasmic reticulum.
The time course of CPH-induced changes in the pancreatic β-cell was studied in 15-day-old rats. Pancreatic IRI in CPH-treated rats was significantly lower than control at 24 hrs after the first dose. The maximum effect of CPH on pancreatic and serum IRI was reached at 24 hrs after the second dose and thereafter returned to on-
A six week toxicity study on red dye #40 was carried out to investigate histopathologic effects in male Sprague Dawley mice, weighing 18-22 g. Twenty four mice were divided into 4 equal groups. All mice were conditioned to drink water between 10:00 AM to 10:30 AM. Group I received only drinking water, Group II received drinking water containing 50 mg of bouillon per 100 ml of water, and Group III and IV received red dye #40, 0.25 mg and 0.05 mg/ml, respectively, in water containing bouillon. The water intake was measured after each period and mice were weighed once a week. At the end of six weeks, mice were sacrificed and tissue slices from brain, kidney, liver and spleen were fixed by immersion in a fixative containing 2% glutaraldehyde, 1% formaldehyde, and 1% acrolein in 0.05 M cacodylate buffer at pH 7.2. The samples were rinsed in 0.07 M cacodylate buffer containing 10% sucrose. Post fixation was done in 2% osmium prepared in 0.05 M cacodylate buffer containing 10% sucrose. The samples were rinsed in cacodylate buffer, dehydrated in acetone series and were embedded in Spurr's plastic. Thin sections were stained with uranyl acetate and lead citrate and examined in Siemens Elmiskop 101 at 80kV accelerating voltage. Chronic ingestion of dye #40 had no significant effect on water consumption, growth and development of mice. Also no major histopathologic alterations were observed in the brain, kidney, liver and spleen following chronic ingestion of red dye #40.

Although toxicants are generally reported to decrease homecage food and water consumption, subchronic administration of acrylamide was found to increase episodic milk-licking even in severely intoxicated mice. Two strains of mice, C57BL/6J and CD-1, were injected i.p. 5 times weekly with either saline, 20 mg/kg or 60 mg/kg of acrylamide. Mice were weighed daily and tested for milk-licking (10% sweetened milk) during a 15-min session. Hindlimb grip-strength, a measure of neuropathy, was measured weekly. Mice receiving 60 mg/kg of acrylamide showed a significant increase in milk-licking by day 2 which persisted throughout the 16 day dosing period. There were no qualitative differences in this effect between the two strains of mice and this effect was replicated in two studies using CD-1 mice. The increase in milk-licking occurred before the decrease in hindlimb grip-strength (week 3) or the slight decrease in body weight which actually oscillated around control values. The recovery of grip-strength was complete 4 weeks after dosing stopped. In contrast, the milk-licking of some mice had not recovered even 9 weeks after dosing. The 20 mg/kg dose of acrylamide produced a decrease in grip-strength after 5 weeks but no change in milk-licking or body weight even after 7 weeks of dosing. The new appetite behavioral procedure used in this study has documented a robust behavioral effect of acrylamide which occurred before other signs of toxicity. (Supported in part by PHS Grant OH-00973 from NIOSH, by training Grant ES-07065 and Center Grant ES-00260 from N.I.E.H.S.)

The concern of asbestos carcinogenesis has intensified from an occupational hazard to a general public health hazard. In our attempt to develop a simple and accurate analytical technique for asbestos-form mineral, we observed that many of these naturally occurring minerals were thermoluminescent. Asbestos thermoluminescence presumably results from the thermally-induced release of electrons "trapped" in lattice imperfections. We hypothesized that asbestos thermoluminescence is related to the presence of electron traps and is responsible for its unique biological toxicity. We have found that the thermoluminescence produced upon heating chrysotile asbestos is distinctive and that heated asbestos exhibits decreased biological toxicity. Bovine alveolar macrophages, used to assess asbestos cytotoxicity, were exposed to varying concentrations of untreated, heat-treated and acid-leached Canadian chrysotile asbestos. Compared to untreated asbestos, macrophage viability was significantly higher in both the heat-treated and acid-leached asbestos fiber groups. Macrophage elastase activity, indicative of cell secretion, was increased in all groups compared to unexposed, control macrophages. Additionally, elastase activity from the heated and acid-leached asbestos groups were higher than the untreated asbestos groups. Concurrent with decreased macrophage cytotoxicity, heat-treatment of chrysotile asbestos (≥ 4000°C) reproducibly decreased the amount and rate of bovine serum albumin bound to asbestos. These observations indicate that asbestos interactions with biological materials were reduced after heat treatment and are consistent with the hypothesis that exoelectrons are involved in asbestos fiber toxicity.

Gossypol is a polyphenolic naphthylalddehyde found in the meal and oil of cotton plants (Gossypium sp.) which has been shown to be a potent male contraceptive. It has been postulated that gossypol acts through inhibition of
spasm and motility. We have examined the effects of gossypol on adrenylate cyclase, a key regulatory enzyme in spermatogenesis. A soluble form of adrenylate cyclase was isolated from the seminiferous tubule of mature bovine testes. The enzyme requires a strict requirement for Mn²⁺. ATP as a substrate. The Km value for ATP in the presence of saturating Mn²⁺ ion is 580 µM. The enzyme is insensitive to calmodulin and only slightly activated by sodium fluoride or the non-hydrolyzable GTP analog Gpp(NH)p. Unlike the membrane associated form of adenylate cyclase found in mature sperm, the soluble enzyme is insensitive or inhibited by spermine and protamine. The soluble form of adenylate cyclase is inhibited by gossypol in a concentration-dependent manner. Half-maximal inhibition occurs at a gossypol concentration of 100 µM. In contrast, adenylate cyclase isolated from bovine cerebral cortex and solubilized with Lubrol PX exhibits an EC₅₀ at 500 µM gossypol. The inhibition of soluble adenylate cyclase from testis is not reversed by sodium fluoride, calmodulin or Gpp(NH)p, however inhibition is reversed by high concentrations of ATP (5 mM). Inhibition kinetic data for adenylate cyclase in the presence of gossypol revealed competitive inhibition with an apparent Kᵢ = 100 µM. We conclude that gossypol inhibits testicular adenylate cyclase by formation of a Schiff base in the active site. Supported by NIH grant GM 22897.

636 THE INTERACTION OF ANTHRAQUINONE DERIVATIVES WITH DNA AND PROTEIN IN VITRO. I. INTERCALATION WITH DNA. B.M. Elliott and J. Ashby, ICI, Central Toxicology Lab., Alderley Park, Nr. Macclesfield, Cheshire, SK10 4TJ, UK. Sponsor: I.F.H. Purchase. Over 100 9,10-anthraquinone (AQ) derivatives have been previously examined in the Ames test, and many found positive in S.typhimurium strain TA1537 in the absence of any added metabolising fraction. This activity profile is suggestive of intercalation, and 12 AQs with hydroxy, amino or nitro groups were therefore studied for their ability to bind reversibly to DNA in vitro. All the AQs showed a bathochromic shift in their visible spectra with calf-thymus DNA at pH 7.2, suggesting a change to a more hydrophilic environment. The ionisation of 1,4-dihydroxy AQ (1 x 10⁻¹⁰M) on increasing the pH, was prevented by DNA (4 x 10⁻¹⁰M(P)) indicating removal from contact with the aqueous environment. These data suggest an association of the AQs with DNA that may be a stacking along the wide groove, or an insertion within the base pairs (intercalation). Only 1,4-diamino AQ however showed competition for the binding sites of ethidium bromide (a known intercalating agent) on DNA and none of the AQs showed any stabilisation of the double helix to thermal denaturation even at a chemical DNA:DNA(P) concentration ratio of 1:3. Equilibrium dialysis of [¹⁵³C]-labelled 1,4-dihydroxy, 1-amino-2-methyl and 1-nitro-2-methyl AQ, showed the greatest affinity for DNA with 1,4-dihydroxy AQ, with an affinity constant of 4.0 x 10⁻¹⁰M⁻¹, 100-1000 times less than that for reference intercalating agents. It is concluded that the reversible interaction of AQ derivatives with calf-thymus DNA is of low affinity (4 x 10⁻¹⁰ to 4 x 10⁻¹² M⁻¹) and that intercalation is unlikely on its own to account for the Ames positive responses.

637 AN INVESTIGATION OF ³⁵S-SULPHATE INCORPORATION INTO RAT BONE MARROW CELL SUSPENSIONS AS A MEANS OF QUANTITATING CELLS WITHIN THE MYELOID SERIES. A F Wright, N M Blackett*, M S Rose*, ICI, Central Toxicology Lab., Alderley Park, Nr. Macclesfield, Cheshire, SK10 4TJ. *Institute of Cancer Research, Chester Beatty Institute, Fulham Road, London SW3 6JZ. +ICI Corporate Biosciences Group, Runcorn Heath, Cheshire, UK.

The selective incorporation of ³⁵S-sulphate, as a result of mucopolysaccharide synthesis in certain bone marrow cells has been identified as a process that may be utilized for quantitation of specific cells. A method for measuring the rate of ³⁵S-sulphate incorporation in bone marrow cell suspensions has been developed. The amount of radiolabel associated with perichloric acid precipitates of bone marrow cells increased with time of incubation, was dependent on energy production and protein synthesis, and represented incorporation into macromolecules. Autoradiography of cells incubated with ³⁵S-sulphate has demonstrated that the radiolabel was associated specifically with bone marrow cells within the myeloid series. When bone marrow cell suspensions were prepared from rats treated with 120µg endotoxin (Salmonella typhosa), the nucleated cell number was reduced (30% at day 1), whilst the rate of ³⁵S-sulphate incorporation was increased (100% at day 2). Determination of the cellularity of the bone marrow by conventional histopathology confirmed that endotoxin predominantly affected the myeloid cell series. Intermediate and late myeloid precursors were depleted (60% at day 1) whilst early myeloid cells increased by 50% at day 3. These data suggest that the incorporation of ³⁵S-sulphate into rat bone marrow suspensions is able to reflect specific myeloid cells. Such methods may provide additional quantitation of chemically-induced myeloid dyscrasias within the bone marrow.


7-Methylguanine (7-MG) is formed in liver DNA during hepatotoxicity induced by a variety of chemicals that cannot directly methylate DNA (L.R. Barrows and R.C. Shank, Toxicol. Appl. Pharmacol. 60, in press). In a study using radiolabeled N-nitroso-pyrrolidine (NP), it was shown this hepatotoxin and hepatocarcinogen is not metabolized in the rat to an agent which methylates liver DNA guanine (Topics in Chemical Carcinogenesis, 213, 1972). 7-MG was detected in liver DNA of adult male Sprague-Dawley rats after administration of a hepatotoxic dose of NP using a highly sensitive high pressure liquid chromatography method which optically detects fluorescent alkylated purines in DNA hydrolysates without the need for radiolabel. Rats were given 14, 28, 57, 113, 287, 587, or 900 mg NP/kg body wt and decapitated 12 hr later; liver DNA was isolated, purified, and hydrolyzed at pH 7.0 or 1.0. The amount of 7-MG in liver DNA obtained at each dose level was 25, 64, 87, 58, 97, 112, and 57 µmol per mol guanine, respectively. No 7-MG was detected in comparable
amounts of liver DNA from rats 3 or 12 hr after treatment with vehicle alone. The detection of 7-MG in liver DNA of rats treated with NP is consistent with similar observations in liver DNA from rats treated with such diverse hepatotoxins as hydrazine, carbon tetrachloride, ethanol, phosphorus, bromobenzene, and puromycin (Shank, unpublished data). Although mechanisms for the indirect methylation of DNA guanine during hepatotoxicity remain undefined, 7-MG produced in liver DNA following treatment with NP may be related to the hepatotoxicity and carcinogenicity of this compound. (Supported by PHS Grant CA 21853 and Air Force Contract F33615-80-C-0512).

639 OXIDATION OF TETRAMETHYL HYDRAZINE BY PEROXIDIZING ENZYMES: AN ELECTRON SPIN RESONANCE STUDY. K. Sivarajah, B. Kalyanaraman, R. P. Mason, and T. E. Elling. Laboratory of Pulmonary Function and Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Hydrazine derivatives are used in medicine, agriculture, and the aerospace industry. Some of them are carcinogenic or toxic. In view of such widespread application, a knowledge of their metabolism is of importance in furthering our understanding of detoxification/toxicification mechanisms. The oxidation of tetramethylhydrazine (TMH) was carried out with horseradish peroxidase and H2O2. Electron spin resonance (esr) spectroscopy identified the one-electron oxidation intermediate as the TMH radical cation. We propose a tentative mechanism involving the TMH radical cation and dication as possible intermediates during the oxidation of TMH. Subsequently, prostaglandin synthetase (PGS) was used to carry out a similar peroxidatic reaction. Aerobic incubation of ram seminal vesicle (RSV) microsomes (a source of PGS), arachidonic acid and TMH yielded the typical esr spectrum of the TMH radical cation, which was indomethacin sensitive. Incubation of RSV, TMH and H2O2 under N2 yielded the same esr spectrum which was indomethacin insensitive. Thus, a peroxidative reaction mechanism was inferred. Demethylation of TMH was measured by measuring the formaldehyde (HCHO) formed from aerobic incubations containing RSV, TMH and arachidonic acid. The HCHO formation increased with increasing enzyme and substrate concentrations. The cation radical or the decay product formed may covalently bind to the tissue macromolecules and result in toxicity.


An homologous series of hair dyes was selected for percutaneous absorption studies with excised human skin. It was of interest to observe the effect of small changes in structure on skin absorption as well as to attempt to correlate the permeability of the compounds with solubility properties. These properties were expressed as (i) the octanol/water partition coefficient (Ko) and (ii) the stratum corneum/water partition coefficient (Km). The compounds selected were: p-phenylenediamine (p-PDA, I), o-PDA, 2-nitro-p-PDA, 2-amino-4-nitrophenol, 4-chloro-m-PDA, and 4-amino-2-nitrophenol (II).

Permeability studies were conducted by standard diffusion cell techniques using an aqueous vehicle. Skin absorption was expressed in terms of a permeability constant (kp). Compounds I and II exhibited the lowest and highest permeability, respectively (kp I=2.4x10^-4 cm/hr; kp II=2.8x10^-3 cm/hr).

Ko values ranged from 0.5 to 13.5, and with one exception (2-amino-4-nitrophenol, Ko=13.5) they increased in the same order as the Ko values. In general, changes in structure which increased the lipophilicity of the molecule were found to result in a corresponding enhancement of permeability. The moderate lipophilicity of the compounds as evidenced by the Ko values is in agreement with the moderate percutaneous absorption observed.

Km values were determined for 3 compounds from the decrease in concentration of dye in distilled water during a 3 day incubation. The values did not rank the compounds in the same order as did their Ko and Km values. It appears that binding to the stratum corneum may occur since a concentration dependence was observed on the resultant Km values.

641 DEPLETION OF MOUSE HEPATIC GLUTATHIONE BY SELECTED ENDOGENOUS COMPOUNDS. R.C. James, S.M. Roberts and R.D. Harbison. Division of Interdisciplinary Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR

Narcotics deplete hepatic glutathione (GSH), induce hepatocellular injury and potentiate the hepatotoxicity of other agents. These effects may be regulated by CNS receptor control or result from a direct chemical interaction of these compounds. The purpose of this study was to determine if other compounds affecting the CNS or hepatic receptors might also produce similar effects. Epinephrine (Epi), 0.5 mg/kg; glucagon, 1.0 mg/kg; dexamethasone, 1.0 mg/kg; and ethanol, 2.0 mg/kg, significantly lowered GSH by 20-50% within the first 3 hours following treatment. Epi increased serum glutamic-pyruvic transaminase (SGPT) levels at least 100%, which was similar to the elevation produced by morphine. Glucagon had no effect on SGPT. The adrenergic blocker propranolol did not alter Epi-induced lowering of hepatic GSH, but phentolamine completely antagonized this effect. Similarly, the α2 blockers, prazosin and phenoxybenzamine had no effect on the Epi induced lowering of GSH, while the α1 antagonist, yohimbine, caused complete blockade. Correspondingly, the α2 agonists guanabenz and clonidine also lowered GSH. Interestingly, phentolamine significantly antagonized the morphine depletion of GSH and conversely naltrexone significantly antagonized Epi, but neither inhibition was complete. These studies suggest that several important endogenous compounds can lower hepatic GSH and therefore potentiate other hepatotoxic agents. This effect may be receptor mediated with some stereochemical specificity and overlap between the narcotic and adrenergic effects. (Supported by USPHS Grant ES00782)

Administration of intravenous doses of 200 mg/kg/day of echinocandin B for 3 days to beagle dogs resulted in signs of toxicity related to an intravascular hemolytic crisis. In vitro incubation of echinocandin B at concentrations achieved in vivo with canine and human erythrocytes produced lysis and a shift in the fragility curve. Analogs of echinocandin B prepared by microbial deacylation of the linoleic acid moiety followed by selective N-acylation were tested for their effects on erythrocytes. The following relationships between structure and erythrocyte fragility were determined. Fragility increased with: 1) length of fatty acid side chain, 2) saturated vs unsaturated fatty acids, 3) trans vs cis configuration of the fatty acid, 4) lesser degree of unsaturation, and 5) position of unsaturation. Evaluation of erythrocyte fragility and antifungal activity led to the selection of the n-tridecanoyl, n-lauroyl-p-aminobenzoyl, and p-n-octyloxybenzoyl analogs of echinocandin B for further toxicity studies in dogs (100 mg/kg/day, 1x5, 5 days). As predicted by the in vitro erythrocyte fragility testing, all compounds were less toxic than echinocandin B.

644 Hypoglycemic effect of the venom from the snake Piscivorus piscivorus. S.A. Taha, Department of Pharmacology, College of Pharmacy, University of Riyadh, Riyadh, Saudi Arabia. Sponsor: K.N. Salman

Venom from the snake P. piscivorus caused a significant reduction in the blood glucose level of both rats and rabbits. In normal rats the hypoglycemic effect was of short duration, reaching a peak 30 minutes after injection, and lasting for 1 hour. The effect was produced by doses as low as 10 µg/kg; doses higher than 20 µg/kg, although causing more pronounced hypoglycemia, were lethal to the rats. No hypoglycemia was produced in alloxan diabetic rats. In moderately and severely diabetic rats, the combined administration of insulin and the venom did not potentiate the hypoglycemia. Dose response curves for the blood glucose level of insulin-treated and insulin + venom treated animals were not parallel. Measurement of plasma insulin showed about 50% increase in insulin level 30-60 minutes after venom injection. It is postulated that the venom acts partly through release of insulin and partly by a direct mechanism.


Hexafluoroacetone (1,1,1,3,3,3-hexafluoro-2-propanone sesquihydrate, HFA) is used as an organic solvent, as an intermediate for organic synthesis, and as a process intermediate. The material has been shown to be both toxic and teratogenic to pregnant rats following dermal exposures. In this study, male Crl-Cr® rats, 3 groups, 10 rats per group, were clipped free of hair over the back area and fitted with Teflon® collars to reduce grooming and ingestion. The material was applied dermally, 5 days/week for 2 weeks at doses of either 65, 130, or 260 mg/kg. Another group of 10 rats treated with water served as controls. Clinical signs and body weights were recorded daily. Five rats from each group were sacrificed 4 hours following the 10th exposure and given a complete pathologic examination, as were the remaining rats following a 14-day recovery period. Rats treated at 260 mg/kg showed severe clinical signs including the 2 deaths following the 5th and 1 death following the 10th dose. Lactation, rapid breathing, lethargy, and weight loss were seen. Rats given 130 mg/kg showed weakness and moderate to slight weight loss with incomplete recovery post-exposure. Rats treated with 65 mg/kg showed slight weight loss which was not apparent during the recovery period. Testicular degeneration and atrophy were seen in all HFA groups with the damage most obvious at the 2 higher levels. These changes were still apparent following the 14-day recovery period. Depletion of lymphocytes in the spleen and thymus along with hypocellularity and hypoplasia of the bone marrow were also detected, both lesions being dose-related and reversible.

645 Intravenous (IV) toxicity study of homoharringtonine (HH) (NSC 141633) in CDF1 mice and Beagle dogs. K.N. Newman, R.G. Meeks, Southern Research Institute, Birmingham, AL; J.P. Ferrell, Experimental Pathology Laboratories, Inc., Herndon, VA; and K.S. Greenspun, Battelle TPO, Vienna, VA.

HH, a drug derived from Cephalotaxus harringtonia, an evergreen native to China, has been shown to have marked antitumor activity against transplanted tumors of mice and is in clinical use in China. Following single (x1) and five daily dose (x5) lethality studies in mice, x1 and x5 studies were performed in mice and dogs to determine target organ toxicity.

Calculated estimates of the LD10, LD50, LD90 in mice were 12.8, 18.0, and 25.3 mg/m² (x1) and 5.8, 8.6, and 12.8 mg/m²/day (x5).

In the mouse toxicity studies anemia, reticulocytopenia, thrombocytopenia, and leukopenia were histopathologically confirmed by degeneration and depletion of the lymphoid and hematopoietic tissues in mice sacrificed on day 4 (x1). In the x5 study, lymphoid depletion of the thymus was observed at the day 8 sacrifice, but increased hematopoiesis in the bone marrow, liver, and spleen was indicative of recovery.

In dogs given x1 and x5 HH IV, gastrointestinal (GI) (emesis, hemorrhagic diarrhea), cardiac (tachycardia, premature ventricular contractions, ventricular arrhythmias), bone marrow (granulocytopenia), and lymphatic (lymphocytopenia) toxicity were observed. Liver and kidney damage (elevated SGOT, SGPT, SAP, and BUN) and electrolyte imbalances were seen. Histopathological changes were seen in the lymphoid system, marrow,
GI tract, and kidneys. Myocardial degeneration was seen in dogs given 1x HH IV at the mouse equivalent (ME) LD50 (12.8 mg/kg). Seven of eight dogs (x1 and x5) that received the ME LD50 died from treatment. ME LD50/2 and ME LD50/10 were not lethal. Supported by NCI Contract NOI-CM-17365.

646 RED CELL KINETICS IN RIBAVIRIN-TREATED MONKEYS. P.G. Canonico, M.D. Kastello, and G. Wannarka. Dept. of Antiviral Studies, U.S. Army Med. Res. Inst. Inf. Dis., Frederick, MD. Sponsor: F. Reno. Man and thesus monkeys may develop anemia during treatment with the broad spectrum antiviral ribavirin (Virazole, 1-8-D-Ribofuranosyl-1,2,4-triazole-3-Carboximide). To assess whether the anemia is due to increased production of erythrocytes, increased destruction, or a combination of both factors, the disappearance of 1,3-3-DI-3-isopropyl fluorophosphate ("H-DFP) labeled erythrocytes was measured in ribavirin-treated monkeys.

Monkeys were divided into 3 groups, their red blood cells (RBC) labeled in vitro with "H-DFP and reinjected into each donor. The first two groups were treated with either 15 or 60 mg/kg of ribavirin (IM) for ten days. The third group served as saline treated controls. Hemograms showed that by day 10 of treatment there were 12-26% and 53-60% decreases in RBC, hematocrit and hemoglobin in the low and high dose groups, respectively. All values returned to normal levels by day 42. A dose-related decrease in RBC survival was observed from day 0 to 28. Thereafter, red cell half-lives were comparable to control values. These data indicate that ribavirin at a dose as low as 15 mg/kg decrease the half-life of RBC. This effect is reversible upon discontinuation of the drug. At 60 mg/kg, ribavirin also inhibited the release of RBC from the bone marrow. Termination of treatment was accompanied by release of RBC from the bone marrow as indicated by a drop in the specific activity of "H-DFP-labeled red cells and marked reticulocytosis. No inhibition of RBC release from the bone marrow was seen in the low-dose group. We conclude that ribavirin can decrease red cell survival as well as inhibit RBC release from the bone marrow. Both effects appear fully reversible when treatment is withdrawn.

647BILE DUCT PROLIFERATION AFTER 4-FLUORO-8-OXOBENZENEPROPANENITRILE ADMINISTRATION IN MONKEYS. R. J. Arceo, M. R. Irving, and M. J. Iatropoulos, American Cyanamid Company, Medical Research Division, Wilbur G. Malcolm Toxicology Laboratories, Pearl River, NY 10965

4-Fluoro-8-oxobenzopenanenitrile (FOBPN), a non-steroidal anti-inflammatory compound, when administered by nasogastric intubation at 10, 60 and 100 mg/kg, continuously for up to 12 months to cynomolgus monkeys, induced hepatobiliary changes. These changes consisted mainly of biliary duct hyperplasia with periductal inflammatory response. Associated were also varying degrees of fibrosis with focal necroses of hepatocytes bordering the fibrosis. No morphologic or clinical evidence of cholestasis was evident. None of the clinical chemical and hematologic parameters showed any deviation from the controls.

Metabolic studies showed that the compound is rapidly metabolised and not present in plasma in measurable amounts 4 hours after drug administration. Furthermore, the glycine conjugate of FOBPN, which is formed by the loss of the cyanide group, in only accounting for 4% in the urine, with significant cyanide containing metabolite present in plasma and urine. It is therefore postulated that the cyanide group present, is responsible for the biliary changes.

648 NEPHROTOXICITY AND KIDNEY CONCENTRATION OF SELECTED FURAN DERIVATIVES IN MICE. L. Gammal, R. Wilex, G. Traiger, and S. Baraban, Departments of Pharmacology & Toxicology and Medicinal Chemistry, The University of Kansas, Lawrence, KS 66045.

The furans are a class of compounds which have widespread occurrence in the environment and produce toxic effects in the liver, lung, and kidney of rodents. The purpose of this study was to determine if a correlation exists between renal concentration of selected furan derivatives and nephrotoxicity. Renal tissue concentrations after 1, 2, and 5 hr and nephrotoxicity were determined in mice for the following furan derivatives: furan, 3-ethylfuran, 3-pentylfuran, 2-ethylfuran, 3-methylthiophene, and 2-furamide. Three indices were used to assess nephrotoxicity: histological observation, blood urea nitrogen (BUN) concentration and renal urine concentrating ability in water-deprived mice. The results demonstrated that 3-ethylfuran (150-300 mg/kg), 2-ethylfuran (150-250 mg/kg) and 3-pentylfuran (250 mg/kg) caused massive proximal tubular necrosis, elevated BUN levels and decreased urine concentrating ability. Furant (175-350 mg/kg) caused proximal tubular necrosis but did not affect either BUN levels or urine concentration. 2-Furamide (150 mg/kg) and 3-methylthiophene (600-900 mg/kg) caused no change in any of the parameters measured. All compounds were shown to reach the kidney in reasonable concentrations, but there did not appear to be a simple correlation between kidney concentration and extent of damage among these substances. Supported by GM-26,366.

649 CARDIOTOXICITY OF BETA-ADRENERGIC AGONISTS IN MICE OF VARIOUS AGE GROUPS. X. Joseph, V. Wakehurst and T. Balazs, Food and Drug Administration, Washington, D.C.

To examine the influence of age on the sensitivity to the cardiotoxicity of beta adrenoceptor agonists, we studied the acute toxicity and the myocardial lesion-inducing effects of isoproterenol and terbutaline in 3 groups of 4-, 8- and 16-week-old CD-1 mice with average body weights of 26, 33 and 38 g, respectively. The LD50 values for isoproterenol were 342.5, 317.7, and 325.1 mg/kg for 4-, 8- and 16-week-old mice.
respective; for terbutaline the values were 206.3, 213.9 and 191.7 mg/kg for the respective groups. Each group was injected ip with 1/10 and 1/100 of the LD50 isoproterenol or terbutaline with appropriate controls, and the hearts were examined histologically. Lesions were graded on a scale of 0 to 3 with 3 being the most severe. Isoproterenol at 1/10 of the LD50 produced an average lesion score of 1.85 in 16-week-old mice as compared to scores of 0.62 and 0.6 for 8- and 4-week-old mice, respectively. Similarly, 1/100 of the LD50 dosage produced a greater score in 16-week-old mice. Terbutaline did not produce myocardial necroses in any age group. These data indicate that even though the LD50 values do not vary significantly among the various age groups, the 16-week-old mice are more sensitive to the lesion-inducing effects of isoproterenol than are younger mice and that terbutaline, a predominantly S2 agonist, is much less cardiotoxic than isoproterenol, a B1 and B2 agonist, in mice. The significance of age vs body weight in the sensitivity to isoproterenol is being investigated.

The primary advantages of this system include: 1) enhance sensitivity - the use of functional parameters should provide a more sensitive index of toxicity than morphological parameters; 2) extensive automation - computer support decreases operator time, increases precision, and permits rapid screening of large numbers of animals; 3) versatility - this system provides the capability to utilize a variety of animals, both anesthetized and unanesthetized, ranging in age from fetuses to geriatrics, and permits studies of block as well as longitudinal design; and 4) ease of replication - standardization of equipment and techniques facilitates replication by other laboratories.

Previously short-term studies with octachlorostyrene (OCS), a demonstrated environmental contaminant indicated that it could produce hepatomegaly, induce mixed function oxidases and cause histological changes in thyroid and liver. The present study was undertaken to determine the effects of subchronic exposure to this chemical. Male and female rats were fed diets containing 0, 0.05, 0.5, 5.0, 50 or 500 ppm OCS in the diet for 13 weeks. No mortality occurred at any dose level. Hepatomegaly was observed in both males and females at 50 and 500 ppm OCS. Increased kidney and spleen weights were observed at 500 ppm OCS. Decreased hemoglobin values occurred with 50 and 500 ppm OCS in male rats and with 500 ppm in females. Decreased hematocrit values were observed at 500 ppm in both males and females whereas decrease red blood cells were observed only in males receiving 500 ppm OCS. Induction of mixed function oxidases was observed in males at 5.0 ppm and higher and in females at 50 ppm and higher. Histological changes in thyroid, liver, and kidney were noted in all treatment groups of both sexes. Octachlorostyrene accumulated in fat and liver in a dose-dependent manner. The data generated in this study suggest that prolonged feeding of OCS can produce biochemical, hematological and histological changes at low levels and that males tend to be more susceptible to the toxic effects of this chemical than females.
vealed severe focal lung edema (31.2 mg/kg) and edema with alveolar hemorrhage (62.4 mg/kg).

Swelling, vacuolization and congestion of hepatocytes occurred at both doses. No pathological changes were seen in the spleen, pancreas, kidney or heart. Humans exposed to DAP should be monitored for lung and liver damage in addition to changes in cardiovascular parameters. (Supported in part by the Research Institute of Pharmaceutical Sciences, University, MS and the Amer. Found. for Pharmaceut. Ed.).

IN VITRO RATES OF COLLAGEN SYNTHESIS IN MOUSE LUNG TISSUE FOLLOWING THE ADMINISTRATION OF BUTYLATED HYDROXYTOLUENE. J.P. Kehrer, Dept. of Pharmacology, College of Pharmacy, The University of Texas at Austin, Austin, TX. (sponsored by D. Acosta)

Treatment of mice with butylated hydroxytoluene (BHT) results in the formation of a dose-dependent lung lesion. At doses of 300 mg/kg or greater pulmonary fibrosis and the deposition of excess collagen became evident within 14 days as shown by an elevation of total lung hydroxyproline. The in vitro rate of collagen synthesis was measured in minced lung tissue as the formation of [3H] hydroxyproline from [3H] proline at 1, 2, 3, and 4 hours of incubation at 37°C in Dulbecco's Modified Eagle's Medium. The rate of synthesis was elevated two days after BHT 400 mg/kg and reached a maximal rate of 150 pmol/mg dry wt/hr at day 7. The rate then declined but was still significantly elevated at day 14. Expressing these data as a percentage of total protein synthesis committed to collagen demonstrated a specific stimulation of collagen synthesis to a level of 1.5% at 7 days after BHT compared to control levels of 0.6%. Both the maximum and the control levels were the same as that reported in vivo following BHT 400 mg/kg. Doses of BHT as low as 200 mg/kg produced a significant increase in both the collagen synthetic rate and the percentage of collagen synthesis. While DNA synthesis was not elevated at BHT doses less than 300 mg/kg, doses greater than this produced a dramatic increase. The rate of collagen synthesis exhibited a more linear dose-response relationship. These data show that the rate of collagen synthesis is elevated at levels of lung damage which do not result in the deposition of excess collagen. This study also shows that percentage of protein synthesis devoted to collagen is the same in vivo and in vitro in both normal and damaged lung tissue and that in vitro rates of collagen synthesis are a sensitive index of acute lung damage. (Supported by NIH-BRSG grant no. RR-07991-15 awarded to the Univ. of Texas at Austin.)

ACRYLAMIDE EFFECTS ON ACTIVITY OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN RAT BRAIN HOMOGENATE. I.L. Vyas, R.D. Howland and R.E. Lowdnes, CMDNJ, NJ Medical School, Newark, NJ.

Acrylamide inhibits several glycolytic enzymes in vivo and in vitro, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and neuron-specific enolase (NSE). We investigated the kinetics of acrylamide inhibition of GAPDH and its ability to antagonize inhibition in tissue homogenates and its ability to antagonize inhibition in tissue homogenates and its ability to antagonize inhibition in tissue homogenates and its ability to antagonize inhibition in tissue homogenates and its ability to antagonize inhibition in tissue homogenates and its ability to antagonize inhibition in tissue homogenates and its ability to antagonize inhibition in tissue homogenates and its ability to antagonize inhibition in tissue homogenates. Its ability to antagonize inhibition in tissue homogenates was also investigated. Enzymatic activities were determined spectrophotometrically at 340 nm. Rat brain homogenate was incubated at 37°C + acrylamide. Inhibition is via an irreversible, noncompetitive mechanism with K_i= 1.12mM. Acrylamide at 1.25mM inhibited 50% of GAPDH activity. Addition of d-glucose-6-phosphate (5mM) resulted in a 48% increase in the concentration of acrylamide required for 50% inhibition of activity. Cysteine was also capable of antagonizing acrylamide inhibition of GAPDH but was only one-tenth as potent. These results indicate that acrylamide can inhibit GAPDH directly in tissue homogenates and therefore the in vivo inhibition may also be a direct inhibition. Supported by NIH Grant ES-20405.

EFFECT OF PHTHALATE ESTERS ON SUCCINATE OXIDATION AND ENERGY COUPLING IN RAT LIVER MITOCHONDRIA. R.L. Melnick and C.H. Schiller, National Toxicology Program and Laboratory of Pharmacology, NIHES, Research Triangle Park, NC 27709

A comparative study was conducted on the effects of monobutyl phthalate (MBP), dibutyl phthalate (DBP), mono(2-ethylhexyl)phthalate (MEHP), and a single dose of DPP and at 1, 2, 3 and 4 days following multiple doses. The testes were excised and fixed and sections made for examination by both light and electron microscopy. After 6hr the lumen of the seminiferous tubules (ST) had disappeared and by 26hr a marked degeneration of spermatids was prevalent, spermatocytes were also affected but to a lesser degree. At 4 days almost all ST showed atrophy with complete disruption of the ST cell organisation.

Ultrastructural studies showed an early effect on Sertoli cells, with an accumulation of autophagic vacuoles. The Sertoli cell cytoplasm in the central area of the ST showed degeneration and detachment from the immature spermatids. At the same time spermatids showed changes in Golgi structure, with mitochondrial damage and a decrease in rough ER. Later stages showed that Sertoli cell cytoplasm continued to atrophy accompanied by an accumulation of lipid. Thus it would seem that DPP-induced damage to the testis is rapid in onset and that this may be due to a disruption of the interactions between Sertoli cell cytoplasm and developing germinal cells. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food).
di(2-ethylhexyl)phthalate (DEHP) on energy coupling and electron transport activities of isolated rat liver mitochondria. Energy coupling was evaluated by 2 approaches: 1) active transport of K+ (plus valinomycin) driven i.p. resulted in the oxidation of two respiratory chain substrates, succinate and ascorbate + TMPD, and by the hydrolysis of ATP; and 2) succinate respiration rates in the absence and presence of ADP (respiratory control ratios). Energy-linked processes were uncoupled most by DBP and MEHP. MDP had a moderate effect on energy coupling and DEHP had no apparent effect. The potencies of inhibition of succinate cytochrome c reductase activity followed the order MEHP > DBP > MDP = DEHP. MDP was found to be a non-competitive inhibitor of succinate dehydrogenase (K_i = 2.1 x 10^{-4} M). It is concluded that phthalate esters affect mitochondrial activities by altering permeability properties of the inner membrane and by inhibiting succinate dehydrogenase activity.


Rats were trained to lever touch for food on a Variable Interval (VI) 15 sec schedule of reinforcement. Dose-response alteration of VI responding by apomorphine, d-amphetamine, clonidine, and chlordiazepoxide was studied alone and following an acrylamide treatment, which, by itself, did not alter VI responding. Male Fischer 344 rats were given 12.5 mg/kg of acrylamide by gavage 24 hrs prior to challenge with the psychoactive drugs. In the absence of acrylamide pretreatment, apomorphine produced responding that was 71%, 52%, and 20% of baseline after i.p. injections of 0.05, 0.1, and 0.2 mg/kg, respectively. Corresponding values after acrylamide pretreatment were 42%, 26%, and 15% of baseline, which is a significant shift in the dose response curve to the left. Likewise, d-amphetamine given i.p. resulted in values that were 81%, 53%, and 14% of baseline after 0.25, 0.5, and 1 mg/kg, respectively. Following acrylamide pretreatment, responding was 59%, 42%, and 11% of control, respectively. No significant effect of acrylamide pretreatment on the behavioral effects of clonidine and chlordiazepoxide was observed. These data suggest that acute exposure to acrylamide increases responsiveness to catecholaminergic agents. The change in responsiveness to apomorphine and d-amphetamine may be related to the effects of acrylamide on the affinity or density of dopamine receptors reported elsewhere (Agrawal et al., Pharmacol. Biochem. and Behav., 14:527, 1987).


Several hypolipidemic drugs and the plasticizer di-(2-ethylhexyl)-phthalate cause proliferation of hepatic peroxisomes and induce peroxisomal enzymes in rats and mice. Since these compounds also induce liver tumours in rodents, a relationship between peroxisome proliferation and carcinogenesis has been suggested. We describe here the induction of peroxisome proliferation in primary hepatocyte cultures.

Hepatocytes were isolated from male rats (180-250g) by a collagenase perfusion technique and were cultured in supplemented RPMI 1640 medium containing the test compounds or DMSO. Electron microscopy of hepatocytes cultured for 48 hours in the presence of clofibrate, monoo-(2-ethylhexyl)phthalate or 2-ethylhexanol (10^{-4}-10^{-5}M) showed markedly increased numbers of peroxisomes. There was a time- and dose-dependent induction of palmitoyl CoA oxidation and carnitine acetyltransferase (CAT), two peroxisomal markers. CAT activity reached 15 times control levels in cells cultured for 72hr with 5x10^{-4}M clofibrate, whereas the mitochondrial marker carnitine palmitoyltransferase was only increased by 3-fold at this point. No effects on peroxisome number or enzyme activity were produced by n-hexanol or by two microsomal enzyme inducers, phenobarbital and 1,2-benzanthracene. These findings indicate that primary hepatocyte cultures may be useful for studying the mechanisms underlying, and the consequences of, peroxisome proliferation. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food).


For the evaluation of long term effects of the oral contraceptive Norlestrin, groups of 16 sexually mature female rhesus (Macaca mulatta) monkeys were studied over 10 years. Norlestrin, a combination of norethindrone acetate and ethinylestradiol (50:1) was given continuously on a cyclic regimen (21 days on drug, 7 days off) at levels of 0.05, 0.51, and 2.55 mg/kg, representing 1, 10, and 50 multiples of the human dose. Selected clinical and laboratory parameters were monitored throughout the study at established intervals. All dose levels were well tolerated, survival was not affected and there were no alterations in coagulation or other laboratory parameters. Ophthalmologically macular pigmentary anomalies were observed in all groups. Treatment associated pathologic findings included ovarian and uterine atrophy and dilatation of acini and ducts in the mammary gland. These findings constituted exaggerated pharmacologic responses together with superimposed advanced senile changes. Periodic vaginal cytologic examinations and mammary gland palpations did not demonstrate drug-related changes. The neoplasms found were three cutaneous papillomas (1 low dose, 2 high dose); an uterine leiomyoma (high dose); a pancreatic duct adenoma, (low dose) and a granulosa cell adenoma of the ovary (control). The overall evaluation of the study indicated an adequate long term safety margin since no malig-
nacies were elicited and 0.51 mg/kg or 10 times the human dose, was considered to be the no-effect dose due to the absence of significant toxicity or pathology.


Ametantrone acetate (NSC 287513) is an experimental antineoplastic agent with activity against a comprehensive panel of solid transplantable tumors in mice. Studies in mice and dogs were carried out to establish tolerable levels. In mice, LD10, LD50 and LD90 values were 26, 35, and 47 mg/kg following single iv injection and 22, 67, and 266 mg/kg after single ip injection. Hemorrhage and necrosis of the small intestine occurred in intercurrent deaths. A five daily iv dose study yielded LD10, LD50, and LD90 values of 18, 21, and 25 mg/kg in mice. Bone marrow hypoplasia, lymphoid depletion and focal cardiac changes were observed only in animals which died during a 28 day post-dose observation period. In dogs, two iv injections 3-8 weeks apart resulted in a toxic dose high (TDH) of 2.71 mg/kg (54.2 mg/m²) and a toxic dose low (TDL) of 0.68 mg/kg (13.6 mg/m²). Five daily iv injections of TDL in dogs induced significant clinical and laboratory signs of toxicity but 1/2 TDL (0.35 mg/kg/day) was tolerated. Following single or multiple iv administration in dogs, bone marrow, lymphoid tissue, and gastrointestinal tract in both sexes and gonads in males were target organs for toxicity. Clinical signs and clinical laboratory abnormalities abated in mice and dogs which survived the post dosing observation period. The spectrum of tissue changes obtained with this compound shows essentially no qualitative difference from those changes elicited with alkylating agents.

**TOXICOKINETICS OF HYDRAZINE AND H70 VIA LIMITED PERCUTANEOUS EXPOSURE IN THE RABBIT.** Keller, W. C., Murphy, J. P., Andersen, M. E., Bruner, R. H., and Back, K. C., AFAMRL/TH, Wright-Patterson AFB, OH.

Hydrazine is used as a propellant in Titan II missiles and H70 (70% hydrazine) is the propellant in the emergency power unit of the F16 fighter creating the possibility that Air Force maintenance personnel may be subject to accidental percutaneous hydrazine exposure. Groups of 4 or 5 New Zealand white rabbits were percutaneously exposed to either anhydrous hydrazine or H70. Exposure was terminated at various times after application by a dilute hypochlorite wash and distilled water rinse. Another group of 5 rabbits was given 10X hydrazine IV. All groups of rabbits received doses of 12 mg hydrazine/kg. Serum hydrazine concentrations were determined spectrophotometrically as described by Reynolds and Thomas. The time course of serum hydrazine concentrations was determined for all groups. Examination of a semilogarithmic plot of the time course of the IV serum hydrazine concentration supports the use of a one compartment model to describe hydrazine elimination. The apparent volume of distribution, serum half-life, and elimination rate constant for hydrazine were 0.63 liter/kg, 2.3 hours, and 0.29 hr⁻¹ respectively. Moderate depression was observed in the IV group rabbits and severe chemical burns were observed in all the percutaneously exposed groups. Selected sections of hydrazine exposed skin from each group of rabbits were examined histopathologically to correlate burn severity with duration of hydrazine exposure. Bioavailability was determined by comparing the areas under the serum hydrazine-time curves of the percutaneously exposed groups with the IV group. Anhydrous hydrazine was absorbed more quickly (28% vs 15% at 2 minutes) and to a greater extent than H70 hydrazine (86% vs 55%).


Sprague-Dawley rats, (45M, 45F, weight range 311-440 grams and 158-260 grams, respectively) were anesthetized by ether inhalation, intraperitoneal (IP) injection of pentobarbital, or IP injection of T-61, prior to blood collection and necropsy, to determine the suitability of these agents as euthanizing agents in toxicity studies. Blood was obtained from the orbital sinus after anesthesia for hematologic and clinical chemistry determinations.

Major differences in AST (SGOT) and ALT (SGPT) between groups were found. Rats given T-61 had a marked increase in AST [220.5 ± 99.7 (males) and 183.5 ± 75.1 (females) vs. 88.3 ± 22.3 and 95.4 ± 31.1 for the groups given ether]; and in ALT [75.4 ± 41.7 (males) and 49.1 ± 24.7 (females) vs. 28.3 ± 6.1 and 24.5 ± 4.3 for the groups given ether]. There were less marked differences in thrombocyte count and serum potassium. Rats injected with T-61 had a brownish discoloration of abdominal fat which interfered with gross examination of the abdomen.

The effects observed made IP injection of T-61 an unsuitable means of pre-necropsy anesthesia of rats when hematology, clinical chemistry and necropsy data are to be obtained.

* T-61® = Euthanasia solution, National Laboratories.

**SOURCES OF INTRALABORATORY VARIABILITY AND THE EFFECTS OF DOSE VOLUME IN OCULAR TOXICITY TESTING.** S. J. Williams, and G. J. Graepel. E. I. du Pont de Nemours & Co., Inc., Haskell Laboratory for Toxicology & Industrial Medicine, Newark, DE 19711. Sponsor: G. L. Kennedy, Jr.
This study investigates the sources of variability in scoring ocular lesions and the sensitivity and reproducibility of the test using a dose volume of 0.01 mL instead of the standard dose of 0.10 mL of test material. A series of 7 previously tested compounds were chosen and assigned letter codes A-H (B and G were the same). Thirteen rabbits were used each week for a total of 6 weeks (72 rabbits). Each week, 7 rabbits were dosed with 0.01 mL of test materials A-H (excluding G) by direct application to the cornea. Compound G, used as a standard to measure test variability, was applied to each of the remaining 6 rabbits at a dose of 0.10 mL. Eyes were scored independently by 2 experienced readers at 1 and 4 hours and at 1, 2, 3, 7, 14 and 21 days post-dosing. Statistical analysis demonstrated that the greatest source of variability was rabbit to rabbit difference. Test group (one week to the other) and reader bias (except at 4 hours) did not contribute to the observed variability. Also, when comparing 0.10 mL of G with 0.10 mL of B, a dose-effect relationship was clearly discernable. When the compounds tested at 0.01 mL were ranked from severest to mildest based on maximal score and duration of effect, the order was the same as that obtained from previous studies using the higher volume, but the effects were lessened. Thus, reducing the dose volume from 0.10 to 0.01 mL has little effect on the sensitivity of the test (some very mild irritants may not be detected), yet the extent and duration of damage to the rabbit eye is lessened.

664 A NOVEL METHOD FOR QUANTITATIVE CUTANEOUS TOXICO­KINETICS. J.M. Holland and J. Kao, Biology Div., Oak Ridge National Lab., Oak Ridge, TN.

The skin is a primary route of exposure to many occupational hazards as well as a surface upon which drugs and cosmetics are intentionally applied. In spite of this there is little systematic information dealing with the translocation and coupled bioconversion of surface applied materials by mammalian skin.

To permit accurate quantitative comparisons across species, while preserving metabolic viability and the unit skin structure, we have designed a compact, multisample, skin permeability chamber. One inch diameter skin discs form the upper seal of a 1 mL chamber, through which fresh perfusate is pumped. The effluent from each chamber passes to a fraction collector. The chamber is water jacketed, permitting incubation at any temperature.

Studies with benzo(a)pyrene on mouse skin reveal that metabolism is linear through 5 micrograms. The rate of benzo(a)pyrene metabolism, as judged by recovery of radioactivity in the chamber effluent, is sigmoid, with the exponential phase occurring approximately two hours after application. The overall extent of metabolism is influenced by strain, sex and status of the mixed group (one week to the other) and reader bias (except at 4 hours) did not contribute to the observed variability. Also, when comparing 0.10 mL of G with 0.10 mL of B, a dose-effect relationship was clearly discernable. When the compounds tested at 0.01 mL were ranked from severest to mildest based on maximal score and duration of effect, the order was the same as that obtained from previous studies using the higher volume, but the effects were lessened. Thus, reducing the dose volume from 0.10 to 0.01 mL has little effect on the sensitivity of the test (some very mild irritants may not be detected), yet the extent and duration of damage to the rabbit eye is lessened.


The reduction of pain and stress on laboratory animals is always a desirable goal. This can be accomplished only to the extent that the method used to relieve pain does not jeopardize test results. This study investigated the effects of treatment of the rabbit eye with anesthetics prior to performing testing for eye irritation. Control rabbits were dosed with one of five model irritants. Test rabbits were dosed with an anesthetic in both eyes and an irritant in only one of the eyes. Eyes were examined and scored with and without an ocular slit lamp. All animals were sacrificed on the 21st day post dosing and the eyes were retained for histopathological evaluation. Of the four anesthetics tested, tetracaine HCl, given in two equal doses 10-20 minutes apart, was found to be the anesthetic of choice for use in the rabbit eye. This drug showed the combination of pain relief and the least alteration in ocular test scores.


The nucleoside, ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), effective against high hazard viruses such as Lassa fever may produce hematological changes in some species. The present study determined the extent of these changes in monkeys given a ribavirin regimen to be used in Lassa fever-infected patients. Rhesus monkeys were randomly assigned to either a ribavirin or saline control group. Drug was administered IV in a 33 mg/kg loading dose, followed with 17 mg/kg q6h for 4 days, then 8 mg/kg q8h for an additional 6 days; control monkeys received an equivalent volume of saline. Blood was drawn from the femoral vein at selected intervals. Red blood cell counts decreased significantly by day 6 but returned to pretreatment levels by the third week post-treatment. Hematocrits were reduced 30% by day 7, reached their nadir on day 11 with a mean value of 22%. Hemoglobin values dropped to 7-8 mg/dl, remained at that level for 10 days, and gradually returned to pretreatment levels. Three days after discontinuance of drug, reticulocytes increased over 400% and returned to control levels by day 44. On day 5 of treatment, platelet levels increased, peaked on day 14 and returned to baseline levels. No significant changes were observed in total and differential WBC counts. Haptoglobin, SGOT, SGPT, total and direct bilirubin, BUN, blood glucose and body weight were not altered. These
data indicate that the proposed therapeutic regimen of ribavirin modifies the normal hemogram of rhesus monkeys. Observed effects appear to be fully reversible with hemograms returning to normal limits upon discontinuance of ribavirin. This is an important consideration in the treatment of Lassa fever in man.


The purpose of this study was to determine the toxic effects of Levair® [NaAl3H14(P04)34H20] in beagle dogs when administered in the diet at concentrations of 0, 0.3, 1.0 or 3.0 percent for 6 months. Levair is widely used commercially as a leavening acid. Six male and 6 female beagle dogs were assigned to each of the four treatment groups. All animals were observed twice daily for physical and behavioral changes. Body weights and food consumption were reported each week. Selected hematological and serum chemistry parameters were measured during the pre-test acclimation period and at 6-week intervals during the study. Urinalyses were performed on samples collected prior to initiation of the study, and at 8, 17 and 25 weeks. After 27 weeks of treatment, all animals were necropsied. Vital organs were weighed and tissues were examined microscopically. No adverse treatment related clinical signs were observed. Mean body weights of all groups of animals fed Levair were comparable to those of the controls at all weekly determinations. Weekly mean food consumption values of all male groups given Levair were comparable to those of the control group throughout the study. Statistically significant (p<0.05) food consumption depression was found sporadically in all groups of female dogs given Levair. No significant absolute or relative organ weight differences were found between any of the Levair treated groups and their respective controls. Evaluation of hematological, blood chemistry and urinalysis data revealed no toxicologically significant trends. Microscopic examination showed no histomorphologic changes considered to be related to treatment.


Abnormal platelet function in patients with chronic renal failure has been associated with elevated levels of phenol and phenolic acids in serum. In vitro studies have demonstrated the inhibition of secondary aggregation by phenol, suggesting the site of action to be at the release reaction. The studies discussed in this paper explored: (1) the dose related effect of one minute exposure to phenol on human platelet aggregation, (2) the effect of time and temperature on the response of platelets to phenol, and (3) the ability to overcome the inhibitory effect of phenol on platelet aggregation.

Platelet aggregation was measured by the loss of turbidity in a Chronolog Aggregometer at 37°C, with the secondary wave quantified as the percent change in light transmission from 1-5 min after ADP induction. A one minute exposure to phenol was found to inhibit the secondary wave of ADP induced platelet aggregation in a dose-dependent fashion. When studied with relation to the time and temperature of incubation of phenol with platelet rich plasma, the inhibition was found to decrease with increasing pre-incubation time at 37°C but not at 0°C. It was also found that the inhibitory effect of phenol can be overcome by the addition of arachidonic acid. Thus, these findings confirm inhibition of secondary aggregation by phenol. Furthermore, they suggest the site of this action of phenol is at the initiation of the secondary wave of aggregation.

This work was supported in part by grant ST-32ES07026 from the National Institute of Environmental Health Sciences.


Octachlorostyrene (OCS) is an environmental contaminant found in the Great Lakes region of North America. In view of the lack of toxicity data on this compound, acute and subacute experiments were carried out. For the acute study, groups of 10 male were administered by gavage single oral doses of OCS at levels of 1300, 1690, 2190, 2850 or 3710 mg/kg and killed 14 days later. No deaths occurred at any dose level. OCS at a level of 1690 mg/kg and higher caused increased liver weights, mixed function oxidase activity and serum cholesterol and uric acid levels. In the subacute study, groups of ten male and female rats were fed diets containing 0.5, 5.0, 50 or 500 ppm of OCS for 28 days. Growth rate and food consumption were not affected by treatment. Liver hypertrophy and hepatic microsomal enzyme induction were observed in animals fed 50 ppm OCS or higher. Elevations in serum cholesterol, total protein, potassium and SDH occurred in rats fed 500 ppm OCS. Histological changes occurred in the liver and thyroid of rats exposed to levels as low as 5.0 ppm of OCS. OCS residues accumulated in the fat and liver in a dose-dependent manner. These data suggest that although OCS has a very low acute toxicity, the administration of low levels in the diet for 28 days can produce both biochemical and histological changes in the rat similar to other organohalogens.


Cadinene, a mixture of isomers with partially unsaturated sesquiterpenoid structures, occurs nat-
urally in essential oils from Junipers and Cedars. It is used as a flavoring agent in foods and as a fragrance in cosmetics and soaps. Cadinene was selected for the NTP Bioassay program because of the lack of toxicity or metabolism data in the literature and its human use pattern. In the 13-week toxicity study, F344 rats (10/sex/group) were given cadinene mixed in the diet at 0 (I), 3125 (II), 6250 (III), 12500 (IV), 25000 (V) and 50000 (VI) ppm. Significant decreases in mean body weight and/or food consumption were seen in all treated groups except the II males. Mortalities (four) occurred only in the VI group. Organ weight changes included increased liver (III - VI groups) and decreased testicular (VI males), and thymic weights (V and VI groups). Histopathological findings included toxic hepatitis: biliary hyperplasia, hepatocellular degeneration, necrosis, regeneration, and intracellular pigment occurred in various degrees among V, VI males and III - VI females. Toxic nephrosis: tubular degeneration (and cortical necrosis in animals that died) and regeneration and pigmentation in tubuliner epithelial cells occurred in various degrees in all male groups and IV - VI females. Testicular degeneration occurred to a trace degree in IV, V males and a severe degree in VI males; there was also atrophy of preputial and prostate glands. VI females revealed ovarian, endometrial and clitoral atrophy. Lymphoid depletion was evident in thymus and mesenteric lymph nodes in the VI group. The findings in liver and kidney were considered to be direct test article effects, whereas, effects on reproductive and hematopoietic tissues may have been secondary to inanition.

**671 THE LEDTOX PROTOCOL SYSTEM: AN AUTOMATED, INTERACTIVE PROTOCOL INFORMATION SYSTEM.**


An automated protocol information system has been developed at the Medical Research Division of American Cyanamid as part of an interactive real time data acquisition and reporting system supporting the operations of Toxicology Research. The Toxicology Project Management Group enters planning information via the planning subsystem. The Study Director then interactively builds the direct test article effects, whereas, effects on reproductive and hematopoietic tissues may have been secondary to inanition.

**672 IDENTIFICATION OF LABORATORY ANIMALS.**


Federal guidelines regarding the conduct of toxicity studies specify that test animals be uniquely identified. Means for identification have included ear tags, ear-toe-clips and tatooes. Another method utilizing a radio transponder non-surgically implanted in each animal and a non-contacting electronic interrogator has been developed. The transponder, which is implanted subcutaneously in unanaesthetized animals, has circuitry for utilizing an external power source to transmit one of a possible thousand different identification codes, thus providing accurate verification of animal identification and ensuring compliance to GLPs. The system also allows simultaneous collection of body weight data when coupled to an electronic balance. In a preliminary evaluation of the system's potential utility in long-term bioassay programs, "dummy" implants of the same size and consisting of transponder encapsulation material were inserted into 10 weanling rats. After 14 months, a grossly-apparent thickening of tissue surrounding the implants was seen in all rats. The thickenings exhibited slow growth and achieved relatively constant size 1-2 months after first being observed. Gross pathological examination of 4 rats that died within 16 months after implantation revealed the presence of fat tissue accumulation around the implants. Except for mild, transient dermatitis in the region of the implant observed in 8 rats early after implantation, no gross abnormalities have been observed. Weight gain and blood chemistries have been normal. Tissue and systemic responses of the rats to the implants presently appears to be favorable. Further progress in evaluating the responses to these implants will be presented.

**673 ACUTE TOXICITY OF URANIUM EXTRACTANT (UE) AND UE CO-SOLVENT.**


Uranium extractant, an organophosphorus, was developed with a dibutyl butyrophosphonate co-solvent system for the recovery of low concentrations of uranium from phosphoric acid waste streams. The UE co-solvent was designed to be used with UE in the recovery process. A Tier I acute battery of tests were employed to establish a data base for these compounds. UE and UE co-solvent produced irreversible ocular damage in rabbits thus being classified as corrosive eye irritants. Both UE and UE co-solvent were also irritant and corrosive to intact non-abraded rabbit skin. UE was a dermal nonsensitizer in guinea pigs. In the acute dorsal toxicity test in rabbits at 2 mL/kg, UE caused necrosis of the application site. Body weight was significantly (p < 0.05) depressed in treated animals for at least one week post dosing. The tests showed evidence of germinal epithelial atrophy. UE was also tested for acute oral toxicity in F-344 rats at dose levels of 1.25, 3.0, 5.0 and 10.0 mL/kg in both sexes. The oral LD50 was 3.9 mL/kg in males and 4.4 mL/kg in females. Females in the 3.0 mL/kg group showed a significant (p < 0.05) depression of body weight. The rate of body weight gain for
both males and females was significantly (p < 0.05) depressed on days 1, 4, 7 and 11 post-dosing. Clinical observations deemed compound related included: mydriasis, lacrimation, cyanosis, prostration, diminished respiration, hematuria and salivation. Necropsy observations associated with UE treatment included: liver discoloration, urogenital staining, hemorrhagic lung, intracranial hemorrhage and porphyrins out of mouth, nares and eyes. In summary, acute exposure to UE resulted in severe ocular and dermal irritation and both oral and dermal systemic toxicity. The co-solvent is similarly a severe ocular and dermal irritant.


Chronic exposure to benzene (B) causes progressive degeneration of bone marrow (BM) and aplastic anemia or leukemia. B-induced anemia might result from covalent binding of a toxic metabolite to BM mitochondrial (mt) DNA and subsequent inhibition of mt transcription and translation. Mt from the livers of partially hepatectomized rats injected ip with B (2200 mg/kg) 10 hr post-hepatectomy show an inhibition of mtRNA synthesis when incubated in vitro. Regenerating rat liver mt, free of microsomal contamination, incubated with B in vitro show a dose-dependent inhibition of all three types of RNA synthesis and an inhibition of protein synthesis. The effects of benzene on mt macromolecular synthesis do not appear to result from solvent effects since equivalent concentrations of toluene do not inhibit. Inhibition of mtRNA synthesis by B requires NADPH suggesting that B is activated by a mono-oxygenase system in the organelle. Mt from rat liver, incubated in vitro with 1 mM B, metabolize the B to hydroquinone, catechol, phenol and two unknown compounds as determined by HPLC; one of these compound may represent the active species which covalently binds to mtDNA. Most importantly, mt from rabbit BM cells incubated in vitro with 1 mM B also show an inhibition of RNA synthesis. Covalent binding of B to mtDNA results in an inability of mtRNA polymerase to transcribe the genome which subsequently results in an inability of translation. Since an inhibition of mt protein synthesis can result in a loss of cellular energy derived from oxidative phosphorylation, the consequence in BM cells might be an inhibition of the maturation of the precursors of the circulating blood cells leading to aplastic anemia. (Supported by ES00322).

675 THE EFFECT OF AN EXPERIMENTAL REDUCTION OF SODIUM IN DRINKING WATER ON THE BLOOD PRESSURE DISTRIBUTION PATTERNS OF ELEMENTARY STUDENTS. E.J. Calabrese and R.W. Tuthill, Division of Public Health, University of Massachusetts, Amherst, MA.

An experimental bottled water study assessed the effect on blood pressure (BP) of lowering sodium concentration in the water of some fourth graders of a "high" sodium community who had previously been shown to have significantly higher blood pressure than comparable students in an adjacent "low" sodium community closely matched for socio-demographic variables of concern. For three months, trios of children matched by sex, school, and baseline BP, used different water for all cooking and drinking purposes, with BP monitored bi-weekly. Pupils were randomly allocated to the three water conditions: (1) high sodium water bottled from their own community distribution system, (2) low sodium water bottled from the distribution system of the comparison community with sodium added to the level of the high sodium community water, and (3) low sodium water bottled from the distribution system of the low sodium community but with no sodium added.

Preliminary findings indicated that BP levels among the girls but not the boys on the low sodium water exhibited marked decreases in BP over the test period when compared to the other two "high" sodium groups.
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