1. THE IMPORTANCE AND RESULTS OF PROPER MORPHOLOGIC EVALUATION OF THE NASAL TURBINATES IN INHALATION TOXICITY STUDIES. G.C. Jersey and R.J. Kociba, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U. S. A., Midland, MI 48640.

Morphologic evaluation of the nasal turbinates and nasal mucosa of animals used in inhalation toxicity studies provides a sensitive method for detecting the irritant properties of volatile chemicals. Historically, evaluation of these tissues has often been overlooked or was inadequate. The acceptable methodology is simple and readily available. Inhalation exposure to an irritating chemical typically results in degeneration, necrosis and inflammation of the nasal mucosa; this may lead to hyperplasia and metaplasia of the mucosal epithelium. Dose-response relationships are readily assessed by grading the extent and severity of the lesions. The nasal mucosa is composed of two distinct types of epithelium, olfactory and respiratory. Differential toxicity has been demonstrated in which a chemical injured primarily the olfactory epithelium while exposure to another chemical resulted in injury to both types of epithelium. The significance of these differential effects on the nasal mucosa in long-term inhalation studies is presently being pursued.

2. SENSORY IRRITATION WITH TOLUENE DIISOCYANATE IN SINGLE AND REPEATED EXPOSURES. G.K. Sangha and Y. Alarie, University of Pittsburgh, Dept. of Ind. Env. Hlth. Sci., GSPH, Pittsburgh, PA 15261.

Various concentrations of 2,4 toluene diisocyanate (99.7%) were studied to establish RD50 values from concentration-response relationships for exposure periods of 10, 30, 60, 120, 180, and 240 minutes using male Swiss-Webster mice as the test animal. It was established that the sensory irritation response in the animals (i.e., decrease in respiratory rate) develops slowly at all concentrations tested, reaching a maximum in about 180 minutes. Repeated exposures, 3 hours/day for 5 consecutive days at exposure concentrations from 0.0072 ppm to 2 ppm revealed that a cumulative effect could be obtained at concentrations above 0.023 ppm. Recovery rates were dependent on both exposure concentrations as well as exposure durations within the ranges given above.

Supported by the Mobay Chemical Fellowship in Toxicology and in part by NIOSH Grant #2RO1-08-00367.

The sensory irritation potential of sulfur dioxide/acrolein mixed atmospheres were evaluated. Three series of mixtures were studied. Series I used constant SO\textsubscript{2}/acrolein conc. ratios; Series II used fixed acrolein conc. and various SO\textsubscript{2} conc; and Series III used fixed SO\textsubscript{2} and various acrolein conc. The conc used were 0.85-3.4 ppm for acrolein and 9-140 ppm for SO\textsubscript{2}. The results demonstrated that, depending upon the concentration ratios of these sensory irritants either can alter, or completely block, the effect of the other during the exposure. Following exposure to these mixtures, the recovery phase was delayed, and almost always showed an additional response, although the exposure had ended. This effect has not been previously observed. A possible mechanism is proposed involving the formation of acrolein bisulfite adduct. Additional inhalation studies supported this hypothesis. Thus, two sensory irritants have been shown to exhibit an antagonistic effect that is ratio-dependent, and can lead to a secondary response following exposure.


Deposition efficiency and site of deposition within the respiratory tract were determined for sulfuric acid mist. Controlled parameters were droplet size, external relative humidity and aerosol concentration. Deposition efficiency was determined in the rat, dog and guinea pig by exposing the animals via the nose-only route to sulfur-35 labeled sulfuric acid mist and determining deposition by measuring excreted radioactivity. Sites of deposition in the dog were determined by monitoring appearance of radioactivity in the blood and comparing to observed rates of appearance following instillations in various portions of the respiratory tract. The deposition efficiency of sulfuric acid was determined to be 3-4 times that for a dry aerosol and is largely independent of droplet size or external relative humidity. Deep lung deposition of sulfuric acid was determined to be negligible for mists with mass median aerodynamic diameters of 0.4 \mu m or greater. (Research supported by the EPA under an Interagency agreement under DOE Contract EV-76-C-04-1013.)
5. DIFFERING TOXICITY OF 0.4 AND 0.8 μm SULFURIC ACID AEROSOLS IN GUINEA PIGS. R.K. Wolff, S.A. Silbaugh, D.G. Brownstein and J.L. Maunderly. Inhalation Toxicology Res. Inst. Albuquerque, NM. Sponsor: R.O. McClellan

Acute mortality studies of 8 hr exposures to sulfuric acid aerosols were carried out in Charles River Hartley guinea pigs. Groups of 16 animals were exposed at a relative humidity of 80% to graded concentrations of two different well-defined particle sizes - 0.8 μm mass median aerodynamic diameter (MMAD), σg 1.4; and 0.4 μm MMAD, σg 1.3, based on measurement by cascade impaction. Exposure to 0.8 μm aerosols at concentrations of 22, 32 and 43 mg/m³ resulted in mortalities of 6, 56 and 95%, respectively, giving an LC50 of 30±3 mg/m³. In contrast, exposure to the 0.4 μm aerosols at concentrations of 43, 83 and 109 mg/m³ resulted in mortalities of only 4, 13, and 31%, indicating an LC50 of greater than 109 mg/m³. Higher concentrations could not be achieved with the 0.4 μm aerosols because the coagulation limit had been reached. Animals that died had lungs which collapsed marginally, inflated poorly, and for the 0.8 μm group were congested and hemorrhagic although the 0.4 μm seemed less so. The extraordinary difference in toxicity for the two particle sizes may be related to differing deposition sites of the inhaled material. (Research supported by DOE Contract EY-76-C-04-1013.)


People are continuously exposed to effluents from fossil fuel combustion. These exposures occur through direct inhalation of airborne effluents, inhalation of contaminated soils after resuspension, and by ingestion of contaminated foods or water. The metabolism of one trace pollutant, selenium, was studied in rats after inhalation and ingestion of different chemical forms. A simulation model of selenium metabolism was developed from the organ distribution and retention data obtained from these studies. This model was modified to account for the deposition and absorption of selenium in people by using the ICRP Task Group on Lung Dynamics recommendations for inhalation and the gastrointestinal model of Dolphin and Eve for ingestion. Predictions of the composite model for the uptake and organ retention of selenium from the environment were compared to measured organ burdens in humans. (Research supported by the EPA via DOE Contract EY-76-04-1013 under an Interagency Agreement.)
7. THE PULMONARY RETENTION OF COAL DUSTS IN DOGS

P.E. Morrow, F.R. Gibb, H. Beiter and P. Amato. Department of Radiation Biology and Biophysics, University of Rochester, Rochester, NY

Investigations into the retention of inhaled coal dusts have been undertaken in dogs using neutron-activated coals. Several radionuclides of trace metals were identified, studied independently after inhalation and by in vitro tests, and subsequently utilized for the serial measurement of retained coal dust. Subjects were exposed briefly (1 - 2 hours) to either an anthracite or bituminous coal each having markedly differing pneumoconiotic potential, at a concentration > 100 mg m⁻³ and an AMAD of 1.0 μm, og 2.9. Histologic examination of lungs and pulmonic lymphoid tissues was emphasized. Additionally, the differential properties of the radionuclide labels were studied in relation to particle size distribution and leaching characteristics. Completed studies indicate pulmonary retention half times in excess of 1,000 days.


The purpose of this study was to determine the retention kinetics of fly ash particles in the rat lung following inhalation. Fly ash from the baghouse of a fluidized bed coal combustor was neutron-activated for 40 hr with a neutron flux of 9 x 10¹³ cm⁻² sec⁻¹. The activated fly ash particles contained several radionuclides including ⁴⁰Sc and smaller amounts of ⁵⁹Fe, ⁶⁰Co, ¹⁵²Eu and ⁵⁴Mn. Rats were given a nose-only exposure to an aerosol of fly ash with a mass median aerodynamic diameter and geometric standard deviation of 3.8 μm and 2.9, respectively. The animals were whole-body counted periodically and groups were sacrificed at 0, 2, 4, 8, 16, 30, 63 and 127 days after exposure. High resolution gamma spectra of the lungs were obtained using a Ge(Li) detector. The whole-body retention was described with a 3-component exponential function with a terminal half-life of 88 days. Comparisons among the radioactive components suggested that Mn and Co were preferentially dissolved from the fly ash particles in vivo while Sc, Fe and Eu appeared to remain associated with the ash particles. (Research supported by DOE Contract EY-76-C-04-1013.)

The deposition and internal distribution of the TPM of cigarette smoke has been characterized in BC3F1/Cum mice. Smoke was generated using a newly developed Smoke Exposure Machine (SEM) which has the capacity to expose 480 mice to smoke at one time. Using a radioactive tracer for the TPM in smoke, the quantity of TPM found throughout the body of the mouse has been determined after exposure to varying smoke concentrations and smoke exposure times. Thirty mice were evaluated for each exposure condition. At conditions favoring maximum TPM deposition, 70% of the TPM was found in the lung and 80% was contained in the entire respiratory tract. The deposition in the lung was 80-90 µg TPM per cigarette. Thus, the smoke generated penetrated to the rodent lung and was not trapped in the upper respiratory tract. As the exposure time or smoke concentration increased, the particulate deposition increased proportionately in the lung and only slightly in the head, larynx, stomach and other body tissues.

10. ACUTE MORTALITY DUE TO INHALATION OF THERMAL DECOMPOSITION PRODUCTS OF SYNTHETIC AND NATURAL POLYMERS. R.C. Anderson and Y. Alarie, University of Pittsburgh, Dept. of Ind. Env. Hlth. Sci., GSPH, Pittsburgh, PA 15261.

The acute mortality (LC₅₀) due to inhalation of decomposition products of polymeric materials was determined using a method described by Anderson and Alarie (J. Combustion Toxicology 5, 54, 1978). Materials were heated at 20°C/minute. Four male Swiss-Webster mice were exposed (head only) to various concentrations of the decomposition products for 30 minutes from the time of initial weight loss of each sample. Deaths occurring during this period and 10 minutes following exposure were included in the LC₅₀ calculations. LC₅₀ values, expressed in grams of each polymer were: polytetrafluoroethylene 0.4, polycarbonate 8.3, polyvinylchloride 11.3, polyurethane foam 8.3, polystyrene foam 5.8, fiberglass reinforced polyester 67.5, Douglas fir 63.8, and ureaformaldehyde 2.5. Using Douglas fir (wood) as a reference material, the LC₅₀ data were grouped as follows: less toxic than wood >200 g., as toxic 20-200 g., more toxic 2-20 g., and super-toxic < 2 g. Major toxicants responsible for acute mortality varied for each polymer from CO, HCN, or HCl.

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11. THE EFFECTS OF METHYLENE CHLORIDE INHALATION ON LUNG PHOSPHOLIPIDS IN THE RAT. F. L. WIESENFELD AND A.G. ULSAMER, DIRECTORATE FOR ENGINEERING AND SCIENCE, CONSUMER PRODUCT SAFETY COMMISSION, BALTIMORE, MD.

Methylene chloride is a widely used solvent whose lung effects have been little studied. The present work utilizes male and female Wistar Lewis rats exposed to 4000 ppm CH₂Cl₂ for four hours per day for three weeks. Lung effects were monitored by changes in tissue weight, phospholipid (PL) concentration and composition, and incorporation of radioactive precursors. Total lipid phosphorous analysis of whole lung indicated a significant increase in lung PL concentration for male rats exposed to CH₂Cl₂ but not for females. Lung PL composition in exposed rats of both sexes showed significant increases in phosphatidylcholine (PC). A significant decrease was found in phosphatidylinositol in both sexes. Total lung PL specific activity in exposed males increased 1.5 times over control male values when ³²P was used as a precursor. The incorporation of choline 1,2 ¹³C into PC increased twofold in exposed males over that found in controls. The incorporation of ethanolamine ¹⁴C into either PE or PC was not augmented by CH₂Cl₂ exposure. The data show that CH₂Cl₂ induces significant changes in lung lipid that are reflected by metabolic alterations.


Various attempts have been made to design an inhalation chamber for conducting metabolic studies on volatile compounds in small laboratory animals. We report here on the design and construction of a 27 liter closed cycle, recirculating inhalation system for such studies. The main component of the system, a 25 liter all glass exposure chamber, is capable of housing 4-6 rats and is designed for the separate collection of urine and feces. Other components of the system include: (1) a diaphragmatic pump for recirculating chamber air, (2) a vacuum transfer system for introducing known quantities of volatile chemicals into the system (3) a rotameter for monitoring air flow, (4) a differential pressure gauge and (5) an ascarite-chromosorb trap for removing CO₂ and moisture from the chamber air. Material balance studies using ¹⁴C-labeled ethylene oxide have been conducted and greater than 95% of the radioactivity introduced into the system was recovered. Exposures have been conducted for up to 6 hours at relatively constant concentrations of ethylene oxide. The advantage of this type of system is in the ability to quantitate the uptake of chemical vapor by the animals.

Twenty young, normal male volunteers were exposed to clean air (sham) and to NH₄NO₃ aerosol (exposure) (200 μg/m³, mass median aerodynamic diameter 1.1 μm, geometric standard deviation 2.3). Pulmonary function tests were performed at the start (pre) and after (post) each 2-hour exercise-sham or exercise-exposure period. Pre-exposure to post-exposure changes in flow volume functions (mean FVC, max FVC, mean FEV₁, max FEV₁, V50, and V25) did not differ significantly on the exposure day as compared to the sham day. Nor did single-breath ΔH₂, RV, and TLC differ. The function CC/TLC (%) did not show a significant change and the function, CV/VC (%) significantly decreased (a change in the direction of improvement) on exposure day. No significant change was noted for respiratory resistance. The data do not indicate that exposure to worst case concentrations of NH₄NO₃ aerosols, in the absence of other contaminants, significantly alters the lung function of normal human volunteers. We expect to continue this study in Fall 1978, using asthmatic volunteers.

14. THE BIOTRANSFORMATION AND BILIARY EXCRETION OF ETHALFLURALIN IN MALE RATS. G.K. Hanasono and C.L. Moss, Toxicology Division, Lilly Research Laboratories, Greenfield, IN. Sponsor: J.S. Wold

Ethalfluralin (E), N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine, a dinitramine herbicide, is rapidly metabolized by rats. The peak plasma concentration of E (150 ng/ml) 2 hours after a single 100-μg/kg oral dose of ring-labeled [¹⁴C]-E represented less than 2% of the total plasma radiocarbon content. Peak tissue concentrations of radioactivity at 8 hours in liver, fat, and kidney were all 2 to 3-fold higher than that in plasma. Bile-fistula rats excreted about one-third of the radiolabeled dose of E in the bile as metabolites during a 24-hour period. Almost half of the biliary radioactivity collected was characterized as glucuronide conjugates. GC-MS and NMR analyses showed that a diol metabolite of E, i.e., N-ethyl-N-(2-methyl-2,3-dihydroxypropyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine, was a major glucuronide metabolite in bile. Approximately half of the biliary radioactivity infused into the duodenum of bile-fistula rats underwent enterohepatic circulation and reappeared in the 24-hour bile collection.

The comparative metabolism of lindane was studied in the rat following administration by gavage or incorporation in the diet. Lindane was administered to weanling female rats at the following dosages for 14 days: 1.96 mg/day by gavage or 130 ppm incorporated in the diet. Appropriate controls were run for each route of exposure. The rats receiving lindane in the diet excreted significantly more conjugated 2,4,6-trichlorophenol and 2,3,4,6-tetrachlorophenol as well as total urinary metabolites than rats receiving lindane by gavage. Rats receiving lindane by gavage excreted more unchanged lindane than rats receiving lindane in the diet. In vitro dehydrogenation and dechlorination of lindane as well as conversion of 1,2,3,4-tetrachlorobenzene to 2,3,4,5-tetrachlorophenol was significantly greater in rats receiving lindane in the diet than in rats receiving lindane by gavage. The route of oral administration had a significant effect on the metabolism of lindane. This was probably associated with the longer period of exposure, the greater absorption, and greater bioavailability of lindane in rats receiving the pesticide in their diet, which in turn, led to a significantly greater induction of specific metabolic pathways.

16. PHARMACOKINETIC STUDIES ON A NEW CANDIDATE ANTIMALARIAL 3-DI-n-BUTYLAMINO-1-[2,6-BIS(4-TRIFLUOROMETHYLPHENYL)-4-PYRIDYL]PROPANOL METHANESULFONATE, WR-172435.CH3SO3H, IN RHESUS MONKEYS. J. R. Hodgson, J. L. Minor, C. C. Lee, and H. Chung, Midwest Research Institute, Kansas City, Missouri and Walter Reed Army Institute of Research, Washington, D.C.

The pharmacokinetics and metabolism of a single oral dose of 35 mg/kg 14C-WR-172435.CH3SO3H were studied in male rhesus monkeys. The primary route of elimination was via the feces, accounting for about 82% of the dose after 24 days. Urinary excretion was minimal (<2.0%). The elimination of 14C in the feces appeared to be biphasic. The half-lives for the two phases were 19 hr and 282 hr, respectively. Analysis of blood level versus time data indicated the data described a single absorption phase and two elimination phases. The half-lives were 2.8 hr, 8.5 hr and 525 hr, respectively. Significant amounts of 14C were recovered in the liver, lung, bile, fat, and bone marrow 9 hr after dosing. After 92 days, these tissues still had appreciable 14C. TLC analysis of 14C in fecal and on tissue extracts indicated 14C-WR-172435.CH3SO3H was metabolized extensively. (This research was supported by U.S. Army Medical Research and Development Command under Contract No. DAMD-17-76-C-6059.)
17. PHARMACOKINETICS AND METABOLISM OF METRONIDAZOLE (FLAGYL) IN RAT AFTER INTRAVENOUS AND INTRAVAGINAL ADMINISTRATION.
Sponsor: K.S. Khera.

Metronidazole (MTZ) is highly effective against human trichomoniasis. Metronidazole-2 [14C], 10 mg/kg, was administered in rats intravenously (iv) or intravaginally (ivg). Upon iv administration, the disappearance of 14C from blood followed the kinetics of a two compartment open-system model. The apparent volume of distribution was 55% of the body weight which suggested rapid transfer of MTZ from plasma to tissues. The elimination t1/2 of 14C from blood during the β-phase was 9.7 ± 1.0 hr and the blood clearance rate was 106 ± 3.1 ml/kg/hr. After ivg administration, the MTZ-derived radioactivity was detected in tail blood at 5 min, peaked at 1 hr, declining rapidly in 6 hr, and more slowly thereafter. About 2.7% of the administered dose was found in the vagina after 4 hr and 1.2% after 24 hr. The 24 hr recoveries of 14C in the urine and feces were 58 and 15%, respectively, of the dose given iv, and 37 and 40%, respectively, of the dose given ivg. Unchanged MTZ and its five metabolites were detected in the urine. MTZ was rapidly absorbed following ivg application. The total excretion of 14C in the urine and feces was similar after iv and ivg administration.

18. PHARMACOKINETICS AND METABOLISM OF ETHYLENEDIAMINE IN THE RAT FOLLOWING ORAL, ENDOTRACHEAL AND INTRAVENOUS DOSING.

The fate of ethylenediamine (EDA) in relation to po, endotracheal and iv dosing were studied in rats. Male Hilltop Wistar rats were dosed with [14C]EDA at 5, 50 and 500 mg/kg. For the two types of studies (material balance and plasma kinetics), the experimental periods were 48 hr and 24 hr, respectively. The routes of elimination of radiochemical(s) were via urine (42–65%), feces (5 to 32%) and 14CO2 (6 to 9%). At the end of the 48 hr experimental period, a large portion of the radioactivity (11 to 21%) remained in the organs and the carcass. The plasma concentration vs time curves from rats receiving EDA at 5 and 50 mg/kg could be resolved into 3 exponentials. Similar curves at 500 mg/kg suggested dosage mixing problem and saturation kinetics. Preliminary analyses on the results from animals dosed with 50 mg/kg yielded the following estimates for po, endotracheal and iv routes, respectively: bioavailability, 97.5, 102.8 and 100%; total clearance, 211.1, 211.6 and 221.0 ml/hr; and terminal t1/2, 5.8, 0.2 and 9.1 hr. These data suggest that the fate of EDA in the rat following po or endotracheal dosing is similar.
19. THE PHARMACOKINETICS AND METABOLISM OF INHALED METHYLENE CHLORIDE IN RATS. M.J. McKenna, J.A. Zempel and W.H. Braun, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical USA, Midland, MI.

The pharmacokinetics and metabolism of inhaled $^{14}$C-methylene chloride ($^{14}$CH$_2$Cl$_2$) was studied in male Sprague Dawley rats after single 6 hr exposures to 50, 300 and 1500 ppm $^{14}$CH$_2$Cl$_2$. The major metabolites of $^{14}$CH$_2$Cl$_2$ were $^{14}$CO and $^{14}$CO$_2$, both of which were found in expired air. Over the range of exposure concentrations evaluated, the net uptake and metabolism of $^{14}$CH$_2$Cl$_2$ by rats was disproportionately less than the incremental increase in $^{14}$CH$_2$Cl$_2$ exposure concentration. This observation was accompanied by an increase in unchanged $^{14}$CH$_2$Cl$_2$ in expired air as the $^{14}$CH$_2$Cl$_2$ exposure concentration was increased from 50 to 1500 ppm.

The relationship of the total metabolism of $^{14}$CH$_2$Cl$_2$ to the exposure concentration followed apparent Michaelis-Menten kinetics. The use of a Michaelis-Menten model to describe the metabolism of inhaled CH$_2$Cl$_2$ allowed a reliable prediction of the body burden achieved in an inhalation exposure. It is concluded that such predictions are a valuable asset in the design and interpretation of experiments for the assessment of dose-response relationships in inhalation toxicology.

20. THE DISPOSITION OF THREO-$\alpha$-(2-PIPERIDYL)-2,8-BIS(TRIFLUOROMETHYL)-4-QUINOLINEMETHANOL HYDROCHLORIDE (WR 777,602·HCl) IN MICE. H. Chung, V. Jimmerson, D. Bounds, R. Keller and R. Rozman, Dept. of Pharmacology, Walter Reed Army Institute of Research, Washington, D.C.

The disposition of this promising candidate antimalarial drug was studied over a 168 hr period after 10 mg/kg of the $^{14}$C labeled drug was administered orally to female albino ICR mice. About 70% and 23% of the radioactivity was excreted via feces and urine respectively, with 2.1% of the radioactivity recovered in carcasses. The excretion of the radioactivity in the urine and feces peaked between 24 and 36 hr. The elimination half-life ($T_{1/2}$) of urine and feces were 13.9 hr and 13.8 hr respectively, as calculated by linear regression analysis of the "percent remaining radioactivity versus time" plot. This drug was well absorbed.

Two hr after dosing, the radioactivity was distributed throughout the animal body with large percent of the radioactivity found in the eyes, submaxillary salivary glands, lungs, spleen, liver, gall bladder plus bile, kidney and carcass. About 69% of the administered dose was absorbed 2 hr after dosing. The disappearance T$_{1/2}$ of the parent drug in plasma was approximately 16 hr.

Ethyleneurea (EU) and ethylenethiourea (ETU) have been identified as plant and animal metabolites of the Ethylene-bisdithiocarbamate fungicides. Previous work in the rat has shown that major urinary metabolites of orally administered $^{14}$C-EU are EU and 4-imidazol-2-one (Imidazolone). The present study indicates that dehydrogenation of $^{14}$C-EU, administered po, is extensive, yielding Imidazolone as the major urinary metabolite. This indicates that the major urinary metabolites arise after oxidative desulfuration of ETU. However, qualitative HPLC analysis of urine from rats given ETU po, reveals the presence of thioimidazole, which indicates that dehydrogenation can occur without formation of EU. The HPLC analysis also revealed the presence of additional, minor, sulphur containing metabolites, namely thiohydantoin, N-methyl EU and N-methyl thioimidazole. These results indicate a complex pattern of metabolism for ETU in the rat.

22. CARBON DISULFIDE AND CARBONYL SULFIDE METABOLISM IN ISOLATED HEPATOCYTES. C.P. Chengelis and R.A. Neal. Center in Environmental Toxicology, Dept. of Biochemistry, Vanderbilt Univ., Nashville, TN.

A possible relationship between the metabolism and hepatotoxicity of thionsulfur compounds was explored in isolated rat hepatocytes, employing carbon disulfide (CS$_2$) as a model compound. Cells were incubated (1-3 mg cell protein/ml) at 37°C with either $^{14}$C-CS$_2$, or $^{35}$S-CS$_2$ (0.05-0.4 mM) in sealed 25 ml flasks. No carbonyl sulfide (COS) (the predominant product of isolated microsomal CS$_2$ oxidation) was found. CO$_2$ was the only detectable volatile metabolite ($V_m$=1.3 nmole/min/mg protein). A yet unidentified metabolite was formed from $^{14}$C-CS$_2$, which was not volatilized from acid or alkaline evaporates, and moved as a single band on TLC. With $^{35}$S-CS$_2$ all nonvolatile label was recovered as sulfate. No protein binding was discernible. The disappearance of exogenously added COS was rapid (30-40/min/mg protein) and not altered by CS$_2$. With $^{35}$S-COS, sulfate formation was 15 nmoles/min/mg protein) and inhibited 90% by 0.1 mM p-nitrobenzyl bromide, a substrate for glutathione S-transferase. We conclude that CS$_2$ is oxidized by the microsomal monooxygenase (MM) to COS, which is subsequently metabolized by both MM and, primarily, an additional system, possibly involving glutathione.
23. METABOLISM OF 1,3-BENZODIOXOLES TO CARBON MONOXIDE.
C.P. Wilkinson, L.S. Yu and M.W. Anders, Dept. Entomology,
Cornell Univ., Ithaca, NY and Dept. Pharmacology, Univ. of
Minnesota, Minneapolis, MN

In studies of the spectral interactions of several 1,3-
benzodioxoles (BD) with cytochrome P-450, difference spectra
showed the formation of a peak at 450nm when NADPH reduced
microsomes were allowed to become anaerobic or when dithio-
nite was added. Disappearance of the 450nm peak and appear-
ance of a peak at 419nm following addition of hemoglobin
suggested metabolic conversion of these compounds to CO and
further studies were conducted to define this conversion.
5-CN-BD and 5,6-dICl-BD served as major substrates and CO
production was confirmed and measured by gas chromatography.
The enzymes catalyzing the conversion of BD to CO were
localized in rat hepatic microsomal fractions and required
NADPH. The pathway was characterized with respect to time,
PH and protein concentration. Microsomal fractions from
phenobarbital-, but not methylcholanthrene-treated rats
showed increased CO production from 4-CN-BD. Cobaltous
chloride treatment decreased CO formation. The reaction was
inhibited by SKF 525A, 1-phenyl- and 1-(2-isopropylphenyl)imi-
dazoles. These studies suggest that BD are metabolized
to CO by a cytochrome P-450 dependent enzyme system.
(Supported by NIH Grants ES 01082 and ES 00400).

24. Metabolism of 17alpha-Ethynylestradiol (EE2) in the Rhesus
and E. D. Helton. NCTR, Jefferson, AR and University of
Arkansas Medical School, Little Rock, AR. (Spon. T. E.
Sheilenberger).

To establish the rhesus monkey as a metabolic model for the
study of estrogen hepatotoxicity and ethynyl cleavage,
metabolites of EE2 were obtained from urine of 3 female
monkeys following oral administration of [9,11-3H] EE2 and
[20,21-14C] EE2 in specific ratios (3H/14C). Forty percent
of the dose was excreted in the urine by 96 hrs while the
feaces accounted for less than 15%. Characterization of
urinary activity on Sephadex LH-20 showed four fractions
with the glucosiduronate fractions comprising the dominant
excreted activity (95%). The urinary conjugates were
chromatographically resolved for 3 consecutive 24 hr
intervals and the products did not change with time. Beta-
gluconuronidase hydrolysates of the two major glucuronide
radioactive fractions were separated by HPLC utilizing a
Chromegabond diol column with a mobile phase gradient of
hexane to hexane/LEA (65/15). Ethynyl cleavage was indi-
cated by loss of 14C in two of the isolated metabolites
which co-chromatographed with authentic estrone and
estradiol. The metabolism of EE2 in the monkey and the
human female appears to be similar.2 (Steroids Vol 25, 2).
25. IN VITRO AND IN VIVO EFFECTS OF PENICILLIC ACID ON ADENOSINE TRIPHOSPHATASE ACTIVITIES IN MOUSE. P.K. Chan, T.D. Phillips and A.W. Hayes. Dept. of Pharmacol. and Toxicol., University of Mississippi Medical Center, Jackson, MS

The effects of penicillic acid (P.A.), an environmental hazardous mycotoxin and a potential carcinogen, on mouse tissue ATPase was examined both in vitro and in vivo. P.A. inhibited in vitro the mitochondrial (B) fraction of brain and kidney (Na⁺K⁺)-ATPase activities in a dose-dependent manner with calculated IC₅₀ values of 2.5 and 3.3 x 10⁻⁵M, respectively. Similar inhibition in the microsomal (C) fraction also was observed with IC₅₀ values of 5.97 and 1.0 x 10⁻⁵M in brain and kidney tissues, respectively. Brain and kidney (Na⁺K⁺)-ATPase activities also were inhibited by P.A. in vivo. A time response of inhibition of (Na⁺K⁺)-ATPase in brain and kidney from mice receiving 80 mg/kg of P.A. in normal saline was observed and a dose-dependent relation in brain (C) fraction and in kidney (B) and (C) fractions was demonstrated. Mg²⁺-activated ATPase activities in both fractions of brain, kidney and liver tissues were not impaired significantly with the exception of oligomycin-insensitive Mg²⁺-activated ATPase in the brain (B) fraction. Thus, in vitro and in vivo results suggested a possible correlation between the inhibition of (Na⁺K⁺)-ATPase activity and P.A.-mediated toxicity. (Supported by ES 01351 and Training Grant ES 07045).


Penicillic acid (P.A.), which can exist in tautomeric forms (open ring substituted ϕ-keto hexenoic acid or corresponding αβ-γ-hydroxy lactone), inhibits selectively mouse (Na⁺K⁺)-ATPase both in vitro and in vivo. It also is a potent inhibitor of swine brain microsomal (Na⁺K⁺)-ATPase (IC₅₀ = 1.8 x 10⁻⁶ M) with inhibition at concentrations as low as 1 x 10⁻¹₂ M. Kinetic evaluation of P.A. on (Na⁺K⁺)-activated ATPase indicated competitive antagonism of inhibition of monovalent cationic Na⁺ activation and noncompetitive inhibition with regards to K⁺ activation. K⁺-activated p-nitrophenyl phosphatase activity was not significantly altered by P.A. Preliminary binding studies indicated that inhibition of ATPase activity can be partially restored by washing and by incubation with sulfhydryl reagents. A linear correlation of free enzyme sulfhydryl (SH) and ATPase activity in the presence of varying concentrations of P.A. also was noted. It is postulated that P.A. binding occurs via critically exposed or accessible thiol receptors regulating Na⁺-dependent phosphorylation of the membrane transport enzyme. (Supported by ES01351.)
27. RENAL AND HEPATIC CLEARANCE STUDIES OF CITRININ IN RATS.

The purpose of these experiments was to determine the renal excretory mechanisms and the extent of biliary excretion for citrinin administered acutely to rats. Renal clearance studies of the handling of acutely administered $^{14}C$-citrinin were performed on pentobarbital-anesthetized rats. The concentration of ultrafilterable plasma citrinin (citrinin_{ul}) was estimated using Amicon filter cones having a molecular weight cutoff of 50,000. Citrinin_{ul} was only 1.65 ± 0.15% of total plasma citrinin when 6 mg/kg of $^{14}C$-citrinin was administered to rats i.v. in 5% Na bicarbonate. The ratio of citrinin_{ul} to inulin clearance was 9.22 ± 0.39. The ratio of total plasma citrinin to inulin clearance was 0.15 ± 0.01. Rats administered a single dose (6.0 mg/kg, i.v.) of $^{14}C$-citrinin excreted 32% of total administered radioactivity in bile after 3 hr. These experiments demonstrated that following acute administration of citrinin, most of the citrinin was bound to plasma protein and the renal elimination of citrinin_{ul} was by glomerular filtration and tubular excretion. Although extensive biliary excretion was observed, fecal excretion was modest. This indicated that citrinin undergoes considerable enterohepatic circulation. Supported by ES 01351, ES 01645 and ES 07045.


Toxicity of secalphonic acid-d was examined using lethality, growth retardation and histopathology as indices. IF LD50 values of 36, 32 and 28 mg/kg were obtained for Charles River CD-1, Texas (ICR) and (CF-1) Sprague Dawley strains of mice, respectively. A po LD50 of 22 mg/kg was obtained for neonatal Charles River rats. Doses up to 20 mg/kg were not lethal to CD-1 mice, whereas 30 mg/kg or higher produced death between 2 and 8 days, often with oro-nasal bloody discharges. A dose-dependent growth retarding effect up to 14 days after 20 mg/kg or higher was observed. Histopathology included focal periostitis, involving capsule surfaces of kidneys and liver, with limited subjacent hepatic parenchymal necrosis. Pulmonary atelectasis and hemorrhages were frequent. Animals that died also showed massive atrial dilation thus signifying a probable cardiac-pulmonary death. Five daily sublethal doses (10 or 20 mg/kg) indicated cumulative toxicity. I.V. doses of 25 mg/kg resulted in portal necrosis of the liver at 48 hr which appeared repaired by 21 days. Doses of 40 mg/kg resulted in nearly 100% mortality with the above described pulmonary-cardiac lesions being major observations. (ES 01351 and ES 01352).
29. Comparative effects of toxins from Clostridium difficile and Clostridium sordellii. Marion Ehrich, Roger Van Tassell, James E. Aswell, and Tracy D. Wilkins, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia (Sponsor: S. D. Cohen).

The toxin produced by *C. difficile* has been implicated as a causative agent of pseudomembranous colitis associated with antibiotic chemotherapy. There has been some confusion in the literature, however, as toxin found in patients with pseudomembranous colitis can be neutralized with the antitoxin of *C. sordellii*. These two microorganisms are taxonomically very different, but we demonstrated that *C. difficile* (strain 10463) antitoxin was capable of neutralizing the LD_{100} of the toxin of *C. sordellii* (strain 8825) at dilutions up to 1:64. This antitoxin neutralized the LD_{100} of *C. difficile* toxin at dilutions up to 1:1000. The molecular weights for both toxins were approximately 600,000. Symptoms in mice and rats included loss of muscular coordination and death by respiratory failure. Intraperitoneal inoculation of either toxin caused severe hemorrhagic effects in the liver and small intestine. The toxin of *C. sordellii* caused more severe hemorrhagic effects, which encompassed the entire length of the small intestine and included the lungs and brain. Administration of atropine (1mg/Kg) after the toxin but before onset of symptoms increased survival in mice administered the toxin of *C. sordellii*. Studies such as these provide information on properties of bacterial toxins associated with human disease and may be useful in the determination of their mechanisms of action.


Purified T-2 mycotoxin was examined for toxicity to 7 day old male broiler chickens. Toxin dissolved in dimethylsulfoxide: saline (1:9 v/v) was administered by crop intubation. Single dose 72 hour LD_{50} was 2.55 mg/kg. Necropsied survivors had microscopic lesions of atrophy of the cortex of the bursa of Fabricius (BF) and thymus. Birds administered 7 consecutive daily doses of 1.25 mg/kg had significantly reduced (P<.01) weight gain and hematocrit. Necropsied survivors had yellow or pale red bone marrow, yellow friable livers, and white foci in the crop mucosa. Microscopic lesions were atrophy of the cortex of the BF and thymus, cecal tonsil necrosis, hepatocellular degeneration, bile ductule hyperplasia, and focal necrosis of crop mucosa. Birds consuming 4 ppm dietary T-2 for 21 days had oral ulcers covered with yellow caseus exudate on the tongue, oral mucosa, palate, and beak. Microscopic lesions were hydropic degeneration, erosion, ulceration, and inflammation of the oral cavity and squamous epithelium of the nasal turbinates.
31. ALTERATIONS IN COVALENT BINDING OF AFLATOXIN B<sub>1</sub> TO LIVER MACROMOLECULES AS INFLUENCED BY ROUTE OF ADMINISTRATION. J. R. Hayes and T. C. Campbell, Div. of Nutri. Sci., Cornell University, Ithaca, NY 14853.

Studies examining the toxicity, carcinogenicity and mode of action of aflatoxin B<sub>1</sub> (AF<sub>B1</sub>) have utilized various routes of administration. To ascertain the effect of route of administration, <sup>14</sup>CH<sub>3</sub>-AF<sub>B1</sub> (1 mg/kg) was injected i.p. or intubated i.g. to Sprague-Dawley rats. Radioactivity levels were determined in blood and liver at various times after dosing. Liver DNA, RNA and protein were extracted and covalently bound aflatoxin (AF) determined. Maximal levels of AF occurred in blood and liver at 0.5-1.0 hr after i.p. dosing and 6 hr after i.g. dosing, with AF levels lower when dosed i.g. DNA, RNA and protein showed maximal levels of covalently bound AF at 0.5 hr after i.p. dosing and 6 hr after i.g., paralleling blood and liver values. Except for the 6 hr period, the quantity of AF-macromolecular adducts was lower after i.g. dosing, but tended to converge at 72 hr. These data indicate that route of administration produces significant alterations in the time course of AF-macromolecular adduct formation. (Supported by Grant #PDT-104 from American Cancer Society and NCI Grant #RO1 CA 20079.)

32. THE IN VIVO AND IN VITRO EFFECTS OF RUBRATOXIN B ON HEPATIC MICROSOMAL ENZYMES IN MALE MICE. Mohamed Y. Siraj and A. Wallace Hayes. Dept. Pharmacol. & Toxicol., Univ. Miss. Medical Center, Jackson, MS 39216.

The in vivo and in vitro effects of rubratoxin B (RBT) on hepatic drug metabolizing enzymes were examined. Ethylmorphine N-demethylase (EM) and aniline hydroxylase (AN) were measured 48 hr after a single ip dose of 0.25, 0.5, 1.0 or 1.5 mg RBT/kg. The in vivo enzyme activity was suppressed significantly at all 4 dose levels with maximum inhibition of 85% at 1.5 mg/kg. At 0.25, 0.5 and 1.0 mg/kg EM (32, 58 and 64%) was inhibited more than AN (21, 25 and 37%). The in vitro effects of RBT on kinetic constants were examined by adding 1, 10 or 100 μM RBT to microsomal suspension from untreated mice. The microsomal suspension was incubated with either 0.2 to 1.5 mM EM or 80 to 180 μM AN. The apparent K<sub>m</sub> for EM increased 61% while the apparent V<sub>max</sub> remained constant at 10 μM RBT. For AN, the apparent K<sub>m</sub> decreased 29, 31 and 44% while the apparent V<sub>max</sub> decreased 31, 33 and 55% with 1, 10 and 100 μM RBT, respectively. The in vivo effects of the mycotoxin on these enzymes, as suggested by the in vitro study, may be a secondary effect. (Supported by ES 01351.)
33. EFFECT OF ALTERED MICROSONAL METABOLISM ON RUBRATOXIN TOXICITY. S. A. Watson and A. W. Hayes, Dept. Pharmacol. & Toxicol., Univ. Miss. Med. Ctr., Jackson, MS 39216

Two forms of microsomal monooxygenase, cytochrome P450 and cytochrome P448, can be preferentially induced by pentobarbital and 3-methylcholanthrene (3MC), respectively. The LD50 of rubratoxin B in DMSO in male mice was 0.34 mg/kg. In animals pretreated with pentobarbital, the LD50 was increased to 0.44 mg/kg. In animals pretreated with 3MC the LD50 was reduced to 0.172 mg/kg. Pretreatment with SKF-525A at a dose which preferentially inhibited P450 mediated activity resulted in a decreased LD50 of 0.20 mg/kg. These results suggest the formation of a less toxic metabolite by P-450 mediated enzymes while a more toxic metabolite is formed by P-448 mediated enzymes. A protective role by glutathione conjugation is suggested by LD50 studies following pretreatment with cysteine (a glutathione inducer) or diethyl maleate (an inhibitor of glutathione formation). Cysteine pretreatment increased the LD50 to 0.70 mg/kg while diethyl maleate pretreatment decreased the LD50. Data indicated that the protective effect of induced glutathione was overcome with time. (Supported by ES 01351, ES 01352 and ES 07045).

34. THE AFLATOXIN INTAKE OF VARIOUS AGE GROUPS OF CANADIANS. T. Kuiper-Goodman, D. C. Kirkpatrick and D. Krewski, Health Protection Branch, Ottawa, Canada.

In Canada, peanut products represent the most significant dietary source of aflatoxins. This may be of particular concern in children because of their high intake of peanut butter relative to body weight. Monitoring data generated by the Health Protection Branch from 1974 to 1977 indicate an average level of total aflatoxins in peanut butter of approximately \(4\) (range 1-7) ng/g. Estimates of intakes of peanuts and peanut butter for various age groups of the Canadian population were derived from the food frequency recall portion of the Nutrition Canada Dietary Intake Survey. The average intake of these foods was 0.4 g/kg body weight for children up to 10 years of age and declined progressively thereafter to less than 0.1 g/kg body weight. From these data estimates of aflatoxin intake were derived. Various factors were examined to determine if these levels of aflatoxin intake are of concern.

Balkan nephropathy is more common in older individuals and predominates in females below middle age. The purpose of the present study was to examine in the rat sex and age as factors in the action of ochratoxin A and citrinin, on the renal transport of organic compounds in vitro. Transport was examined after the direct addition of the nephrotoxins to fresh renal cortex slices and after pretreatment of animals. With lactate present, p-aminomethylurate (PAH) uptake was altered significantly by both compounds in older animals. That is, an age-inhibitor interaction occurred such that the older animals showed a greater response to the toxins. Without lactate, effects on PAH were more pronounced in younger animals. With ochratoxin pretreatment, renal N-methyl-N-nicotinamide (NMN) transport in female rats was altered more than with males. This significant sex-inhibitor interaction indicated an unusual sensitivity of NMN transport to ochratoxin A in the female. The age and sex-inhibitor interactions support the proposition that citrinin and/or ochratoxin A might cause the Balkan nephropathy. (Supported by ES 01351 and ES 01643).

36. ALLYLAMINE INHIBITION OF MONOAMINE OXIDASE: ANTAGONISM BY SEMICARBAZIDE. T.J. Nelson and P.J. Boor, Dept. of Pathol., Univ. of Texas Medical Branch, Galveston, TX. Sponsor: Mary Treinen Moslen

Monoamine oxidase (MAO) activity has been implicated as a factor in the cardiotoxicity of allylamine (AA) because the simultaneous administration of various MAO inhibitors including semicarbazide (SC) virtually abolishes AA-induced myocardial fibrosis in rats (Boor and Reynolds, Fed. Proc. 36(3), 397, 1977). Therefore the interaction of AA and SC were investigated on MAO activity for tyramine using a rat heart homogenate or purified MAO. Initial velocity studies were done with 2 mM NaCl (control), 2 mM AA, 2 mM SC, or 2 mM SC added after 20 min preincubation with 2 mM SC. AA exhibited a time-dependent, uncompetitive and apparently irreversible inhibition of MAO, similar to that observed for SC. The I_{50} for AA (after 1 hr) was 1.5 mM. The preincubation with SC diminished the inhibiting effect of AA, indicating similarities in the mechanisms of MAO inhibition by AA and by SC.
37. PATHOPHYSIOLOGIC PROFILE OF ACUTE PHENCYCLIDINE LETHALITY IN THE CONSCIOUS DOG. R.B. Hackett, I.W. Waters, K.W. Obrosky and R.F. Borne, Departments of Pharmacol. and Med. Chem., Sch. Pharmacy, University of Mississippi, University, MS 38677. Sponsor: W.M. Davis

Phencyclidine HCl was infused intravenously (1.0 mg/kg/min) to conscious mongrel dogs until death. All animals convulsed (mean convulsive dose: 4.7 ± 0.3 mg/kg) and died approximately 48 minutes after the beginning of the infusion (mean lethal dose: 49 ± 3 mg/kg). All animals exhibited significant increases in heart rate, mean arterial pressure, cardiac output, body temperature, and arterial pO₂. Statistically significant reductions from pre-drug control levels were observed in total peripheral resistance, arterial pH, arterial pO₂, and respiratory minute volume. Plasma glucose, blood lactate and O₂ uptake exhibited significant increases during the early minutes of drug infusion then declined to values below pre-drug control levels during the latter phase of the experiments. (Supported in part by the Research Institute of Pharmaceutical Sciences, University of Mississippi, and National Research Service Award 5 T32 GM 07099-04).


APAP appears to be metabolized to several products, at least one of which is an electrophile that combines with glutathione (GSH). After depletion of GSH the electrophile presumably binds to cellular components in the initial step of toxicity. To estimate the ability of the kidney to generate electrophilic metabolites, GSH concentrations were measured in kidneys from Fisher 344 rats perfused with several concentrations of APAP. Perfusate alone reduced renal GSH in comparison to non-perfused controls (17±1% depletion in cortex, 17±8% in medulla and 22±7% in papilla). Treatment of rats with polybrominated biphenyls enhanced the effect of 3x10⁻⁵M APAP to deplete GSH (30% and 51% compared to 13% and 14% in cortex and medulla with APAP alone). Treatment with piperonyl butoxide reduced the effect of 3x10⁻⁵M APAP to deplete GSH (12% and 16% compared to 30% and 34% in cortex and medulla with APAP alone). Dose-related APAP-induced depletion of GSH in the IPK, enhancement by microsomal enzyme induction and blockade by microsomal enzyme inhibition all suggest that an electrophilic metabolite of APAP is formed in the kidney. (Supported by NIH grant ES00560 and a grant from the Mich. Dept. of Agric.)
39. THE COMPARATIVE NEPHROTOXICITY OF AMINOGLYCOSIDE ANTIBIOTICS IN THE RAT. J.S. Wold, D.O. Robbins, C.L. Gries, B.L. Miller and S.A. Turnipseed, Toxicology Division, Lilly Research Laboratories, Greenfield, IN.

The nephrotoxicity of tobramycin (TOB), gentamicin (GEN), amikacin (AMI), kanamycin (KAN) or dibekacin (DBK) was studied after the s.c. administration of 8 doses (twice daily for 4 days) to female Fischer 344 rats. Nephrotoxicity was evaluated by the determination of urinary N-acetylglucosaminidase activity (NAG), serum urea nitrogen, relative kidney weight, renal cortical slice function and metabolism (measured by PAH and NAG accumulation and glucosoneogenesis) and renal histopathology. NAG excretion measured on day 4 was the most sensitive indicator of nephrotoxicity. DBK produced a significant increase in NAG excretion at 40 mg/kg while TOB or GEN caused an increase at 80 mg/kg and AMI and KAN significantly increased NAG at 600 mg/kg. Consideration of each of the parameters led to the conclusion that in this model, the various aminoglycosides could be ranked (in order of decreasing nephrotoxicity) as follows: DBK > GEN > TOB > AMI = KAN.


Clinical use of the chemotherapeutic agent, CDDP, is limited by its renal toxicity as measured by increased blood urea nitrogen (BUN). This study was designed to determine the effects of CDDP on the in vitro accumulation of the organic anion, p-aminohippurate (PAH), and the organic base, tetraethylammonium (TEA) by renal cortical slices. CDDP, when added to incubation media at concentrations of 500 or 600 μg/ml, depressed both PAH and TEA transport in kidneys from both male Sprague-Dawley and male Fisher 344 (F344) rats. To determine the effects of in vivo administration on organic ion transport, CDDP (1.25, 2.50 or 5.0 mg/kg) was injected i.v. Four days following treatment, there was a dose-related elevation of BUN in animals treated with CDDP. Similarly, glucosuria was also detected in treated animals. However, accumulation of neither PAH nor TEA by renal cortical slices from the CDDP treated animals was depressed. Thus, although accumulation of PAH and TEA is inhibited by CDDP in vitro, alterations in these transport systems may not be involved in the nephrotoxicity of CDDP in vivo. (Supported by USPHS grant ES00560.)
41. **THE TOXIC EFFECTS OF HEXACHLOROBUTADIENE ON THE RAT KIDNEY.**

E A Lock and J Ishmael, Imperial Chemical Industries Ltd., Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire, SK10 4TJ, UK. Sponsor: M S Rose.

Hexachlorobutadiene (HCBD) is a by-product of certain processes associated with the chlorination of hydrocarbons and has been shown to produce renal tubular damage in the rat. Urinary analysis 24 hr after a single i.p. injection showed diuresis, proteinuria and an increase in the excretion of the enzymes alkaline phosphatase and N-acetyl-β-D-glucosaminidase at doses of 100 mg/kg and above. Histological examination of the kidneys indicated damage to the straight portion of the proximal convoluted tubule with many hyaline casts. Measurement of water, non-protein sulphydryl (mainly glutathione), protein and DNA content of the rat kidney up to 48 hr after 300 mg/kg HCBD i.p., showed a significant increase in kidney water by 4 hr, glutathione (GSH) by 16 hr and protein at 24 hr compared with controls. No changes in DNA content were seen. The livers of these rats also showed a significant increase in water content whilst GSH was initially depleted and then increased compared with controls. These preliminary studies indicate that HCBD may be metabolised in the liver and excreted as a GSH conjugate. It is interesting that no depletion of GSH occurs in the kidney, which is the major site of damage.

42. **CARDIOVASCULAR TOXICITY OF BHT (Butylated Hydroxytoluene) AND BHA (Butylated Hydroxyanisole)** - S. C. Gad, B. H. Chin, J. A. McKelvey, D. A. Acosta* and S. W. Leslie*, CHF-CMIR, Pittsburgh, Pa. and Univ. of Texas, Austin, Texas.

BHT, in concentrations of 1 to 500 mg/l significantly (P < 0.05) depressed the frequency and amplitude of contractions of isolated atrial preparations. BHT at 500 mg/l terminated spontaneous contracting of isolated rabbit atrial after 20 min and after 72 min when tested on rat atria. Concentrations below 500 mg/l of BHT did not terminate atrial beating activity, but did produce a concentration-dependent depression of rate and force of atrial contractions.

Similar significant dose-related depressions were seen at the same concentration levels in intact perfused rat heart preparations. Additionally, perfused hearts treated with BHT (in concentrations from 1 to 500 mg/l) showed significant dose-related increased in creatinine phosphokinase (CPK) leakage into the perfusion solutions. Correlation of changes in LDH-2 and α-HBDO levels with these changes was examined, and the interaction of aerobic vs. anaerobic cardiovascular metabolism with the effects of BHT was explored.

Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) at concentrations of 0.01% produced a marked leakage of lactic dehydrogenase (LDH) from cultured myocardial and endothelial cells into the culture medium. The results suggest that both BHT and BHA produce injury to myocardial cells.
43. BIPHASIC RESPONSE OF RENAL 5'-PHOSPHODIESTERASE FOLLOWING FOLIC ACID ADMINISTRATION IN THE RAT. R.R. Gaddis and R.T. Louis-Ferdinand, College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, MI.

Renal enlargement induced by Folic Acid (FA) is attributed to the production of non-specific damage to the proximal tubules, tubular regeneration, increased DNA, RNA and protein. Previous work from our laboratory has shown that FA inhibits renal microsomal 5'-phosphodiesterase (PDE) in vitro (Gaddis and Louis-Ferdinand, 1978). The present investigation was conducted to determine the influence of FA (100,150,250 mg/kg, ip) administration on PDE activity during renal enlargement induced by FA in adult male Sprague Dawley rats. Renal PDE activity was decreased by 13, 23 and 33% respectively and 101, 114 and 115% increases in total kidney protein were produced by 24 hrs. However kidney/body wt ratios were increased to 119, 134 and 156% of controls respectively. Folic Acid (250 mg/kg, ip) decreased total PDE per kidney by 17% by 16 hrs. followed by an increase (13% of control) at 72 hrs. The results indicate that direct inhibition of PDE by FA is only partially responsible for initial decreases in PDE and that this enzyme responds in a biphasic manner during renal enlargement induced by FA. (Supported by Michigan Kidney Foundation.)

44. A COMPARATIVE STUDY OF THE EFFECTS OF THREE HALOGENATED METHANES ON CARDIOVASCULAR DYNAMICS AND MYOCARDIAL ENERGY METABOLISM, R.N. Terpolilli, R.A. Davis, K.C. Back, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH 45433

The purpose of this study was to determine if the negative inotropic effect of bromotrifluoromethane (1301), bromochlorodifluoromethane (1211), and bromochloromethane (1011) was accompanied by altered myocardial metabolism. Guinea pigs were urethane anesthetized, respired on 100% O2, and instrumented to record various cardiological parameters. Following a control period and 30 min exposure, myocardial ATP, ADP, AMP and creatine phosphate levels were determined by enzymatic assay. Agent blood levels were measured by gas chromatography. A concentration of 75% 1301, 20% 1211, and 3.5% 1011 produced a 34%, 54% and 20% decrease in contractility, respectively. 1301 and 1211 blood levels stabilized within 2.5 min while 1011 blood levels stabilized after 10 min at levels 10 times greater. 1301 and 1211 decreased blood pressure and heart rate while 1011 produced the opposite effect. High energy phosphate levels were not altered by any of the agents in a fashion commensurate with the theory that a block in energy metabolism was responsible for the production of the induced negative inotropic effect.

Male CD rats inhaled 0, 19, 39, or 89 ppm of EDB 7 hr a day, 5 days a week for 10 weeks. Mortality, reduced weight gain, and reduced feed consumption occurred at the high concentration. These males had reduced serum testosterone levels, atrophy of the testes, epididymis, prostate, seminal vesicles, and failed to impregnate females. Reproduction was normal in males exposed to 19 or 39 ppm of EDB. Since these reproductive changes occurred with adverse effects on well-being, it was not possible to attribute reproductive effects directly to EDB. Female CD rats inhaled 0, 20, 39, or 80 ppm of EDB 7 hr a day, 7 days a week for 3 weeks. Mortality, weight loss, and reduced feed consumption occurred at the high concentration. These females did not cycle normally until several days after exposure ended and fewer females mated during a 10-day period than in other groups. Pregnant females in all groups produced normal litters. No lesions were observed in the ovary or uterus that would impair reproductive performance. (Supported by EPA Contract No. 68-0-3242).


Polybrominated biphenyls (PBBs) cross the placenta and have been detected in milk from several species including man, cow and rat. Therefore, it was of interest to determine the effects of PBBs on developing mammals. Rats were fed diet containing 0 or 100 ppm PBBs (Firemaster BP-6) from day 8 of gestation until 28 or 90 days of age. At 28 days of age, pretreatment with PBBs reduced the increase in seminal vesicle weight produced by testosterone and decreased the duration of anesthesia following progesterone. At 90 days of age, rats exposed to PBBs had a higher concentration of coproporphyrin in urine. Treatment with PBBs did not modify hematocrit, estrus cycle length or ovary, uterus, testes or kidney to body weight ratios. However, at 28 and 90 days of age, liver was enlarged, activity of hepatic and renal AH and hepatic epoxide hydratase was increased, and both pentobarbital sleeping time and body wt gain were decreased by PBBs. These results demonstrate that exposure to PBBs reduces the action of certain exogenous steroid hormones and, by implication, may produce subtle alterations in endogenous compounds. (Supported by USPHS grant ES00560 and a grant from the Mich. Dept. of Agriculture.)
47. Some Non-Priority Pollutants Commonly Found in Industrial Effluents. Satu M. Somani, Robert C. Teece, and David Schaeffer, School of Medicine, Southern Illinois University, and Illinois Environmental Protection Agency, Springfield, Il. 62708.

Organic analysis of industrial discharges and their mutagenicity testing are becoming increasingly important. We have carried out the analysis of waste water effluents representing specialty chemical, resin and plastic, petrochemical, industrial chemical, surfactant and polyurethane industries. Acid and base-neutral fractions were isolated after pH adjustment by liquid-liquid extractions. They were analyzed by gas chromatography-mass spectrometry. Compounds were semiquantitated using d-10 anthracene. Few consent degree compounds were detected in the samples. The compounds present in significant quantities were benzo-fluorene, fluoranthene, methylated naphthalenes, phenanthrenes, pyrenes, indanes, complex phenols, phthalates, and many of their analogs. Extracts from industrial effluents were tested for mutagenicity against S. typhimurium and many indicated toxic effects on the bacterial strains. The isolation of possible mutagen(s) are being carried out. Supported by EPA Grant No. 100517077.


The effects of environmental contaminants on the reproductive capacities of avian species have been evaluated for a limited number of pesticides. Regulations under FIFRA and TSCA will increase the demand for these studies, however, limited control data in the literature makes interpretation of these studies difficult. This paper summarizes the results from 75 control female mallard ducks representing a data base of 2926 eggs. Adult ducks, 1 male and 5 females, were housed in clean pens. Tap water and control diet (Agway® Game Bird Breeder Ration) were freely available to birds for 10 weeks prior to egg laying and during the 8-week laying season. After 8 weeks on diet, the photoperiod was increased from 7 to 17 hours to induce egg laying. Eggs were collected daily and incubated. Shell thicknesses were measured on one egg from each pen biweekly. The mean reproductive indices are as follows: Eggs cracked/Eggs Laid=2.2%; Viable 11 Day Embryos/Egg Set=85.2%; Live 21-Day Embryos/Viable 11 Day Embryos=98%; Hatchlings/Live 21-Day Embryos=80.0%; and 14-day Survivors/Hatchlings=93.6%. The mean egg shell thickness for 60 eggs is 0.378 millimeters.

Sprague Dawley rats were exposed to filtered air or 20 ppm or 1,2-dibromoethane (EDB) with or without 0.105% disulfiram (DS) in the daily diet. The rats were exposed to EDB 7 hr/day, 5 days/week for 3 or 6 months. At 3 and 6 months, rats sacrificed and liver, spleen, kidney and reproductve organs removed for histopathological examination. The tissue lesions in the EDB + DS rats included hepatocellular dysplasia, splenic atrophy, and atrophy of the testes at both 3 and 6 months. The incidence of these lesions was higher in the EDB + DS rats than the other treatment groups. At both 3 and 6 months, the EDB + DS rats were found to have reduced hematocrits, hemoglobin concentration and red blood cell counts. These data indicate that concurrent treatment with DS during EDB inhalation increases EDB toxicity. The changes in hematological parameters may serve as an early indicator of the toxicity of the EDB + DS combination.

50. ACUTE AND SUBCHRONIC TOXICITY OF SOLVENT REFINED COAL-RELATED HYDROCARBONS. D.D. Mahlum and P.D. Andrew, Biology Department, Pacific Northwest Laboratory, Richland, WA.

Acute toxicities of materials from solvent refined coal processes I and II were determined in fasted adult and fasted weanling and newborn rats after gavage. The LD₅₀'s were determined in the adult for materials designated light oil (LO), wash solvent (WS) and process solvent (PS) from SRC I and light, middle and heavy distillates (LD, MD and HD) from SRC II. Newborns and weanlings were tested only with PS. Maximum tolerated doses (MTD=no overt toxicity) were estimated in fasted pregnant females dosed from either 7-11 or 12-16 days of gestation. Adult LD₅₀'s of 0.7, 2.9 and 2.8 g/kg were obtained for undiluted WS, LO and PS; dilution in corn oil increased the LD₅₀ for WS to 1.7 but did not alter values for LO and PS. Values for LD and HD were similar to those for LO and PS, respectively, while the value for MD was about 4 g/kg. The lethal dose of PS for weanlings and adults was similar but was about twice as high for newborns. MTD values were about 1.8, 1.3, and 0.8 g/kg/day for LO, WS and PS, respectively, but are not yet available for LD, MD and HD. The results of this study establish rodent acute and subchronic toxicity for a number of SRC-related materials. (Supported by the U.S. Department of Energy under contract EY-76-C-05-1830.)

The post-mitochondrial (S9)/Salmonella (Ames) system was used to evaluate the mutagenicities of light oil (L0), wash solvent (W8), and process solvent (PS) from SRC I; light, middle, and heavy distillates (LD, MD, and HD) from SRC II, and 3 shale oils (Parahoe 16, Parahoe 504, and Livermore L01). The shale oils, HD and PS, were all mutagenic in S. typhimurium strains TA98, TA100 and TA1538, but not in TA1535 while L0, W8, LD and MD were without activity in any strain; metabolic activation by S9 was required for mutagenesis. Using the same conditions and TA98 as the reference strain, we obtained the following activities (revertant colonies per μg crude material): HD, 87 ± 23; PS, 12 ± 1.9; Parahoe 16, and 504, and L01, 0.6 ± 0.2. After fractionation of crude materials into acidic, basic and neutral components, highest activities resided in the basic fraction. Thin layer chromatography of the basic fraction of HD and PS revealed a single mutagenic peak. These data demonstrate that much of the mutagenic activity associated with crude SRC and shale oil materials resides in the basic fraction. (Supported by the U.S. Department of Energy under contract EY-76-C-06-1830.)


The in vitro cytotoxicity of materials from two solvent refined coal processes were compared with those from other fossil fuel products. The SRC I process solvent (PS) and SRC II heavy distillate (HD) caused a 50% reduction in the relative plating efficiency (RPE50) of VERO cells at concentrations between 30 and 50 μg/ml. Other materials (including other SRC by-products, diesel oil, and several crude oils) were slightly less toxic, producing RPE50's at concentrations between 50 and 500 μg/ml. These materials were assayed for their ability to transform Syrian hamster embryo cells into potentially neoplastic cells; no transformation was produced by the basic fractions of PS and HD in the absence of exogenous activating enzymes (S9). Addition of S9 significantly increased the transformation rate. These data demonstrate that certain fossil fuel components are toxic and capable of transforming mammalian cells but they are less hazardous than many chemicals presently in industrial use. (Supported by the U.S. Department of Energy under contract EY-76-C-06-1830.)
53. DEVELOPMENTAL TOXICITY OF SOLVENT REFINED COAL-RELATED HYDROCARBONS. F.D. Andrew, D.D. Mahlum, and M.R. Petersen, Biology Dept., Pacific Northwest Laboratory, Richland, WA.

The developmental toxicity potential of materials from two solvent refined coal processes (SRC I & II) were evaluated. Pregnant rats were exposed to 1 of 3 materials from SRC I or II. Light oil (LO), wash solvent (WS) or process solvent (PS) from SRC I, or light, middle or heavy distillate (LD, MD, or HD) from SRC II was given undiluted or in corn oil by gavage once daily from either 7-11 or 12-16 days of gestation (d.g.). Rats were sacrificed at 21 d.g. for evaluation of embryotoxicity or were permitted to deliver offspring for postnatal monitoring of growth, physical maturation, and reflex ontogeny. Some maternal lethality and embryolethality of ≥ 50% were seen in groups dosed on 7-11 d.g. with LO, WS or PS at 3.0, 1.4, or 0.7 g/kg/day, respectively. Similar results were seen after dosing on 12-16 d.g. Malformations and low fetal weights were seen only after LO at 3.0 g/kg/day on d.g. 7-11 or after PS at 0.7 g/kg/day on 12-16 d.g. No effects on postnatal maturation were seen. Comparable results were obtained for LD, MD, and HD. Embryotoxic effects were induced in rats after ingestion of ≥ 0.7 g/kg/day of SRC-related hydrocarbons. (Supported by the U.S. Department of Energy under contract EY-76-C-06-1830.)

54. DISPOSITION OF PROCESS SOLVENT FROM SOLVENT REFINED COAL IN TISSUES OF THE RAT AFTER ORAL DOSING. F.G. Burton, R.E. Schirmer, D.D. Mahlum, and F.D. Andrew, Biology Department, Battelle, Pacific Northwest Laboratory, Richland, WA.

The tissue distribution of the aromatic compounds of complex mixtures associated with solvent refined coal (SRC) processes is being studied to aid in developing dose-effect relationships for these materials. In this study, female rats were gavaged with 0.5 mL of process solvent (PS) from the SRC I process. Animals were sacrificed at 2, 4, 8, 24, and 48 hr after dosing and tissues taken for analysis. Blood was obtained at 0.5, 1, 1.5, and 16 hr as well as at sacrifice. Urine and feces were also analyzed. Samples were homogenized, extracted with pentane, and chromatographed on a 50 meter SP 2100 glass capillary column. The highest levels of aromatic PS components at all times were in the gastrointestinal tract with significant amounts in the liver, kidney, and fat. There were traces in the lung and no detectable residues in brain or heart. The relative distribution of different aromatic components varied according to tissue and time after administration. (Supported by the U.S. Department of Energy under contract EY-76-C-06-1830.)
55. FATE OF N-BUTANOL IN RATS AFTER ORAL ADMINISTRATION AND ITS UPTAKE BY DOGS AFTER INHALATION OR SKIN APPLICATION. G.D. DiVincenzo and M.L. Hamilton, Health, Safety & Human Factors Laboratory, Eastman Kodak Co., Rochester, NY.

[1-14C]n-Butanol was administered by gavage to male Charles River C.D. rats in doses of 4.5, 45 or 450 mg/kg of body weight. Rats dosed with 450 mg/kg excreted 83.3% of the dose as 14CO2 at 24 hrs; 4.4% was excreted in the urine; less than 1% was eliminated in feces and 12.3% remained in the carcass. n-Butanol was excreted in the urine apparently as an O-sulfate and as an O-glucuronide both of which accounted for 75% of the radioactivity. Urea accounted for the remainder. Rats dosed with 4.5 or 45 mg/kg showed a similar excretion pattern to that of rats dosed with 450 mg/kg. Excretion studies were performed to quantify the percutaneous absorption of n-butanol in male beagle dogs. n-Butanol was absorbed through the skin of dogs at a rate of 8.8 µg min⁻¹ cm⁻². Dogs exposed by inhalation to 50 ppm of n-butanol vapor absorbed about 55% of the inhaled vapor. Dogs dosed with ethanol (200 mg/kg, po) and then exposed to 50 ppm of n-butanol vapor for 6 hrs showed no evidence that the exposure to n-butanol vapor inhibited the metabolism of ethanol. These findings suggest that occupational exposure to n-butanol vapor at the current TLV is not likely to affect the metabolism of low doses of ethanol taken concurrently.

56. NIAAX CATALYST ESN - SUBCHRONIC NEUROPHARMACOLOGY AND NEUROTOXICOLOGY - S. C. Gad, R. R. Maronpot, J. A. Mckelvey, and R. A. Turner, Chemical Hygiene Fellowship, CMIR, Pittsburgh, PA

NIAAX catalyst ESN (a mixture of 95% DMAPN/dimethylaminopropionitrile and 5% A-99/bis-dimethylaminomethyl ether) and its two components were tested by a variety of short term tests for neurologic activity. In vivo (rat and mouse) and in vitro assays were employed.

ESN at high doses produced tremors, convulsions and weight losses in rats and mice. Rats treated with ESN at dose levels as low as 0.01 ml/kg also displayed a loss of their micturition reflex. DMAPN-treated animals displayed all these same effects except the weight losses. ESN-treated animals showed a gradual reversal of effects one week into the dosing regimen. ESN caused decreased heart rate and increased blood pressure in treated rats, and rats treated with ESN at 0.25 ml/kg displayed no normal central cardiovascular reflexes. These cardiovascular effects were reversible within two hours of dosing of animals. DMAPN showed the same cardiovascular and reflex effects as ESN, while A-99 did not. ESN and DMAPN were lethal at 0.50 ml/kg.

A-99 caused weight loss but no neurologic signs in rats or mice in the daily dose range of 1.0 to 0.01 ml/kg. TP-A-99 showed a delayed (5-24 hours after dosing) lethality in rats and mice dosed at daily levels of 0.5 ml/kg or greater.

The rapid expansion of solar technology will result in the introduction of new materials into large numbers of dwellings. While some of these materials have been evaluated toxicologically for industrial applications, their use in residential solar energy systems will require that they be more closely scrutinized for human health hazards. Of particular concern are heat transfer fluids used in active solar designs. Leak in the heat exchanger used in solar hot water heaters could result in contamination of the potable water supply. Twenty-four commercial fluids were evaluated for acute oral toxicity in the rat, dermal and ocular irritation and mutagenic potential (Ames test). The fluids included various hydrocarbon oils, ethylene and propylene glycol formulations, polydimethylsiloxanes (silicones), and others. Most fluids were non-irritating to rabbit skin and eyes. None of the fluids were mutagenic in the Ames test (Strains TA 1535, 1537, 1538, 98, 100 with or without S-9). Oral LD50’s ranged from 2 g/kg for ethylene oxide formulations to greater than 24 g/kg for most hydrocarbon and silicone oils and propylene glycol formulations. (Supported by DOE Contract ET-76-C-04-1013.)


Methyl methacrylate monomer (MMA) has been assessed for potential carcinogenic and mutagenic properties using several in vitro predictive bioassays and in vivo studies. The potential carcinogenicity was assessed in tests including the Ames test and a mammalian cell transformation assay. MMA was negative in both of these assays which is consistent with published metabolic studies and the negative carcinogenicity data. It is therefore considered unlikely to represent a carcinogenic risk to humans. The mutagenic properties of the compound were assessed using cytogenetic analysis of rat bone marrow cells the Ames test and a dominant lethal assay in mice. Although there was evidence of some non-dose-related chromosome damage in the cytogenetic study no effect was observed in the bacterial mutation test and the dominant lethal assay. The unusual cytogenetic effects observed are difficult to understand particularly considering the overall negative biological profile of this chemical. The present communication will describe the various tests used.

Methyl Methacrylate (MMA) is a monomer, which in addition to surgical and dental use, has wide application in the manufacturing of plastics and coatings. To confirm the safeness of repeated exposure to MMA under conditions of use (TWA 100 ppm), we exposed Fisher 344 rats and Syrian Golden hamsters of both sexes to 0, 25, 100 or 400 ppm MMA vapors, 6 hrs/dy, 5 dys/wk for 24 and 18 months respectively; male beagle dogs were exposed to 0, 100 or 400 ppm MMA vapors 6 hrs/dy, 5 dys/wk for 3 months. We observed the animals daily for signs of pharmacologic and toxicologic effects and periodically evaluated hemograms of rats, hamsters and dogs, clinical chemistries and urine of rats and dogs, and EKG's and blood pressures of dogs. Gross and histopathologic evaluations were made after exposures of 3 months to dogs, 18 months to hamsters, and 3, 12 and 24 months to rats. With the exception of mild rhinitis in rats, we observed no exposure related toxic effects.

60. DIETHANOLAMINE INDUCED CHANGES IN THE NEONATAL RAT. G.A. Burdock and L.W. Masten, Dept. of Pharmacol., Sch. of Pharmacy, Univ. of Mississippi, University, MS. Sponsor: W.M. Davis.

Previous studies have demonstrated the incorporation of diethanolamine (DEA) into microsomal phospholipids and changes in liver enzyme activity for various strains of adult rats. Our studies involved daily oral dosing (1.0, 2.0, and 3.0 mM DEA/kg) of neonatal S-D rats from 5 to 15 days postpartum. The liver, heart, kidneys and brain of these rats were monitored for changes in organ/body weight ratio (OBWR) organ moisture content, as well as membrane bound enzyme activity (tissue cholinesterase and succinic dehydrogenase) in various cell fractions. Significant increases in OBWR were noted for the liver and kidneys at the two highest doses while the remaining organs showed significance only after 3.0 mM/kg. No changes in moisture content were observed for any organs. However, the succinic dehydrogenase activity in the nuclear and mitochondrial fractions were elevated for the liver and kidneys, respectively, following the highest dose. Significant changes in cholinesterase activity were also noted in the microsomal fraction of these organs. Supported by NIH Training Grant GM 07099-02 and by the Univ. of Miss. Research Institute of Pharm. Sci.
61. DISPOSITION PROCESSES IN MUSSELS MYTILUS CALIFORNIANUS.
Env. Tox. and Med. Pathol., Univ. Calif., Davis, CA.

Uptake and accumulation of antritive chemicals including
organochlorine pesticides and PCBs, heavy metals, radio-
uclides and petroleum chemicals by marine mussels has been
frequently observed and tissue levels measured as part of
environmental monitoring programs. Studies of disposition
processes are being conducted to determine biological con-
sequences of antritive chemical exposures. Mytilus calif-
ornianus are being obtained from large uncontaminated pop-
ulations near Bodega Bay, CA. Using neutral red in static
bioassays containing 4 mussels in 1 liter Instant Ocean®
v ventilation rates of 0.5 - 1 1/hr/mussel have been recorded.
Antipyrine (6µM) is distributed in body water, rapidly
cleared (0.1 1/hr/6 mussels), and metabolized to a small
extent (less than 4%) to 4-hydroxyantipyrine during test
periods up to 6 hrs. In vivo biotransformation of aldrin
to dieldrin has been measured under field and laboratory
conditions. Mussel tissue pieces of mantle, gill and green
gland transformed more substrate than corresponding homogen-
ates. Histological preparations of active mussel tissues
will be presented.

62. A UNIQUE SYSTEM FOR QUANTIFICATION OF INCAPACITATION TIME
FOR RATS EXPOSED TO PRODUCTS OF COMBUSTION - J.B. Reid,
T.F. Brecht, Chemical Hygiene Fellowship, CMIR, Pittsburgh,
PA. Sponsor: E.R. Roman

The determination of incapacitation time is crucial in
design of a model system to evaluate survival in a real fire
situation. In our system, an exercise wheel linked to a
torque measuring transducer produces an activity trace
throughout the exposure. After base line activity is deter-
minded, the exposure is begun. Thermolysis products are
passed from a furnace, via the central shaft, to a cylindri-
cal exposure chamber and exercise wheel containing 6 animals.
The behavior of the animals, as altered by the combustion
products, produces a change in the torque measurement. This
measurement reaches a plateau at incapacitation. The gross
body movement of each animal is recorded in the fine struc-
ture of the trace. The trace allows a determination of
degree of incapacitation and ultimately, death, and a
"fingerprint" for the exposure which is, thus far, unique to
the material being tested. Two wood samples and two plastic
materials have been thoroughly investigated in this system.
Distinction between wood and the tested plastics is readily
discernible. This unique solution provides quantitative,
objective data in a system which, in our opinion, is closest
to the real case, i.e. escape from a fire.
63. TERATOGENICITY OF DIPHENYLHYDANTOIN IN NEW ZEALAND WHITE RABBITS. R.M. McClain and L. Landhoff, Department of Toxicology, Hoffman-La Roche, Inc., Nutley, NJ. Sponsor: E. Pfister

Diphenylhydantoin sodium (DPH) was given po to pregnant New Zealand White rabbits on days 7-18 of gestation at dosages of 0, 25, 50, 75, 100 and 150 mg/kg/day. No pharmacological or toxicological effects were noted in does except for reduced maternal weight gains at 100 and 150 mg/kg. Average fetal weights and crown rump lengths were unaltered. Dose-related increases in resorptions and malformations were observed. DPH resulted in fetal resorptions of 15, 10, 25, 57 and 76% at 25, 50, 75, 100 and 150 mg/kg, respectively, compared to 6% in controls. The percentage of fetuses with abnormalities (total) was 6% (8/126) in controls vs 17% (18/106) at 25 mg/kg, 9% (9/100) at 50 mg/kg, 11% (13/114) at 75 mg/kg, 41% (20/49) at 100 mg/kg, and 9% (1/11) at 150 mg/kg. A number of litters (1/14 with viable fetuses at 75 mg/kg and 3/8 at 100 mg/kg) exhibited a similar syndrome of malformations that included some or all of the following: open eyes, cleft palate, limb abnormalities with shortened and/or curved long bones, pes cavus, syndactyly and hypoplasia of the phalanges. DPH is fetal toxic and teratogenic in the rabbit at dosages of 75 mg/kg/day or more.

64. TERATOGENIC EFFECTS OF ASPIRIN IN THE FISCHER 344 RAT. L. R. DePass, W. M. Snellings, and M. D. Woodside, Chemical Hygiene Fellowship, CMIR, Pittsburgh, PA. Sponsor: E. R. Howan

The Fischer 344 rat has received increasing interest in recent years as a model for toxicology studies. However, this strain has rarely been used in teratology studies. To validate the Fischer rat as a good teratology model we administered aspirin in single oral doses of 500 mg/kg on day 9 or 625 mg/kg on day 10 of gestation. The dams were sacrificed on day 20 at which time the pups were examined. Aspirin treatment was associated with reduced fetal length and weight. A wide variety of skeletal malformations were also observed in the aspirin-treated groups. These included extra 14th and 15th ribs, poorly ossified sternabrae, extra thoracic and lumbar vertebrae, fused or missing ribs, split vertebrae centra and missing vertebrae. A small number of aspirin-treated fetuses also exhibited visceral malformations such as hydrocephalus, atrial enlargement, moderate hydronephrosis and ureteral dilation. The frequency of visceral malformations was much smaller than that reported in aspirin-treated Wistar (Kimmel et al., Teratol. 4, 15, 1971) or Sprague-Dawley (Goldman et al., Proc. Soc. Exp. Biol. Med., 118, 857, 1965) rats.
65. EFFECTS OF MONOETHYLHEXYL PHTHALATE (MEHP) ON PREGNANT RABBITS AND THEIR OFFSPRING. J.A. Thomas, P.R. Felice, L.G. Schein, P.K. Gupta and R.E. McCafferty, West Virginia University Medical Center, Morgantown, WV.

Female rabbits weighing 3.9 ± 0.5 kg were artificially inseminated and subsequently injected with varying doses of MEHP (1.14, 5.69 or 11.38 mg/kg daily x 13 – i.v.) beginning on the 6th day of gestation; on the 30th day, fetuses were removed by caesarian section. Regardless of dose, MEHP caused no significant changes in the number or size of the litters, the sex ratio or fetal weights. The incidence of live fetuses in control groups was 94% while in the 1.14 mg/kg dose it was 89%; 5.69 mg/kg, 87%; and 11.38 mg/kg, 77%. MEHP had no significant effect upon either fetal crown–rump or trans–umbilical measurements. The number of corpora lutea or resorptions in MEHP-treated groups was not significantly different from controls. While the 5.69 and 11.38 mg/kg doses led to higher maternal mortality (22% and 33% respectively), those pregnant animals continuing to gestation failed to reveal any differences in gravidometric responses in adrenal, liver, kidney, heart or lungs when compared to control organs. Skeletal and visceral examination as well as histological assessment are currently under investigation. Supported in part by Travenol Laboratories, Chicago, IL.


There are a number of pesticidal agents to which human exposure is highly likely and whose teratological potential remains to be determined. From these the following were investigated at the given doses; Bipheny1 (99.9%), ethoxyquin (unknown purity), piperonyl butoxide (80%), Karmex (80% diuron) or Perfect (45% thiabendazole) at 0, 125, 250 or 500 mg/kg; Zolone TM (30% phosalone) at 0, 12.5, 25 or 50 mg/kg; and Benesan (50% lindane) at 0, 6.25, 12.5 or 25 mg/kg. These doses were given orally by intubation as single daily administrations to rats from the sixth to the 15th day of pregnancy. The rats were necropsied at term. Fetuses were examined to obtain values on body weight, survival, intrauterine deaths and anomalies (external, visceral and skeletal) by using standard methods. Diuron was found associated with an increased incidence of wavy rib anomaly at the 250 mg/kg, and at the masculinizing dose of 500 mg/kg. Otherwise, in all the remaining test groups, no adverse effects were observed on fetal development.
67. TERATOGENICITY EVALUATION OF COMMERCIAL FORMULATION OF
DIMETHOATE (CYGON 4E) IN THE CAT AND RAT. K.S. Khera,
New Research Centre, National Health and Welfare, Tunney’s
Pasture, Ottawa, Canada.

Dimethoate (Rogor), an extensively used insecticide
whose residues occur in a number of foods, was investigated
for teratogenic activity, since such a study was not
available.

A commercial formulation containing 47.3% of dimethoate
and 52.7% of unknown ingredients (Cygone 4E) was
administered orally in single daily doses to cats from
the 14th to the 22nd day of gestation and to rats from
the sixth to the 15th day of gestation. The cats and
rats were necropsied on the 43rd and the 22nd day of
gestation, respectively. Fetal values on survival, body
weight, and skeletal and visceral anomalies, were obtained
by using standard methods. The highest dose, 12 mg/kg,
was found associated in the cat with polydactyly in eight
of the 39 fetuses. In the rat, an increased incidence of
fetuses deformed with wavy ribs and other anomalies, was
noticed at 12 mg/kg, and at a maternally toxic dose of
24 mg/kg. However, 3 and 6 mg/kg doses of Cygone
4E, compared with control, produced no evidence of
teratogenicity or embryotoxicity in the two species.

68. A CORRELATION BETWEEN ELEVATED AMNIOTIC FLUID OSMOLALITY AND
THE DEVELOPMENTAL TOXICITY OF TRYPAN BLUE. J. Rogers, Dept.
of Biology, Univ. of Miami, Coral Gables, FL. Sponsor: J.
Radomski.

Trypan blue is one of many teratogenic agents known to
cause embryonic edema, neural and cardiovascular distention,
blisters and hematomas. The objectives of this study were:
to establish data on the osmotic and ionic characteristics
of amniotic fluid in control and Trypan blue treated em-
byos; to look for the factor(s) responsible for any differ-
ences found; and to correlate these differences with the
incidence and types of abnormalities found. Female Long-Evans
rats were injected s.c. on day 8.5 with 50 mg/Kg Trypan blue.
Individual samples of amniotic fluid were drawn on day 11.5,
and melting point depression analysis was done on 1 μl sam-
pleS. Amniotic osmolality was greatly elevated in treated
embryos (467 mosm/Kg vs. 347 in controls), and showed a much
wider range (316-1069 vs. 332-396 in controls). The eleva-
tion was not due to sodium or potassium. Elevated amniotic
osmolality was closely correlated (r= .79) with gross swel-
ling and rupture of the neural tube, and swelling of heart
and pericardium. Thus, embryonic swelling induced by osmotic
imbalance is probably one mechanism of Trypan blue terato-
genesis.
69. THE EFFECTS OF 2-NITROPROPAINE, NAPHTHALENE, AND HEXA-
CHLOROBUTADIENE ON FETAL RAT DEVELOPMENT. S.J. Harris,
G.P. Bond, and R.W. Niemeier, ASTS, DBBS, NIOSH, Cincinnati,
OH. Sponsor: T.R. Lewis. Adult female Sprague Dawley
rats were injected ip with either 170 mg/kg bw 2-nitro-
propane (2-NP), 395 mg/kg bw napthalene (N), 10 mg/kg bw
hexachlorobutadiene (HCBD), or 1 ml/kg bw corn oil (C) on
days 1-15 of gestation. Litters were collected one day
prior to parturition and examined by the techniques of
Wilson and Dawson. Retarded heart development (1-2 days)
was observed in pups from 9 out 10 litters from mothers
treated with 2-NP (P < 0.001). Thirty to 86 percent of
the pups examined within a litter were affected. Retarded
cranial ossification and heart development were observed
in twice as many pups from mothers treated with N as in
pups from mothers treated only with corn oil (P < 0.001).
The number of soft tissue anomalies in pups from mothers
treated with HCBD was three times as high as in pups from
control mothers; no specific anomaly prevailed. External
hydrocephaly was observed in one HCBD litter. Effects on
postnatal growth and behavior were not determined.

70. "Teratology Studies of Benzethonium Chloride, Cetyl Pyridinium
Chloride and Chlorhexidine In Rats". M. R. Gilman, S.
J. de Salva, Colgate-Palmolive Company, Piscataway, NJ.

Quaternary compounds are widely used in preventative and
therapeutic applications. The potential for the exposure of
pregnant women increases the importance of determining
the teratogenic effect in rats. Benzethonium chloride,
cetyl pyridinium chloride and chlorhexidine were chosen as
representative quaternary chemicals. The materials were
administered by gastric intubation to pregnant rats on days
6 through 15 of gestation, sacrificed on day 20 and the
fetuses examined. Doses administered ranged from 0.059 to
68.541 mg/kg/day. No adverse effects were observed with
chlorhexidine. Benzethonium chloride and cetyl pyridinium
chloride produced lower mean body weights at the high dose
(35.58 and 27.33 mg/kg/day respectively). Benzethonium
chloride produced delayed ossification at the high dose
only; this finding was confirmed in a second study. No
clinical manifestations of skeletal deformity were observed
in either Segment I, Rat Fertility, or Segment III, Rat
Perinatal and Postnatal, studies. The relevance of delayed
ossification does not appear expressible for skeletal
teratogenic changes and most likely is related to reduction
in fetal maturation secondary to maternal toxicity.

Daily injection of 300 mg/kg sc of streptomycin sulfate (S) to rats from 2 to 22 days of age resulted in dyskinesia, including backward movement, circling, head bobbing, and hyperactivity, and in deficiency of the free-fall righting reflex; these effects lasted for months after dosing was stopped (Allewa and Balazs, Toxicol. Appl. Pharmacol., in press, 1978). Although vestibular damage in rats by S is known to result in impaired righting reflex, the other abnormalities are suggestive of a central injury. Histologic examination of brains of S-treated rats revealed no changes. In the present experiment we tested the effect of graded doses of various agonists and antagonists of central neurotransmitters in groups of 5 dyskinetic rats. Only the dopaminergic agonists amphetamine, apomorphine, and methylenidate reversed the dyskinesia. Paradoxically, S-treated rats of both sexes had a significant increase of dopamine in the nucleus accumbens whereas females also had significant increases in the caudate nucleus and olfactory tubercle. Data reveal that the neurotoxic syndrome may involve central and vestibular changes.

72. CHARACTERIZATION OF A DIETHYLSILBESTROL (DES) BINDING COMPONENT IN FETAL RAT UTERUS. C.L. Kimmel and J.R. Harmon, National Center for Toxicological Research, Jefferson, AR. Sponsor: L. Fishbein.

The fetotoxicity of DES and other estrogens suggests the existence of an estrogen binding component through which such agents can interact with target cells. Using $^3$H-DES, we have begun to characterize such a component in the near-term, fetal rat uterus. Uteri from day-20 rat fetuses were homogenized and the supernatant (cytosol) from a 150,000xg centrifugate was analyzed for $^3$H-DES binding by sucrose density gradient, saturation analysis, and competitive protein binding. A binding component was identified by gradient analysis. Addition of unlabeled DES lowered the level of binding to the component, and a shift to a larger sedimentation coefficient was evident when salt was omitted from the gradient buffer. Saturation analysis indicated a limited number of binding sites (<100 fmols/mg protein) with an apparent dissociation constant of approximately $1 \times 10^{-8}$ M. DES and estradiol-17β competed for $^3$H-DES binding in a similar fashion, while progesterone did not compete significantly. This indicates a specificity of the binding component for estrogen. Our results demonstrate a DES binding component in fetal rat uterus which has characteristics similar to the cytoplasmic estrogen receptor described in the postnatal animal. Thus, it appears that an estrogen binding component exists in the fetus which could provide a mechanism for the interaction of DES and other estrogens with target cells.
73. Craniofacial Teratogenicity of an Experimental Anti-allergy Agent. Fred Martz, Leo Arslanoglou, and Roger D. Hemm, Toxicology Section, Ayerst Research Laboratories, Chazy, NY.

AY-25,674 (N-[2-oxo-3,5,7-cycloheptatrien-1-yl]aminooxaoacetic acid ethyl ester) is an orally active anti-allergy agent which suppresses antigen-mediated reactions by inhibition of histamine release. Studies were conducted with CRCD male rats and CD-1 mice to test the teratogenic potential of AY-25,674 given by gavage on gestation days 6 through 15 (plug day = 0) at doses of 250 or 500 mg/kg. Litters of 500 mg/kg rats delivered by hysterotomy on day 21 had high incidences of multiple craniofacial, rib, and spinal deformities including acrania, exencephaly, anophthalmia, chelognathopalatoschisis, and spina bifida. One complete litter had retarded ossification of the sternum and xyphoid. All 500 mg/kg rat dams had alopecia, polydipsia, and markedly reduced food intake and weight gain, and 28% died before day 15 with oxalate-induced nephrosis. In contrast, the 250 mg/kg rats and the 250 or 500 mg/kg mice showed no significant toxicity and all carried litters free of drug-related malformations. These results implicate maternal toxicity as a contributing cause of teratogenic effects. Differential susceptibility between mice and rats is speculated to involve different thresholds for toxicity or differences in the disposition of AY-25,674.

74. STUDIES ON THE EMBRYOTOXICITY OF PHOSPHONACETYL-L-ASPARTIC ACID (PALA) IN MICE. S.M. Sieber, D. Cooney, C. Botkin and R.H. Adamson, NCI, NIH, Bethesda, Md. 20014

PALA is a new antitumor agent which inhibits L-aspartate transcarbamylase (ATCase), the second enzyme in de novo pyrimidine biosynthesis. It is a potent embryotoxin in mice, with embryolethal and teratogenic effects related to the day of gestation on which it is administered. The peak of embryonic sensitivity to the teratogenic effects of PALA occurs on day 7 of gestation, when 70% of fetuses surviving a dose of 12.5 mg/kg were malformed. Embryolethality was most marked on day 8; embryonic LD50 doses of PALA on day 4, 6, 8 and 10 were 111, 31, 9 and 164 mg/kg, respectively. The marked difference in sensitivity to PALA noted between day 8 and 10 embryos may be due to differences in ATCase levels or to changing permeability to PALA. Thus, ATCase levels were about 4-fold higher in placental and embryonic tissue on day 8 than on day 10 of gestation. Four hours after pregnant mice were injected ip with (acetyl-14C)-PALA, the placenta/maternal plasma 14C-ratio was about 6 on both day 8 and 10 of gestation. The embryo/maternal plasma 14C-ratio on day 10 approximated unity, whereas it exceeded 12 on day 8. Although uridine reverses the antitumor effects of PALA, it does not prevent PALA-induced embryolethality.
75. DISPOSITION OF AN ORAL DOSE OF O-ETHYL-O-p-NITROPHENYL
PHENYLPHOSPHONOTHIOATE (EPN) IN THE RAT. J.M. Charles and
J. Farmer, Toxic Effects Branch, HERL, Environmental Protec-
tion Agency, Research Triangle Park, N.C. Sponsor: M.B.
Abou-Doria
The distribution, excretion, and metabolic profile of a
single, oral 1.5 mg/kg dose of $^{14}$C-EPN in rats was investig-
gated. The plasma half-life for the initial process was
approximately 5 hr in both sexes. The second slower kinetic
process had a half-life of 33.6 hr in males and 38.6 hr in
females. Initially the highest levels of radioactivity in
both sexes were found in the highly perfused liver, kidney,
and lung. While concentration levels declined steadily in
all tissues during the course of the study (8 days), the
lungs and liver demonstrated a 4X increase in concentration
when compared to that in the blood of both sexes. Urinary
excretion was the predominant mode of elimination represent-
ing 68.2% of the dose after 48 hr in the males and 60.6% in
the females. The total amount excreted by the males in the
feces was 4.9% for the first day, 6.7% by the second day,
and 7.4% by the third day. In the females, the values were
13.8%, 17.2%, and 21.1%, respectively. Biliary excretion
studies performed for up to 8 hr demonstrated a significa-
ntly higher rate in females versus males. The tissue and ex-
cretion EPN metabolite profiles were similar in both sexes.

76. The Oral and Dermal Uptake of Radiolabeled Fyrol FR-2 by
Rats and Rabbits. A.G. Ulsgaard, P.L. Weisenfeld, and R.C.
Collins, Consumer Product Safety Commission, Bethesda, Md.
The oral and dermal absorption of tris (2,3-dibromopropyl)
phosphate was presented at the 17th Annual S.O.T. Meeting.
The present study utilizes the structurally related flame
retardant Fyrol FR-2 (tris 1,3-dichloroisopropyl) phosphate
New Zealand white rabbits (2.0-2.5kg) and Osborne-Mendelrats
(200-250g) of both sexes were used. For dermal absorption
of single dorsal application of Fyrol-14C was made (either
0.9 ml/kg or 0.05 ml/kg). For oral uptake a single dose of
Fyrol-14C was administered by gavage. Fyrol-14C was also
administered i.v. (1 mg/kg). Urine, feces and CO$_2$ were
monitored for 96 hrs. Dermal rabbits absorbed 4 times as
much radiolable as did rats (6% at 0.9 ml/kg and 20% at
0.05 ml/kg) with radiolabel appearing in the urine at 4 hrs.
Liver and kidney had the highest specific radioactivity.
Following oral or i.v. administration in both species most
of the radiolabel appeared in the urine within 24 hrs. (80-
85%); 3-8% of the dose remained in body tissue at 96 hrs.
The half-life in both species was 4.5-7.4 hrs. with signif-
icant amounts of Fyrol-14C was converted to CO$_2$. Fyrol
FR-2 appears to penetrate skin more rapidly than TRIS by a
factor of two and has a similar half-life.
77. EFFECTS OF N-(PHOSPHONOMETHYL)GLYCINE ON SOME ENERGY-LINKED FUNCTIONS OF RAT LIVER MITOCHONDRIA. O.O. Olorunsogo, E.A. Bababunmi, and O. Bassir, Dept. of Biochemistry, Univ. of Ibadan, Ibadan, NIGERIA.

Very few compounds derived from α-amino acids are able to produce deleterious effects on the inner mitochondrial membrane functions such as oxidative phosphorylation. N-phosphonomethyl derivative of glycine (PMG), a unique product with broad-spectrum, non-selective, with post-emergence herbicidal properties has been shown to uncouple mitochondria when administered intraperitoneally to rats. Polarographic measurements of the rates of oxygen uptake by isolated liver mitochondria, respiring on either succinate, or 1,3-dihydroxyacetone-phosphate and in the presence of varying concentrations of PMG, revealed that respiratory control ratio (RCR) of hepatic mitochondria was significantly reduced by at least 10% at 3.95 x 10^{-5} M PMG. Maximal reduction was obtained at 1.25 x 10^{-3} M concentration of PMG. Adenine concentrations ranging from 1.25 x 10^{-3} M to 3.12 x 10^{-3} M PMG restored respiration of isolated rat liver mitochondria (RLM) previously inhibited by oligomycin. Latent adenosine triphosphatase activity (ATPase) was stimulated at least 3-fold by the addition of 6.25 x 10^{-3} M PMG. These results suggest that PMG uncouples mitochondrial oxidative phosphorylation.

78. EFFECT OF PARTIAL HEPATECTOMY ON BENZENE METABOLISM AND TOXICITY. D. Sammett, E.W. Lee, J.J. Kocsis and R. Snyder, Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA

To determine the role of the liver in producing toxic 3H-benzene (3H-B) metabolites, the toxicity and metabolism of 3H-B was studied in partially hepatectomized (PH) and sham operated rats. Toxicity was based on the reduction of 59Fe uptake into red cells in 3H-B treated (2200 mg/kg, sc) rats by the method of Lee et al. (Toxicol. Appl. Pharmacol. 27, 431-436, 1974). 3H-B metabolites in urine were used as a measure of 3H-B metabolism. PH rats were protected against 3H-B toxicity and also displayed reduced 3H-B metabolism. These observations suggest that the metabolism of benzene by the liver is a necessary step in the production benzene-induced bone marrow toxicity. (Supported by ES-00322.)
79. BENZENE INHIBITS OVARIAN HYPERTROPHY IN THE HEMISPAYED RAT. M. D'Souza, R. Snyder and J.J. Kocsis, Thomas Jefferson University, Philadelphia, PA 19107

The finding that benzene (B) can block liver regeneration in the partially hepatectomized rat (Sanmert et al. 1978) suggested that B may also inhibit growth and development in other organs. The well-known model of ovarian hypertrophy in the hemispayed rat was chosen to test this concept. B was administered (1 ml/kg, sc) daily to unilaterally ovarectomized (ULO) and sham operated rats starting at the beginning of diestrus. B caused persistant diestrus, inhibited hypertrophy and reduced the numbers of corpora lutea and Graafian follicles. Covalent binding of $^3$H-B was seen in the ovary. It is suggested that increased levels of FSH normally seen after ULO were not reduced by benzene but estrogen secreted by the follicles was inadequate to trigger LH release. The mechanism by which B inhibits growth and development in the ovary may be due to a common cytostatic action of benzene or its metabolites. (Supported by ES-00322.)

80. THE KINETICS AND EFFECTS OF CHLORINE DIOXIDE IN DRINKING WATER ON RAT AND CHICKEN BLOOD. M. S. Abdel-Rahman and D. Couri. Ohio State University Toxicology, Columbus, OH.

Since chlorination of drinking water produces organochlorinated substances (some possibly carcinogenic) the use of chlorine dioxide disinfectant would avoid halogenation. There is scarcely any data published on the effects of ClO$_2$ in drinking water on human or animal health. The kinetics of $^{36}$ClO$_2$ was studied in rats. Radioactivity was rapidly absorbed from the gastrointestinal tract following the administration of (0.7 uCi) $^{36}$ClO$_2$ orally. $^{36}$Cl in plasma reached a peak at 1 hr. The half life for the elimination of $^{36}$Cl from the rat was 44 hr corresponding to a rate constant of 0.016 hr$^{-1}$. After 72 hrs radioactivity was highest in plasma followed by kidney, lung, stomach, duodenum, ileum, liver, spleen, thymus and bone marrow. $^{36}$Cl excretion was greatest at 24 and 48 hrs after the administration of $^{36}$ClO$_2$. 43% of the total initial dose was excreted at 72 hrs in the urine and feces. No $^{36}$Cl was detected in expired air throughout the 72 hrs studied. ClO$_2$, ClO$_{-2}$ and ClO$_{-3}$ (1,10,100,1000 ppm) given daily in drinking water decreased blood glutathione, decreased osmotic fragility and changed the morphology of erythrocytes in both chicken and rat after two months. Methemoglobin was not detected throughout these studies.

An African arrow poison of plant origin was separated using the following ion exchangers and eluants: Amberlite (IR-120) cation exchanger with 1.0 M NaCl - 0.1 M NaOH (pH 11) as eluant; Dowex (1 X 2) anion exchanger with 0.1 M NH₄OH (pH 11) as eluant and Dowex (2 X 2) with NaOH (0 to 1 M) - NaCl (1 to 0 M) gradient elution. The recovery (%) of the toxin was 38, 13, and 50 respectively, thereby suggesting that gradient elution gave a relatively superior separation. The absorbance of the separated fractions were read at 250 nm. Acute toxicity studies of the isolated fraction in male Sprague-Dawley mice showed some signs of toxicity similar to some of the symptoms observed with the crude poison. The solution of the toxin gave a negative test with Mayer's reagent, indicating that quaternary nitrogen might not be part of the structure. Gibb's reagent gave a positive test with the toxin and some of the separated fractions, thereby suggesting the presence of phenolic alkaloids in the poison. (Supported by NIH Grant RR-8008-851.)


In our search for possible antidotes for victims of arrow poisoning, we have studied the acute toxicity of the poison in Sprague-Dawley mice and rats. Doses of the toxin were injected iv in the tail vein of male mice (15-20 g). The LD50 was 27 mg/kg. There was an initial increase in respiration prior to death, and, in the surviving animals, the increased respiration was followed by slowed respiration with pronounced excrusion of the thorax. The front limbs and head trembled violently prior to death. The treated animals showed prominent signs of muscle paralysis. Treatment of mice with the toxin caused cholinergic syndrome. An iv injection of a lethal dose of the toxin in rats led to an immediate decrease in respiration which occurred within one minute. Concomitantly, the heart rate decreased from 420 to 170 beats per minute, stopping completely in 1½ minutes. The apparent cause of death was respiratory paralysis and cardiovascular failure. (Supported by NIH Grant RR-8008-851.)
83. The Importance of Dose Frequency to Zinc Pyridinethione-Induced Paralysis. W. B. Gibson, Miami Valley Laboratories, The Procter & Gamble Company, Cincinnati, OH. Sponsor: E. V. Buehler

Zinc pyridinethione (ZPT) is an antidandruff agent used in commercial shampoos. It has been reported (Snyder, Buehler, and Winek, Toxicol. Appl. Pharmacol. 7, 425, 1965) that ZPT causes hind limb paralysis in rats and rabbits when administered subchronically in the diet, but not when it is dosed by stomach tube five days a week. An investigation of the metabolism of ZPT in rats after they were dosed by stomach tube, or after they ingested ZPT mixed with diet, showed no metabolic basis for the dose route difference in toxicity. These results prompted us to do further subchronic studies which showed that ZPT-induced hind limb paralysis could be produced by stomach tube dosing. In these experiments the frequency of dosing proved to be as important as the quantity dosed for production of a readily observable toxic symptom. Rats that were dosed for three weeks with 10 mg/kg/day seven days a week (210 mg/kg total exposure) were more severely affected than rats dosed with 20 mg/kg/day five days a week (300 mg/kg total exposure). It is therefore important, when planning subchronic studies, to consider the effect of interrupting the dosing schedule on weekends.

84. THE EFFECT OF THE ACIDIC AND BASIC FRACTIONS OF MARIHUANA WHOLE SMOKE CONDENSATE ON THC-INDUCED HYPOTHERMIA IN MICE. J. M. Johnson, L. Lemberger, W. S. Dalton, and R. B. Forney, Dept. of Pharmacology and Toxicology, Indiana Univ. Sch. of Med. and Lilly Research Laboratories, Indianapolis, IN.

The activity of marihuana cannot be accounted for solely by its tetrahydrocannabinol (THC) content. Body temperatures (BT) of mice were measured following iv administration of acid and base fractions obtained from marihuana smoke, both alone and in combination with THC. In studying the acid fraction (A), mice were assigned to the following groups: 1) vehicle, 2) 1.0 mg/kg THC alone, 3), 4), and 5) received 1.4, 2.8, and 5.6 mg/kg A, respectively, 6), 7), and 8) received the above doses of A in combination with 1.0 mg/kg THC. A alone had no effect on BT. However, at 1 hr post injection, mice receiving 5.6 mg/kg A plus THC had a significantly lower BT than animals receiving THC alone. Similar experiments were done using the base fraction (B). A dose of 3.0 mg/kg B in combination with THC also caused a significant depression of BT when compared to THC alone at 1 hr. B alone had no effect on BT. These results suggest an alteration of THC metabolism, excretion, and/or distribution by marihuana acids and bases. (Supported in part by USPHS Grant T32 GM 7097 and R01 DA 00069.)
85. A 90-DAY INHALATION TOXICITY STUDY OF EPICHLOROHYDRIN IN
LABORATORY RODENTS. J.F. Quast, J.W. Henck, M.J. McKenna,
Toxicology Research Laboratory, Health and Environmental
Research, Dow Chemical, USA, Midland, MI. Sponsor:
P.J. Gehring

Groups of 20 male and 20 female B6C3F1 mice, Fischer 344
rats, and Sprague-Dawley rats were exposed to epiclorohy-
drin vapors. Exposure concentrations were 0, 5, 25 or 50
ppm for 6 hours/day for 5 days/week during a 90-day period.
The following parameters were evaluated: clinical signs,
body weight, hematology, urinalyses, blood urea nitrogen,
serum alkaline phosphatase activity, serum glutamic pyruvic
transaminase activity, serum glutamic oxaloacetic transam-
инase activity, serum glucose, gross pathology, organ
weights and organ/body weight ratios of brain, heart, liver,
kidneys, testes, spleen and thymus. Microscopic examination
of tissues from these animals revealed the most sensitive
target organ to be the nasal turbinates. Microscopic
changes characterized by hyperplasia and metaplasia with an
increased inflammatory cell infiltrate were seen in the
nasal turbinates at 25 and 50 ppm in a dose-related fashion.
Other parameters evaluated showed minimal treatment-related
effects at the 50 ppm level. No treatment-related effects
were detected at the 5 ppm level of exposure. (Supported
by the Manufacturing Chemists Association).

86. COMPARATIVE TOXICOLOGY OF BUTYL NITRITES IN MICE.
D.P. McFadden and R.P. Maickel, Dept. of Pharmacol. & Toxi-
col., School of Pharmacy & Pharmacal Sciences, Purdue
Univ., W. Lafayette, IN 47907.

Administration of "Rush" (a street preparation containing
83% isobutyl nitrite by GLC analysis) to mice (i.p.) mark-
edly increased heart rate, an action typical of organic
nitrites. Commercially available n-butyl nitrite (nBN),
sec-butyl nitrite (sBN), tert-butyl nitrite (tBN) and
isobutyl nitrite (iBN) were found to be 79%, 44%, 96%, and
63% pure by GLC analysis. Administration to mice of pure
compounds (>99% by GLC analysis) synthesized in this
laboratory yielded LD50 values (30 min., i.p., 95% C.L. in
parentheses) of 158 mg/kg (127,197) for nBN, 169 mg/kg
(139,199) for iBN, 592 mg/kg (476,734) for sBN, and 625
mg/kg (520,750) for tBN. No deaths were observed after 30
min. post-administration for nBN or iBN; however, deaths
occurred up to 6 days following the administration of
sBN and tBN. LD50 values at 6 days were 187 mg/kg (112,
312) for tBN and 496 mg/kg (403,611) for sBN. (Supported
by USPHS grant 1-T-32-GM-07095)

Single oral doses of 950, 790 and 550 mg/kg α-picoline were administered to male Sprague-Dawley rats. Treatment related toxicity was observed only at the highest dose level. Four of the 10 rats given 950 mg/kg died within 2 days of treatment. The remaining 6 showed decreased body weights throughout the study and 2/6 showed signs of encephalomalacia (areas devoid of neuronal elements, neurons and supportive cells, normally found in brain tissue). The data indicate that α-picoline given to rats at a dose which causes death to some animals results in pathologic alterations in the central nervous system (CNS). Since the primary route of occupational exposure to α-picoline is via inhalation, male and female Sprague-Dawley rats (10/sex/level) were exposed to 0, 5, 35 or 100 ppm α-picoline vapor 5 days/wk for 6 months to determine whether CNS lesions or other toxic manifestations would be induced. Despite the CNS effects observed after oral administration of a lethal dose, histopathologic alterations of the CNS were not observed at any exposure level following repeated inhalation exposure. In addition no other parameters indicative of toxicity were noted at any exposure level.


Measurement of chemical induced DNA damage in cultured mammalian cells has been proposed as a predictive test for mutagens/carciogenens. The predictive value, reproducibility and practicality of three methods of measuring DNA damage were compared using ten compounds. These systems included: the measurement of unscheduled DNA synthesis (UDS) by autoradiography or liquid scintillation counting in CRFK cells, hamster embryo cells (SHE) and rat hepatocyte cultures (PRH); and measurement of DNA fragmentation in CRFK and SHE cells by alkaline sucrose gradient centrifugation. Liquid scintillation counting of UDS proved unreliable and successful DNA fragmentation required the inclusion of liver microsomes for compounds requiring metabolic activation. Positive autoradiographic response for UDS occurred in CRFK and SHE cells with direct acting compounds, while PRH cells were positive with compounds requiring activation.
89. CHARACTERIZATION OF Ames MUTAGEN ASSAY USING AFLATOXIN B\(_1\) AS A MODEL CARCINOGEN. Cheng-I Wei and D.P.H. Hsieh, Dept. of Environmental Toxicology, Univ. of Ca., Davis, CA.

The effects of the initial bacterial number undergoing mutation and the activity of activation enzyme system on the outcome of the Ames Mutagen Assay were studied using aflatoxin B\(_1\) (AFB\(_1\)) as a model carcinogen. In the presence of a rat hepatic microsomal system (S-9), AFB\(_1\) is converted to metabolites that are both lethal and mutagenic to S. typhimurium TA 98. Within the linear range of the mutagenicity dose-response curve (up to 1 \(\mu g\) AFB\(_1\) per plate), the initial number of bacteria was not reduced, indicating that the killing effect is insignificant. The optimum amount of liver S-9 protein per plate is 1.8 \(\mu g\). Preincubation of the activation system at 37\(^\circ\)C for 1 hour resulted in an 80% increase in the enzyme activity which led to a substantial increase in the sensitivity of the assay. At doses greater than 1 \(\mu g\) per plate, AFB\(_1\) is mutagenic to strains TA 98 and TA 100 without activation. The results indicate that AFB\(_1\) is also a direct acting mutagen possibly due to its ability to intercalate the bacterial DNA structures. (Supported by USPHS Grant ES 00612.)

90. MUTAGENIC N-NITROSO AMADORI COMPOUNDS FORMED FROM MODEL MAillard NON-ENZYMATIC BROWNING PRODUCTS IN THE PRESENCE OF NITRITE. J.R. COUGHLIN, C. F. RUSSELL, DEPT. FOOD SCI & TECH., AND C. I. WEl, D.P.H. HSIEH, DEPT. ENV. TOX., UNIVERSITY OF CALIFORNIA, DAVIS, CA

Ames mutagen assays on S. typhimurium TA98 and TA100 showed positive dose-response results in the absence of rat liver microsomal activation system (S-9) for an aqueous solution of the Amadori compound fructose-L-tryptophan (Pru-trp) incubated with excess NaNO\(_2\) for 24 hr at 37\(^\circ\)C. The mutagenicity to both strains was reduced in the presence of S-9 mix, although a dose-response was still obtained. A Pru-trp control incubated without nitrite showed no evidence of mutagenicity in either strain, and a much lower revertant frequency than seen in the nitrosated Pru-trp was observed for an equimolar nitrite control. Since Amadori compounds are 2\(^\circ\) amines occurring widely in browned, heat-processed foods, beverages and unburned tobacco, our finding that an N-nitrosated Amadori compound acts as a direct mutagen suggests the possibility that this previously unrecognized group of potential carcinogens/mutagens may contribute to the causation of human GI tract cancers either by ingestion of the preformed compounds or by in vivo nitrosation of precursor Amadori compounds.
91. MUTAGENICITY OF 1, 2-DIBRMO-3-CHLOROPROPAINE (DBCP): IN VIVO CYTOGENETICS STUDY IN THE RAT. R.W. Kapp, Jr., Hazleton Lab. America, Inc., Vienna, VA. Sponsor: M. Steinberg

The halogenated aliphatic hydrocarbon 1, 2-dibromo-3-chloropropene (DBCP) has been widely used as a soil fumigant. Studies have shown that DBCP is harmful to humans by various routes. The genetic toxicity of DBCP in humans has been demonstrated in previous studies by the author. (Kapp et al. Mut. Res., in the press). To more clearly define the mutagenicity of this pesticide, three groups of 20 male rats were administered 0.73, 7.3 and 73 mg/kg/day of DBCP by gavage for 5 days. The high dose level was selected since it was the highest non-toxic dose (LD_{50} = 146 mg/kg).

Positive and negative groups were utilized as reference controls. Colchicine (2 mg/kg) was administered intraperitoneally to all animals 24-hours after the last dose. The rats were sacrificed 2 hours after the colchicine injection and spermatagonial and bone marrow cells were extracted, fixed in Carnoy's Solution, and flame-dried onto slides. Fifty cells from each tissue were examined from each animal that produced analyzable cells. Cytogenetic analysis was performed with the scorer unaware of the dosage levels or group assignments. A dose-related increase in the incidence of aberrant cells was evident in both cell types. These data indicate DBCP is a clastogenic agent in the rat.


Short-term tests utilizing bacteria and hepatic microsomal activating enzymes are widely used to determine the mutagenicity of xenobiotics. Because little is known concerning the disposition of test compounds under conditions of these procedures, we have examined the disposition of \(^{3}H\)-benzo(a) pyrene (BP, 80nM) in a suspension containing \textit{S. typhimurium}. TA98 (10^9 ml^{-1}) and cholate-solubilized rat hepatic microsomes (0.5, 1.0, and 2.5 mg ml^{-1}). After incubation for 30, 60 or 120 min., bacteria and medium were analyzed for BP and metabolites by HPLC. Under the conditions used, BP was metabolized to 8 major products, including 3 dihydrodiols, 3 quinones and 2 phenols. Each of these metabolites were found to be associated with the bacteria; the amounts of each metabolite increased with an increase in both incubation time and protein conc. and reflected an increase in the amounts present in the medium. The amount of BP associated with the bacteria increased and that remaining in the medium decreased in proportion to length of incubation. These data indicate that the amounts of BP metabolites associated with the bacteria are proportional to the amounts formed in the medium.
93. MUTAGENICITY OF HALOGENATED AND OXYGENATED 3-CARBON COMPOUNDS. S.J. Stolzenberg and C.H. Hine, Dept. of Pharmacology, Toxicology Activity, University of California, School of Medicine, San Francisco, CA 94143

A series of three-carbon compounds, known for their antifertility activities in males, and structurally related analogs, were tested against three strains of Salmonella typhimurium (TA1535, TA100 and TA98). Compounds active against the strains sensitive to base-pair substitution were: technical and purified DBCP, α-chlorohydrin, α-bromohydrin, epichlorohydrin, epibromohydrin and glycidol. S-9 addition was required for activation of DBCP whereas epichlorohydrin and epibromohydrin were less active in the presence of S-9. The bromine analogs were more active than the respective chlorine analogs. Five of the seven compounds showed low level activity against the frame-shift tester strain; α-bromohydrin and glycidol had no activity at the concentrations tested.

94. MUTAGENICITY STUDIES OF FLAVONOIDS IN VIVO AND IN VITRO. J.T. MacGregor, USDA, Western Regional Research Center, Berkeley, CA.

Quercetin and certain other flavonols occurring naturally in the human diet are mutagenic in Salmonella typhimurium. In mice, quercetin, neoheesperidin dihydrochalcone (NDHC, a new sweetening agent derived from a flavonoid in citrus peels), and hesperetin dihydrochalcone did not increase the normal frequency of micronucleated polychromatic erythrocytes in bone marrow at doses up to 1 g/kg, p.o. The same result was obtained with quercetin, i.p. Rat urine was mutagenic to Salmonella strains TA98 and TA100 after doses of 1 g/kg quercetin, i.p. or p.o., demonstrating that derivatives mutagenic to Salmonella are present in vivo under dosage conditions comparable to those employed in the mouse study. In the B. subtilis multi-gene (forward mutation) sporulation test, quercetin was nonmutagenic in strain 168 both with and without S-9 and in strain hcr-9 (hcr-) with S-9. NDHC is itself nonmutagenic in Salmonella strains TA100, TA1535, TA98, TA1538, TA1536 and TA1537. At levels of 500-5000 µg/plate, NDHC enhances (2-5 fold) the mutagenicity of 2-amino-fluorene (in TA1538, TA98) and of MNG (in TA1535, TA100), but not that of quercetin (in TA98) nor of 4-nitroquinoline-N-oxide (in TA100, TA98).

The mutagenesis of NF, NOF and NHOF was investigated in the Salmonella test system for a bacterial tester strain containing nitroreductase (TA 100) and for the same strain lacking nitroreductase (TA 100 FR). The standard plate assay of Ames et al. (Mut. Res. 31, 347, 1975) was used with the exception that 9,000xg supernatant of rat liver was omitted. Results are taken from linear portions of dose-response curves. Methylmethane sulfonate was a positive mutagenic control (500 revertants/1 µg) for both TA 100 and TA 100 FR. Nitroreduction is required for the mutagenic effects of NF (TA 100 = 850 revs/10 µg; TA 100 FR = 50 revs/10 µg) and of NOF (TA 100 = 80 revs/0.2 µg; TA 100 FR = 0 revs/0.2 µg). However, NHOF is mutagenic to the same extent in both TA 100 (450 revs/2 µg) and TA 100 FR (465 revs/2 µg). Thus NHOF does not require activation by the reductase. Glutathione (0.5 mM) or cysteamine (7 mM) but not methionine (2.7 mM) or DNA (0.6 mg/ml) completely blocked the mutagenesis observed with NHOF. Thus sulfhydryl compounds inactivate the mutagenic species.

96. THE MUTAGENIC PROPERTY OF CHAMUVARITIN, A CHEMICAL ISOLATED FROM AN AFRICAN MEDICINAL PLANT. A.O. Uwaifo, D.A. Okorie, and E.A. Bababunmi, Depts of Biochemistry and Chemistry, Univ. of Ibadan, Ibadan, NIGERIA.

Some plants which are used as native medicines in West Africa contain substances which possess effective therapeutic properties but are poisonous when they are ingested in large quantities. The presence of certain hepatocarcinogens in some tropical plants has previously been reported (IARC Monographs 10, 1976; J. Toxicol. Environ. Hlth. 4, 691-699, 1978). Mutagenic effects of chamuvaritin, a dihydrobenzylchalcone which was isolated from the medicinal plant (Uvaria chamae), have been observed on Salmonella typhimurium Ames tester strains TA 100 and TA 1538. The mutagenic action of the phytochemical required activation by the hepatic S-9 micronomal enzyme fraction.
97. THE MUTAGENICITY OF PALMOTOXIN B_0 RELATIVE TO AFLATOXINS B_1 AND M_1. A.O. Uwaifo, G.O. Emerole, O. Basir, and E.A. Bababunni, Dept. of Biochemistry, Univ. of Ibadan, Ibadan NIGERIA.

The mycotoxins, palmotoxin B_0 and G_0 are quite lethal to chick embryos. Palmotoxin B_0 (PB_0) is as toxic as aflatoxin B_1 (AFB) or aflatoxin M_1 (AFM) in experimental animals (J. Pathol. 102, 49-51, 1970; Xenobiotica 5, 649-655, 1975). The structures of the palmotoxins could be heterocyclic and are probably similar to those of the aflatoxins (J. Agr. Food Chem. 25, 1218-1219, 1977). The mutagenic potency of AFB relative to that of AFM is 3.3% using Ames tester strain TA 98 (Proc. Natl. Acad. Sci. (U.S.A.) 73, 2241-2244, 1976).

Our present results confirm that AFM is very positively mutagenic in S9 mix mediated system for three bacterial tester strains, in the increasing order TA 1538, TA 98 and TA 100. The prediction of the relative carcinogenicity of AFM to AFB posed by the mutation of TA 100 is probably more authentic than that by TA 98. The extent of mutation caused by PB_0 is much less than those induced by either AFB or AFM. The authors are grateful to Dr. Ames for the gift of the bacterial tester strains.


The mutagenicity of oxidative hair dye components has been studied in several test systems, but the mutagenic potential of the products to which they are oxidized in the dyeing process has not been investigated. In our study five products of p-phenylenediamine (PDA) were separated and isolated by thin-layer chromatography and tested for mutagenicity by Ames assays. The products were Randowski's base (BB), 2-(4'-aminoaniline)-5-hydroxy-1,4-quinonedimine (HQD), 4,4'-azodiylaniline (ADA), p-nitroaniline (NA), and p-dinitrobenzene (DNB). These compounds induced frameshift mutations in Salmonella TA 1538 and TA 98. In TA 1538, BB was the most mutagenic and NA the least. ADA and DNB also caused mutations in TA 100. HQD acted directly while the others required metabolic activation. The increased mutagenicity of the oxidation products over the parent compound was also observed when PDA was oxidized with hydrogen peroxide in the presence of m-aminophenylamine such as 2,4-toluenediamine and 2,4-diaminoanisole. These results indicate that the oxidation of aromatic amines can increase the mutagenicity of oxidative hair dyes.
99. Enzymatic alteration of the mutagenicity of extracts from human fecal samples. James E. Aswell, Marion Ehrich, and Tracy D. Wilkins, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia (Sponsor: S. D. Cohen).

Previous studies in our laboratory and others have demonstrated the presence of mutagens in ether extracts from human fecal samples which induce the reversion of the histidine requirement of Salmonella typhimurium tester strain TA-100. In studying properties of these mutagens we have examined the possibility that they may be enzymatically altered by mammalian and bacterial enzyme systems. When the mammalian microsomal enzyme system (S/9 mix) from rat livers induced with Archlor 1254 was included in the mutagenicity test there was complete inhibition of 70% of 27 mutagenic samples. An S/9 mix prepared from control rat livers was not as inhibitory, reducing mutagenic activity of 7 of 9 mutagenic samples tested by an average of 54%. An enzyme system prepared from the 30,000 x g supernatant of a lysate of Bacteroides fragilis was capable, however, of increasing the mutagenicity of 5 of 10 samples, with the average increase being 165%. These results indicate that fecal mutagens may be enzymatically altered, either in mammalian or bacterial systems, and capacity for alteration may be of importance in the determination of risk for colon cancer in individuals excreting fecal mutagens. (Sponsored by NCI grant number 1 R26 CA 23857-01 and NCI contract number NO1 CP 55685).

100. THE EFFECTS OF ETHANOL ON HUMAN PSYCHOMOTOR PERFORMANCE: I CALIBRATION OF A BEHAVIORAL TOXICOLOGY TEST BATTERY.

This study evaluated the ability of a new behavioral test battery to detect the CNS depressant effect produced by ethanol. Auditory and visual reaction times, eye-hand coordination, stability while standing and estimation of the speed of a moving target were quantitatively determined. Nine adult male volunteers received three alcohol doses and a placebo drink. Blood alcohol concentrations (BACs) determined periodically after drinking were divided into three ranges for statistical analysis: <.01, .01-.03 and >.03%. Analysis of the data revealed that visual reaction times were significantly longer when BACs were >.03 compared to control (p <.05) and that the dose-response relationship was linear. Statistically significant shifts in balance while standing were observed when BACs were >.03%. Also, estimation of the speed of the moving light was significantly altered at the highest BACs tested. Eye-hand coordination was not affected. The results of this study demonstrate the sensitivity of three of the four tests and indicate the potential utility of these measures for detecting subtle CNS changes produced by other chemicals.

Treatment of dams during 21 days of gestation (G) or gestation and lactation (GL) with oral doses of 0, 1, 5 or 10 mg/kg of Δ⁹-THC induced protein and nucleic acid decreases in neonate brain at the two high doses (Nat. Meet. Fr. Soc. Toxic. 1978). As part of the same study to assess neurotoxicity of Δ⁹-THC, glucose and lactate levels were also measured. The findings (P<.01) were: G treatment caused 12-20% lactate decline at 5 and 10 mg/kg in less than one day old pups. Seven days old sucklings from dams treated during G had 13-19% drop in glucose at the two high doses whereas lactate levels fell by 18-20% at all doses tested. GL induced up to 18% decline in both the parameters at all the dose levels in 7 days old offsprings. Therefore, it appears that Δ⁹-THC affects carbohydrate metabolism in neonate brain. Decrease in glucose may occur as a result of increased rate of utilization and/or inhibition of its access to the brain. Fall in lactate could be related to its use as one of the oxidisable substrates to maintain brain metabolic activity.


The behavioral effects of orally administered d-amphetamine and paraquat were evaluated in rats by using an un-signalled single spatial alternation (SSA) procedure which allows assessment of both speed and accuracy of responding. SSA requires non-food deprived animals to obtain a 45mg sucrose pellet by alternating responses between two retractable levers presented every 40sec on a discrete trial basis. Rats were trained until a criterion of at least 70% accuracy in each daily session was attained. Low doses (0.5-10mg/kg) of d-amphetamine were administered orally for 2hr pre-session via drinking water. Dopamine dependent decreases in response speed (increases in latency) were found, but no significant effects on accuracy were seen. Response latencies returned to pre-drug values within two sessions after drug access. The effects of acute access to paraquat were also studied. Results from the d-amphetamine study indicate that this procedure provide toxicologically relevant, sensitive, and stable behavior for assessing the effects of drugs and environmental agents.

Since cocaine is used as a stimulant medication in racing horses studies to determine the relationship between plasma levels and the behavioral effects of cocaine in horses were performed. After rapid IV injection of cocaine (0.75 mg/kg), plasma levels peaked at 950 ng/ml, and fell biphasically, with an alpha phase half-life of 4.5 minutes, and an apparent beta phase half-life of 45 minutes. After IM injection (3.0 µg/kg) plasma levels reached 700 ng/ml at 30 minutes post dosing, and then declined with a t 1/2 of about 40 minutes.

Two methods were used to study the behavioral response to cocaine: spontaneous locomotor activity, using a step counting (S.C.) method and operant conditioning using a variable interval reinforcement schedule (V.I.). With the S.C. method large doses of cocaine were needed to stimulate locomotor activity. In contrast the V.I. method was sensitive to low doses, and allows accurate determination of the behavioral response to low plasma levels of cocaine.

Supported by the KY Equine Drug Research Fund.

104. NECROSIS PRODUCED IN FETAL MOUSE BRAIN BY TRANSPLACENTAL EXPOSURE TO THE MYCOTOXIN, OCHRATOXIN A. G.M. Szczep and R.D. Hood, The UpJohn Co., Kalamazoo, MI and The University of Alabama, Tuscaloosa, AL.

While studying teratogenic potential of ochratoxin A in CD-1 mice, we discovered that certain doses cause necrosis of cells in fetal cerebrum. Pure crystalline ochratoxin A was dissolved in 0.1 N NaHCO₃ and given to groups of 10 pregnant mice by i.p. injection at doses ranging from 2 to 5 mg/kg. Ochratoxin A was given for 1 to 3 days during organogenesis and the fetuses were removed on the 18th day of gestation (I-insemination). Parasagittal sections through fetal bodies fixed in formalin and transverse sections through fetal eyes and cerebriums were examined by light microscopy. There were no lesions in organs regarded as target organs (kidney, liver, lymphoid tissue). There were many necrotic cells in cerebriums in fetuses from dams given 3 or 4 mg/kg ochratoxin A on days 15-17. They were most numerous adjacent to ventricles. There was no necrosis in fetuses from dams given ochratoxin A on days 8-10 when it is teratogenic or in fetuses from dams given a single dose on day 17 or 18. Necrosis was reproduced in fetuses from ICR mice given 5 mg/kg per os on days 15-17. This lesion could be associated with behavioral deficits or neurooncogenesis.
105. PROTECTIVE EFFECTS OF ATROPINE AND/OR MORPHINE AGAINST THE LETHALITY DUE TO DL-MUSCARINE AND FURAN ANALOGS OF MUSCARINE

It has been postulated that a bulk release of Ach and blockade of multiple cholinergic synapses is the cause of death due to furan analogs of muscarine (Toxicol. Appl. Pharmacol. 43: 73-83, 1978). Endogenous morphine-like compounds are known to decrease the release of neurotransmitters at synapses. Therefore, we used different pretreatment regimens involving morphine and atropine to prevent the lethality of muscarine and its furan analogs. These studies lead us to classify these compounds into 3 groups: 1) DL-muscarine acts at post synaptic receptors, and atropine prevents both respiratory depression and death; 2) Fur-methide and 5-methylfurumethide seem to act presynaptically to increase Ach release, and their lethality can be prevented by a combination of atropine and morphine; 3) 5-Hydroxymethylfurumethide, 5-methylthyltrimethylammonium, tetrahydrofurumethide and 5-methoxyfurumethide may release endogenous morphine-like substances to decrease the release of Ach and/or other substances, and their lethality was not prevented by any of these regimens. (Supported by King Abdulaziz Univ., Saudi Arabia and USPHS Grants NS-04699 and 14989.)

106. THE BEAGLE DOG AS AN INDICATOR OF EXPERIMENTALLY-INDUCED RETINOPATHY
U. Schaeppi, Ciba-Geigy Limited, CH-4002 Basle, Switzerland

The dog electroretinogram (ERG) was recorded with the technique of Ganzfeld stimulation (Schaeppi U. and Liverani F., Agents and Actions 7, 347-351, 1977).

Dogs treated with known retinotoxic agents exhibited treatment related ERG changes demonstrating that the dog was a sensitive indicator for treatment induced retinopathy.

The Beagle dog model has the following limitations: While the function of the rod system in the Beagle is similar to that in man, the cone system has 1/30 only of man's sensitivity. The Beagle dog displaying signs of a relative cone dysfunction is therefore of limited value for the evaluation lesions of the cone system.

The occurrence of progressive canine retinal atrophy which also may occur in Beagle colonies demands that dogs are observed for retinal function prior to initiation of testing.
107. Studies on Hexane 2,5-dione Neurotoxicity: No Effect on Microtubular Properties. J.P. Nachtmann and D. Courti. Ohio State University Toxicology, Columbus, OH.

Hexane 2,5-dione (HDO) is a common metabolite of n-hexane and 2-hexanone. HDO has been shown to produce a peripheral neuropathy indistinguishable from either parent compound. Rats were maintained on drinking water containing 0.5%, 0.1% or 0% HDO. Clinical signs of neuropathy are hind limb weakness and loss of body weight. Microscopic observations of sciatic nerves from treated animals showed swelling with neurofilaments, loss of myelin and intact microtubules (MT). Because other MT associated properties are HDO sensitive (Axoplasmic flow), studies were undertaken to detect altered MT function. 3H-colchicine binding activity of brain and sciatic nerve was used as a marker for tubulin since this parallels MT function. Monomeric and polymeric forms were separated by centrifugation to determine HDO induced changes in equilibrium. Half life of 3H-colchicine binding was also measured. Neither property showed any change through the treatment groups. Experiments in membrane microviscosity indicate that HDO treated nerves have a lower viscosity than untreated nerves and that the decrease is dose related.

108. STUDIES ON THE MECHANISM OF ACRYLONITRILE NEUROTOXICITY. M. Abreu and A.E. Ahmed, Dept. of Pathol., Univ. of Texas Medical Branch, Galveston, TX. Sponsor: W. Decker.

Two phases of acute toxicity are observed in fasted rats given an oral LD50 of acrylonitrile (VCN) (90 mg/kg). The first phase is cholinomimetic in nature and the second phase appears to be CNS dysfunction. In order to test whether the first phase is mediated by the reactive VCN molecule while the second is due to its capacity to metabolically liberate cyanide, we examined pretreatments which alter cholinergic or metabolic activities. Aroclor 1254 pretreatment abolished the first phase but accelerated and intensified the second, and increased cyanide levels in blood 3 fold. CoCl2 enhanced the cholinomimetic effect of VCN and blocked the second phase. Phenobarbital (PBT) pretreatment protected against both phases of toxicity. Neither CoCl2 nor PBT pretreatment altered cyanide liberation from VCN. VCN lowered catecholamine levels and cytochrome oxidase activity. Atropine pretreatment blocked the first phase only while atropine plus Na2SO3 protected against both phases of toxicity. These findings suggest that the cholinomimetic phase of VCN toxicity could be mediated by the entire molecule while the CNS phase is a function of cyanide liberation.
109. THE STRUCTURE-ACTIVITY RELATIONSHIP OF ALIPHATIC DIKETONES AND THEIR POTENTIAL NEUROTOXICITY. J.L. O'Donoghue and W.J. Krasavage, Health, Safety, and Human Factors Laboratory, Eastman Kodak Company, Rochester, NY

The specific structural properties that allow 2,5-hexanedione, a metabolic derivative of n-hexane and methyl n-butyl ketone, to interact with neural tissues are unknown. To examine this problem, male rats were administered either 2,5-heptanedione or 2,6-heptanedione five days per week by gavage. 2,6-Heptanedione given in ascending doses from 500 to 1000 mg/kg/day for 13 weeks did not produce clinical or neuropathological evidence of neurotoxicity. 2,5-Heptanedione at doses of 1000 and 2000 mg/kg/day produced clinical signs of neuropathy and neuropathological alterations identical to those produced by 2,5-hexanedione. Thus the specific-structural properties of aliphatic diketones which are necessary for neurotoxicity do not include a six-carbon chain or molecular symmetry but do require a γ-diketone.

110. COMPARATIVE NEUROTOXICITY AND METABOLISM OF ETHYL BUTYL KETONE AND METHYL N-BUTYL KETONE OF RATS. G.V. Katz, J.L. O'Donoghue, G.D. DiVincenzo and C.J. Terhaar, Health, Safety, and Human Factors Laboratory, Eastman Kodak Company, Rochester, NY

Methyl n-butyl ketone (MnBK) and n-hexane are metabolized by oxidation of their subterminal carbons leading to the production of 2,5-hexanedione (2,5Hxdn). Each oxidative step increases the neurotoxic potential so that 2,5Hxdn is the most active compound in the metabolic series. The most likely 7-carbon compound commercially available that would produce a metabolite analogue to give a diketone [2,5-heptanedione (2,5Hpdn)] analogous to 2,5Hxdn is ethyl n-butyl ketone (EBK). Rats were exposed to 700 ppm MnBK by inhalation for 11 weeks or EBK for 24 weeks or twice the exposure period needed to produce a severe clinically evident neuropathy with MnBK. No clinical or neuropathological evidence of neurotoxicity was evident after EBK exposure. These findings appear to be due to the low serum concentrations of 2,5Hpdn (6.8 ± 4.0 µg/ml) formed from EBK in comparison to the much higher concentrations of 2,5Hxdn (133.2 ± 36.7 µg/ml) formed from MnBK. This argument is strengthened by the fact that in a companion study, 2,5Hpdn at much higher levels was found to produce the same type of neurotoxicity as seen with 2,5Hxdn.
111. SIMULATION STUDIES OF BLOOD CARBOXYHEMOGLOBIN LEVELS ASSOCIATED WITH INHALATION EXPOSURE TO METHYLENE CHLORIDE. C.L. Hake, Dept. of Env. Med., Med. Coll. of Wisc., Milwaukee, WI.

This study was undertaken to obtain the computer-predicted blood carboxyhemoglobin levels that will be attained when a physically active worker is exposed to the vapors of methylene chloride (dichloromethane). Existing programs were adapted to include the uptake by hemoglobin of the carbon monoxide metabolite derived from methylene chloride and its subsequent release into the exhaled breath. Simulated values of methylene chloride in alveolar breath and blood carboxyhemoglobin were first compared with experimental values obtained from sedentary humans previously exposed in our laboratory. When increased ventilation and cardiac output values consonant with exercise were applied to the simulated exposures at 50, 100, 250 and 500 ppm for 3 and 7.5 hr, blood carboxyhemoglobin levels did not increase in proportion to uptake. At the highest exposure level of 500 ppm, simulated carboxyhemoglobin values were higher without exercise than with exercise, while at 100 ppm they were almost equal. These studies indicate that physical work will not have the same effect of greatly increasing blood carboxyhemoglobin levels during methylene chloride exposure as occurs during exposure to carbon monoxide.


Adaptation to high altitude hypoxia has been well-documented but mechanisms that could lead to an increased tolerance for CO-induced hypoxia have not yet been studied. Sprague Dawley rats weighing 175 g were exposed to 450 ppm CO for 6 hr/day, 5 days/week for 5 weeks. Each exposure resulted in a final carboxyhemoglobin level of 28 ± 2%. The hematopoietic reaction to repeated hypoxia stimulus was observed in reticulocyte count (Ret) at the end of the first exposure day (4.4% vs 6.6% CO-exposed) and peaked during the first week. In spite of the continued intermittent hypoxic stimulus and net deficit in hemoglobin (Hb) and hematocrit (Hct) no Ret response was observed in the remaining 3 weeks indicating that the reactivity of the organism to hypoxia had changed. Bone marrow stimulation resulted in an increased Hb and Hct in the exposed group during the third week, and persisted for the rest of the experiment (16.2±0.3 g/100 ml and 48.8±0.9% vs 18.2±0.3 g/100 ml and 53.4±0.7% CO-exposed). By the end of the 5th week signs of cardiac hypertrophy were evident in the heart (1.87±0.03 and 0.53±0.01 g/kgBW vs 2.16±0.05 and 0.63±0.01 g/kgBW for the left and right ventricles, resp.). The results indicate that as in high altitude, repeated intermittent CO exposure results in increased tolerance to partial hypoxia.
113. ACUTE INHALATION TOXICITY OF PYROLYSIS PRODUCTS OF POLYMERIC CABLES, R.R. Raje, Arnold & Marie Schwartz College of Pharmacy and Health Science, L.I.U., Bklyn, NY. Sponsor: W.H. Lawrence

The purpose of this investigation was to determine relative acute inhalation toxicity of different polymeric cable materials pyrolyzed at 200, 400, 600 and 900°C. Five male and five female SD rats were exposed to pyrolysis products for one hour and chamber atmosphere analyzed for toxic gases by means of colorimetric tubes. Behavioral symptoms were recorded during exposure and animals were ultimately autopsied. LD₅₀ values were calculated for chamber, 24 hr, 48 hr and 14 day mortality. To date, seven materials have been investigated. Halar, a fluoropolymer has been found to be the most toxic with significantly low LD₅₀ values at 600 and 900°C. It also produced severe lung damage and caused hematemesis in the animals surviving exposure. Ethylene propylene rubber was found to be the least toxic material tested.

(Supported by Grant from NYC Transit Authority)

114. PULMONARY MECHANICS IN UNANESTHETIZED GUINEA PIGS AFTER ACUTE EXPOSURE TO PLASTICS COMBUSTION PRODUCTS. W. Skornik, R. Heimann and R. L. Jaeger, Harvard School of Public Health, Boston, MA 02115

The effects of the combustion products of polyvinyl chloride (PVC) on pulmonary resistance (R) and compliance (C) were studied in awake male guinea pigs (300-350 g). Breath to breath measurements of C and R were calculated by a digital computer using a zero-crossing algorithm from simultaneous measurements of intra-esophageal pressure and tidal volume. This non-surgical technique permits measurements to be made during and after acute inhalation exposure. Groups of 3 guinea pigs were exposed to the combustion products of pure or technical PVC. Particle size distribution was determined by laser interferometry. Acute exposure to the thermal decomposition products of 0.75 g technical PVC resulted in an immediate fall in C from 0.5 to 0.2 ml/cm H2O and a concurrent increase in R from 0.3 to 2.0 cm H2O/ml/sec. At 24 hr, C decreased by more than 90% and the animals were in severe respiratory difficulty. The method described is a useful non-destructive test method which may be used in the prospective assessment of chemically-induced chronic pulmonary disease. Supported by NHLBI SCOR. RJJ is an NIEHS ROA recipient.
115. BIOCHEMICAL CHANGES IN GUINEA PIG LUNGS FOLLOWING EXPOSURE TO PLASTICS COMBUSTION PRODUCTS, PARAQUAT, OR ANTU R. Heimann, W. Skornik, and R.L. Jaeger, Harvard School of Public Health, Boston, MA 02115

The effects of polyvinyl chloride (PVC) or polystyrene (PS) combustion products on pulmonary aminopyrine demethylase (AD) and aniline hydroxylase (AH) were measured at 24 and 48 hr. Lung and liver to body weight ratio (L/R) and LI/BW and lung non-protein sulfhydryl (NPSH) were determined. Significant increases in L/R ratios were found after exposure to 0.5 and 0.75g of technical PVC at 24 hr (24% and 35% respectively). A 25% increase in L/R occurred at 24 hr. AD and AH activities were highly variable. Lung NPSH levels did not change. PS caused an increase in L/R ratio at 24 hr only. No consistent changes in MPO, NPSH or L/R were seen. Paraquat (15 mg/kg, ip) resulted in no change in L/R at 1 or 4 hr. AD and AH were unchanged at 1 hr; AH decreased by 22% at 4 hr and 71% at 24 hr. At 24 hr AP was 23% of control; NPSH level did not change. After ANTU (140 mg/kg, ip) AP activity increased at 4 hr but decreased to 51% at 24 hr. AH activity was 78% at 4 hr and 53% at 24 hr. No changes in L/R ratio or lung NPSH occurred. Supported by NHLBI SCOR. RJJ is an NIEHS RECA recipient.

116. ACCUMULATION OF PARAQUAT AND DIQUAT BY CULTURED TYPE II PNEUMOCYTES. K. Saito, W.B. Parker, and D.B. Menzel. Departments of Pharmacology and of Medicine, Duke University Medical Center, Durham, N.C. 27710.

The accumulation of 14C-paraquat and 14C-diquat by three types of lung cells was studied. L-2 cells (adult rat lung cells) accumulated paraquat (1 mM extracellular concentration in Tyrode's solution at 37°C) with time to a maximum of 14.3 mmol/mg protein by 60 minutes. Diquat was accumulated slowly but reached a concentration of 7.2 mmol/mg protein by 30 minutes. AKD cells (feline embryonic lung cells) accumulated a maximum of 6.8 mmol paraquat/mg protein by 30 minutes, but did not accumulate further paraquat on incubation up to 2 hours. A-549 cells (human lung carcinoma cells) also reached maximum paraquat accumulation by 30 minutes of 4.0 mmol/mg protein. Both AK-D and A-549 cells accumulated less diquat. Cell viability was unaffected by up to 2 hours incubation with either paraquat or diquat. The site of cellular accumulation of paraquat by the lung may be the Type II cell. Species differences in the ability of Type II cells to accumulate paraquat and diquat may exist. Cultured Type II cells may be particularly useful in the study of lung toxicants. (Supported by EPA Contract 68-02-2436 and by NIH Grant HL16264.)
117. THE EFFECT OF HIGH CONCENTRATIONS OF OXYGEN ON THE TOXICITY OF PARAQUAT AND DIQUAT IN RATS.  I S Pratt, P L Keeling & L L Smith, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire, UK.  Sponsor: M S Rose

It has previously been shown that high concentrations of O₂ enhance the toxicity of paraquat. We have examined the effect of paraquat (2.5 mgs/kg and 20 mgs/kg given sc) on the mortality of rats left in ambient air or placed in 85% O₂. The lethality of paraquat was found to be enhanced approximately ten-fold in 85% O₂. In contrast the lethality of diquat (10 mgs/kg and 20 mgs/kg given sc) was enhanced only two-fold in 85% O₂. The pathology in the lungs of the treated rats 24 hours after dosing with paraquat reflected the mortality data. The primary target cells following 20 mgs/kg in air are the type I and type II alveolar epithelial cells whereas with 2.5 mgs/kg in 85% O₂ the type II cell is the cell type primarily damaged although there is some effect on type I and endothelial cells. Diquat 20 mgs/kg in air produces only very minimal cellular damage as does 10 mgs/kg in 85% O₂ whereas 20 mgs/kg in 85% O₂ more markedly damages type II cells. The importance of lung damage in the death of diquat treated rats in 85% O₂ is still unclear.

118. PERACUTE TOXICITY OF PARAQUAT AND DIQUAT IN 100% OXYGEN.
J. P. Keffer*, Univ. of Tenn-Oak Ridge Graduate School of Biomedical Sci., W. Haschek and H. P. Witschi, Biology Division, ORNL, P. O. Box Y, Oak Ridge, TN 37830

While the enhancement of paraquat toxicity by oxygen has been studied, less is known about the effects of oxygen in acute diquat poisoning. Rats were injected i.v. with either paraquat or diquat and placed into an atmosphere of 100% O₂. The LT₅₀ for 5, 10, 20, 40 or 80 mg/kg of paraquat were 1360, 865, 666, 405 and 254 min. and for diquat 3040, 1132, 703, 98 and 87 min. Following a dose of 10 mg/kg of either paraquat or diquat the plasma concentrations and tissue distribution were the same in animals kept in air or in oxygen. Histopathologic studies showed that the most prominent feature in lungs exposed to oxygen and either herbicide was a severe and general perivascular oedema. Rats pretreated with endotoxin in a manner which makes them tolerant to hyperoxia exhibited decreased mortality to diquat treatment in oxygen. These data suggest that the primary site of the paraquat or diquat - oxygen interaction is the pulmonary endothelial cell. (*Postdoctoral Investigator supported by subcontract #3322 from the Biology Div. to the Univ of Tenn.) (Research supported by Division of Biomedical and Environmental Research, U. S. Department of Energy under Contract W-7405-eng-26 with the Union Carbide Corporation.)
119. **Oxygen Toxicity in the Rat: Correlation between age-dependent histochemical findings and enzyme induction.** J.B. Stevens and A.P. Autor. The Toxicology Center, Dept. of Pharmacology, The University of Iowa, Iowa City, IA 52242.

Neonatal rats, unlike their adult counterparts, have been shown to be inherently resistant to oxygen toxicity. These neonates have also been shown to be capable of rapidly inducing lung tissue superoxide dismutases (mitochondrial and cytosolic), catalase (Cat.) and glutathione peroxidase (G.P.), enzymes which presently are thought to be the cellular defense mechanisms against free radical damage associated with oxygen toxicity. An age-dependent differential induction of these four enzymes was found in neonates, as well as age-related differences in the extent of histologically observed damage following 72 hours exposure to hyperoxia. During the first 12 days after birth, each activity was increased in the lungs during the exposure period with no tissue damage observable upon microscopic examination. In animals 15 through 30 days of age only the lung Cat. and G.P. activities were elevated and focal damage was seen. In animals older than 35 days none of the activities were enhanced. These animals exhibited the same time course of tissue damage and death as did adult animals. The above temporal correlations support the free radical hypothesis of oxygen toxicity. (Supported by GM-12675 and the National Foundation-March of Dimes).


We studied the effect of preexposure to ozone on the respiratory response to histamine. We used the Amdur-Mead technique to measure respiratory mechanics in Charles River Hartley guinea pigs. Following s.c. injection of histamine phosphate at 0.06, 0.125 or 0.25 mg/kg, respiratory measurements were made at 2-min intervals for 16 min by which time resistance and compliance had returned to control values. The respiratory response produced by histamine was dose-related. The increase in resistance was 34, 50 and 100% above control; the decrease in compliance was 19, 31 and 67% below control. Groups of animals were exposed for 1 hr to ozone generated with an electric arc and monitored with a chemiluminescent ozone meter. Control measurements were made 2 hr after exposure and the response to histamine was measured. Although control values did not differ from those of unexposed animals preexposure to 0.8 ppm ozone caused resistance increases of 61, 97 and 132% above controls following histamine. Preexposure to 0.4 ppm ozone produced a 72% increase in resistance in response to injection of 0.225 mg/kg histamine. Lingering sensitivity to bronchoconstrictive agents is a subtle but important manifestation of ozone toxicity.
121. SMOKE EXPOSURE IN RODENTS: RESPONSES OF HEPATIC, RENAL AND PULMONARY ARYL HYDROCARBON HYDROXYLASE IN MALES AND FEMALES OF THREE SPECIES. M.H. Bilimoria and D.J. Ecobichon, Pathology Institute and Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada.

Inhalation of tobacco smoke by various rodent species results in enhanced tissue aryl hydrocarbon hydroxylase (AHH) activity. Using the B.-A.T. inhalation system, which releases a precisely calibrated dilution of tobacco smoke into a chamber, male Sprague-Dawley rats were exposed to a range of smoke concentrations for varying periods of time, and renal AHH activity measured in 9,000 g supernatants of tissue homogenates. Induction of activity was dependent upon concentration and duration of exposure. Male and female rats, guinea pigs and hamsters, were exposed to the optimum smoke concentration found to induce rat renal AHH, and enzyme activities in lung, liver, and kidney were measured. Marked tissue differences in basal AHH were observed between sexes and species. Induction, up to 6-fold was observed only in lung and kidney of male and female rats. AHH was induced only in guinea pig kidney. In hamsters, lung AHH was induced in both sexes, but only liver AHH in males. Comparative studies in a number of species will be necessary for the toxicological assessment of such a complex material as tobacco smoke.

122. SENSORY IRRITATION TOLERANCE DEVELOPMENT TO CHLORINE IN RATS FOLLOWING REPEATED INHALATION. C.S. Barlow, W. Steinhaagen, and M. Phelps, Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Sponsor: J.E. Gibson

The perception of upper respiratory tract irritation (sensory irritation) caused by an airborne chemical is an important respiratory tract defense mechanism. This response can be quantitated in animals by measuring the percent decrease in respiratory rate. The purpose of this investigation was to study sensory irritation tolerance development resulting from repeated Cl₂ exposure. Male, F-344 rats were exposed to a time-weighted average of 9.8 ppm Cl₂, 6 hr/day, 5 days/week for 2 weeks. One day following the final Cl₂ exposure, groups of these rats were exposed for 10 minutes to various Cl₂ concentrations and respiratory rate decrease was measured. A concentration-response curve was obtained and compared to that for non-pretreated exposed (naïve) rats. The RD₅₀ values (concentration to elicit a 50% decrease in respiratory rate) were 454 ppm in the exposed rats versus 23.3 ppm in the naïve rats, a factor of 17.9. These results demonstrate that rats repeatedly exposed to 9.8 ppm Cl₂ became tolerant to the sensory irritation properties of chlorine. Tolerance development to sensory irritation is important because an organism’s ability to detect the presence of lung-damaging concentrations of irritants may be compromised.
123. LUNG SULFHYDRL CONTENT IN RATS FOLLOWING CHLORINE INHALATION. D.E. Dodd, J.S. Bus, and C.S. Barrow, Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Sponsor: J.E. Gibson

Chlorine's (Cl₂) pulmonary irritancy may be associated with oxidation of tissue sulfhydryl (-SH) levels. The purpose of this investigation was to examine the effect of Cl₂ on lung -SH content and the enzymes glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase (GSSG-Red) which maintain -SH levels. Male, Fischer-344 rats were exposed to 12.9 ppm Cl₂ (time-weighted average), 6 hr/day for 1, 5 or 10 days. Following exposure, rats were sacrificed immediately or after a 5-day recovery period. Total -SH, non-protein -SH, G6PD activity and GSSG-Red activity in rats sacrificed immediately after 1, 5 or 10 days of exposure did not differ from control. However, total -SH levels increased 40 and 48% above control in rats given a recovery period after 5 or 10 days exposure, respectively. Upon recovery from 5 or 10 days exposure, G6PD activity rose 40 and 66%, respectively. GSSG-Red activity increased 37% following recovery after a 10-day exposure. In conclusion, oxidation of pulmonary -SH levels immediately following Cl₂ exposure was not observed. An increase in -SH content and enzyme activities upon a 5-day recovery period may reflect reparative processes subsequent to Cl₂-induced lung damage.


There is controversy as to the occurrence, nature and source of non-ionic fluoride fractions in blood of several species including man. In the present studies, the F⁻ ion electrode was used to measure fluoride in ashed (total F⁻) and unashed (ionic F⁻) plasma of rats 6 hours after a 6 hour inhalation exposure to HF. Total plasma F⁻ concentration consistently exceeded ionic F⁻ concentration indicating the presence of fluoride which did not respond to the F⁻ electrode. We term this fluoride the ΔF fraction, whose concentration was obtained by subtracting ionic F⁻ from total F⁻ concentration. Both total F⁻ and ΔF concentrations in plasma increased in a dose-dependent manner with increasing airborne HF levels. The ΔF could be separated from ionic F⁻ by the calcium phosphate adsorption technique indicating that the fluoride in this fraction was non-ionizable. Sephadex G-25 gel chromatography suggested that the molecular weight of ΔF was less than 5000 daltons. The ΔF fraction appears, therefore, to consist of low molecular weight non-ionizable metabolites(s) of HF. The non-ionic fluoride fractions reported in blood of other species may be similar to ΔF.
125. THE EFFECT OF SO₂ ON THE UPPER RESPIRATORY TRACT OF RATS.

Rats were exposed to 200 ppm SO₂ for 5 hrs. 5 days weekly for a maximum of 8 weeks. Histopathological examination showed that with increasing exposure the airways undergo changes which progress through deciliation, goblet cell hyperplasia to squamous metaplasia. Tracheas removed 2, 4 and 8 weeks after exposure were incubated in medium containing ³⁵SO₄⁻ and the incorporation of ³⁵S into the trachea and the non-dialysable portion of medium (macromolecules) was determined and compared with appropriate controls. Although ³⁵S was more effectively incorporated into the tracheas taken from SO₂ treated rats compared with controls there was a reduction in the transport of macromolecules containing ³⁵S from the trachea into the incubation medium. At 2, 4 & 8 weeks treated and control rats were dosed with ³⁵SO₄⁻ before killing. There was no difference in the ³⁵S incorporated into the tracheas of treated and control rats. However, there was a marked increase in the ³⁵S content in macromolecules found in tracheal washings. These results demonstrate that ³⁵S incorporation into the trachea and into macromolecules secreted by the trachea are dissimilar between in vivo and in vitro studies.


The administration of CP to rats results in the accumulation of AM-derived cells of variable sizes in the alveoli. This study was done to characterize the cells following separation into more homogenous populations. Cells were lavaged from the lungs of CP-treated rats (30 mg/kg, 4 wks, 5 days/wk) and fractionated by centrifugal elutriation. Four fractions (frxs) were collected and the percent of total cells in each was: frx (1)-28%, (2)-44%, (3)-21%, (4)-7%. The frxs contained cells of the following approximate mean volumes: frx (1)-340μ³, (2)-3000μ³, (3)-7200μ³, (4)-14,000μ³. Control AMs separated only into frxs (1)-440 μ³ and (2)-640μ³. The protein content of the CP-cells (mg/10⁷) were: frx (1)-1.8, (2)-3.7, (3)-6.8, (4)-14.5. Lysosomal enzyme activities increased with cell size, but because of the concomitant increase in cellular protein, the specific activities (act./mg protein) were not significantly different in the 4 frxs. Therefore, as the cells get larger, they are accompanied by an increase in protein and a proportional increase in lysosomal hydrolase activity. (Support: PMA Research Starter Grant).

Our laboratory has shown the efficacy of screening for acute lung injury by analysis of endobronchial lavage fluid in Syrian hamsters. To investigate what parameters might greatly affect control values and to determine if other laboratory animals could be used in a pulmonary toxicity screening program, we have examined the endobronchial lavage fluid and lung histopathology in young adults of 4 species (Syrian hamster, rat, guinea pig, and rabbit) and in older rats and hamsters. Values measured included dehydrogenases; phosphatases; proteases; anti-proteases; glutathione oxidase and reductase; b-glucuronidase; soluble collagen; soluble protein; sialic acid; and cell counts. Fluid and tissue from lungs lavaged both in vivo and after excision were examined. Baseline biochemical indicators of damage were lower in the fluid from excised lungs except in the hamster, which showed no difference in response to the two methods of lavage. If lavage is done in the excised lung, all the species examined are suitable for this method of screening for lung injury. (Research supported by DOE Contract EY-76-C-04-1013.)

128. REMOVAL OF Ni(II) FROM THE ISOLATED PERFUSED RAT LUNG IN THE PRESENCE OF SULFATE. S.J. Williams and D.B. Menzel. Departments of Pharmacology and of Medicine, Duke University Medical Center, Durham, N.C. 27710.

Ni(II) and sulfate ions occur in the respirable particles of polluted air and may exhibit mutually potentiated toxicity. Therefore, the absorption of $^{63}$Ni(II) by the isolated ventilated and perfused rat lung (IVPL) was studied in the absence and presence of sulfate ion. The IVPL was prepared as previously described (Charles and Menzel, Tox. Appl. Pharmacol. 41: 91-99, 1977). Venous effluent was collected at 1 minute intervals for 90 minutes. The kinetics of absorption of $^{63}$Ni(II) alone was determined following intratracheal instillation of 100 $\mu$g isotonic sucrose (pH 7.4) containing 1, 10, or 127 nmol $^{63}$NiCl$_2$. Removal was diffusionaly controlled, but rate was dependent on concentration. The effect of sulfate ion on the rate of nickel absorption was determined by adding 0.1 nmol Na$_2$SO$_4$ to 100 $\mu$g isotonic sucrose containing 10 nmol Ni(II). No alteration in the absorption rate of nickel was evident. These studies suggest that the interaction of Ni(II) with the lung is independent of the counterionic species. (Supported by EPA Contract 68-02-2436, NIH Grant GM07105, and a Grant from the N.C. Lung Association.)

Pharmacokinetic studies have shown that certain basic amines, e.g. imipramine, accumulate and persist in lung tissue. Using the rabbit IPL this laboratory has previously described a model for the uptake and persistence of imipramine in the lung (Eling, et al, Drug Metab. Disp. 3, 389-399, 1975). In this study we report on the effect of pre-exposure of rabbits to morpholine (250ppm, 6 hrs/day, for periods up to 8 weeks) on $^3$H-imipramine uptake and persistence in the IPL. A pronounced decrease was observed in the "steady-state" accumulation of imipramine in lungs from animals exposed to morpholine. This resulted from a decrease in the size of a pool believed to be responsible for the persistence of imipramine in the lung. This pool is, at least in part, associated with the alveolar macrophage. The decrease in the amount of imipramine associated with this pool was related to the length of exposure to morpholine. After five weeks of exposure, a 95% decrease in the pool size with respect to control was observed. This decrease may be related to the observed decrease in both the number and viability of alveolar macrophages obtained from morpholine exposed animals.


Inhalation studies were conducted to determine the toxicological effects of aerosols of Permethrin (NRDC-143) on dogs, rats and guinea pigs. Animals were exposed for 13 weeks to analytically determined concentrations of 500, 250 and 125 mg/m$.^3$. Dogs showed no toxic signs or changes in pulmonary function and blood chemistries. Rats exposed to the highest concentration showed severe tremors and convulsions throughout the first week of exposure but all toxic signs disappeared by the third week. No changes were seen in oxygen consumption or major organ-to-body weight ratios. Rats exposed to the highest concentration had a significantly prolonged hexobarbital induced sleeping time. Guinea pigs were not sensitized by the 13 week exposure nor did they show any toxic effects. Rabbits were dosed orally with Permethrin for 28 consecutive days at dosages of 3000, 960 and 300 mg/kg. Rabbits receiving the highest dose showed severe tremors and sub-convulsive responses which abated by one or two days. Rats and rabbits apparently adapt to the observable toxic effects of Permethrin, an apparent increase in hepatic enzyme activity occurred in rats during inhalation exposure. Sponsor: M. H. Weeks
131. POSSIBLE PROTECTIVE ROLE OF GLUTATHIONE IN PULMONARY TOXICITY BY 4-IPOMEANOL. M. Boyd, C. Statham, A. Stiko, J. Mitchell, and R. Jones, NIH, Bethesda, MD.

Because glutathione (GSH) may participate in the detoxication of highly reactive electrophilic metabolites, we have studied the role of GSH in the toxicity of 4-ipomeanol (IPO) in rats. Previous investigations have indicated that a highly reactive metabolite, formed in situ, is responsible for selective lung damage caused by IPO. Toxic doses of 4-ipomeanol preferentially depleted lung GSH. Pretreatment of animals with piperonyl butoxide, an inhibitor of the metabolic activation of IPO, prevented both the depletion of lung GSH and the pulmonary toxicity of IPO. Prior depletion of lung GSH by diethylmaleate treatment increased both the pulmonary covalent binding and the toxicity of IPO, whereas administration of cysteine and cysteamine decreased both the covalent binding and the toxicity. These in vivo studies, in conjunction with previous in vitro studies that showed inhibitory effects of sulfhydryl compounds on the covalent binding of IPO, are consistent with the view that pulmonary GSH may play a protective role in lung toxicity by IPO, probably by reacting with toxic IPO metabolite(s) to form nontoxic conjugate(s).


We have previously shown that intravenous transfusion of DEHP solubilized in blood causes severe hypotension, pulmonary damage and rapid death in pre-hemorrhaged rats. In the present studies we investigated the role of histamine and fibrinogen activation in the above pathology. Initially we observed a significant fall in blood histamine following DEHP. Subsequent studies revealed a concomitant increase in plasma histaminase. When the rats were pretreated with the histaminase inhibitor, aminoguanidine, then histamine levels in plasma were markedly elevated by DEHP. Under these conditions, the lethality of DEHP was significantly increased. These studies indicate that DEHP causes the release of histamine, but because of the simultaneous elevation of histaminase no net increase in histamine is seen systemically. These data suggest that histamine can effect the lethal response to DEHP and that, although elevated plasma levels are not seen, histamine release may exert an effect at the local tissue level. Gel filtration chromatography (Sepharose-4B) revealed a rapid in vivo conversion of plasma fibrinogen to fibrin monomer complexes, suggesting a role of disseminated intravascular coagulation in the response to intravenous DEHP.
133. THE EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL AND NABILONE ON THE ISOLATED GUINEA PIG BRONCHUS. R. M. Orzelek, F. R. Goodman, and R. B. Forney. Department of Pharmacology and Toxicology, Indiana University School of Medicine and The Dow Chemical Company, Indianapolis, IN.

Delta-9-tetrahydrocannabinol (THC) has been postulated to possess a bronchodilating effect in clinical trials with normal and asthmatic subjects. This effect is believed to act by a different mechanism of action than bronchodilator agents currently on the market. Nabilone (Nab), a synthetic analog of THC, is of interest since it has been shown to possess many of THC's salutary effects without its undesirable psychotropic effects at therapeutic doses. In view of this, we investigated the bronchoactivity of THC and Nab in vitro utilizing rings of guinea pig bronchi. The bronchi generated substantial force when contracted by histamine, KCl, and carbachol (maximal tension 0.7 to 2.6 g). THC pre-treatment (10^{-5} to 10^{-3} M) did not alter histamine, KCl, or carbachol-induced contractions. Nab (10^{-7} to 10^{-5} M) partially relaxed carbachol-contracted tissues; however, it did not affect KCl or histamine-induced contractions. Neither Nab nor THC had inherent relaxant properties on resting tension in guinea pig bronchi. (Supported in part by USPHS Grant T32 GM 7097 and ROI DA 00069.)


This study was performed to ascertain the effects of inhaled sulfuric acid aerosol on the phagocytic capabilities of alveolar macrophages. The lungs of exposed and control animals were lavaged and the following parameters examined: 1) ability of the cells to phagocytize radiolabelled microspheres in vitro, 2) percent viability and differential cell count of recovered cells, and 3) morphological changes in the bronchi via scanning electron microscopy. Phagocytic indices were determined for alveolar macrophages obtained from New Zealand white rabbits exposed in vivo to sulfuric acid aerosol at concentrations ranging from 46 to 522 mg/m^3 for periods of from 0.5 hours to 4.0 hours. No relationship was observed between percent phagocytosis and a CT index (concentration x exposure duration). Viability and the percent macrophages in recovered populations were not affected by exposure to H_2SO_4 aerosols regardless of concentration and exposure duration. Morphological changes, observed by scanning electron microscopy, revealed shortened and matted cilia in the deeper segments of the superior rami.

SRI has developed procedures to evaluate the toxicity of the pyrolysis/combustion products of many types of materials. This phase of the program assessed the importance of CO in producing mortality and determined the influence of ambient O2 concentrations on CO toxicity in rats. Rats and rabbits had increased carboxyhemoglobin levels after a 30-minute exposure to CO in air; the increase depended on the ambient concentration of CO. Carboxyhemoglobin levels returned to normal over a 30-minute recovery period post-exposure. Recovery rates were greater in rats than in rabbits, but both were more rapid than those reported for humans. Rats were exposed to CO for 30 minutes in ambient O2 concentrations of 15-20%. Mortality occurred only during exposure. The LC50 values for CO varied from 4207 ppm in 15% O2 to 5795 ppm in 20% O2. CO apparently did not damage the lung, since no differences in lung weights were observed between exposed rats and controls. It is suggested that CO, when present, can be a major contributing factor to death, especially in the reduced O2 atmosphere that may be present in fires.


The present studies were initiated to investigate the effects of cobaltous chloride(CO) & its interaction with iron on rat hepatic microsomal enzymes. Male Sprague-Dawley rats were fed iron-deficient(ID) diet mixed with 0,100 & 200 ppm of CO for 4 wks. At the end of 4 wks 3 rats from each group were sacrificed and the remaining rats were fed on iron-sufficient (IS) diets mixed with 0,100 & 200 for another 4 wks in order to determine the role of iron on CO effect on microsomal enzymes. The hepatic NADPH-Cyt.C-reductase, NADPH-dehydrogenase, Cyt.P-450 & aniline binding were determined. The rats fed CO mixed with ID diets for 4 wks showed a 5%, 30%, 35% & 40% decrease in NADPH-cyt.C reductase, Dehydrogenase, Cyt.P-450 & aniline binding respectively. When the diet regimen was reversed to IS diet mixed with CO the inhibition of microsomal P-450 system was reversed completely. CO at 200 ppm when mixed with IS diet has no detectable effect on hepatic Cyt.P-450 system. These results indicate that CO inhibited the rat hepatic microsomal Cyt.P-450 complex in the absence of iron and the inhibition was reversed by iron in vivo. (This work was supported by NIH grant RR 08133 (NBS)).
137. PROTECTION AGAINST ALLOXAN-INDUCED ALTERATIONS IN HEPATIC DRUG METABOLISM AND PLASMA GLUCOSE LEVELS IN THE MALE RAT. G.W. Wolfe and R.C. Schnell, Dept. PCOL/TOX, School of Pharmacy, Purdue University, Lafayette, IN 47907.

Treatment with glucose or diphenylhydantoin (DPH) has been demonstrated to protect against alloxan-induced diabetes. Experiments were undertaken to determine if these agents would also protect against alloxan-induced alterations in hepatic drug metabolism. Two days after alloxan (25, 50, 75 and 100 mg/kg, i.v.) a dose-dependent decrease was observed in ethylmorphine (EM) metabolism, plasma insulin (PI) levels and insulinogenic index while an increase was found in aniline (AN) metabolism and plasma glucose (PG) levels in male rats. Pretreatment with phenobarbital (100 mg/kg, i.p. for four days or 150 mg/kg, i.p. 1 hr prior), SKF 525-A (50 mg/kg, i.p.) or DPH (100 mg/kg, i.p. for four days) protected against alloxan-induced changes in EM metabolism but not in changes in AN metabolism, PG levels or insulinogenic index. Both DPH (100 mg/kg, i.p. 1 hr prior) and glucose (1 g/kg, i.v. 2 min prior) protected against alloxan-induced changes in PG and PI; however, glucose protected against changes in both EM and AN metabolism while DPH protected only against changes in AN metabolism. (Supported by ES-01123).


The different factors which influence the biliary excretion of metals are not understood clearly. This investigation was undertaken to study the effect of changes in the sulfur containing intracellular ligands on biliary excretion of cadmium (BL Cd). The induction of metallothionein synthesis of cadmium, zinc or copper salts markedly reduced BL Cd in rats. A significant decrease in BL Cd was also observed (60%) when the synthesis of glutathione was increased by injecting rats with two daily doses of 10 mg cysteine. On the other hand, depletion of glutathione levels by pretreatment with diethylmaleate had little effect on BL Cd or tissue deposition. Injection of 100 mg/kg EDTA or NTX, thirty minutes after intravenous injection of 1 mg/kg Cd as \textit{109}CdCl\textsubscript{2} caused only a small increase in BL Cd. Injection of cadmium complexed with cysteine, glutathione or DL-penicillamine did not affect BL Cd, though there was an increase in the urinary excretion. Cadmium complexed with selinite increased the plasma level and renal deposition of Cd and decreased BL Cd. These results suggest that the mechanism of BL Cd is complex and factors other than intracellular thiol ligands are involved. (Supported by MRC, Canada and ILZRO)
139. THE EFFECTS OF HEAVY METALS (HMs) ON THE ALVEOLAR MACROPHAGE (AM), IN VITRO. M.J. Reasor and P.R. Miles. W.VA. Univ. Med. Ctr. and ALOSH/NIOSH, Morgantown, WV (Sponsor: J.U. Bell)

Heavy metals are major contaminants of the respirable particulate effluents of coal combustion and gasification. To initially examine the toxicity of this material we studied the effects of PbCl$_2$, NiCl$_2$ and CdCl$_2$ on certain properties and functions of isolated rat and rabbit AMs, in suspension. In rat AMs, the effect of HMs on a number of lysosomal enzyme activities was measured. Of these, acid phosphatase was inhibited non-competitively by Cd$^{++}$ and Pb$^{++}$. The generation of chemiluminescence by rat AMs during challenge with zymosan was inhibited in a dose dependent manner by Pb$^{++}$ (ED$_{50}$=3.0x10$^{-4}$M), Ni$^{++}$ (ED$_{50}$=3.4x10$^{-5}$M) and Cd$^{++}$ (ED$_{50}$=5.1x10$^{-5}$M), and appeared to be due to an interaction of the metals with the cells and not with the zymosan. Phagocytosis by rabbit AMs was assessed by measuring the uptake of an oil suspension containing oil red 0. All three metals inhibited uptake with the effectiveness being Pb$^{++}$>Cd$^{++}$>Ni$^{++}$. From this work, it is concluded that a number of functions of AMs are very sensitive to HMs. (Supported by ERDA contract EY-77-C-21-8087).


Scotopic vision deficits have been reported in animals following exposure to either lead (Pb) or mercury (Hg). To date, no such reports exist regarding cadmium (Cd). Isolated perfused bullfrog retinas were studied to determine if this deficit involved a primary lesion in the photoreceptors. Rod and cone responses to brief light flashes were separated using previously described techniques (Sillman, Vision Res. 14, 1021, 1974). Reversible dose-response (10$^{-6}$M to 5 x 10$^{-3}$M Pb) amplitude decrements were observed in rods, but not in cones. Additionally, Pb treated photoreceptors exhibited an approximate one-half log increase in absolute threshold sensitivity. Similar findings were observed with Hg and Cd. However, on an equimolar basis Cd had more pronounced effects than either Pb or Hg. Furthermore, only the effects of Hg were non-reversible. These observations suggest the involvement of rod photoreceptors as a primary lesion site and are consistent with the reported scotopic visual deficits following Pb and Hg exposure. The results predict that Cd exposure may produce similar effects. (Supported by N.I.E.H.S. ES05094 and PHS EY01839.)

Single doses of 335 (adults only), 78, 15, and 3 mg/kg of lead labelled with Pb-203, and of 8 x 10^-7 mg/kg as carrier-free Pb-203, were administered per os to 10 week and 10 day old Cl29F1 mice (day 0). Retention on day 6 did not differ among dose groups in adult (1.1±0.1 S.E.) or in pups (0.4±0.1 except with the carrier-free dose (47 and 68% retention respectively). Retention in pups significantly exceeded retention in adults for all doses. Ontogenic differences were also observed in organ distribution in kidney, skull, and brain. C.I. absorption and brain uptake were linear in the dose range of 3 to 335 mg/kg. Due to their greater lead retention and brain concentration (8 to 18 times adult values) young animals could manifest toxicity when exposed to doses which are non-toxic in adults. Supported by NIEHS Grant ES-02148 and US/DOE Contract UR-3490-1457.


Mercury is a generally recognized environmental toxicant. In the case of poisoning by methylmercury, there is no accepted specific therapy. The purpose of this study was to develop a new specific mercury chelating agent. A polymer, polyethyleneetriphenolphosphine, was synthesized by reacting ethylene bromide with triphenyl phosphine. The polymer has a median lethal dosage of greater than 1 g/kg. The polymer was able to chelate and retain greater than 20% Hg++ by weight. The polymer was able to antagonize acute methyl mercury-induced toxicity and this effect was further enhanced by phenobarbital pretreatment. Methyl mercury, 15 mg/kg ip, produced a 90% lethality, while the combined polymer and phenobarbital pretreatment protected against the methyl mercury-induced toxicity resulting in only a 60% lethality. Similarly, at lower dosages of methyl mercury the polymer also antagonized methyl mercury-induced toxicity. This new polymer polyethyleneetriphenolphosphine antagonizes methyl mercury-induced toxicity and is a specific mercury chelator. (Supported by USPHS Grants ES01018 and ES00267).
143. LONG TERM BEHAVIORAL EFFECTS RESULTING FROM GESTATIONAL EXPOSURE TO Cd. L. Hastings, H. Choudhury, and G. P. Cooper, Dept. of Env. Hlth., Univ. of Cincinnati, Cincinnati, Ohio.

There is evidence which suggests gestational exposure to Cd results in offspring who show alterations in both trace metal balance and activity levels. To better characterize both the behavioral and biochemical effects of gestational Cd exposure, rat dams were maintained on a purified diet of specified trace metal content and exposed to either 8.6 or 17.2 ppm CaCl in drinking water. There were no differences in litter size or birth weight among the groups. In the 17.2 group, whole body Fe and Zn levels were significantly depressed at birth, while the Cd levels were equivalent to control. By weaning, body weights of the two Cd-exposed groups were significantly depressed. No differences were seen in shock-elicited aggression when tested at 60 days. At 94 days the rats were placed in running wheels for 9 weeks. The activity level of the 17.2 group was elevated while that of the 8.6 group was depressed. All rats were then tested on a simultaneous visual discrimination task with reversal. Again the 17.2 group took longer to acquire the reversal. These results suggest that the behavioral effects observed are due to an indirect action of Cd on trace metals which may be further confounded by altered growth.

144. ALTERATION OF MAMMALIAN UROPOPHYRINGEN DECARBOXYLASE (UD) BY HEAVY METALS. R. Kardish, B. A. Fowler and J. S. Woods, NIEMS, NIH, Research Triangle Park, NC 27709

Recent studies have established the role of metals as regulators of heme metabolism in mammalian tissues. Principal effects are mediated via induction and repression of heme oxygenase and ALA synthetase, rate limiting enzymes in heme degradation and synthesis respectively. In the present studies, the in vitro effects of Hg$^{+2}$, As$^{+3}$, As$^{5+}$, Co$^{2+}$ and Pb$^{2+}$ on the activity of UD, a biosynthetic enzyme implicated in the etiology of porphyria cutanea tarda (PCT), were investigated. A procedure designed in this laboratory to measure UD via enzymatic generation of uroporphyrinogen was used. Hg$^{+2}$ was the most potent inhibitor of UD activity and at $10^{-3}$ M there was 90% inhibition of coproporphyrin formation as measured spectrophotometrically. A dose related decrease in activity of UD also occurred with As$^{3+}$, As$^{5+}$, or Co$^{2+}$. Pb$^{2+}$ had little effect on UD activity. The inhibitory effects of these metals in combination was also shown to be additive and this inhibition was overcome by the addition of glutathione. These studies demonstrate the in vitro sensitivity of UD to a variety of toxic elements and suggest that experimental inhibition of UD may serve as a model for studying porphyria in humans.
145. PERINATAL DEVELOPMENT OF HEPATIC METALLOTHIONEIN IN THE RAT. John U. Bell, Department of Pharmacology and Toxicology, West Virginia University, Medical Center, Morgantown WV.

It has been previously reported [Pharmacologist 20,76,1978] that the hepatic cytosol of the term rat fetus contains a metallothionein-like protein which is associated in-vivo with relatively high levels of zinc. In the present study, the perinatal development of hepatic metallothionein was followed by measuring the total cadmium-binding capacity of the isolated protein at term and at 7, 14, 21, 28 and 35 days after birth. In addition, total cytosolic zinc and metallothionein-bound endogenous zinc levels were determined. Hepatic metallothionein levels which were significantly higher in the term fetus than in the adult, were found to increase during the first week of extrauterine life and then to decline to non-detectable levels by day 28. Total cytosolic zinc and metallothionein-bound zinc followed the same developmental pattern, lending support to the hypothesis that metallothionein can serve as a regulator of hepatic zinc during the perinatal period. [Supported by USDOE Contract EV-77-C-21-8087]

146. FETAL TO MATERNAL CADMIUM MOVEMENTS ACROSS THE PERFUSED HEMOCORIAL PLACENTA OF THE GUINEA PIG. B.J. Kelman and R.K. Walter, Comparative Animal Res. Lab, Oak Ridge, TN. Sponsor: M.A. Evans. Guinea pig placentas (59-61 days gestation) were perfused from the fetal side in situ. Radiolabeled CdCl₂ was added to the perfusing fluid; the dam was injected with tracer amounts of tritiated water. Cd clearance from fetal to maternal circulation (Cd₆₋₅) was 0.063 ± 0.004 ml/min (mean ± SE), 17% of the Cd clearance from maternal to fetal circulation (Cd₅₋₆). Unlike Cd₆₋₅, Cd₅₋₆ was not correlated with flow through the fetal circulation of the placenta. Clearance of tritium from maternal to fetal circulation (T₆₋₅) was measured concurrently with Cd₆₋₅. In most cases there was an initial period where perfusate flow correlated linearly with T₆₋₅ independent of rate of flow through the fetal circulation of the placenta. Even when there was a significant linear relationship between T₆₋₅ and perfusate flow, correlation coefficients were lower in the presence of Cd, indicating increased variability in maternal blood flow to the placenta. There appeared to be dose-related decreases in maternal blood flow to the placenta caused by perfusate Cd. Differences in the effects of Cd on maternal blood flows when administered from fetal or maternal sides of the placenta may be due to differences in binding of Cd by fetal and maternal plasma proteins. (Sponsored by U.S. Dept. of Energy Contract No. EV-76-C-05-0242 with the Univ. of Tn.)

The purpose of this study was to determine the effects of replacing cysteine with the dithiol complexing agent DMSA on the efficiency of removal of methylmercury during extracorporeal complexing hemodialysis in the dog. DMSA was found to be ten times as potent as cysteine in complexing methylmercury in blood. DMSA caused an increase in methylmercury clearance from blood when used to replace cysteine in the dialysis procedure. In addition, systemic effects of DMSA included a shift in distribution of methylmercury from red cells into plasma, an enhanced redistribution of methylmercury from extravascular compartments into blood, and an increased excretion of methylmercury in the urine. Cumulative excretion of methylmercury in the urine was of a similar magnitude to the amount extracted by the dialyzer. These two routes of excretion accounted for a four hundred fold increase in methylmercury removal compared to untreated dogs. The results confirm the utility of a systemically acting complexing agent in enhancing the efficiency of methylmercury removal during extracorporeal complexing hemodialysis in the dog. This work was supported in part by grants from the National Institute of General Medical Sciences (GM 15190 and GM 25329 and by the State University of New York at Buffalo.


Since exposure to Cd occurs in man via the diet, a study was conducted in rats to elucidate the fate of orally ingested Cd. A single dose of 100 μCi $^{109}$CdCl$_2$ (1 μg Cd) was administered by intubation to male Wistar rats (132 ± 8g). The unabsorbed dose was eliminated to a large extent during the first 5 days. Only 0.5-1.5% of the $^{109}$Cd was absorbed. The daily excretion amounted to about 0.4% of the body burden. Whole body retention time was estimated to be 165 ± 12 days. The distribution of the isotope 24 h after the ingestion was in the order: liver > kidneys > pancreas > spleen > testes > heart > lungs. However, its concentration in the kidneys was more than twice that in the liver. Depletion of the $^{109}$Cd was noted in all tissues except the kidneys. In the liver the calculated retention half-time was 74 days. It is concluded that Cd is absorbed poorly, its excretion is negligible and that it is concentrated and accumulated in the kidneys. (Supported by PHS Grants ES 01247 and ES 01448).

The affinity of methylmercury (CH$_3$Hg$^+$) for sites of growth inhibition in cultured L5178Y mouse leukemic cells was studied by titration of binding capacity for the mercurial. With 0.3μM CH$_3$HgCl, 50% growth inhibition was produced in exponentially growing cells. The Lowry protein content of the cell suspension was measured as 1250μg serum protein and 40μg cell protein per ml. Binding capacity of the serum proteins for CH$_3$Hg$^+$ was titrated with 16μmoles Hg/ml of medium, showing less than 2% saturation at 50% inhibition. Given the high association constant of CH$_3$Hg$^+$ to serum proteins (Log$_{10}$K = 17) and the great excess of total protein over the mercurial, the mechanism of growth inhibition involved the partition of CH$_3$Hg$^+$ between inhibitory cellular growth sites and serum proteins, and the association constant of CH$_3$Hg$^+$ to inhibitory sites was calculated at an order of magnitude higher than its association constant to serum proteins. Potentiometric titration thus permitted the affinity of a mercurial to the site of poisoning to be evaluated in growing cells in culture.


Due to the importance of metal binding by plasma proteins in controlling metal distribution and excretion in mammalian systems, the in vitro binding of Cd, Zn and Cu in rat and human plasma was investigated. Metals (2μg/ml) were incubated with plasma for 10 min at 37°C and protein binding evaluated by non-equilibrium gel permeation chromatography on Ultrogel AcA34. In rat plasma, exogenous Cd Zn and Cu was only bound to a fraction characterized as albumin-transferrin (AT) by molecular weight, bromocresol green reaction and electrophoretic-immunodiffusion. In human plasma, Zn and Cu was only bound to the AT fraction, however, 10-15% of the Cd was bound to a high MW fraction which contained endogenous Zn (tentatively identified as α2-macroglobulin). Zinc levels in this fraction were depressed by Cd binding indicating displacement of Zn by Cd. The affinity of human transferrin for Cd was evaluated. Both iron saturated and iron free transferrin did not bind Cd. These studies indicate that at high blood levels of these metals the major plasma protein controlling free metal concentration is albumin. However, at tracer or low dose levels plasma components such as α2-macroglobulin may be quantitatively important.
151. EFFECTS OF PROLONGED ORAL METHYLMERCURY EXPOSURE ON THE
STRUCTURE AND FUNCTION OF MOUSE LIVER MITOCHONDRIA.
B. A. Fowler and J. S. Woods. NIEHS, NIH, Research Triangle
Park, NC 27709

Female C57 mice were given access to deionized drinking
water containing 0, 3, 5, or 10 ppm mercury as methylmer-
curic hydroxide (MMH) for 6 weeks. Combined ultrastructural/biochemical studies were conducted to assess effect of
MMH treatment on mitochondrial structure and function. In
situ mitochondrial swelling was associated with a dose
related decrease in succinate mediated state 3 respiration
to 81% of control and a decrease in respiratory control ra-
tios to 73% of control at the 10 ppm dose level. Specific
activity of monoamine oxidase localized in the outer mito-
chondrial membrane, was found to show a dose-related in-
crease in MMH animals to 161% of control. Cytochrome
oxidase (CO) which is a constituent of the inner mitochon-
drial membrane showed a non-dose related decrease to 86% of
control while 6-aminolevulinic acid synthetase (ALAS) which
is loosely bound to the inner membrane was not affected at
any dose level. These studies demonstrate differential ef-
teffects of MMH on specific mitochondrial functions and suggest
that these changes are in part determined by location of the
function within the organelle.

152. EARLY ACTIONS OF METHYLMERCURY AND THEIR USE IN TREATMENT
EVALUATION. C.A. Lapin and D.E. Carter, Haskell Lab. for
Toxicology and Industrial Medicine, E.I. du Pont de Nemours
& Co., Newark, DE.; and Toxicology Program, Univer. of
Arizona, Tucson, AZ. Sponsor: I.G. Sipes

The early actions of methylmercury (MM) on body weight,
food consumption, and protein synthesis were examined as
possible criteria for evaluating treatments used in MM poi-
soning. Rats received po 40 mg MMOH/kg which produced neuro-
logical symptoms 8-13 days later. Before symptoms appeared,
MM decreased food consumption (50-75%) and body weight (14-
30 g), increased protein synthesis in liver (18-39%), kid-
ney (27-85%), and blood (45-125%), and decreased it in cere-
bellum (15-19%). Treatment with D-penicillamine (DPA) (sc 1.2
g DPA/kg daily for days 2, 3, and 4 after MM) prevented neu-
rological symptoms, restored food consumption and protein
synthesis to control levels, and increased body weight to-
wards control levels. The addition of vitamin B-6 (sc 70 mg/
kg) improved DPA's reversal of MM's action on body weight
and food consumption, but antagonized it in protein synthe-
sis. Therefore, while food consumption, body weight, and
protein synthesis were indicators of MM toxicity, only food
consumption and body weight predicted treatment efficacy.
(Supported by NIEHS Grant 5RO1 ES01004).

Cadmium chloride has a relatively long half life of retention in Syrian hamster lung following inhalation exposure. Studies were conducted to investigate the role of a metallothionein-like protein (MT) on retention of Cd in lung following exposure by bronchopulmonary lavage. Eight 10-14 week-old Syrian hamsters were lavaged with 0.15 mM CdCl₂ containing 0.02 µCi 115mCd/mL. In addition, 2 groups of 8 hamsters were lavaged with 1 µm CdCl₂ once or twice at 3-day intervals prior to lavage with 0.15 mM CdCl₂. Two animals from each group were sacrificed at 1, 2, 7 and 21 days following exposure. Lungs were processed for isolation of MT. After 21 days, 66±8% of the initial Cd body burden remained in the lung, while 14±1% and 10±1% were present in liver and kidney, respectively. Pre-administration of 1 µm CdCl₂ had no significant effect on retention or distribution of Cd. Greater than 80% of the 115mCd in supernatants of lung homogenates was associated with a protein eluting from Sephadex 6-75 with a Vₑ/V₀ characteristic of MT. This protein did not bind Se, Ni, or Cr. This data suggests that a MT is present in hamster lung which may be responsible for retention of inhaled Cd. (Research supported by EPA and the DOE under DOE Contract EY-76-C-04-1013.)

154 ALTERED REGULATION OF HEPATIC HEME METABOLISM BY INDIUM CHLORIDE. J. S. Woods, G. T. Carver, and B. A. Fowler. NIH, NLM, Research Triangle Park, NC 27514

Regulation of heme metabolism by trace metals in mammalian tissues is well known. The present studies investigated the effects of indium, a hepatotoxic trace metal, on heme biosynthesis and degradation in rat liver. Indium chloride (InCl₃), administered IP to male rats at 0, 10, 20 or 40 mg/kg, 16 hrs prior to sacrifice, produced a dose-related decrease in the activity of δ-aminolevulinic acid synthetase (ALAS) to 48% of control levels, and a 5-fold increase in heme oxygenase (HO) activity, at the highest dose level. Levels of microsomal heme and mixed function oxidase (MFO) parameters (cytochrome P-450 and aminopyrine demethylase) decreased to 70% of control at 40 mg/kg. In time course studies HO was increased 2-fold at 8 hrs after InCl₃ injection, and a 2-fold increase persisted for 72 hrs after treatment. ALAS activity was maximally depressed at 16 hrs, but increased to 2 times control levels by 24 hrs post-treatment. Depression of MFO parameters persisted for at least 72 hrs. These studies demonstrate that indium modifies the regulation of cellular heme levels, and thus of heme-dependent oxidative processes, by altering the rates of heme biosynthesis and degradation in mammalian cells.
155. CHLORPHERTERNINE UPTAKE BY ISOLATED PERFUSED RABBIT LUNG. L.S. Angevine and H.M. Mehendale, Dept. of Pharmacology and Toxicology, Univ. of Miss. Med. Ctr., Jackson, MS 39216

Studies have shown that many amines accumulate in the lung both in vivo and in vitro. Chlorphterine (CP) is a phenylethanolamine type of amorexigenic agent in use, that is known to be accumulated in the lung and to cause phospholipidosis. These studies were undertaken to characterize the uptake and accumulation processes in the lung. Uptake and accumulation of 14C-CP were followed in artificially ventilated isolated perfused lung preparations using either autologous blood or artificial perfusate at initial CP concentrations of 12.5 to 200 μM. Perfusate and lung homogenate samples were analyzed for the parent compound and possible metabolite(s). Steady-state uptake was reached after 25 min of perfusion. Higher extraction of CP was observed from artificial medium than from whole blood. No metabolism of CP was observed. Lung uptake was concentration dependent and was saturable at 100 μM. Higher concentrations resulted in edematous deteriorating preparations. Experiments with partly Na+-depleted media as well as perfusate containing 10-4M harmaline suggest CP uptake is partly Na+-dependent. Similar studies were carried out with lung slices. (Supported by Miss. Heart Assoc. and PHS grant HL-20622).


Recent investigations have shown that daily treatment of 1 day-old rats for 1 week with chlorphterine results in dose-dependent morphological alterations and an increase in incorporation of thymidine into pulmonary DNA. In order to examine whether inhalants interfere with chemical-induced lung repair processes, newborns were administered 20 or 60 mg/kg/day chlorphterine for 6 days followed sequentially by 95% oxygen for 3 days. Whereas O2 produced reduction in incorporation of thymidine into lung DNA without any change in aoeolar histiocyte number, a significant increase in DNA synthesis and these cells was seen with chlorphterine, suggesting that incorporation into DNA might partially be related to proliferation of these cells or their precursors. Oxygen significantly decreased the chlorphterine-stimulated increase in incorporation of thymidine into lung DNA, a 45% reduction observed in the case of 60 mg/kg. In contrast, oxidant-inflicted inhibition of DNA synthesis rose significantly higher than control in rats subsequently administered chlorphterine. Our data demonstrate that O2 may interfere with repair of chemical-inflicted processes. (Supported by Medical Research Council of Canada).

High conc. of O2 in vivo potentiate paraquat (Pq) toxicity while low conc. may protect against the lung damage. Pq accumulation (accum.) into 0.50 mm slices of fresh rat lung was not altered by incubation for up to 4 hr in 85% O2 (Po2 = 645 mm Hg) compared to room air (Po2 = 160 mm Hg). Reducing the incubation Po2 below 100 mm Hg inhibited Pq accum.; 50% of maximal accum. was observed at Po2 = 50 mm Hg. Significant Pq accum. was detected after incubation for 1 hr at Po2 = 10 mm Hg; below this O2 tension no accum. was observed. Incubation for up to 3 hr in atmospheres (atm.) of 100% N2 completely inhibited Pq accum. When slices were placed in Po2 > 10 mm Hg following preincubation for 2 hr in 100% N2, control rates of accum. (no preincubation in N2) were obtained. Production of 14CO2 from 14C-glucose was also completely inhibited by 100% N2 but progressed at control rates when slices were incubated in Po2 = 160 mm Hg after preincubation in 100% N2 for up to 2 hr. These values suggest that active accum. may require relatively high O2 tensions compared to other O2-dependant metabolic processes. Lung slices are resistant to anoxia when returned within 2 hr to atm. containing O2. Supported in part by NIH Grant ES01365.

158. PARAQUAT (PQ) TREATMENT ALTERS DISPOSITION OF 5-HYDROXYTRYPTAMINE (5-HT) AND ANGIOTENSIN I (AI) BY RAT LUNG. R.A. Roth, K.B. Wallace, R.H. Alper and M.D. Baille, Depts. of Pharmacology & Toxicology, Physiology, Michigan State Univ., East Lansing, MI. Sponsor: T.M. Brody

Although morphologic damage to both Type I and Type II alveolar pneumocytes has been commonly observed after PQ administration, disagreement exists as to whether PQ treatment alters vascular endothelium of lung. Since pulmonary disposition of 5-HT and AI is a function of vascular endothelium, we examined the disposition of these agents by lungs of rats treated with PQ. Isolated lungs from rats treated with PQ dichloride (25 mg/kg, i.p.) 3 or 4 days before perfusion removed less perfused 14C-5-HT (0.1 mM) than did saline injected controls. This effect was not caused by PQ directly since perfusion of lungs from untreated animals with PQ did not alter removal of co-perfused 14C-5-HT. Monoamine oxidase activity of 600 x g supernatant fractions of lungs from PQ-treated rats was also reduced compared to controls. Although removal of perfused AI (1 mg/ml) by isolated lung was not altered by PQ pretreatment, angiotensin-converting enzyme activity in 600 x g supernatant fractions of lung was significantly reduced. These results suggest that PQ damages pulmonary endothelium and impairs the metabolic function of lung. (Supported by NIH grants ES01861 and HD02690.)
159. STRUCTURE-ACTIVITY CORRELATIONS OF PARAQUAT HOMOLOGUES IN THE INHIBITION OF ALDRIN EPOXIDATION BY RAT LIVER MICROSOMES

To clarify the mechanism of paraquat's toxic action, the N-alkyl homologues of paraquat (viologens) were investigated for their ability to inhibit rat liver microsomal aldrin epoxidation and produce rat toxicity. Viologens were uncompetitive inhibitors of aldrin epoxidation. Lethal dose of viologens in rats can be related to the uncompetitive inhibitor's kinetic constant (Ki). Ki was closely related to the inductive effect of the alkyl side chains of the viologens. A correlation of Ki and the ability of the viologens to inhibit paraquat uptake into rat lung slices (Toxicol. Appl. Pharmacol. 41 134, 1977) was excellent indicating dependence of both lung uptake and microsomal inhibition on a common physicochemical parameter of the viologens i.e. charge on the quaternized nitrogen. An alternative mechanism of paraquat's toxic action that does not require lipid peroxidation is proposed based on current findings concerning the ability of viologens to shunt electrons from critical biochemical reactions. Supported in part by NIH-ES 00125.


Rat and mouse lung microsomes were found to be relatively resistant to lipid peroxidation as promoted by Fe²⁺/NADPH, Fe²⁺/ascorbate, carbon tetrachloride, paraquat, or xanthine oxidase, when compared to brain (B), kidney (K), and liver (LV) microsomes. Production of malondialdehyde and disappearance of polyunsaturated fatty acids both occurred at a several-fold lower rate in lung microsomes than in B, K, or LV microsomes incubated under optimal conditions for lipid peroxidation. The extracted lipid fraction of lung microsomes (liposomes) is also resistant to peroxidation relative to B, K, or LV liposomes. Addition of untreated or boiled lung microsomes or liposomes inhibits peroxidation of LV microsomes or liposomes. Ethanol extracts of lung microsomes are similarly inhibitory. These results suggest the presence of a heat-stable lipid-soluble antioxidant factor in lung that may function in vivo to protect against peroxidative damage. Consequently, lung injury incurred by paraquat or CCl₄ appears less likely to be mediated by a lipid peroxidation mechanism.
161. THE EFFECT OF ALCOHOL ON THE UPTAKE OF XENOBOTICS BY THE ISOLATED PERFUSED RABBIT LUNG. S. Lock, H.P. Mitschi and G.L. Plass, Dépt. de Pharmacologie, Univ. de Montréal, Montreal, Canada.

Previous studies have shown that the isolated perfused rabbit lung is capable of accumulating and metabolizing xenobiotics. It has also been shown that intratracheally administered compounds will enter the blood stream. It was of interest to know whether the addition of toxic amounts of ethanol (EtOH) to the perfusate of an isolated lung would alter the accumulation of model compounds by the lung or their passage from the airways into the blood stream. Blood and lungs from male New Zealand white rabbits (3-5 kg) were employed. Imipramine-^{14}C (3 μmol/lung) or paraquat-^{14}C (3 μmol/lung) were added to the perfusate containing 300 mg EtOH/100 ml. No changes in the rates of pulmonary accumulation of the compounds were observed. The lung is also susceptible to airborne xenobiotics. A lipid-insoluble compound (mannitol-^{14}C) (1 μmol/lung) and a lipid-soluble compound (sulfamide acid-^{35}S) (5 μmol/lung) were administered intratracheally to determine whether EtOH could influence their absorption. High EtOH concentrations did not affect their absorption. (Supported by the Medical Research Council of Canada).


Among the novel side chain congeners of the potent, lung-selective toxin, perilla ketone (Science, 197, 573, 1977), all exhibit lung toxicity at high doses and some display renal and/or hepatic damage at pulmonary subtoxic doses (ip, 21-26 g male Notre Dame strain Swiss mice, 50 μl, DMSO). Among series I, increased polarity at R₃ decreases lung selectivity and reaches the lung-based LD₅₀. In series II, lung selectivity reaches a maximum at n=4, then declines. With both series, the lung toxic dose seems determined by hydrophobic and partitioning factors, yet the common 3-carbonyl-furan linkage is implicated as a key determinate of lethal lung effects by these agents.

I (3-Furyl)COCH₂CH₂CHRCH₃  (3-Furyl)CO(CH₂)ₖCH₃  II


The carbonyl-modified congeners of perilla ketone (pk) in series I and II were injected ip into 21-26 g male Notre Dame strain Swiss mice (50 μl, DMSO) and were evaluated primarily for pulmonary toxicity. All agents in series I have distant lung toxicity at variable, but higher doses than pk(X=CH₂) with some displaying renal effects at pulmonary subtoxic doses. In series II, one (R₁=R₂=H, R₃=Am) was without lung effects at ≥100X the dose for pk. Another (R₁=H, R₂=OH, R₃=Am) was lung toxic and lung selective, but was probably converted in vivo to pk, since the deuterob alcohol (R₁=H) was significantly less toxic. As with the side chain derivatives (vide infra), the carbonyl group seems critical for the most significant lung toxicity in these series.

I (3-Furyl)COCH₂Bu (3-Furyl)CR₁R₂R₃  II

* Present address: Dept. Animal Sci., Coll. Agric., Univ. IL., Urbana, IL, 61801. Supported by NIH grants #ES 01732-01, ES 00569-14, and ES 00267.


Most ring-modified congeners of perilla ketone (I and II), when injected ip into 21-26 g male Notre Dame strain Swiss mice (50 μl, DMSO) produce lung toxicity, although some also cause renal or hepatic damage at lung sublethal doses. Among series I, alterations of X, which increase ring stability, result in decreased pulmonary toxicity. Similarly, with series II, alterations of substituent R, which increase ring electron density, result in decreased lung toxicity. Aromatic systems with electron-withdrawing groups (e.g. carbonyl) seem most capable of inducing pulmonary edema with pleural effusions provided hydrophobic-partitioning factors are relatively constant (vide infra). Possible mechanisms for bioactivation of the electron-deficient ring will be presented.

I ArCOCH₂CH₂CH(CH₃)₂ (3-Furyl)R  II

* Present Address: Dept. Animal Sci., Coll. Agric., Univ. IL., Urbana, IL 61801. Supported by NIH grants #ES 01732-01, ES 00569-14, and ES 00267.
165. ON THE PATHOGENESIS OF TOXIC LUNG FIBROSIS. Wanda Haschek and H. P. Witschi, Biology Division, Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, TN. 37830

We have found earlier that in lung dividing epithelial cells are more susceptible to the cytotoxic effect of O₂ than are interstitial cells. Interference of O₂ with epithelial cell repair could thus produce fibrosis. To test this hypothesis, mice were injected i.p. with 400 mg/kg of butylated hydroxytoluene (BHT), placed into 70% oxygen for 6 days and kept in air for another week. Extensive interstitial pulmonary fibrosis developed and total lung collagen was twice as high as in controls. Animals placed in O₂ only did not develop fibrosis and animals treated with BHT alone showed a slight increase only in lung collagen. The combined effects of BHT and O₂ were therefore synergistic and not simply additive. No fibrosis developed if O₂ exposure was delayed until 6 days after BHT. It is concluded that toxic lung fibrosis develops if epithelial repair, following lung injury caused by a bloodborne agent, is compromised by a toxic inhalant. (Research sponsored by Division of Biomedical and Environmental Research, U. S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.)


Alumina fibre has been synthesised with a narrow range of diameter. The median diameter selected was such that the fibres are above the range predicted for mesothelioma induction and below the range at which glass fibres show greatest irritant potential. The fibre has been tested in the rat for potential to induce neoplasia or lung fibrosis. Intrapleural injection of UICC chrysotile produced the expected incidence of mesothelioma with asbestos but there was no case of mesothelioma or significant excess of other tumours in the group which received alumina fibres. Parallel experiments where fibres were administered by intratracheal injection or inhalation also produced no excess of tumours with alumina fibres. These results reinforce previous observations of the importance of fibre diameter in mesothelioma induction using material with a narrower range of diameter than previously. The alumina fibres induced a mild 'foreign body' reaction with minimal production of fibrous tissue after administration by a variety of routes. We predict that alumina fibres within this size range will be biologically inert.
167. TERATOLOGY AND REPRODUCTION STUDIES WITH RATS EXPOSED TO 10, 33 OR 100 PPM OF ETHYLENE OXIDE (EO). W.M. Snellings, J.L. Pringle, J.D. Dorko and W.J. Kintigh, Chemical Hygiene Fellowship, CMIR, Pittsburgh, PA. Sponsor: C. S. Weil

A teratology and one-generation reproduction study were performed at the same exposure concentrations as those in a chronic rat study, 0, 10, 33 and 100 ppm. From Day 6 of gestation through Day 15, bred Fischer 344 rats were exposed 6 hr/day. Fetuses were delivered by caesarean section at Day 20 and evaluated for growth and visceral and skeletal development. Only male and female fetuses of rats exposed to 100 ppm had body weight significantly lower than that of the control. No developmental effects attributable to EO exposure were observed; therefore, EO was judged not to be teratogenic at these exposure concentrations. In the reproduction study, male and female weanling rats were exposed 6 hr/day, 5 days/week for 12 weeks. These rats were mated, and exposure was continued through Day 19 of gestation. The dams were not exposed during the first 5 days of lactation. From Day 5 through Day 21 postpartum, they were separated from the pups and exposed to EO. Only the female rats exposed to 100 ppm of EO had a longer gestation period, reduced fertility index and significantly fewer pups. No differences were noted in any treatment versus control level for gestation survival or postpartum survival indices.


2'-Deoxycoformycin (2'-dCF, NSC 218321) is a potent inhibitor of adenosine deaminase that enhances the biochemical, toxicologic, and antitumor activity of some adenosine analogs. 2'-dCF also exhibits immunosuppressive activity; selective lymphoid toxicity has been reported (Cancer Chemother. Pharmacol. 1:49-51, 1978). We administered 2'-dCF ip to male BDF1 mice (26-28 g) daily for 5 days in dosages of 5, 7, and 9 mg/kg/day. These dosages approximate 0.5, 0.9, 1.2, and 1.5 LD10 based on concurrent 30-day lethality data. Ten mice from each dosage group were killed on Days 6, 8, 11, 15, 19 and 26 (day of first treatment = Day 1) for hematologic, clinical chemistry and histopathologic evaluation. Lymphocyte counts in the 5th percentile of the reference range on Day 6 and lymphocytosis on Days 15-19 suggested lymphocytic toxicity and recovery during treatment. A second experiment revealed lymphopenia on Days 4 and 5. Plasma total bilirubin, alkaline phosphatase, GPT, and GOT exhibited dose-related elevations that were sustained throughout the study. Plasma glucose concentrations declined. Histopathologic evaluation confirmed hepatomegaly. Supported by Contract No1-CM-57000, DCT, NCI, NIH, DHEW.
169. TOLUENE DI-ISOCYANATE (TDI) AND p,p-DI-PHENYL METHANE DI-ISOCYANATE (MDI) ON GUINEA PIG RESPIRATORY SMOOTH MUSCLE. J E Doe and Gillian M Horspool, ICI, CIL, Alderley Park, Macclesfield, Cheshire, UK. Sponsor: I F H Purchase

TDI may cause asthma in some of those exposed to it, but there is no conclusive immunological evidence for the asthma. TDI inhibits cAMP increase by catecholamines in human leucocytes, suggesting β-blockade as the basis of TDI asthma. To investigate the effect of isocyanates on respiratory smooth muscle, strips cut from guinea pig lung parenchyma were suspended in a 90 ml bath in Tyrode at 37°C and tone monitored by an isotonic transducer. Drugs were injected into the bath (TDI and MDI dissolved in dimethyl-sulphoxide). Isoproterenol (IPR) (10 µg) consistently relaxed methacholine induced tone and was inhibited by propranolol. 1 mg of either TDI or MDI was added to the bath 0, 5, 10 or 15 minutes before IPR. TDI significantly inhibited the IPR relaxation after 5 and 10 minutes contact time, and also significantly relaxed the methacholine tone after 15 minutes. MDI was without effect. These results suggest that TDI has a non-specific effect on smooth muscle and is not a specific β-blocker.


The immune complement system is a cascade series of 11 enzyme-proteins, the activation of which has many biological consequences. This includes the generation of chemotactic factors for inflammatory cells and factors which effect vascular permeability. Since complement components are present in lung fluids and the inhalation of complement activating agents can result in lung injury, complement consumption in vitro was explored as a rapid screening assay for potential in vivo biological activity and toxicity. Fly ash, particulate effluents from coal combustion processes, from different coal-fired power plants were incubated with pooled normal dog serum at 37°C for 1 hour. The resulting supernatants were assayed by a 50% hemolytic (CH50) endpoint method. Results indicate that ash from fluidized bed combustors activated complement with 40% of total available complement depleted at 10 mg/ml serum. This activation was concentration dependent. In contrast, samples from power plants utilizing conventional combustion methods had little if any activity. Experiments are under way to determine if this activity is related to combustion technology, coal type or chemical composition of the ash. (Research supported by DOE Contract EV-76-C-04-1013.)
171. HEXYL ISOCYANATE PULMONARY HYPERSENSITIVITY IN GUINEA PIGS.

Aliphatic and aromatic isocyanates are of considerable importance in the manufacture of plastics, foams, paints and coatings. However, allergic asthmatic reactions to diisocyanates have been noted in workers. In order to assess the potential of isocyanates as sensitizers towards the respiratory tract, an animal model of pulmonary hypersensitivity to low molecular weight industrial chemicals was developed (Am.Ind.Hyg. Assoc.J. 39,546,1978) for toluene diisocyanate. Using the same procedures, we now report respiratory sensitization in guinea pigs as a result of repeated inhalation of aerosolized hexyl isocyanate-ovalbumin conjugate. Sensitivity was directed exclusively toward the hexyl isocyanate hapten portion of the conjugate antigen. PCA tests and gel diffusion techniques revealed solely hapten directed antibodies. These results, together with our prior account of guinea pig sensitivity to tolyl isocyanate, provide a method whereby the sensitizing potencies of isocyanates can be compared.

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172. PCB AND HCB INDUCED ALTERATION OF LYMPHOCYTE BLASTOGENESIS.
J.B. Silkworth and L.D. Loose, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY, 12208
Sponsor: F. Coulston

To extend the previous findings of our laboratory that polychlorinated biphenyl (PCB) and hexachlorobenzene (HCB) suppress humoral immunity and host defense, the influence of these chemicals on cell mediated immunity was assessed. C57Bl/6 mice were fed 167 ppm PCB 1016 or HCB for 41 weeks. Spleen cells were isolated and examined for their ability to respond to H-2 alloantigens (Bab/c, BDF1) in a one way mixed lymphocyte culture (MLC) and to respond to mitogen (PHA-M, LPS) stimulation. Spleen cells from mice fed PCB for 24 weeks demonstrated a 56% enhancement of the MLC response to BDF1 lymphocytes, whereas spleen cells from HCB treated mice demonstrated a 46% depression of the MLC response to Bab/c and BDF1 cells. Spleen cells from mice fed PCB for 13, 24 or 41 weeks demonstrated a time dependent enhancement of up to 64% above control values of mitogen responsiveness, whereas spleen cells from mice fed HCB demonstrated a transient suppression of up to 71% below control values and which returned to control values after 41 weeks. These findings indicate that chronic exposure to these chemicals can modify cell mediated immune mechanisms. JBS is a Monsanto Fund Fellow.
173. INFLUENCE OF POLYBROMINATED BIPHENYL ON IMMUNOLOGICAL AND HOST DEFENSE PARAMETERS. S. Muzinski, J.B. Silkworth, N.M. Wilson and L.D. Loose. Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY, 12208
Sponsor: F. Coulston

The influence of 3 and 6 week dietary administration of 5 or 167 ppm of polybrominated biphenyl (PBB) on endotoxin sensitivity and resistance to a challenge malaria infection was evaluated in male Balb/c mice. Control mice manifested an endotoxin LD50 of 373 µg whereas the LD50 for mice which received 167 ppm PBB for 3 weeks was 64 µg, representing a 5 X increase in endotoxin sensitivity, and mice which received 5 ppm PBB had no significant change in endotoxin sensitivity. Mice fed a diet containing 5 ppm PBB for 6 weeks manifested an LD50 of 413 µg of endotoxin, similar to that observed in controls, i.e., 403 µg. However, the animals which received 167 ppm PBB had an LD50 of 217 µg, suggesting a return toward control endotoxin sensitivity. The mean survival time of mice fed 5 or 167 ppm PBB for 3 or 6 weeks and then challenged with an intravenous inoculum of Plasmodium berghei, a murine malaria, was essentially unaltered from that observed in control mice. These immune alterations were not as profound as those demonstrated following exposure to the same dietary concentrations of PCB or HCB suggesting that the response to the organohalides is influenced by the halide. Supported by NIEHS 1T32-ES-0705801

174. THE ANTIBODY AND DELAYED TYPE HYPERSENSITIVITY RESPONSE OF MICE FED POLYBROMINATED BIPHENYLS. P.J. Fraker and S. Aust. Department of Biochemistry, Michigan State University, East Lansing, MI.

In order to determine the effects of polybrominated biphenyls (PBB) on the immune response, Balb/c female mice were fed meal diets containing 0, 1, 10, 100 and 1000 ppm PBB. The results were as follows: (1) After 30 days, the in vivo IgM and IgG primary antibody response of mice fed 1, 10 and 100 ppm PBB was about 80%, 30% and 12% respectively of control values. Determinations were done by the Jerne hemolytic plaque assay. Histopathology indicated preferential wasting of the cortex of the thymus. (2) After 14 days, 63% of the mice fed 1000 ppm PBB were dead. The remainder were athymic and essentially unable to mount an antibody mediated response. (3) The delayed type hypersensitivity response of mice fed 1, 10 and 100 ppm PBB for 31 days was normal. In contrast to other reports on the effects of PBB on rodents, these results indicate that PBB may have a deleterious effect on B-cells and helper T-cells. Whether or not PBB has an effect on those subsets of T-cells involved in cell mediated reactions remains open to question.

Nitrogen dioxide (NO₂) exposure results in the killing of Type I cells which are replaced by a Type II cell hyperplasia. The loss of the Type I cell barrier could allow excess antigen to pass into the interstitium of the lung and to the lung-associated lymph nodes (LALN) resulting in hyperimmunity. Adult male rats were immunized by intratracheal instillation of 1 x 10⁸ sheep red blood cells (SRBC) 24 hr before, and 24, 48 and 72 hr after the onset of inhalation to 25 ppm NO₂ for 24 hr. The number of anti-SRBC antibody-forming cells (PFC) in LALN, cervical lymph nodes (CLN) and spleen were evaluated 7 days after immunization. A sevenfold increase in the number of IgG anti-SRBC PFC (6,400 PFC/10⁶ lymphoid cells) in LALN were observed in rats immunized 24 hr after NO₂ inhalation. This enhanced IgG response decreased in time following NO₂ exposure and was suppressed at 7 hr. Rats immunized 24 hr before NO₂ inhalation exhibited an enhanced number of IgM anti-SRBC PFC in LALN. Mechanisms by which the immune response is altered may include an increased load of antigen to the LALN because of lung damage or alteration of a regulatory population of immune cells. (Research supported by DOE Contract EY-76-C-04-1013.)

176. PERSISTENT POSTNATAL EFFECTS ON THE IMMUNE SYSTEM FOLLOWING PRENATAL EXPOSURE TO THE ANTICHOLINESTERASE PESTICIDES DIAZINON OR CARBOFURAN. J. Spyker Cranmer, D. L. Avery and J. B. Barnett, Department of Pharmacology and Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Progeny derived from hybrid mice treated with 0, 0.18 or 9.00 mg/kg/day Diazinon or 0, 0.01 or 0.50 mg/kg/day Carbofuran were overtly normal at birth. When sacrificed at 3, 15 or 24 months of age, changes were recorded in the serum concentrations of IgG₁ and IgG₂a immunoglobulins of the offspring of mothers receiving either pesticide. The IgG₁ and IgG₂a concentrations of male offspring were generally increased, while those of female offspring were decreased. No systematic dose-effect relationship was observed for the range of doses tested. Treatment effects were most apparent at 3 months of age. No effects of pesticide exposure on IgM or IgG₂b concentrations were found, but the IgA levels of female offspring exposed to 0.01 mg/kg/day Carbofuran were found to be elevated at 15 months of age.

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177. INFLUENCE OF POLYBROMINATED BIPHENYL ON THE SPLENIC ANTIBODY RESPONSE IN THE MOUSE. N. Wilson, S. Mudzinski, J. Silkworth, and L.D. Loose. Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY, 12208
Sponsor: K-F. Benitz

Male Balb/c mice were fed diets containing 5 ppm or 167 ppm of polybrominated biphenyl (PBB) for 3 and 6 weeks. The primary (1⁰) and secondary (2⁰) antibody responses to sheep erythrocytes (SRBC) were determined following intravenous injection of 0.1cc of a 10% SRBC suspension by the direct splenic plaque-forming cell (PFC) method. The 1⁰ response was measured on days 3, 4, 5, and 6 and the 2⁰ response was measured on days 2, 3, 4, and 5 following immunization. PFC's/10⁶ spleen cells were reduced by 40% from controls in 167 ppm treated mice at the peak 3 week 1⁰ response period but only 15% in the peak 6 week 1⁰ response peak, possibly indicating immunological recovery. Spleen cell yields in the 167 ppm treated mice were 30% and 40% less than in controls at the 6 week 1⁰ and 2⁰ response peaks, respectively, contributing to significantly diminished PFC's/spleen in these mice. PFC's/spleen in 167 ppm treated mice also were less than in controls at the 3 week 1⁰ response peak. No impairment in antibody formation was observed in mice administered 5 ppm PBB suggesting a dose-related alteration. Supported by NIEHS 1T32-ES-0705801

178. SPECIES DIFFERENCES BETWEEN RATS AND MICE ON THE METABOLISM AND HEPATIC MACROMOLECULAR BINDING OF TETRACHLOROETHYLENE. A.M. Schumann, F.G. Watanabe, Dow Chemical, H&EER, Midland, MI 48640.

Tetrachloroethylene (Perc) has been reported by NCI to induce hepatocellular carcinoma in B6C3Fl mice (but not rats) following long-term exposure to high oral doses. Species differences regarding the metabolism and hepatic macromolecular binding of Perc were therefore evaluated in B6C3Fl mice and Sprague Dawley rats exposed to 10 or 600 ppm of ¹⁴C-Perc vapor for 6 hr or orally to 500 mg/kg. At 10 ppm, 63% of the total recovered radioactivity from the mouse appeared in the urine as nonvolatile metabolite(s) and 12% was excreted unchanged in expired air (19 and 68% respectively for the rat). The mouse metabolized 7-8 times more Perc per kg of body weight than did the rat following 10 ppm and 1.6 times at 500 mg/kg. Approximately 7-9 times more radioactivity was irreversibly bound to hepatic macromolecules in the mouse than in the rat at all exposure levels. No radioactivity was detected bound to purified hepatic DNA at times of peak macromolecular binding in the mouse. These results support the view that mice are more sensitive than rats to the hepatic effects of Perc due to greater metabolism of Perc to a reactive intermediate(s).
179. COVALENT BINDING OF HALOGENATED VOLATILE SOLVENTS TO SUBCELLULAR MACROMOLECULES IN HEPATOCYTES. M.L. Cunningham, A.J. Gandolfi, K. Brendel, and I.G. Sipes, University of Arizona, Health Sciences Center, Tucson, Az.

The bioactivation of $^{14}$C-labeled trichlorethylene (TCE), carbon tetrachloride (CT), and methylene chloride (MC) was studied with isolated rat hepatocytes (IRH). After a 2 hr incubation of the compounds with IRH under $O_2$ or $N_2$, lipids, proteins, RNA, and DNA were isolated and analyzed for non-extractable $^{14}$C. Maximal binding of TCE and MC to protein (0.41 and 0.19 pmole/mg/µmole substrate) and lipids (0.83 and 0.34 pmole/mg/µmole substrate) occurred under $O_2$, while CT bound highest under $N_2$ (5.6 pmole/mg lipid/µmole CT and 0.75 pmole/mg protein/µmole CT). Binding to protein and lipid for TCE and CT was linear for over 2 hrs and exhibited a dose response relationship. IRH from phenobarbital pre-treated rats showed increased lipid binding of TCE (300%) and CT (130%), and decreased binding of MC (30%). Decreasing GSH levels had no effect on MC binding, but decreased the binding of CT to lipid (50%) and TCE to protein and lipids (35%). While TCE and CT bound to DNA and RNA in the range of 200-900 fmol/mg/µmole substrate, MC bound in the range of 0.3-0.6 fmole/mg/µmole MC. (Supported by NIH # CA21820-01)


The relative in vivo hepatotoxicity of bromobenzenes bearing cyano, hydrogen, methyl, and trifluoromethyl substituents decreases in that order. To determine whether rate of metabolism and covalent binding in vitro would also correlate with substituent electronic character, these were measured in rats. It was found, as expected, that metabolic rate was faster with compounds bearing electron releasing substituents (methyl, hydrogen). All compounds became covalently bound to rat liver microsomes. The nontoxic methyl-bearing compound was bound fastest, followed by materials bearing cyano, bromo, and hydrogen substituents. It was also established that o-methylbromobenzene was metabolized primarily via the arene oxide pathway. It appears that substituted bromobenzenes are metabolized at rates predictable on the basis of substituent electronic character, but that there is a poor correlation between in vivo hepatotoxicity and the rate of covalent binding to rat liver microsomes among this series of compounds.
181. STUDIES ON THE METABOLISM OF ALIPHATIC NITRILES. A.E. Ahmed and K. Patel, Dept. of Pathol., Univ. of Texas Medical Branch, Galveston, TX. Sponsor: J. Palmer Saunders. The toxicity of aliphatic nitriles could be due to their ability to liberate cyanide and/or to alkylate cellular nucleophiles. To test these possibilities we compared the capacity of a series of aliphatic nitriles to liberate cyanide and to interact with the nucleophile GSH. Fasted rats were orally fed comparable LD<sub>50</sub> doses of KCN (10 mg/kg), propionitrile (40 mg/kg), butyronitrile (50 mg/kg), malononitrile (60 mg/kg), acyronitrile (VCN) (90 mg/kg), allyltcynamide (115 mg/kg) and fumaronitrile (150 mg/kg). Blood cyanide levels 1 hr after treatment ranked: KCN (6.6 ± 1.4 μg/ml) = malononitrile (7.4 ± 2.3 μg/ml) > other nitriles (0.4 to 2.3 μg/ml). Hepatic GSH levels at 1 hr after treatment were: VCN (30% control) > fumaronitrile (60%) > allyltcynamide (70%) with no depletion following KCN or any saturated nitrile. S-Cyanoethyl-N-acetyl-cysteine was detected in the urine of VCN treated rats. In vitro studies using liver homogenates showed that the enzymic rates of cyanide liberation from malononitrile, VCN and acetonitrile were 1865, 840, 390 nmoles/mg protein/min respectively. These findings imply that neither the relative extent of cyanide liberation nor of GSH depletion corresponds to relative toxicity of this series of nitriles.


To better understand the relationship between dose and neuropathy caused by acrylamide (AAm) and to search for a bio-monitoring technique, the fate of AAm-1,3-<sup>14</sup>C was studied in tissues including red blood cells (RBC) of rats. During 48 hr after a 50 mg/kg iv dose the concentration of radioactivity decreased in selected tissues but increased in RBC to a plateau which was from 10 to 90-fold greater than the concentration in other tissues examined. When AAm-1,3-<sup>14</sup>C was given daily by gavage or in drinking water at 30 mg/kg/day, the concentration in RBC rose to a plateau of about 400 μgE/g. During this regimen a foot-splay test indicated neuropathy developed at day 9, when the RBC concentration had plateaued. When 0.05 mg/kg/day was given by gavage the RBC concentration rose to about 1 μgE/g and no neuropathy was noted. Urinary excretion of radioactivity during 14 days of dosage was nearly constant from day-to-day, and most of the dose was excreted as two major metabolites and a small amount of the parent compound. The results suggest that a metabolite associated with RBC or one of the urinary metabolites may reflect the average daily exposure to AAm by all routes.
183. DECREASED INDUCTIVE RESPONSE OF MICROSOMAL ENZYMES TO PHENO-BARBITAL (PB) IN GENETICALLY OBSESE RATS. Charles Litterst, Lab. Toxicol., NCI, NIH, Bethesda, MD, 20014

The hepatic microsomal drug metabolizing enzyme (MFO) system in genetically obese (ff) Zucker rats is significantly less active than in either Sprague Dawley or in genetically homozygous normal (FF) Zucker rats. Hexobarbital sleep time is 73.8 min in obese rats and 43.4 min in FF controls. P-450 levels in obese rats are 60% of controls, and specific activities of aminopyrine (AmPy) demethylase and aniline hydroxylase are 30% of controls. 3-MC produced approximately equal increases in benzpyrene hydroxylase activity (600-700% of control) and P-450 levels (150-200% of controls) in both obese and control Zucker rats. PB (60 mg/kg daily x 4) was lethal to 1/2 the treated obese rats. There were no deaths in controls treated at 75 mg/kg. At 45 mg/kg daily x 3 control rats showed an 86% increase in P-450 levels vs only 7% increase in obese rats. Both aniline hydroxylase and AmPy demethylase activities in control rats increased (28% and 10% respectively) more dramatically than in obese rats (9% and 45%, respectively). The MFO system in FF Zucker rats generally responded as did the MFO system in Sprague Dawley rats. Microsomal enzymes in weanling obese rats responded to PB similarly to the enzymes in weanling controls.


Purified malathion insecticide is relatively non-toxic to mammals, but technical formulations contain several impurities that may inhibit malathion detoxification. In this study, four compounds that were previously isolated from technical malathion effectively inactivated rat serum malathion carboxylesterase, rat serum cholinesterase and rat liver carboxylesterase in vitro and in vivo. These inactivators are, in increasing order of potency, 0,0,5-trimethyl phosphorodithioate, 0,0,5-trimethyl phosphorothioate, 0,0,5-trimethyl phosphorodithioate, and 0,0,5-dimethyl S-(1,2-dicarboxyethyl)ethyl phosphorodithioate (the latter also known as isomalathion). In vivo, three of these compounds produced transient depressions of serum esterase activities, but one compound, 0,0,5-trimethyl phosphorothioate, produced long-lived esterase activity diminutions. Isomalathion, the most potent of the rat esterase inactivators, also inactivated a human liver malathion carboxylesterase preparation. These results support the view that isomalathion is a toxicologically-significant impurity of technical malathion.

The uptake of hexachlorobenzene (HCB) from the gastrointestinal tract by lymph was studied in rats and rhesus monkeys. In both species, lymphatic absorption is the predominant uptake mechanism. An equilibrium between lymph and adipose tissue concentrations was established. A steady increase in the fecal excretion of unmetabolized HCB could not be accounted for by biliary excretion. A parallel increase of HCB concentrations in the intestinal content and in adjacent lymph nodes from the small intestines to the colon, which was found two weeks after a single oral dose of HCB, indicates direct excretion of HCB into the intestinal lumen via the lymph. Based on this excretion mechanism, an explanation is proposed for the greatly enhanced excretion of stored HCB by rats and monkeys on a paraffin-fortified diet.


Due to extensive environmental contamination involving kepone, information regarding the effect of exposure to this pesticide, during gestation and lactation, on the metabolism of other toxicants is important. Timed pregnant Sprague-Dawley rats, individually housed, received daily p.o. doses of either 0, 0.1, 1.0, or 1.5 mg kepone/kg from day 2 of gestation to day 21 postparturition. At day 21, 5 female pups from each treatment group were transferred to individual metabolism cages and all were dosed with 1.66 mg of lindane (containing 1.81 uCi of 14C-lindane). Within 24 hours there was a significant dose-related increase in excretion and a decrease in the tissue storage of radioactivity. Exposure to kepone also resulted in dose-related increases in the proportions of PCCOL, 2,3,4,6- and 2,3,4,5-tetrachlorophenol and a decrease in the proportion of 2,4,6-trichlorophenol excreted. Results of this study indicate that exposure to kepone during gestation and lactation can significantly alter the detoxification of other environmental chemicals.
187. ALLYLAMINE CARDIOTOXICITY AND CARDIAC MAO: MODULATION BY MAO INHIBITORS. P.J. Boor and T.J. Nelson, Dept. of Pathol., Univ. of Texas Medical Branch, Galveston, TX. Sponsor: Mary Treinen Moslen

Monoamine oxidase (MAO) may play a role in allylamine (AA) cardiotoxicity as evidenced by the virtual elimination of AA-induced myocardial fibrosis in animals simultaneously given MAO inhibitors (Fed. Proc. 36(3), 397, 1977). We studied MAO activity in rats given 1.1 mM AA, 1.4 mM Semicarbazide (SC), 2.2 mM hydroxyamphetamine (HY), AA + SC or AA + HY in drinking water for 3 weeks. Activity of MAO for three substrates (5-hydroxytryptamine, tyramine and \( \beta \)-phenylethylamine) was assayed in heart, liver and brain homogenates. All hearts were graded for histopathologic lesions. AA produced severe fibrosis whereas little or no histologic evidence of myocardial damage was found in the other groups. In AA animals, MAO was depressed in both brain and liver, and was usually markedly increased in heart tissue. In SC and HY animals, MAO was relatively unchanged in all tissues. In the AA + SC and AA + HY groups, MAO was decreased in both brain and liver similar to the AA group while, in contrast to the AA group, heart MAO was unaltered. The extent of MAO increase found in AA animals directly correlated with the degree of histologic lesion (p<.02). The increased MAO appears to be localized in scar tissue.

188. TRANSPORT OF BIS-(p-CHLOROPHENYL)-ACETIC ACID (DDA) IN THE ISOLATED, PERFUSED RAT KIDNEY. E. J. Koschier, M. F. Stokols, P. Cattrall, M. Acara and S. K. Hong. School of Medicine, SUNY at Buffalo, Buffalo, N. Y. 14214.

Since DDA has been shown to be transported and concentrated by the renal proximal tubule, this metabolite of DDT has been postulated to be a potential nephrotoxic agent. The present study explored the renal transport of DDA in the perfused rat kidney. When DDA (0.6μM) was present in a dextran perfusate which eliminated DDA-colloid binding, the DDA/insulin clearance ratio was ≈0.05. In addition, DDA had no effect on the glomerular filtration rate and the fractional reabsorption of Na, K or H₂O. To determine the concentration of DDA which would produce an effect on renal cellular function, studies were performed with renal cortical slices. DDA at media concentrations >0.1μM were needed to produce significant alterations in tissue oxygen consumption and intracellular electrolyte composition. In conclusion, DDA at a 0.6μM perfusate concentration undergoes net tubular reabsorption without affecting renal function. Only high concentrations of DDA were shown to produce alterations in cellular function. (Supported by USPHS NIH Grants AM05437, AM18918 and AM17201.)
189. **KIDNEY LESIONS IN HAMSTERS FED MODIFIED FOOD STARCH**  

The objective of this study is to determine how the hamster responds to the ingestion of modified food starch. 100 Syrian Golden hamsters (50 male, 50 female) were divided equally into five groups and fed one of four modified food starches or a control unmodified food starch at 30% of the diet. Histologic examination of the kidneys revealed an incidence of lesions consisting of tubular dilation and cortical scarring with the severity dependent on the modification of the starch and the degree of substitution. Animals fed the hydroxypropyl starch (the most lesion producing) had significantly higher BUN values, serum calcium and serum phosphorus values. Current studies which supplement the diet with magnesium appear to inhibit the development of the lesion indicating that the mechanism involves complex interactions between starch modification treatment and mineral status of the animal. (Supported in part by grants from Staley, Natl. Starch and American Maize Companies).

190. **PRECLINICAL TOXICOLOGY OF (8β)-(8-[(METHYLPHTHO)METHYL]-6-PROPYLERGYLINE METHANESULFONIC ACID SALT (MPE) - A NEW PROLACTIN INHIBITOR.**  

MPE is a semi-synthetic tetracyclic compound derived from the ergot alkaloids. It is a dopamine agonist with potent prolactin inhibiting activity and minimal CNS and blood pressure effects. The oral LD₅₀ values for mice and rats respectively were 54 and 15 mg/kg for males and 07 and 34 mg/kg for females. The oral LD₅₀ in dogs and monkeys was greater than 25 mg/kg. Ninety-day oral studies were conducted in rats and dogs. Dose levels were 0, 0.002, 0.004, 0.008, 0.012 and 0.02% in the diet of Fischer 344 rats and 0, 1, 2, and 5 mg/kg by capsule in beagle dogs. Dose-related growth retardation occurred in rats of all MPE dose groups. All females given MPE had increased ovarian weights, the result of accumulated corpora lutea due to inhibition of luteolysis. The main effect in dogs was emesis which occurred shortly after dosing during the first few days and occasionally toward the end of the study.

The effects of diethylstilbestrol (D.E.S.) and cholesterol on the abdominal aortic wall were studied in ovariectomized Fischer rats. One group was kept as control, a second as ovariectomized control, and the third received 20 mg of D.E.S. and 50 mg of cholesterol for 160 days. Both compounds were implanted subcutaneously as a pellet. Light microscopy was conducted, and tritiated leucine (3H leu) uptake was monitored as a marker of newly synthesized protein. Neither lipid deposition nor any change in the mucopolysaccharides level was seen anywhere on the aortic wall. The combined administration of D.E.S. and cholesterol does not cause any hypertrophy or hyperplasia of the smooth muscle of the abdominal aorta. Instead, a reorientation and degeneration of the muscle fibers were found. There was also loss of the normal corrugation of the elastic fibers. All the rats of group three had anterior pituitary tumors.

192. ENHANCEMENT OF HEXOBARBITAL HYPOnosis BY DIPHENHYDRAMINE METHYLAMMONIUM BROMIDE AND DIPHENHYDRAMINE. R T. Louis-Ferdinand, F. Beuthin, E. Roginski and M. Stout. College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, MI

Previous studies from our laboratory have suggested that inhibition of hepatic hexobarbital (H) metabolism by diphenhydramine (D) contributes to the mechanism by which this antihistamine enhances H hypnosis. The present studies were conducted to determine the effect of D on CNS sensitivity to H measured by the 'silent second' method. The administration of D (10, 25 or 50 mg/kg, i.p.) did not alter the dose of H required to abolish EEG activity for one second. Although 121, 136 & 154% increases in H sleep time were produced. Pretreatment with Diphenhydramine methylammonium bromide (DMAB) (12 mg/kg, i.p.) did not produce a significant increase in H sleep times of male Swiss Webster mice. However DMAB (30 mg/kg, i.p.) increased H sleep time by 137% of control. The results suggest that the mechanism responsible for the potentiation of H hypnosis by D may be pharmacokinetic rather than pharmacodynamic in nature.

Fischer-344 rats of both sexes were studied for toxicologic responses to aniline HCl when fed 10, 30, 100, 300, and 1,000 mg/kg/day. The lowest dosage caused little toxic response after one year. Otherwise, the spleen was primarily affected in a dose- and time-related manner and was grossly enlarged and, histologically, the capsule was diffusely thickened. Bands of fibrous hyperplasia extended centrally into the parenchyma. The red pulp was hyperplastic, contained excess hemochromatin and showed signs of extramedullary hematopoiesis at six months which became progressively more severe at twelve months. Dose-related circulatory responses, i.e., methemoglobinemia, reticulocytosis, Heinz bodies, anemia and marrow erythroid hyperplasia suggested that erythropoiesis was stimulated. Separate pharmacokinetic studies with 14C-aniline suggested that systemic accumulation should not have occurred since radiolabel was largely recovered in urine and plasma radioactivity fell to less than 2% at 24 hr in naïve rats and rats pretreated with aniline at the same dose for 7 days. However, the RBC's showed a significant and progressive bioretention of radioactivity compared with plasma which may be related to the splenic changes noted.

194. LIGHT MICROSCOPIC AND ULTRASTRUCTURAL CHANGES IN MOUSE LIVER AFTER REPEATED ORAL LAAM ADMINISTRATION. R.S. Nair, I.T. McClurkin, and L.W. Masten, Depts. of Pharmacol. and Biol., Univ. of Mississippi, University, MS. Sponsor: W.M. Davis

The effect of 1-α-acetyl-methadol (LAAM) on liver morphology was investigated since it is an inducer of liver microsomal metabolism. Male ICR mice (27-30 g) were intubated daily with 28 mg/kg LAAM HCl and sacrificed 24 hrs after 1, 2, 3 and 6 days of administration; the livers quickly removed and placed in Mallonig's fixative. The liver was embedded and stained for light (LM) and electron microscopy (EM). On day 1, there was marked fatty accumulation compared to controls, which was confirmed by Sudan IV stained sections. At the EM level, large lipid vacuoles were surrounded by mitochondria (Mt) and glycogen granules (GG). Cells showed diffuse vacuolization but lacked lipids on day 2 with LM. Ultrastructurally, Mt swelling, increased smooth endoplasmic reticulum (SER), and decreased GG were observed. At day 3, centrolobular accumulation of lipids was noted. SER content was still increased, a few Mt were swollen, small lipid vacuoles were present and GG reduced. There appeared to be some reversal of the earlier observed LM and EM changes by day 6. (Supported by the Univ. of Miss. Res. Inst. of Pharm. Sci. and NIDA Grant DA 01331-02)
195. **EIGHT YEAR TOXICITY STUDY IN MONKEYS AND REPRODUCTION STUDIES IN RATS AND RABBITS WITH A NEW HYPOCHOLESTEROLEMIC AGENT, PROBUCOL.** J. A. Molello, D. J. Thompson, and J. E. LeBeau, Toxicology Laboratory, Health and Consumer Products Dept., Dow Chemical U.S.A., Indianapolis, IN.

Probucol, 

\[4,4'-(\text{isopropylidenedithio})\text{bis}(\text{2,6-di-t-butylphenol})\]  

was given po to 40 male and female rhesus monkeys at doses of 0, 60, 125, 250 and 500 mg/kg/day. A total of 14 monkeys were killed at 18 and 24 months; 21 remained on test for 8 years. Monitored parameters included growth rate, hematology, clinical chemistries, urinalyses, organ weights, gross, histopathologic and EM studies. Five deaths occurred, none related to treatment. No significant differences were observed between treated and control groups. This study provides data not generally available for rhesus monkeys.

To evaluate the effects of probucol on fertility and postnatal development, doses of 0, 100, 500 or 1000 mg/kg were given po to Sprague-Dawley rats during the appropriate time intervals. In teratological studies, the same dose levels were given during organogenesis or, in some cases prior to breeding and throughout organogenesis, to S-D rats and NZW rabbits. No adverse effects on fertility or postnatal development were observed in rats, and no evidence of fetotoxicity or teratogenicity was observed in either species.


The effect of probucol on liver cell ultrastructure was studied. Sprague-Dawley rats were divided into 4 groups and given 0, 250 or 500 mg probucol/kg/day or 250 mg clofibrate/kg/day po for 91 days, during which time randomly selected animals from each group and sex were periodically sacrificed. The clofibrate group was used as a reference control. Light microscopy revealed granulation in hepatocytes from clofibrate controls whereas the morphology of hepatocytes of probucol-treated rats was comparable to untreated controls. EM indicated the granularity to be peroxisomal enlargement and proliferation. Hepatocytes from probucol-treated rats displayed normal profiles including typical numbers and sizes of peroxisomes. Similarly, comparisons of liver ultrastructure from probucol-treated and control rhesus monkeys revealed no significant differences. Opinions differ regarding the meaning of peroxisomal alterations, i.e., whether they represent pathologic or physiologic changes. In either event, these data indicate noninvolvement of probucol with this organelle.

A previous communication (Fed. Proceed, 37, 505, 1978), described the actions of milk and water, in contrast to weak acids (citric acid and vinegar), in protecting against the lesions induced by alkali on the tongue, esophagus and stomach in a rabbit model. A scoring system was developed to measure effectiveness against chemical burns caused by 1 ml NaOH (1, 2.5, 5 and 10% conc.) administered onto the posterior tongue and compares the histological changes that occurred at 24 and 72 hours after first-aid treatment with these agents. Two basic processes are involved: inflammation, characterized by mucosal congestion, edema and inflammatory cell infiltration and corrosion, manifested by coagulation necrosis or ulceration with or without thrombosis and in the stomach, hemorrhage and pigmentation. Lesions were graded according to intensity (mild, moderate, or marked) and degree of penetration (mucosal, muscularis, or soft tissue serosa). In general, the intensity and degree of penetration of the lesions were reduced by the rapid administration of milk or water within 30 seconds of the alkali poisoning as judged from the lesion scores 24 and 72 hrs. after exposure and treatment.


A method has been developed allowing the use of rodents for predicting the vaginal irritancy of materials intended for retention in the vagina. Test materials, in the shape of a cylinder, were permanently retained in the vagina by surgically altering the vulva. To investigate the sensitivity of the guinea pig model, chemicals of known irritancy were exposed to the vaginal mucosa for 7 days and a gross and histopathological evaluation was conducted on the reproductive tract. A nonirritating medical grade silicone rubber elicited minimal microscopic inflammatory lesions in some animals apparently due to mechanical trauma. Addition of dibutyltin diacetate, a strong tissue irritant, to the silicone rubber at concentrations of 0.2 to 2.0% (w/w) elicited a dose-dependent ulcerative vaginitis. Selected surfactants, hexadecyltrimethylammonium bromide (CTAB), sodium lauryl sulfate (SLS), and nonoxynol 9 (N-9), were administered to the vaginal mucosa in a slow release fashion. The responses predicted from a knowledge of skin irritancy were confirmed: (most irritant) CTAB>SLS>N-9. We conclude that the model will have value in determining the vaginal irritancy potential of selected chemical materials.

The use of quality controlled diets in toxicology studies has been proposed by advisory and regulatory authorities. The levels of 16 contaminants and the variation in the levels of 17 nutrients have been assayed in 39 batches of a rodent diet (PCD) manufactured over a 10 month period. PCD is a fixed formula diet based on natural ingredients. The maximum levels of contaminants detected were: nitrite 4.1 mg/kg, lead <1.0 mg/kg, arsenic 0.6 mg/kg, cadmium 0.9 mg/kg, mercury 0.08 mg/kg. Aflatoxins, PCB, Salmonella and E.coli were never detected. DDT was detected 16 times (<0.1 mg/kg), diefenrin 5 times (once 0.02 mg/kg), lindane 38 times (twice <0.1 mg/kg), heptachlor 3 times (<0.005 mg/kg) and malathion 28 times (<0.75 mg/kg). T.V.O. was between 5-100 x 10³/g. The content of macronutrients was usually within ±20% of specification. The micronutrients were within the nutritionally desirable range. These data illustrate the quality of diet that has been achieved by selection of ingredients and dedication of a mill to the production of quality assured laboratory diets.


Many toxicological studies require continuous monitoring to fully evaluate the subtle yet significant effects that may be manifested when testing compounds to ensure safety. The method most commonly utilized is interim clinical chemistry determinations. However, diverse techniques for the collection of blood may affect the values for the various parameters. This study was designed to evaluate the differences between 3 of these techniques: segmental tail amputation, orbital sinus puncture under ether anesthesia, and orbital sinus puncture without anesthesia. Sixty male and 60 female Fischer 344 [C57B1] derived rats were used in the study. Blood samples were taken at initiation, at 4, 13, and 26 weeks and were analyzed for hematology and clinical chemistry. The results of these experiments indicate variability between experimental groups. Interestingly, the results may be confounded by an age factor since the initial blood collection (6-8 weeks of age) showed little variability between groups. However, as the animals matured the differences between parameters became more evident.
201. THE USE OF VENTILATED CAGES IN RAT STUDIES. 
E.R. Adams, K.S. Newman, W.R. Gibson, D.C. Hoffman, and 
D.M. Morton, Toxicology Division, Lilly Research 
Laboratories, Greenfield, IN.

An animal cage/rack in which room air is exhausted 
through individual cages, was designed to reduce 
exposure of technicians to animal dander, and 
lessen allergic reaction. The use of ventilated 
cages in rat studies in which test chemicals are 
given in the diet was evaluated. In comparison 
with a conventional rack using Fischer 344 rats, 
no differences in survival, growth, food intake, 
or health status occurred over 10 months. Using 
sodium fluorescein as a tracer, differences in 
contamination of personnel and/or environment 
were studied. The greatest contamination oc-
curred during feeding. Of the 19 types of sam-
pling sites, only 1 showed a statistically signif-
icant difference. The number of contaminated 
control rats was higher in conventional hanging 
cages, suggesting that ventilated cages may 
reduce cross-contamination between test groups. 
In these studies, ventilated cages did not ad-
versely influence animal health; neither did they 
limit non-volatile test chemical contamination.

202. THE TOXICOLOGY DATA BANK--EVALUATION OF DATA FOR AN ON-LINE 
DATA RETRIEVAL SERVICE. H.M. Kissman, National Library of 
Medicine, Bethesda, MD.

The Toxicology Data Bank (TDB)--a newly-developed, on-line, 
interactive data retrieval service provided by the National 
Library of Medicine--includes data describing the chemical, 
physical, biological, and usage properties of known or 
potentially toxic compounds to which people are exposed. 
Data, which can be numeric or verbal, are extracted from 
some 80 monographs, handbooks, criteria documents, or other 
"evaluated" sources. The extracts are further evaluated for 
accuracy, completeness, and currentness by a Peer Review 
Committee composed of pharmacologists, toxicologists, and 
analytical chemists, who are also members of the NIH Toxi-
cology Study Section. The TDB is searched on-line by look-
ing for terms in data categories, index fields or anywhere 
in the text of records. The TDB contains some 1,500 com-
pound records and references to another 1,000 records that 
are being processed. The paper describes the design and use 
of the TDB with particular emphasis on peer review evalu-
ation as a consensus-forming process.
203. A DATA ACQUISITION SYSTEM FOR TOXICOLOGY LABORATORIES. 
R. Kahlow and L. Parker, Hazleton Laboratories America, 
Inc., Vienna, Va. Sponsor: M. Steinberg

Following a review of various data processing configurations, Hazleton has developed a new data acquisition system combining the advantages of multi-locational processing with intelligent terminals and the power of a central computer. A direct on-line approach was avoided in favor of intelligent terminals because of the comparatively large distance between the seven laboratories involved.

Critical updating of toxicological data takes place in the laboratory with immediate response time. More sophisticated editing is achieved by dial-up communication between the distributed terminals and the central computer. Problems such as security of data, automatic balance accuracy, technician error, animal identification, data base integrity and scientific supervision have been addressed and resolved.

204. A NEW APPROACH TO AN OLD METHOD — A BASIC PROGRAM FOR THE CALCULATION OF THE ED50 OR LD50 USING PROBIT ANALYSIS.
J. D. O'Neil, D. J. Brown, and R. S. Forney. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

Several methods used in evaluating bioassays where quantal responses are involved use graphical approximations to the more detailed and precise calculations involved in probit analysis. With the availability of digital computers, there is no reason to continue using graphical methods developed to make a mathematically laborious technique more accessible to investigators using only a pencil and slide rule. The BASIC program described provides estimates of the parameters in the probit regression equation using an iterative maximum likelihood method outlined in Appendix I of Finney's Probit Analysis. Output of the program includes an estimate of the ED50 or LD50 and its 95% fiducial limits. Information is also provided which allows one to obtain potency ratios and fiducial limits when multiple assays are performed. A Chi-Square is calculated to test the statistical validity of the procedure. The complete analysis requires only a few minutes at a terminal; the computer time required is less than one second. (Supported in part by USPHS Grant T32 GM 7097.)

The US Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) have completed the first operational year of joint data audits and Toxicology laboratory inspections performed under the terms of an Inter-Agency Agreement (FR 43, No. 65, 4/4/78). This Agreement was initiated as a result of the concern expressed publicly by both Agencies regarding the reliability of toxicity data generated to fulfill federal regulatory requirements.

Approximately 70 joint inspections/audits were conducted during FY'78. The results of this survey relative to the quality of toxicity data submitted to US regulatory agencies will be discussed.

Although the emphasis during the first year of the joint program has been primarily on health effects testing for pesticides, the EPA will be utilizing FDA personnel to carry out their health effects audits under the Toxic Substances Control Act. The health effects standard for Quality Assurance will be similar to those prepared by the FDA under their CLP regulations. Environmental effects and chemical fate testing audits will be carried out by EPA. Standards for carrying out these practices are now being developed and promulgated by EPA. International implications for Quality Assurance standards will also be discussed.


The H-2PI computerized toxicology system consists of a DEC PDP-11/55 computer with 128K words of memory and a RP06 disk storage unit. It uses the FORTRAN IV-Plus language and the RSX-11M (time critical, multi-user) operating system. In the animal weight and food consumption area, a data collection station consists of a Hazeltine Modular One CRT terminal and an electronic balance, interfaced to the computer on a single serial line. The station is mobile and designed to be used in any of the animal rooms. The animal weight and food consumption programs allow for real-time entry of weights, validation of scale and animal or food weight accuracy, and calculation of a variety of consumption parameters including the amount of drug and food needed for mixing for the next feeding period. Both areas have outputs that include statistical analyses, printed raw data for archival storage and report-ready copy of individual and summary data. Historical edit files are maintained for each study and are used for validation of final reports.

The clinical observation programs are designed to be used in conjunction with or separate from the body weight and/or food consumption programs. They allow for entry of weekly observations in serial, random or subsample sequence, or entry of PRN observations on individual animals around-the-clock. The data collection station is a Hazeltine Modular One CRT terminal utilizing a menu format for observational display and tabulation. Frequently occurring findings can be entered using coded keys within observational categories. Categories and coded findings are study-specific and may be expanded during the study if needed. Findings may be qualified with free text observations or specified location codes. A palpable nodule program which can be used in conjunction with or separate from the observation programs is used to maintain detailed accounts of nodules to allow for correlation of information obtained at necropsy. Outputs include tabulated observations by individual animals or study groups.


We investigated the effects of a cholinergic blocker given during pregnancy on the autonomic nervous system post partum in the dam and in the progeny. Holtzman albino rats were injected sc with atropine sulfate at 0.1 or 0.5 mg/kg/day from days 11 to 18 of pregnancy; controls were given saline. Their progenies were cross-fostered. Ten to 20 rats were used for each test of autonomic nervous system function. In the dams, statistically significant effects that lasted about a week after the end of atropine treatment were 1) bradycardia and hypothermia and 2) an enhanced negative chronotropic response to sc injections of pilocarpine at 40 mg/kg. In the progeny, significant effects were tachycardia lasting for 2 weeks and mydriasis lasting for a week after eye-opening; sensitivity to pilocarpine was not altered. Data are consistent with the development of a cholinergic receptor supersensitivity in the dams. The changes in the progeny suggest enhanced development of the adrenergic system.
209. EXPOSURE OF MICE TO CH₂Cl₂ AND CH₃OH ALONE AND IN COMBINATION
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Biophys. Univ. of Rochester Sch. of Med. and Dent.,
Rochester, NY

Biological effects of exposure to single toxic agents
have been far more frequently studied than have effects of
exposure to combinations of such agents. Mice inhaling 500
ppm CH₂Cl₂ together with 1000 ppm CH₃OH 6 hrs daily, 5 days
per week for 3 weeks showed a lesser increase in hepatic tri-
glyceride concentrations than was seen in mice exposed to
CH₂Cl₂ alone at the same concentration. Histological changes
were not induced in the liver by either agent at these con-
centrations, alone or in combination. The 24 hr LC50s for
30 g male mice exposed 6 hrs to CH₂Cl₂ only or to CH₃OH only
were 16100 ppm and 41000 ppm resp. The LC50 for CH₂Cl₂ in
the presence of 40600 ppm of CH₃OH was significantly reduced;
the LC50 for CH₃OH in the presence of 15700 ppm CH₂Cl₂ was
lowered, but not significantly. Keratoconjunctivitis was
seen in mice exposed acutely to CH₃OH alone or with CH₂Cl₂.
Morphological changes in the liver were seen in mice exposed
acutely to either agent alone, or to both in combination. It
is concluded that chronic concurrent exposure to CH₃OH offers
some protection against the hepatic effects of continued ex-
posure to CH₂Cl₂ at concentrations approximating the OSHA
standard for CH₂Cl₂.

210. GLUTATHIONE, EPOXIDES AND MULTIPLE DETOXIFICATION PATHWAYS
IN THE METABOLISM OF 1,1-DICHLOROETHYLENE. M. Andersen,
O. Thomas and L. Jenkins, NMRI/TD, Wright-Patterson AFB OH.

Toxic metabolites of 1,1-dichloroethylene (1,1-DCE) react
with hepatic glutathione (H-GSH). Pretreatment of rats with
epoxypropanol (EP) or styrene oxide (SO) increases 1,1-DCE
toxicity. Epoxides also deplete H-GSH. We studied the role
of H-GSH in epoxide-induced enhancement of the oral and
inhalation toxicity of 1,1-DCE in fasted, male rats. SO,
EP, cyclohexene oxide (CHO) and butadiene monoxide (BMO)
all caused similar, prolonged depletion of H-GSH. Neither
CHO nor BMO were as effective as EP or SO in increasing
1,1-DCE toxicity. The 2 hr LC50 of 1,1-DCE in rats pre-
treated with CHO, SO and EP were, respectively, 569, 163
and 92 ppm. Using kinetic constants of 1,1-DCE metabolism
estimated from a gas uptake studies, fasted rats were found
to die after metabolizing 26-28 mg of 1,1-DCE/kg. The above
LC50 values are equivalent to 20.0, 10.4 and 6.8 mg metabo-
lized/kg, respectively. Epoxide-induced enhancement of 1,1-
DCE toxicity depends on H-GSH depletion, but more impor-
tantly on the particular epoxide used. GSH-Independent
pathways (inhibitable by certain epoxides) may play a major
role in detoxifying reactive metabolites of 1,1-DCE.
211. INFERENCE CONCERNING METABOLISM OF INHALED TOXICANTS BASED ON RATES OF TOXICANT DEPLETION FROM RECIRCULATED ATMOSPHERES. M.E. Andersen, M.L. Gargas and L.J. Jenkins, Jr., NMRI/TD, Wright-Patterson AFB OH.

During exposure to low-solubility gases, blood:gas equilibrium is rapidly achieved. Metabolism, tissue loading and excretion reduce in vivo levels causing further uptake of toxicant. Kinetic parameters for metabolism can then be inferred from the concentration-dependence of rates of gas uptake from recirculated atmospheres. We measured rates of uptake in rats for (1) CH₂=CH₂, (2) CF₃=CH₂, (3) CH₃Cl, (4) BrCH=CH₂, (5) Cl₂C=CH₂, (6) CH₂=CHOCH₃, (7) Cl(CH)=CHCl, (8) halothane, (9) methoxyflurane and (10) CH₃Br. With (1&2), which have very low H₂O solubilities, rates of uptake cannot be accurately measured. Rate curves of (3-6) were described by \( v = \frac{V_m}{(K_m+ppm)} \), equ(I). With (7-10) they had a mixed form, \( v = \text{equ}(I) + k_1 \text{ppm} \). Saturable uptake appeared to be enzymatic metabolism. First-order processes could be tissue loading, or with (10), non-enzymatic metabolism. Gas uptake studies will be of limited use for certain toxicants (1&2). When properly applied (3-10), they provide a convenient means to determine kinetic parameters of metabolism in vivo.

212. POTENTIATION OF CARBON TETRACHLORIDE-INDUCED HEPATOXICITY BY 1,3-BUTANEDIOL. C.L. Plaga, W.R. Hewitt, and M.G. Côté. Dépt. de Pharmacol., Univ. de Montréal, Montréal, Canada.

Rats with uncontrolled alloxan-induced diabetes exhibit an increased sensitivity to the hepatotoxic action of CCl₄. This effect has been suggested to result from the induction of a metabolic ketosis after alloxan treatment. To test this hypothesis, the hepatotoxicity of CCl₄ was evaluated in rats rendered ketogenic with 1,3-butanediol (BD). Male Sprague-Dawley rats were treated (po) with BD (5 g/kg) 3 times daily for 2 days. On Day 3, rats received CCl₄ (0.1 ml/kg, ip) 1 hr after the initial dose of BD. BD treatment was continued and liver injury assessed 24 hr after CCl₄ treatment, using plasma GPT and OCT activity, and hepatic G-6-Pase activity. BD pretreatment alone did not produce liver injury. The CCl₄ challenge dose alone did not alter plasma GPT or OCT activity, but reduced G-6-Pase activity 17%. In contrast, treatment with BD plus CCl₄ markedly elevated plasma GPT and OCT activity, and reduced G-6-Pase activity 44%. These results support the concept that a state of uncontrolled diabetic ketosis contributes to the potentiation of CCl₄ hepatotoxicity. (Supported by Health and Welfare Canada and the Chemical Industry Institute of Toxicology).

Twenty-four hours following administration of CCL₄ (1 ml/kg, i.p.), plasma levels of aspartate aminotransferase were elevated in adult rats and in rats as young as 4 days old. Similarly, treatment with CCL₄ produced the same degree of triglyceride accumulation in liver of adult rats and rats as young as 4 days old. The presence of microsomal conjugated dienes, a measure of lipid peroxidation, was observed in adult and developing rats treated with CCL₄. An vitro binding of ¹⁴CCL₄ to hepatic microsomal protein and lipid was significantly lower in 4 or 14 day old rats than in adults. The results demonstrate that young rats are as susceptible as adults to CCL₄-induced hepatotoxicity even though they have a lower capacity to metabolize CCL₄. The mechanism for the high degree of CCL₄-induced liver damage in young rats is not known but may be similar to the mechanism by which alcohols and diabetes potentiate CCL₄ toxicity. Whole blood concentrations of acetoacetate were 3-5 times higher in young rats than in adult animals and potentiation of CCL₄-induced hepatic damage by alcohol pretreatment and diabetes may be due to the ketosis produced by these treatments (Supported by USPHS grants AM-14513, ES-01142, GM-30996).


The dose-dependent release of alanine aminotransferase and aspartate aminotransferase into plasma twenty-four hours following CCL₄ (i.p.) was markedly lower in rats pretreated for 3 days with zinc chloride (150 μmoles/kg/day). This dose of zinc produced a 2100% increase in hepatic concentration of MT. Following administration of ¹⁴CCL₄ to zinc-treated rats, radioactivity was present in the same elution volume as MT following gel filtration (G-75) of liver cytosol. Metallothionein may protect against CCL₄-induced liver damage by sequestering reactive metabolites of CCL₄. However, treatment with zinc also resulted in a 34% decrease in the concentration of cytochrome P-450 in hepatic microsomes and a 50% decrease in irreversible binding of ¹⁴CCL₄ to hepatic microsomal protein and lipid. The relative importance of decreased metabolism and MT in reducing CCL₄-induced hepatotoxicity following zinc pretreatment is not presently known. (Supported by USPHS grants ES-01142 and GM-30996).

Hexachloroethane (HCE), at dose levels of 0, 1.5, 20 or 80 mg/kg body weight, was fed to 40-45 day old CDF Fischer 344 rats for 110 days. Males given 20 or 80 mg/kg/day had histopathologic alterations of the liver and kidneys and increased urinary excretion of uroporphyrin, creatinine and delta-aminolevulinic acid; the latter two parameters were also increased in males at the 1.5 mg/kg level. In contrast, effects in females were limited to slight histopathologic changes of the liver at the high dose. Liver, kidney, blood and adipose tissue was analyzed from male rats on test for 57 days. The concentration of HCE in the kidneys of male rats was significantly higher at all dose levels when compared to females (μg HCE/g of kidney with increasing dose - males: 1.4, 24.3, 95.1; females: 0.4, 0.7, 2.0); this is consistent with the more pronounced renal toxicity noted for male rats. However, the results of the tissue analysis indicated that HCE was cleared in an apparent first-order manner with a half-life estimated to be 2-3 days.

216. TOXICOLOGY OF 2-CHLORO-1,3-BUTADIENE (CHLOROPRENE): ACUTE EFFECTS IN LIVER AND LUNG AFTER INHALATION EXPOSURE IN RATS. H. Plugge and R.J. Jaeger, Inhalation Toxicology Laboratory, Harvard School of Public Health, Boston, MA 02115

Chloroprene (CBD), used in synthetic rubber, is acutely hepatotoxic, produces pulmonary irritation and is associated with occupational cancer. Charles River or Holtzman rats were exposed to concentrations of 100, 150, 225, and 300 ppm CBD for 4 hr, and killed at 24 hr. Liver injury as measured by serum sorbitol dehydrogenase was apparent in fasted animals exposed to 225 and 300 ppm CBD. Nonprotein sulfhydryl (NPSH) levels in liver were increased 24 hr after all exposures. Polychlorinated biphenyl (PCB, 54% chlorine content) pretreatment prevented both liver injury and increased liver NPSH levels after exposure to 100 and 300 ppm CBD. Decreased lung NPSH was also prevented by PCB treatment. Serum lactate dehydrogenase (LDH) activity increased after exposure of rats to 300 ppm CBD. Acute lung injury was quantitated by determining LDH activity in lung lavage fluid. No significant increase in lavage LDH activity was found. The data suggest that CBD and Vinylidene chloride bear a similarity with respect to the effect of PCB pretreatment. Supported by NIEHS ES-00002. RJJ recipient of RCDA from NIEHS (ES-00027).
217. INHALATION TOXICITY OF MONOCHLOROMONOFUOROMETHANE. W.B.
Coate, R. Voelker and R.W. Kapp, Jr., Hazleton Laboratories
America, Inc., Vienna, VA; J. Anderson and J. Charm, Allied
Chemical Company, Morristown, N.J. Sponsor: M. Steinberg.

The toxicity of monochloromonomofluoromethane was studied in
rats and cynomolgus monkeys. Rats exposed to 2% v/v for 4 hr
showed CNS depression as did monkeys exposed to 1%. One of 2
monkeys so exposed died 4 days later. Rats, but not monkeys,
exposed to 0.5% 6 hrs/day for 20 days showed minimal to slight
hypospermato genesis. This was not seen at 0.1%. Five of 8
monkeys exposed to 0.5% for 19-20 days died with severe
epistaxis and 5 had centrilobular to diffuse hepatocytic
swelling. These effects were not seen in monkeys at 0.4%.
Rats exposed to 0.1% for 6 hrs/day for 13 wks (65 exposures)
showed no toxic effects except for hypospermato genesis which
was not reversed after a 4-wk recovery period. Teratological
evaluation of fetuses from rats similarly exposed during ges-
tation Days 6 through 15 only showed cervical ribs in 8 of
208 but no other effects. Matings of male rats exposed to
0.1% for 10 wks and then mated weekly with new pairs of unex-
posed females over a 16-wk period produced no unequivocal
dominant lethal effect; however pregnancy rate was reduced
throughout this period. Bone marrow cells harvested from male
rats exposed for 13 wks to 0.1% showed no cytogenetic effects.

218. PROTECTIVE EFFECTS OF (+)-CYANIDANOL-3 AGAINST
CCl₄-INDUCED HEPATOTOXICITY. K. Kappus and
J. Zentner, Med. Institut f. Lufthygiene und Sili-
koseforschung, Universität, D-4000 Düsseldorf, G.W.

(+)-Cyanidanol-3 (C-3), a flavonoid drug is able
to inhibit lipid peroxidation induced by halogene-
ted hydrocarbons like CCl₄ in vitro and in vivo.
We were interested, whether it also protects the
liver from the toxic action of CCl₄. Rats were
-treated chronically with daily doses of CCl₄ simulta-
aneously with C-3 for 3-4 weeks. The control ani-
mals received only CCl₄. The histological examination
showed significantly less cell necrosis and
less fatty infiltration in the livers of the ani-
mals treated with C-3 plus CCl₄ compared to the
controls. On the other hand, the drug metabolizing
hepatic microsomal enzymes decreased in both groups
to the same extent. However, the NADPH-dependent
lipid peroxidation was significantly lower in the
microsomes obtained from C-3 treated animals.
Our results indicate that although C-3 does not
prevent the decrease in liver microsomal enzymes
which was induced by CCl₄, this drug should be view-
ed as an antihapatototoxic agent. Hepatotoxicity and
enzyme decrease might depend on different processes.

The hepatotoxin, chloroform (CHCl₃) has previously been shown to be metabolized to phosgene (COCl₂) by liver microsomes of rat. We have investigated the in vivo metabolism of CHCl₃ to COCl₂. In a typical experiment, phenobarbital pretreated rats were treated with cysteine (1 gm/kg, i.p.) followed by CHCl₃ (4.98 mmole/kg, i.p.) 30 min later. After 1 hr, livers were removed and analyzed for COCl₂ as the cysteine conjugate, 2-oxothiazolidine-4-carboxylic acid by GCMS. A fraction with the same retention time and MS as the synthetic standard was detected in an extract of liver. The identity of the fraction as trapped COCl₂ was confirmed by repeating the study with [¹³C] CHCl₃. An additional investigation was performed using a 1:1 mixture of CDCl₃ and [¹³C] CHCl₃. Approximately twice as much COCl₂ was trapped from [¹³C]CHCl₃ as from CDCl₃ a finding which is consistent with studies using rat liver microsomes and indicates that a deuterium isotope effect is involved in the formation of COCl₂. Since CDCl₃ is also less hepatotoxic than CHCl₃ these observations suggest that COCl₂ is a hepatotoxic metabolite of CHCl₃.


The effect of Cd, Co, Pb and Se on hepatic microsomal monooxygenase enzyme system (MOES) and glutathione (GSH) levels was studied in mice. At 1 mM concentration, Cd and Pb significantly inhibited the in vitro metabolism of benzphetamine by MOES, while it was markedly stimulated by Co and Se. The incubation of Cd with 9,000 x g supernatant fraction of mouse liver homogenate resulted in the inhibition of microsomal membrane lipid peroxidation. This effect of Cd was reversed by EDTA, whereas similar effect of Pb remained almost unaffected by EDTA. While Se showed no effect on the lipid peroxidation, Co prevented the formation of lipid peroxides regardless of EDTA. The in vivo data demonstrated that there was a concomitant decrease in both hepatic GSH concentration and benzphetamine metabolism in Cd- and Pb-treated animals. In contrast, Co and Se caused an increase in the hepatic level of GSH, but there was a decrease in the rate of drug metabolism only in the Se-treated mice. (Supported by the DHEW-NIH Grant RR08091)
221. DOSE-RELATED DISTRIBUTION OF VANADIUM IN RAT TISSUES.
R.P. Sharma, S.G. Oberg, and R.D.R. Parker, Toxicology Program, Utah State University, Logan, UT.

Vanadium (V) is both an essential and toxic trace metal, encountered in small amounts in ores and fossil fuels. Male Wistar rats (ca. 200 g) were given vanadyl chloride (VOCl₂) ip in doses ranging from 0.1 to 8 mg/kg and their tissues collected 1 and 5 days later to determine V residues by gamma scintillation spectrometry. Vanadium was distributed in the order: bone > kidney > liver > spleen > intestine > stomach > muscle > testis > lung > brain > blood, with levels declining rapidly at 5 days after administration. The relationship of V residues was linear in most organs when the dose of V was below 2 mg/kg. At 8 mg V/kg, however, liver and kidney showed consistently higher amounts than in expected linear dose-concentration relationships. Sub-cellular distribution of V in liver indicated that this element accumulated in nuclei, mitochondria, microsomes, and in the soluble fraction associated primarily with large molecular weight proteins. Results suggested that V was distributed in rat tissues in a dose-dependent fashion except for liver and kidney at the high V dose, and it was found to be selectively associated with various cell organelles.

222. TIME- AND DOSE-DEPENDENT DISTRIBUTION OF VANADIUM IN MOUSE TISSUES: R.D.R. Parker, R.P. Sharma, and S.G. Oberg, Toxicology Program, Utah State University, Logan, UT.

Vanadium (V) is a toxic element known to be found in various fossil fuel sources. The distribution of V was determined in male Swiss-Webster mice after a single ip administration of 2 mg/kg at different time intervals. The highest amounts of V were observed in kidney at time periods <1 day, whereas after 1 day bone showed the highest levels of all tissues. Levels of V in liver were equal to those in the kidney at 2 days after the injection but were considerably higher than kidney after this period. In most tissues the residues of V declined rapidly but not in a first-order fashion. The spleen, however, showed a significant increase in V from day 5 to 12 after the injection. Groups of mice were injected ip with V in doses ranging from 0.1 to 8 mg/kg and tissues collected at 1 and 5 days after the treatment. At 5 days, all tissues exhibited a linear dose-concentration relationship. At 1 day after V injection, however, most tissues showed higher amounts of V at dose levels of >5 mg/kg than would be expected from a linear pattern.
223. ACUTE TOXICITY OF RUTHENIUM SALTS AND COMPLEXES. R. P. Smith, H. Kruszyna and J. H. Hurst. Dept. Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.

Ruthenium (Ru) compounds are of interest because radio-Ru is a common fission product, because some Ru compounds may have antitumor activity and because the metal may be used in catalytic converters to control automobile exhaust emissions. Except for Ru-red which may have unusual properties studies on the biological activity of Ru compounds are almost nonexistent. Commercially available RuCl₃ was used to synthesize a series of complexes including:
Na₂[Ru(CN)₅(NO)₂], K₂[RuCl₅(NO)₂] and (Ru(NH₃)₅(NO)Cl)Cl₂. The acute ip LD₅₀ in mice ranged from 108 mg/kg for RuCl₃ to 8.9 mg/kg for the pentamine complex. Most deaths occurred on day 4 after RuCl₃ and on day 6 after the pentamine complex. Histopathologic studies on mice given lethal doses of RuCl₃ were essentially negative and dimercaprol, deferroxamine, D-penicillamine and EDTA all failed to prevent death. In toxic doses RuCl₃ decreased urine pH and increased urine bilirubin, hemoglobin and protein. SGPT was also increased. The pentamine complex also decreased urine pH and increased urine protein, glucose and ketones as well as the SGPT. Abnormal values in both groups returned to normal after one week. (Supported by Grant HL 14127 NIH, USPHS and the Ryan Foundation).


Mercurials inhibit enzyme activity at doses below the titer of total available ligands (sulfhydryl groups). Concentrations of free mercurial are negligibly low, and existing concentration-effect models not applicable. New models were developed in terms of partition of mercurial between enzyme and alternate ligands. Effective concentration was derived from saturation of measured binding capacity. Inhibitor partition equations used v₀ and v₁ (activity without and with the mercurial) to derive the stoichiometry of inhibition, the number of non-inhibitory binding sites per enzyme, and the relative affinity of mercurial to inhibitory site. Tests of human red blood cell ghosts showed that methylmercury inhibition of Mg,Ca ATPase was one-hit, but changed to two-hit by further washing of ghosts. Inhibition of Na,K ATPase involved one inhibitory and six non-inhibitory binding sites and was not changed by washing. Affinity to inhibitory sites was 3 to 5 times greater than to alternate ligands in membranes. Inhibitor partition equations are a new tool for defining mechanisms of inhibition by mercurials and for comparing effective doses in vitro with doses in vivo.
225. MODIFICATIONS OF TESTICULAR TOXICITY OF CADMIUM (Cd) BY NEONATAL EXPOSURE TO HORMONALLY-ACTIVE CHEMICALS. E. M. K. Lui, E. E. McConnell, I. P. Lee and G. W. Lucier. NIEHS, Research Triangle Park, NC 27709

We investigated the effect of neonatal exposures (day 2, 4 and 6) to testosterone propionate (TP) and diethylstilbestrol (DES) on the rat testicular response to acute CdCl₂ treatment in subsequent adult animals. Histological examination of testes of control rats obtained 72 hours following Cd treatment revealed hemorrhages, fibrous materials in the interstitium, and the destruction of some seminiferous tubules. Exposures to higher doses of Cd resulted in complete infarction of testes. The degrees of Cd-induced testicular damage in rats which had received neonatal TP treatment were greater than those of controls (neonatal propylene glycol-treated). In contrast, testes of adult rats exposed neonatally to DES exhibited decreased susceptibility to the toxic effects of Cd. Biochemical studies (using acid phosphatase, glucuronidase, carboxylesterase, LDH-X, and 6-glucuronidase as indicators of testicular function) verified the histological findings in controls and rats receiving neonatal TP or DES. These data suggest that the Cd-induced testicular toxicity in the rat may be modified by neonatal exposure to certain hormonally-active chemicals.

226. TOXIC EFFECTS OF CADMIUM ON THE DEVELOPMENT OF THE RAT LUNG AND PULMONARY SURFACTANT SYSTEM, G.P. Daughton, Dept. of Biology, Univ. of Miami, Coral Gables, FL. Sponsor: J. Radomski

The effects of cadmium on the growth and biochemical maturation of the lung were studied. Pregnant rats received sc injections of 8.0 mg/kg/day of cadmium chloride on days 12-15 of gestation. Animals were sacrificed throughout late gestation. Pulmonary surfactant was extracted from fetal lungs and assayed for lecithin and sphingomyelin. Some animals were allowed to give birth, and the neonates were observed for signs of respiratory distress. The treatment resulted in high fetal mortality and retardation. Lung/body weight ratios were reduced in treated fetuses. Sphingomyelin content was not affected by cadmium. Lecithin, the most important surfactant component, was reduced in absolute quantity, but not in lecithin/lung weight ratio. Parturition was delayed by the cadmium treatment, and neonatal weights were reduced. Of the treated neonates, 8% developed respiratory distress syndrome (RDS), died, and had lungs with hyaline membranes. Prenatal cadmium treatment induces hypoplastic lungs and alters pulmonary surfactant. This leads to death with RDS.

The ability of some thiol compounds to mobilize a tracer dose of $^{109}$Cd into the bile was investigated in rats. Of the compounds tested, BAL appeared to be the most effective at the 24 hr. time point after Cd dosing. BAL produced a rapid, transient enhancement of biliary Cd excretion at relatively high doses. The appearance of Cd correlated with the appearance of excess biliary SH groups. The enhancement of Cd excretion could result in the release of 27% of the administered dose within 1 hr. after a single injection of 75 mg/Kg BAL. A subsequent dose of BAL resulted in renewed biliary excretion. Urinary Cd was also slightly increased. Indirect evidence suggests that the stoichiometric ratio of the Cd/BAL complex in bile may be 1:3 and this may explain the efficacy of the higher doses of BAL. BAL glucoside, a more water soluble form of BAL, was less effective in enhancing biliary Cd excretion. However, comparison of the BAL compounds with some other thiols suggests that vicinal sulfhydryl groups on an aliphatic chain are the most effective in mobilizing cadmium.


Synthesis of metallothionein (MT) can be induced in the liver and kidney by cadmium or zinc salts and MT has been found in a number of other organs. MT synthesis was also induced in a clonal rat liver cell line (RLC-GA1). MT synthesis was investigated in a human cell line, HeLa-S, derived initially from a cervical carcinoma. Cells were grown in a serum-free medium containing $1.5 \times 10^{-6}$M Zn$^{2+}$. Confluent cells were labeled with $^{65}$ZnCl$_2$, $^{109}$CdCl$_2$ or $^{35}$S-cysteine + Zn acetate ($5 \times 10^{-5}$ M) and CdCl$_2$ ($10^{-8}$ - $10^{-5}$ M) for 18-24 hr. Soluble proteins were chromatographed on a Sephadex G-75 column. Up to 4.7 nmol Cd$^{2+}$/mg total protein was found associated with MT. Concentrations of $10^{-6}$ and $10^{-5}$ M Cd$^{2+}$ stimulated the incorporation of $^{35}$S-cysteine into MT. Addition of $5 \times 10^{-5}$ M Zn$^{2+}$ produced 3.6 nmol Zn$^{2+}$/mg protein in MT and stimulated the incorporation of $^{35}$S-cysteine. That cycloheximide did not completely inhibit $^{109}$Cd binding indicates that MT is normally present in this cell line. The results indicate that the induction of MT synthesis by Cd$^{2+}$ and Zn$^{2+}$ is not limited only to cells originating from the liver.

Antibodies to rat hepatic Cd-metallothionein (MT) were produced in rabbits, using three different antigen preparations: (a) MT obtained after Sephadex G-75 chromatography, (b) MT-2 obtained after disc electrophoresis and (c) MT-2 conjugated with rabbit serum albumin. Each preparation (0.5-1.0 mg MT/injection) was injected subcutaneously and intradermally with or without Freund's complete and incomplete adjuvants at frequent intervals during 5 to 7 months. Sodium azide was used as a preservative for the serum. The 109Cd-MT-2 and rabbit antiserum mixture was incubated for 5 hr at 4°C and the bound antigen was precipitated by 50% ammonium sulfate. Using the above procedure, the production of specific antibody to rat hepatic MT-2 was demonstrated in the three groups of rabbits. However, marked individual differences in antibody titer were found. Cross-reactivity of the antiserum with rat renal MT, rabbit hepatic MT and human renal MT was also observed. The titration curve of human renal MT indicated that the antiserum to rat hepatic MT can be employed for measuring MT in the human tissues and fluids. (Supported by PHS Grants ES 01247 and ES 01448).


Preliminary observations indicated that hepatic concentration of metal binding protein varied with age in rats, with the concentration in 1-4 day old neonates being twenty times that in rats 4-10 weeks old. Biochemical purifications were performed to characterize the metal-binding protein in newborn liver. Gel filtration of the hepatic cytosol fraction on Sephadex G-75 demonstrated that the major Zn-binding fraction of newborn had a relative elution volume similar to the metallothionein fraction of Zn-treated adults as well as a low absorbance at 280 nm. These fractions were further purified by DEAE A-25 and two major subfractions were obtained in both the newborn and Zn-treated adult rats, each being eluted with buffer of similar conductivity. These subfractions from newborn liver also exhibited similar mobilities on polyacrylamide-gel electrophoresis as the corresponding subfractions from Zn-treated adults and amino acid composition characteristic of metallothionein. These data suggest that the metal-binding protein which is highly concentrated in newborn liver is metallothionein. This observation may be of value in determining the physiological role of metallothionein. (This work was supported by funds from U.S. Public Health Service Grant ES-01142).
231. **LOW LEVEL LEAD: CONCURRENCE OF THRESHOLDS FOR HEMATOLOGIC TOXICITY – P5N, Na/K ATPase, FEP AND ALA-D. C.R. Angle and M.S. McIntire, Dept. of Ped., Univ. of Nebraska Coll. of Med. and Creighton Univ. Sch. of Med., Omaha, Ne.**

Decisions concerning an acceptable level of blood lead have often been based on the observation that many enzymatic effects of lead exposure are only biochemical effects in contrast to health effects. Examination in children of the activity of red cell pyrimidine 5' nucleotidase (P5N; GMPase; UMPase) shows a decrease at blood lead levels of 20-30 µg/dL. Red cell membrane Na/K ATPase as measured in 63 subjects shows a similar threshold. A clustering effect is noted with changes in P5N, Na/K ATPase, delta amino levulinic acid dehydratase (ALAD) and fluorescent erythrocyte protoporphyrins (FEP) all occurring at blood leads of 15-30 µg/dL. The concurrence of thresholds supports the probable biologic significance of these enzymatic effects of low level lead.

232. **Age Differences in Mouse Methylmercury (MeHg) Retention and Demethylation. T.D. Landry, R.A. Doherty, & A.H. Cates; Toxicology Program & Environmental Health Sciences Center, University of Rochester, Rochester, N.Y. Sponsor: T.W. Clarkson**

MeHg demethylation is an important step in total Hg (Hg) excretion; inorganic Hg (I-Hg) is preferentially excreted in feces of mice. Whole body Hg retention and total MeHg demethylation were examined to determine whether the known age difference in Hg retention relates to possible differences in demethylation. Mice (C129F1) aged 4, 10, and 20 days were given .56 mg MeHgCl/kg p.o. (203Hg). The 4 day age group retained .84 FID (Fraction Initial Hg Dose) 12 days after dosing. In contrast, the whole body Hg elimination % of the 20 day age group was 4.8 days (2 week experiment). Feces and urine were collected bi-daily. Fecal ratios (I-Hg/Hg) on day 8 were similar, but fecal Hg excretion was much greater after 18 days of age. FID excreted in feces as I-Hg by 8 days after dosing were .021, .044, and .41 in the 4, 10, and 20 day age groups. I-Hg production in excreta and whole body (relative to body burden of organic Hg) averaged 9.7 times greater in the 20 vs 4 day age group. Differences in demethylation might contribute to the age differences in Hg excretion seen in mouse pups. Information about MeHg demethylation may be useful in human Hg pharmacokinetics and hazard assessment. (NIEHS ES 7026, ES 2148; US/DOE UR 3490 1456)
233. INFLUENCE OF NICOTINE AND TOBACCO GASES ON THE UPTAKE OF NEUTRAL AMINO ACIDS BY ISOLATED HUMAN PLACENTAL VILLUS.

Children born to mothers who smoke cigarettes weigh less than those born to nonsmokers. Therefore, we have subjected isolated human placental villi to components of cigarette smoke (nicotine, CO, nitrite, and cyanide). We measured the capacity of villi to concentrate 14C-alpha-amino isobutyric acid (AIB) from Krebs-Ringer bicarbonate medium. Under control conditions, villi concentrated AIB 5-10 times higher than the concentration in the medium (5 x 10^-5 M) during 2 hours. When exposed to 5% CO, a tendency to decrease the concentrating capacity of the tissue was noted, but it was not significant. Nicotine (2.5 x 10^-3 M) decreased the uptake of AIB by 41%. Nicotine at this concentration produced cholinergic blockade in this tissue. Sodium nitrite (150 8 x 10^-3 M) and sodium cyanide (150 32 x 10^-3 M) decreased the uptake of AIB. When the tissue was exposed to these agents, the return of the uptake capacity was 75% of control for NaNO2 and 45% with NaCN. A combination of all components of tobacco smoke may have a greater effect on the uptake of neutral amino acids by placenta. (Supported by The Council for Tobacco Research, U.S.A. Inc., PMAF Inc. and USPHS-NIH Grant HD-08561.)

234. EFFECTS OF PRENATAL EXPOSURE TO CHLORPROMAZINE ON POSTNATAL DEVELOPMENT AND BEHAVIOR OF RATS. R. Robertson,* J. Majka, D. Zololsman, Merck Sharp & Dohme Res. Labs, West Point, PA.

A variety of behavioral changes have been seen in rats and mice exposed to chlorpromazine (C) pre- or perinatally. Previous studies did not determine if behavioral effects occurred in the absence of physical changes. In order to evaluate the effects of prenatal exposure on postnatal growth, development, and behavior, pregnant rats were administered C at 1, 3, or 9 mg/kg/day from Days 6 to 15 of gestation. Fetuses from half the dams were examined for terata, and the remaining litters were delivered at term. The growth, morphologic and reflex development and reproductive performance of pups was recorded, and selected males were tested on the rotated and for open-field activity. At maturity selected pups were necropsied and mean organ weights recorded. There was no evidence of teratogenicity; F1 pup growth, development, organ weights, mating performance, litter size, and F2 pup weights were unaffected. There were significant (P* ≤ 0.05) increases in activity and decreases in latency time in the open field in off-spring from mothers treated with 3 and 9 mg/kg/day of C compared to controls. Therefore, effects on the behavior of offspring exposed prenatally to 3 and 9 mg/kg/day of C occurred in the absence of significant changes in growth or development.

We report here attempts to define a methodology aimed at identifying biochemical activities whose normal developmental patterns are most sensitive to, and hence altered in response to toxic agents. Known teratogens have been used including hormonally-active compounds (DES and zearanol), and heavy metals (lead and cadmium). We have also studied structure-activity relationships in pure halogenated biphenyls (3,4-3',4'-tetrachlorobiphenyl and its brominated analog). The fetotoxic dose in rats exposed through days 6 to 20 of gestation has been established for each of these compounds. In addition, the activity level of major metabolic pathways in liver and brain has been monitored by measurement of radioactive CO\textsubscript{2} evolved from appropriate substrates. Following this preliminary screening, tissues from a sequence of fetal and postnatal endpoints have been assayed for a number of individual biochemical activities. Included are enzymes which are tissue specific, of regulatory significance, or function during detoxification. The biosynthesis of macromolecules has also been followed. Changes in these parameters have been correlated with teratological, histological, and behavioral assessment.

236. PREGNATAL DETECTION OF CARDIAC PATHOLOGY IN MIREX-FED RATS USING FETAL ELECTROCARDIOGRAPHY. C.T. Grabowski, Dept. of Biology, Univ. of Miami, Coral Gables, FL. Sponsor: J. Hadomski.

The insecticide, Mirex, induces conspicuous fetal edema but little visible teratology. It also reduces perinatal viability. Evidence of considerable developmental toxicity was found using a physiological criterion. Sperm-positive rats were intubated with Mirex in oil (5 to 10) mg/kg on days 8-1/2 to 15-1/2. Controls were untreated or oil-fed. Fetuses were sequentially exposed and ECG's obtained with fetus attached to the uterus. Swollen fetuses were rated on a scale of 1 to 5. ECG's from 51 controls and 205 Mirex-fed fetuses were obtained on day 18-1/2. Mirex-fed fetuses exhibited edema-related tachycardia. The heart rate was 150/min in controls, 180 in the slightly swollen and 224 in swollen fetuses. PR intervals increased with degree of swelling and with dose. The frequency of 1\textsuperscript{st} heart block was dose-related, ranging from 20% to 77%; 8% had 2\textsuperscript{nd} heart blocks; 5% showed sinus arrhythmias; one had atrial flutter. ECG's from treated newborn rats show that the prenatally problems can lead to perinatal pathology. These data demonstrate the usefulness of prenatal electrocardiography to detect developmental toxicity.

Teratogenic insult during the fetal period in rats was evaluated for postnatal effect on gastrointestinal function. Rats were treated (IP) during the fetal period (days 15, 16 and 17; day 0 = sperm) with teratogens which alter cell proliferation, actinomycin-D (0.05 mg/kg/day), hydroxyurea (250 mg/kg/bid or methotrexate (1.0 mg/kg/day). The effect of teratogenic insult was evaluated in postnatal functional tests of (1) gastrointestinal transit time (opaque X-ray contrast media); (2) rectal emptying (antidiarrheal screen) and (3) gastric emptying (altered gastric emptying screen).

Significant (p<0.05) alteration of postnatal gastrointestinal function occurred in rats exposed to teratogens during the fetal period. These rats had more rapid transit times and rectal emptying and slower gastric emptying postnatally than control rats. Sex-related differences occurred.

These data demonstrate that postnatal functional teratology of the gastrointestinal tract can be revealed using simple physiological tests in rats. These tests may provide an animal model to detect functional alteration not revealed in a strict morphological evaluation and aid in determining potential hazards of fetal exposure.


Nabilone (N) was studied as a polyvinylpyrrolidone co-precipitate. Rats were fed N at dietary levels of 0.001, 0.004, or 0.012% w/w (ca. 1, 4, or 12 mg/kg/day) prior to mating and throughout gestation and lactation. Fertility was unaffected, but mean liveborn litter (LL) number was 16% lower than control at the 0.012% dietary level. N given p.o. to rats at daily doses of 1, 4, or 12 mg/kg on gestation days (GD) 6-15 and to rabbits at doses of 0.7, 1.6, or 3.3 mg/kg on GD 6-18 produced no teratogenic effect. In both species LL values declined in a dose-related manner: 19% lower than control in rats and 31% lower than control in rabbits at the highest doses. Abortion rates in rabbits increased at the 3.3 mg/kg dose. In a perinatal-postnatal study, rats were dosed p.o. with 1, 4, or 12 mg/kg/day of N from GD 14 through postpartum day 20. At 12.0 mg/kg, LL number was 23% lower and neonatal survival was 82% lower than control. There was no dominant lethal effect when male rats were given 25 mg/kg of N prior to mating, however LL number was slightly reduced during the first 4 weeks of mating.
239. STUDIES ON THE MECHANISM OF NEONATAL TOXICITY IN RATS INDUCED BY NABILONE, A SYNTHETIC 9-KETOCANNABINOID. C.L. Moss, J.K. Markham, G.K. Hanasono, Toxicology Division, Lilly Research Laboratories, Greenfield, IN. Sponsor: J.S. Wold

Nabilone (N), 12 mg/kg, given to female rats orally as a (polyvinylpyrrolidone) (PVP) coprecipitate from gestation day 14 through postpartum day 20 caused mortality and toxicity in neonates. Litters from ½ of the N-treated and control (PVP-treated) dams were cross fostered (CF) at parturition to determine if neonatal toxicity was associated with fetal and/or postpartum toxicity. All neonates (non-CF and CF) reared by N-treated dams had lower mean body weights, rectal temperatures, survival, and fewer observations of milk in the stomach than neonates reared by control dams. The greatest depressions of these parameters were observed in non-CF offspring from N-treated dams. Smaller but significant differences in body weight, rectal temperatures, and survival occurred between non-CF offspring of control dams and CF offspring with control dams; the latter group having the lower values. Nabilone treatment of dams causes postpartum and, to a lesser extent, prepartum effects resulting in toxicity and decreased survival in neonates.


Chlordiazepoxide (CDO) is a commonly used tranquilizer. The pre- and postnatal effects of CDO were evaluated in Wistar rats in separate experiments by dosing on days 1 through 21 of pregnancy. Single daily doses of 10, 25, 50, or 100 mg/kg of CDO in aqueous solution were administered orally. The controls were given distilled water. Treatment with three larger doses of CDO was associated with a significant reduction in fetal weight as well as increased incidences of wavy and extra ribs and a variety of skeletal defects in the term fetuses. In the postnatal study, adverse effects on pup survival, litter size, and weight, increased incidence of runts, reduced body weight gain, alopecia and generalized tremor of the body were the significant findings.
241. PATHOGENESIS OF INHALED 1,2-DIBROMO-3-CHLOROPROPAINE (DBCP)
INDUCED TESTICULAR ATROPHY IN RATS AND RABBITS.* J.D. Burek,
F.J. Murray, K.S. Rao, A.A. Crawford, J.S. Beyer, R.R. Albee and
H.A. Schwetz, Toxicology Research Laboratory, Dow Chemical
U.S.A., Midland, MI 48640.

The effects of DBCP on spermatogenesis were evaluated in
rabbits and rats by inhalation exposure to 0.0, 0.1, 1.0 and
10.0 ppm of DBCP for up to 14 weeks. The onset, severity
and pathogenesis of testicular atrophy were studied by light
and electron microscopy, by fertility breeding studies and
by correlating these findings with semen evaluation. Rab-
bbits exposed to 10 ppm had nearly complete atrophy by 8
weeks. All stages of spermatogenesis were absent; semin-
iferous tubules were lined by relatively normal Sertoli
cells; there were no germinal cells in the seminiferous
tubules; lipid within the Leydig cells was increased. Rab-
bbits exposed to 1.0 ppm for 14 weeks had a 50% reduction in
testicular size, decreased spermatogenesis, and increased
abnormal spermatocytes within the seminiferous tubules.
Rats exposed to 10 ppm showed approximately a 50% decrease
in testicular weights and a patchy decrease in spermatog-
genesis. Rats exposed to 0.1 and rats exposed to 0.1
and 1.0 ppm did not show any treatment-related testicular
or reproductive alterations.

*Supported in part by Shell Chemical Co.

242. VALIDATION OF A PHARMACOKINETIC MODEL FOR RAT EMB-
RYONAL DOSIMETRY OF SALICYLATES DURING A TERATOGEN-
ERICALLY SUSCEPTIBLE PERIOD. J.F. Young, C.A. Kimmel,
and J.F. Holson. National Center for Toxicological Research,
Jefferson, AR.

Extrapolation of teratogenesis data to man historically has
been based on maternal dose but would be more appropriate-
ly based on time-course exposure (dosimetry) of the embryo.
The value of pharmacokinetics as a tool in predicting embry-
onal dosimetry of teratogens is being explored for the
purpose of extrapolating between species and ultimately to man.
Sodium salicylate was chosen because of its widespread use
as a drug, food additive and positive control in teratology.
CD rats were injected iv with 500 mg/kg sodium salicylate
and blood, urine and fecal samples were analyzed for salicy-
lie, salicyluric and gentisic acids using a simple and sensi-
tive HPLC method developed in our laboratory. A three-
compartment model was generated and validated in nonpreg-
nant and pregnant animals (day 11 of gestation) and by de-
terminations of embryonic salicylate levels. Correlations be-
tween embryonal dosimetry and teratologic response were
made for dose level, route of administration and species.
Temporal and dose-response relationships of the morphologic and biochemical effects of dietary caffeine (C) were studied in immature and mature rats. Levels of 0, 0.125, 0.25 and 0.50% C were fed to 4-week-old male Osborne-Mendel rats for periods up to 6 months. Depressed weight gain and food intake were seen for the mid- and high-dose groups. Severely and irreversibly impaired spermatogenesis was seen as early as 1 month in a majority of animals receiving 0.50% C. Slightly impaired spermatogenesis was seen at 6 months in 27% of the rats receiving 0.25% C. Testicular atrophy was also seen in adult rats fed 0.5% C for 8 months. Spermatogonial chromosome damage was not seen, but a trend toward decreased mitosis (0.65 vs 1.14%) and DNA synthesis (449 vs 606 DPM/mg) was evident at 3 months with 0.50% C. Testicular c-AMP was unchanged, but dry wt., RNA, DNA, protein and zinc decreased 16, 51, 31, 18, and 62%. Serum cholesterol was elevated at 1, 3, and 6 months and testosterone at 3 and 6 months with 0.50% C. None of the effects were seen in pair-fed controls. Thus caffeine at high levels produced changes in testicular morphology and composition and in serum composition not explained by malnutrition.

244. UNUSUAL EXPRESSION OF TERATOGENICITY IN THE RAT. J.L. Schardein and J.A. Pettross, Dept. of Tox., Warner-Lambert/Parke-Davis, Ann Arbor, MI.
In standard fertility studies in rats, a methaqualone-diphenhydramine drug mixture (10:1) given in the diet induced a low incidence (3.5%) of cleft palate in the offspring of parents treated prior to conception and through gestation. Further studies were done to determine under what conditions cleft palate could be induced with the drug mixture, which drug in the mixture was responsible for the induction of the teratogenic response, and the role parental treatment played in the effect.
Components of the drug mixture were fed separately to male and female rats for varying intervals prior to mating and during gestation. The results of the experiments indicate that the teratogenic effect was associated with the methaqualone component of the mixture, and only when females were treated. At doses about 25-fold the human dose, the drug increased the frequency of cleft palate to almost 20%. Diphenhydramine alone was not teratogenic. The unique feature of the teratogenic response by methaqualone in these experiments was that cleft palate resulted when dams were treated for a 6 or 15 day period in addition to the period of fetal organogenesis.
245. TERATOGENICITY OF PYRIMETHAMINE. S. Schwartzman, Instituto da Crianca Hospital das Clinicas, School of Medicine, Sao Paulo, Brazil.

Pyrimethamine, used in therapeutic doses in experimental toxoplasmosis of rats, mice and rabbits showed a damaging action to concept viability and teratogenic effects to the offspring. The effects were more intense with doses of 10 mg/kg given from the 9th to the 11th day of rat pregnancy, 18 mg/kg from the 8th to the 14th day of mice pregnancy, and 40 mg/kg from the 8th to the 16th day of rabbit pregnancy. The anomalies observed were not specific ones, but dental anomalies were relatively frequent. The teratogenic effects seem to be due to the antifolic characteristics of the medicament.


In the IPL of Sprague-Dawley male rats, the SRII consisted of 40 µl of N-methyl-14C-morphine (M) followed by 110 µl of saline wash in. The % recoveries were in recollected bile 7.1 ± 2.1 (M), 10.0 ± 1.8 (morphine glucuronide, MG) and in venous outflow 18.1 ± 2.3 (M), 18.8 ± 1.7 (MG). SKF 525A (20 mg/kg b.w. intraperitoneally) decreased recovery of MG in bile to 4.6 ± 1.7 and in venous outflow to 4.4 ± 2.3; M in venous outflow increased to 25.1 ± 4.0. Although SKF525A had no effect on mean transit time of mannitol (116.1 ± 1.3 vs. 115.1 ± 24.0) (control vs. SKF525A) or morphine (137.1 ± 4.9 vs. 113.0 ± 6.7). The outflow pattern from which the difference in volumes of distribution, Q, were calculated indicated that morphine was taken up by hepatocytes beyond the mannitol space (Q = -1.63 ± 0.35) and SKF525A decreased this volume of distribution of morphine to Q = +0.45 ± 0.21. In addition, we found that SKF525A increased intrabiliary pressure. The total evidence therefore suggests that SKF 525A decreases membrane permeability to produce the variety of effects reported here. (Supported by NIH grant GM 16503).
247. EFFECTS OF HIGH LEVELS OF DIETARY VITAMIN A ON DRUG METABOLISM IN GUINEA PIG AND RABBIT. R.S. Chhabra and C.L. Miranda, Lab. of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The activities of various drug-metabolizing enzymes were measured in the liver of guinea pigs and in the liver, lung and small intestine of rabbits fed diets high in vitamin A content for 6 and 7 weeks respectively. Excess dietary vitamin A increased the activities of aminopyrine demethylase and aniline hydroxylase in guinea pig liver but not in rabbit liver. In contrast, feeding excess vitamin A increased the activities of hepatic 7-ethoxycoumarin diethylase and glutathione S-transferase in rabbit but not in guinea pig. In rabbit, the activities of aminopyrine demethylase and aniline hydroxylase of both lung and small intestine as well as the benzyrene hydroxylase activity of lung were lowered by the high vitamin A diet. Excessive intake of vitamin A reduced the formation of dihydrotolols from rabbit liver microsomal benzyrene metabolism but did not affect epoxide hydrolase activity. It is concluded that (a) animal species, type of tissue and substrate used determine the effect of excess dietary vitamin A on drug metabolism and (b) alteration of carcinogen metabolism may be one of several possible mechanisms for the protective effect of vitamin A in chemical carcinogenesis.

248. EFFECT OF CAPROLACTAM ON HEPATIC TYROSINE AMINOTRANSFERASE AND TRYPTOPHAN OXYGENASE ACTIVITIES. M.A. Friedman and A.J. Salerno, Dept. Biochemical Toxicology, Corporate Medical Affairs, Allied Chemical Corporation, Morristown, NJ.

Since caprolactam (CL), the monomer used in Nylon 6 synthesis has a hydrolyzable peptide bond, significant deviations in liver amino acid metabolism might be induced consequent to loss of the free amino group. When Fisher 344 rats were dosed po with 1500 mg/kg CL, tyrosine aminotransferase (TAT) and tryptophan oxygenase were induced to 350% and 650% of control levels at 3 and 6 hours, respectively. These enzymes were also inducible in adrenalectomized rats. Simultaneous administration of 1 mg/kg actinomycin D had no effect on the induction of TAT in adrenalectomized rats. Feeding rats diets containing 0.5% caprolactam resulted in TAT levels 240% higher than corresponding controls. In none of these experiments was the level of glucose-6-phosphatase or fructose 1,6 diphosphatase different from controls. The authors conclude that CL serves as a non-specific nitrogen source which can modify patterns of liver amino acid metabolism.
249. EFFECTS OF DI(2-ETHYLHEXYL)PHTHALATE (DEHP), AND ITS 
PRIMARY METABOLITES, MONO(2-ETHYLHEXYL)PHTHALATE (MEHP) AND 
2-ETHYL HEXANOL (2-EH) ON HEPATIC LIPID METABOLISM. S.J. 
Morton and R.J. Rubin, Dept. Env. Hlth. Sci., Johns Hopkins 
Univ., Sch. of Hyg. & Pub. Hlth., Baltimore, MD 21205

Previously, we have demonstrated the ability of dietary 
DEHP to prevent orotic acid (OA) induced hepatic triglyceride 
(TG) accumulation and to induce specific enzymes involved in 
lipid transport and metabolism. We report here the comparative 
effects of the parent compound DEHP, and its primary metabo-
lites MEHP and 2-EH on hepatic lipid metabolism. DEHP 
and MEHP, but not 2-EH, at dietary levels of 50-2500 ppm for 
7 days significantly lowered hepatic TG accumulation follow-
ing a subsequent 6 day dietary addition of 1% OA. Dietary 
DEHP and MEHP, but not 2-EH, for 7 days were also able to sig-
nificantly increase hepatic enzymes involved in lipid trans-
port (Carnitine Acetyltransferase and Carnitine Palmitoyl-
transferase) and metabolism (–oxidation). Our data indicate 
a high correlation between the protection from the lipogenic 
effects of OA by DEHP and MEHP and the increased activity of 
hepatic enzymes involved in lipid transport and oxidation.
We further conclude that the metabolite MEHP is capable of 
producing the same lipotropic effects as the parent compound 
DEHP. In another study, dietary DEHP prevented the devel-
opment of fatty liver in the genetically obese Zucker rat.

250. Induction of hepatic α-glycerophosphate dehydrogenase and 
peroxisomal enzymes by hypolipidaemic agents. B.R. Holloway 
and J.M. Thorp (M.S. Rose), TCI Pharmaceuticals, Alderley 
Park, Macclesfield, Cheshire, U.K.

Atromid-S chronically dosed to rats produces a hyperthyroid 
state in liver, resulting in increased mitochondrial α-
glycerophosphate dehydrogenase activity (GPDH). Atromid also 
produces an increase in liver/body weight ratio (L/B) and 
proliferation of peroxisomes. We have investigated the 
effects of hypolipidaemic compounds fed by diet (%w/w) to 
male rats for 14 days, on GPDH, L/B, catalase (CAT) and 
palmitoyl-CoA oxidation (PCO), results are expressed as % 
control.

Atromid-S 0.05/0.15/0.20/0.30/0.40/0.60 % w/w: L/B - 110,131, 
139,163,162,167; GPDH - 133,137,472,460,375,421; CAT - 103, 
124,136,147,151,130; PCO - 135,465,615,999,1568,1672.

Lipentyl 0.01/0.05/0.15 % w/w: L/B - 107,151,163; GPDH - 577, 
1019,1240; CAT - 109,134,145; PCO - 414,2247,3149.

Triclosan acid 0.01/0.05 % w/w: L/B - 146,175; GPDH - 455,782; 
CAT - 137,136; PCO - 309,392.

There is a dose dependent increase in L/B, GPDH, CAT and 
PCO for hypolipidaemic compounds of different structural 
types. Peroxisome proliferation may be secondary to a 
hyperthyroid state in the liver.
251. THE EFFECT OF HYPOLIPIDAEMIC AGENTS ON LIVER DRUG METABOLISING ENZYME SYSTEM. T.C. Orton and J.E. Higgins (M.S. Rose), ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, U.K.

Chronic administration of hypolipidaemic agents causes hepatomegaly in the rat. As the liver is the major site of drug metabolism we have investigated the relationship between this liver enlargement and the activity of the cytochrome P450 dependent drug metabolising enzyme system. The compounds (% in diet) were fed to male rats for 2 weeks and liver:body weight ratio (L/B); Microsomal aminopyrine N-demethylation (APDM); Ethoxyresorufin O-deethylation (ERDE) and cytochrome P450 were examined and expressed as % control.

ATROMID-S 0.2/0.4/0.6 w/w%: L/B - 150,163 and 168; APDM - 115,120 and 103; ERDE - 54,50 and 31; P450 - 185, 148 & 154

ICI 55,695 [Ethyl 4-{(4-chlorophenyl)phenoxyisobutyrate] (0.005/0.05/0.1 w/w%): L/B - 145, 162 & 147; APDM - 92, 68 & 70; ERDE - 50, 13 & 12; P450 - 119, 120 & 144

LIPANTYL 0.01/0.05/0.15 w/w%: L/B - 115, 151 & 163; APDM - 106, 91 & 82; ERDE - 84, 26 & 25; P450 - 165, 224 & 225

There was a dose dependent increase in L/B ratio cytochrome P450. APDM (and also biphenyl 4-hydroxyladon) was either unaffected or inhibited whilst ERDE was consistently inhibited.

252. THE ROLE OF MICROSONAL MIXED FUNCTION OXIDASE SYSTEMS IN HEPATIC TOXICITY OF COCAINE. M.A. Evans, Department of Pharmacology, Univ. of Illinois Medical Center, Chicago, I1.

Cocaine produces a chemically-induced hepatotoxicity in phenobarbital (PB) or 3-methylcholanthrene pretreated mice. Toxicity is species dependent and characterized by an acute periporal necrosis of the liver. Dose response studies with radiolabelled cocaine in vivo or in isolated mouse hepatocytes demonstrates that toxicity is associated with a decrease concentration of glutathione and increased covalent binding of C-14 material to hepatic protein. Studies with norcocaine showed similar results but other known metabolites of cocaine including benzoylgonine, eckonine methyl ester and egonine failed to produce any evidence of toxicity. Studies with microsomes from PB pretreated mice showed an NADPH-dependent and esterase independent covalent binding of both cocaine and norcocaine. Covalent binding but not esterase activity was prevented when incubations were conducted under nitrogen. Metabolism studies in microsomes using HPLC and GC-MS demonstrated the formation of a N-oxide from cocaine. This new metabolite of cocaine may represent the proximal intermediate responsible for cocaine-induced hepatic dysfunction and necrosis. (Supported in part by USPHS DA 1877).

It is well established that reducing equivalents from NADH can support mixed-function oxidation (MFO) in microsomes via an electron transfer chain involving cytochrome b5. Ethanol (E) oxidation produces NADH but has been shown to inhibit MFO. The purpose of these experiments was to determine whether NADH-supported MFO operates in the perfused rat liver. The effects of E and sorbitol (S) on p-nitroanisole (PNA) O-demethylation and surface fluorescence of pyridine nucleotides were compared. Both E and S increased p-nitrophenol formation from PNA by 35 and 70%, respectively, at low concns. in livers from fasted rats (half-maximal activation = 50 μM for E; 170 μM for S). Similar values were obtained for half-maximal reduction of pyridine nucleotides. In livers from fed rats, the activation was much less. MFO activation by either E or S was not additive. With E (0.2 mM), the activation was abolished by pyruvate but not by amino-oxyacetate. At concns. of 20 mM, S stimulated MFO whereas E inhibited. These data indicate that increases in NADH redox state stimulate MFO in the perfused liver.

CA-20807; CA 23080; AA-03624.

254. SELECTIVE CHANGES IN THE HEPATIC MICROSONAL ELECTRON TRANSPORT SYSTEM (HEETS) FOLLOWING REPEATED ORAL METHADONE ADMINISTRATION IN THE MOUSE. T.B. Barnes and L.W. Nesten, Dept. of Pharmacol., Sch. of Pharmacy, Univ. of Mississippi, University, MS. Sponsor: W.M. Davis

Since we and others have demonstrated hepatic microsomal enzyme induction by methadone, it was decided to examine the effects of this narcotic on the HEETS to characterize the phenomenon further. Male ICR mice (27-30 g) received daily doses of 12.5, 25 and 50 mg/kg methadone HCl and were sacrificed 24 hr after 1, 2, 3, and 6 days of treatment. All livers were perfused with ice-cold 0.05M Tris-0.15M KCl. Cytochromes P-450 and b5 and two NADPH-dependent enzymes, cytochrome c reductase and NADPH oxidase were monitored in microsomal suspensions according to standard procedures. By day 2, there were significant increases in both cytochromes and both enzyme activities. Each elevation appeared to be dose-dependent and remained so through the sixth day of administration. However, these changes were much smaller than those reported by us for 1-α-acetylmethadol (LAAM) in the same system. Supported in part by the University of Mississippi Research Institute of Pharmaceutical Sciences and by NIDA Grant DA 01331-02.
255. EFFECTS OF ETHYLAMINOETHANOL ON PHOSPHOLIPID METABOLISM AND HEPATIC MICROSOMAL ENZYME ACTIVITY. A.C. Stevens and R. Hartung, Toxicology Program, Sch. of Pub. Health, Univ. of Michigan, Ann Arbor, MI.

In vitro treatment of rat liver tissue with ethylaminoethanol (EAE) produced significant inhibition of both choline and ethanolamine incorporation into phospholipids. CFN rats were given EAE (neutralized, pH 7.2) in their drinking water at 0.25, 0.5, and 1.0 mg/ml and sacrificed 2 weeks after dosing. Abnormal phospholipids were found in liver by TLC which had an Rf greater than that of phosphatidylethanolamine. The proportions of sphingomyelin and lyssolecithin in total phospholipids were unchanged, but phosphatidylcholine was decreased and phosphatidylethanolamine was increased. Aniline hydroxylase and 0-demethylase activities were depressed at the higher dose levels. Cytochrome P-450 content was reduced only at the highest dose level. These studies suggest that EAE may impair the normal functioning of the microsomal enzyme system by interfering with phospholipid metabolism.


In conjunction with the study of neurotoxic response to acrylamide in large animal species, acrylamide carbonyl-$^{14}$C was administered per os to Beagle dogs and miniature swine. A blood elimination half-life of 5-6 hr. was observed in dogs and ca. 10 hr. for swine. Approximately 60% of the administered radiolabeled dose was excreted in the urine in 2 weeks, and smaller amounts (10% and 25%, dogs and swine, respectively) were excreted in the feces. Tissues were analyzed for radioactivity at various times following oral administration of acrylamide to both dogs and swine. The material is distributed to a high degree in muscle (35% of dose at 6 hr; 5-7 % of dose at 2 weeks.) Although the nervous system is the primary target area of acrylamide monomer toxicity, less than 1% of the label was found in the CNS after oral administration of acrylamide-$^{14}$C to both species. Analysis of discrete areas of the CNS for radiolabel revealed that levels of penetration of acrylamide-$^{14}$C in brain paralleled the vascularization pattern of the brain.
257. PREDICTIVE VALUE OF COVALENT BINDING IN TARGET ORGAN TOXICITY: AGE-RELATED DIFFERENCES IN TARGET ORGAN ALKYLA-
TION AND TOXICITY BY 4-IPOMEANOL. R. Jones, C. Allegra, and M. Boyd, NIH, Bethesda, MD.

We have found striking differences in target organ necrosis caused by the metabolically-activated toxin, 4-ipomeanol (IPO), in C57BL/6/N mice of different ages. Covalent binding of IPO, but not the tissue distribution of the parent compound, was a good predictor of the differences in target organ toxicity. At comparable i.p. doses, the amount of IPO covalently bound was 15-20 times higher in kidney, but 2-3 times lower in lung, in older mice (20-25g) compared to young mice (5-8g). IPO caused pulmonary bronchiolar necrosis in both young and older mice, but comparable doses produced less severe bronchiolar necrosis in older mice. In contrast, IPO caused striking renal cortical necrosis in older mice but not in young mice. These age-related differences were consistent over a wide range of doses (15-60 mg/kg). Similar age-related differences were not observed in Sprague-Dawley rats or Golden Syrian hamsters. These results indicate that, with certain species, animal age can be an important determinant of target organ specificity of cytotoxins, and possibly also carcinogens, that require metabolic activation.

258. COVALENT INTERACTIONS OF BROMOBENZENE WITH HEPATIC MACROMOLECULES. J.D. Sun and J.C. Dent, Chem. Ind. Inst. of Tox., Res. Tri. Pk., NC. Sponsor: J.E. Gibson

Covalent interactions of chemicals have previously been studied by classical exhaustive extraction methods. A new approach has been developed and used to study the covalent interactions of bromobenzene with hepatic macromolecules. Primary hepatocytes isolated from Fischer-344 rats were incubated (37°C) with 14C-bromobenzene (80μM, 5mCi/mmol.). After various times, the reaction was stopped and covalently bound radioactivity was assessed by equilibrium dialysis (ED) and exhaustive extraction (EE). The amount of covalently bound label was 26.5 nmoles/10^6 cells and 0.102 nmoles/10^6 cells at 60 minutes, as determined by ED and EE, respectively. Disc gel electrophoresis (10% SDS-PAGE) indicated that all of the 14C covalently bound was associated with small macromolecules migrating with the dye front. Using 15% acrylamide and a high percentage of crosslinking, these low molecular weight macromolecules could be separated. A macromolecule (ca. 20,000 Daltons) became maximally labeled after 5 minutes, while many smaller macromolecules (<13,000 Daltons) were alkylated linearly with time up to 60 minutes. These low molecular weight macromolecules, discovered by ED and SDS-
PAGE, may be involved in the mechanism of bromobenzene-induced hepatotoxicity.
259. IN VITRO METABOLIC ACTIVATION OF THE PULMONARY TOXIN, 4-IPOMEANOL, BY LUNG SLICES AND ISOLATED WHOLE LUNGS. N. Longo and M. Boyd. NIH, Bethesda, MD.

The pulmonary toxin, 4-ipomeanol (IPO), was actively metabolized and covalently bound both in incubation mixtures containing mouse lung slices and in isolated whole mouse lungs suspended in, and perfused intratracheally, with oxygenated Kreb's solution. In both systems the rates of covalent binding were linear for up to 30 min and maximal rates were obtained with concentrations of 0.25-0.50 M IPO. Heat-denatured tissues did not mediate the covalent binding of IPO. There was no active uptake of unmetabolized IPO in lungs or lung slices. Covalent binding of IPO was markedly decreased both in lungs and lung slices from mice treated in vivo with piperonyl butoxide, an inhibitor of IPO metabolism. In lung tissues from animals treated with diethylmaleate, which depleted lung GSH, covalent binding of IPO was markedly enhanced. These results are fully consistent with previous in vivo studies that indicated the involvement of in situ metabolic activation in the pulmonary toxicity of IPO. The use of isolated lungs and lung slices appears to offer a useful new approach for the in vitro study of IPO activation in systems containing intact pulmonary cells.


Tissue concentration of acetaminophen (APAP) and covalent binding of the reactive metabolite were determined in mice after oral administration of 14C-APAP, 750 mg/kg. The effects of these parameters produced by antitodal intervention with single doses of N-acetyl-cysteine (NAC), 750 mg/kg p.o., given at different times after toxic insult were evaluated. NAC given at the time of APAP overdose (early) significantly decreased the covalent binding of reactive metabolite to liver tissues measured at 2 and 24 hours and caused a similar but nonsignificant decrease in binding to kidney tissue. This coincided (2 hr.) with a lower level of radioactive drug in red cells and brain and less metabolized APAP in kidney. Administration of NAC at 2 or 4 hours (late) did not alter the degree of covalent binding in liver and kidney nor were the levels of total radioactivity or of unmetabolized APAP different from controls in the tissues examined. It is concluded that the marked protection afforded by NAC against APAP mortality in mice observed after late antitodal administration (2-4 1/2 hr.) is not a result of decreased covalent binding of reactive metabolite to liver or kidney, nor is it due to a redistribution of APAP. The reported success of early NAC (within 1 hour of toxic insult) against mortality may be attributable to the decreased liver binding observed here, however, the overall importance of covalent binding per se in terms of antitodal success appears questionable.
261. EYE CONTAMINATION: A POISON CENTER PROTOCOL FOR MANAGEMENT. K. M. Rost, R. W. Jaeger, and F. J. deCastro, Cardinal Glennon Hospital, St. Louis Poison Center.

The large number of calls regarding plants, drugs and other chemical-causing ophthalmic injuries prompted us to develop and evaluate an eye injury protocol. During 1977 our Center was consulted in more than 600 patients with eye contamination. The protocol developed is divided into a general section for all substances (outlined in poster) and a specific section for ophthalmic burns (outlined in poster). No permanent eye damage was observed in patients injured by substances other than alkali burns. No results worse than expected (judged by Ophthalmology) was observed in alkali burns. The approach developed is not intended to be all inclusive but it provides the Poison Center personnel with a concise protocol for the treatment of eye injury.


Cruciferae vegetable diets, e.g. cabbage, cauliflower, Brussels sprouts etc. have been shown to induce hepatic (Babish and Stoewsand, 1975) and intestinal (Wattenberg, 1971) mixed function oxidase enzymes, probably via their indigenous glucosinolates and other natural compounds. Semi-purified diets containing 25% freeze-dried cauliflower fed to Sprague-Dawley rats depressed hepatic residues of polybrominated biphenyls as well as total liver lipids. This diet enhanced hepatic aminopyrine N-demethylase and p-nitranisole 0-demethylase activities. Estimation of \( K_a \) for aryl hydrocarbon hydroxylase in liver, kidney, and intestine of these rats were significantly lower than the purified diet fed animals. Feeding this cauliflower diet with 2 ppm aflatoxin B1 for 41 weeks to Fischer 344 rats enhanced survival and reduced liver pathology as compared to rats consuming 2 ppm of aflatoxin in a purified diet. Detoxication through enhanced metabolism of these two food contaminants indicates that common dietary vegetables may have a significant effect in environmental health.

The practicality of using isolated liver preparations for developing an assay to prescreen for hepatotoxicity has been examined. Hepatocytes were prepared from young-adult rat livers by a nonperfusion method that involved a 60-minute digestion with collagenase and hyaluronidase. Cell impermeability to trypan blue was > 90%, and background GOT and GPT in the medium were low. Viability of > 70% remained after two hours incubation at 37° C in Hanks buffer. Of 16 hepatotoxins known to release transaminases in vivo, 4 failed to do so in the assay. Of the four, one—acetaminophen—did release GOT from mouse hepatocytes, which agrees with its greater hepatotoxicity in mice. The remaining three—tetracycline, thioacetamide, and allyl alcohol—were ineffective at the highest concentration used (10 mM), and additional modifications of the assay are required. Of the seven nonhepatotoxins, two tested positively in the assay; upon reexamination in vivo at higher doses, they were also found to promote elevated SGOT and SGPT in rats. These results mandate continued effort to improve the predictivity and utility of the method.

264. MUTAGENICITY TESTING OF INDUSTRIAL WASTE WATER EFFLUENTS BY AMES' S. TYPHIMURIUM METHOD. Satu M. Somani, Jerry Mack, and David Schaeffer, School of Medicine, Southern Illinois University, and Illinois Environmental Protection Agency, Springfield, IL 62708.

The Ames test is being extensively used for detecting the mutagenicity of individual compounds. Very little is known about the detection of mutagens in complex mixtures. We have attempted to determine the mutagenicity of mixtures of organic compounds in industrial waste waters. Base-neutral and acid fractions were obtained after pH adjustment followed by liquid-liquid extraction. These mixtures were suspended in dimethyl sulfoxide or water and tested against S. typhimurium strains TA 100, TA 98 and TA 1537. The strains were cultured in Vogel-Bonner medium E modified broth or in nutrient broth. Individual compounds (12) were screened to determine the most effective and dependable standards for mutagenesis control. The results from these media were also compared. Most waste waters effluent extracts from various types of industries were toxic to bacterial test strains. Any mutagens which were present were at levels below those at which mutagenicity could be observed. Work is underway to isolate possible mutagen(s) from the mixtures. Supported by EPA Grant No. 100517077.
265. STUDIES WITH INHIBITORS OF EPOXIDE HYDRATASE IN FORWARD AND REVERSION BACTERIAL MUTAGENESIS ASSAY SYSTEMS. D. Guest and J.C. Dent, Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Sponsor: J.E. Gibson

Cyclohexene oxide (CCHO) and 1,1,1-trichloropropene-2,3-oxide (TCP0) are inhibitors of epoxide hydrolase and are used in studies of the mechanisms of mutagenesis in bacterial mutation assays. Little is known regarding the genetic activity of these epoxides in bacterial assay systems. CCHO and TCP0 were tested in S. typhimurium for their ability to induce both reversion to histidine prototrophy (TA98, 100.1535, 1537, 1538) and forward mutation to 8-azaguanine resistance (TM677). In the reversion assays TCP0 produced base-pair substitutions while CCHO showed no mutagenic activity at concentrations up to 10^{-9} M. In the forward assay, TCP0 (5 x 10^{-9} M) was a potent mutagen without metabolic activation and was also toxic, while CCHO showed no mutagenic activity (5 x 10^{-9} M). Under forward mutation assay conditions 85% inhibition of epoxide hydrolase activity was observed with 10^{-9} M TCP0, a concentration which caused a 45-fold increase in the mutation frequency. CCHO (3 x 10^{-9} M) produced a 60% inhibition of epoxide hydrolase activity without exhibiting mutagenic effects. Thus, CCHO may be the more suitable inhibitor of epoxide hydrolase in these assays and the forward mutation assay may be superior for studies of the mutagenic potential of halogenated alkenes and their epoxides.


A series of phthalic acid esters, including dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), mono(2-ethylhexyl)phthalate (MEHP), di(2-ethylhexyl) phthalate (DEHP) and butyl benzyl phthalate (BBP), as well as phthalic acid (PA) itself, were examined for their mutagenic effect in the Ames Salmonella system. TA 98 (a frame shift mutant) and TA 100 (a base substitution mutant) were used. The liver microsomal fraction (S9) was prepared from Arochlor-pretreated rats. All test substances were negative in TA 98 in the presence or absence of S9 at concentrations up to 1000 µg/plate. In TA 100 all test substances were negative at concentrations up to 1000 µg/plate except DMP and DEP which exhibited dose-related responses, but only in the absence of S9. The addition of S9 eliminated the positive mutagenic response of these 2 diesters. This effect of S9 was seen even in the absence of the cofactor, NADP, and is presumably due to the microsomal esterase activity. These results suggest further testing of the carcinogenic potential of these commercial products which are widely used as insect repellents and perfume diluents. (Supported by ES34 and ES454)
267. EFFECTS OF ACETONE OR AROCLOR-1254 ON MICROSONAL MEDIATED DMN MUTAGENESIS. S. Haag and I.G. Sipes, Toxicology Program, Univ. of Az., Tucson, Az.

Pretreatment of mice or rats with acetone (A) enhances the microsomal N-demethylation of dimethylnitrosamine (DMN) at low substrate concentrations (< 5 mM), while pretreatment with Aroclor-1254 (PCB) represses the activity at low, but enhances the activity at high (> 35 mM) DMN concentrations. To relate the activity of DMN-demethylase with the mutagenicity of DMN, liver microsomes were isolated aseptically from mice 18 hrs after A (3 ml/kg ip), 5 days after PCB (500 mg/kg ip), or the appropriate controls, and incubated with Salmonella typhimurium (TA-1538), NADPH, and DMN (1 or 70 mM) for 30 min. After a 48 hr incubation on minimal media, revertants per plate were determined. Microsomes from A pretreated mice bioactivated DMN to a mutagen at significantly higher (p<0.001) levels when incubations were performed at 1 mM DMN. PCB-microsomes exhibited a decreased ability to convert DMN to a mutagen at 1 mM DMN, but a significantly higher (p<0.05) ability at 70 mM DMN. These and published reports suggest multiple microsomal enzymes for DMN bioactivation and that acetone may enhance the enzyme that operates at environmentally important levels of DMN. Supported by ACS # IN-110.

268. ENHANCEMENT OF CHEMICALLY INDUCED MUTAGENESIS IN CULTURED V79 CHINESE HAMSTER CELLS BY CERTAIN FATTY ACID METHYL ESTERS. Howard E. Wey and C. Stuart Baxter, Department of Environmental Health, Univ. of Cincinnati Medical School, Cincinnati, OH. Sponsor: Carl C. Smith

Closely similar relative activities have been shown for the linear alkanes both as tumor promoters, and as enhancers of mutagenesis in V79 cells in vitro. In both systems maximum activity was apparent at chain lengths of 12-14 carbons. We now report that saturated linear fatty acid methyl esters show analogous behavior to their structurally similar alkane counterparts toward mutagenesis in V79 cells. At 0.12 and 0.072mM, myristic acid (C_{14}) ester caused enhancements of methylazoxymethanol-induced frequencies of mutation to ouabain resistance of 104 and 28% respectively, and lauric acid (C_{12}) ester 50 and 36% respectively. Other fatty acid esters showed less, usually nonsignificant, activity. The tumor-promoting activities of phorbol-12-acyl-13-acetates have been shown to be dependent on the length of the fatty acyl substituent in a manner analogous to that shown for the activity of fatty acyl esters in the above mutagenesis system. Our results thus suggest that the relative activities of the phorbol diesters are governed by their respective fatty acyl substituents, and that saturated fatty acids only of specific chain length have promoting activity.

Certain strains of mice with a high background level of hepatic neoplasia show an increased incidence of liver nodules after the administration of halogenated pesticides; other test species, including the rat, are often refractory. The objective of this study was to investigate the nature of these nodules by comparing the AHH activity and morphology of KERB (a halogenated pesticide) induced nodules with that of spontaneous and 2-AAF-induced mouse liver nodules. 1146 male mice (C57 B/6 x C3H/AnF P1) were separated into three groups and fed one of the following diets for 12 or 18 months: NIH open formula chow; chow with 20 to 2500 ppm KERB or chow with 300 ppm AAF. Liver nodules and adjacent normal tissue were taken for morphological and AHH analysis. The following AHH activity ratio was determined for each pair of specimens: (adjacent normal-nodule)/nodule. In AAF and control groups, the ratios were positive ($R=7.6$ and $5.9$, respectively) due to a significant decrease ($p<.05$) in nodular AHH activity. However, in KERB-treated mice, the ratios were significantly lower ($R=2.7$; $p<.05$) than those of control and AAF-treated groups, with 50% of the nodules having activity $\leq$ that of adjacent normal tissue. (Supported in part by a grant from Rohm and Haas Co.).

270. INFLUENCE OF HEPATIC MICROSONAL ENZYME INDUCERS ON THE ACUTE TOXICITY OF MONSENIN IN RATS. C.S. Probst and L.E. Hill, Lilly Research Laboratories, Greenfield, IN.

Sponsor: J.S. Wold

Monensin, a carboxylic ionophore, shows differential toxicity in male and female rats. This observation prompted an investigation of the influence of hepatic drug metabolism on the expression of acute monensin toxicity. Metabolism of $^{14}$C-monensin was measured in vitro using the 9000 x g liver supernatants from male rats pretreated with phenobarbital, hexobarbital, $p$-methylcholanthrene, benzo (a)pyrene or aroclor 1254. Similarly pretreated rats were challenged with a lethal dose of monensin and survival was correlated with in vitro metabolism. $p$-Nitroanisole O-demethylase activity increased in all pretreatment groups, while monensin metabolism was enhanced only in the phenobarbital and aroclor 1254 groups. Upon monensin challenge (LD$_{50}$ dose), 100% survival was observed in the phenobarbital pretreatment group. Pretreatment with SFK-252A potentiated acute monensin toxicity and inhibited in vitro metabolism of monensin. Monensin pretreatments modified neither toxicity nor metabolism.

Sprague-Dawley rats ingesting 30 mg of purified 2,4,5-T/kg/day for up to 2 years had decreased weight gain, increased kidney weights, increased urinary volume and porphyrin, plus slight morphologic changes in kidney, liver and lungs. Parameters unaffected included mortality, food consumption, palpable masses, hematology, urinalysis, serum chemistry, tumor incidence and gross and microscopic morphology of all organ systems (except as described above). At the intermediate dose level of 10 mg/kg/day there were only minimal effects, limited primarily to the kidney. Rats given 3 mg/kg/day had no discernible effects as a result of the 2-year treatment period.

Thus, the results of this study revealed no oncogenic response in rats, even when administered a sufficiently high dosage of 2,4,5-T to induce toxicity.

272. THE SUBACUTE AND CHRONIC TOXICITY OF FENITROTHION TO MALE RATS. D.J. Ecobichon and D. Zelt, Dept. of Pharmacology & Therapeutics, McGill University, Montreal, Que., Canada.

Reports conflict concerning the chronic toxicity of fenitrothion (0,0-dimethyl-O-(4-nitro-m-toly)phosphorothioate). Weaning male rats received peanut oil (control) or technical fenitrothion in oil at 0.5, 1, 5, 10 and 50 mg/kg/day by oral gavage for 12 months. Ten control and 5 treated rats from each group were killed monthly for analysis of cholinesterase (ChE) (brain, plasma and erythrocytes) and carboxylesterase (CE) (liver, kidney) activities and fenitrothion residues (liver, kidney, perfrenal fat). Brain, spinal cord and sciatic nerve were preserved for morphological examination. At 50 mg/kg, fenitrothion was acutely toxic to the rats within 3 days of starting treatment, a marked inhibition of tissue esterases being observed. Overt toxicity was not observed at the other doses though a dose-dependent inhibition of ChE and CE was measured at 1 month and persisted afterward. At 3 months, slower weight gain, excitability, piloerection and exophthalmos were observed at the 10 mg/kg/day dosage. Tissue residues of agent remained at the limit of detection by GLC. Tissues appeared normal by gross examination but histopathology in progress will assess possible central and peripheral nervous tissue damage. To-date, there has been no physical evidence of neuro-toxicity.
273. EFFECTS OF INHALED 1,2-DIBromo-3-CHLOROPROPAne (DBCP) ON THE
Semen Of Rabbits And THE FERTILITY OF MAle And FeMaL Rats.
K.S. Rao, F.J. Murray, A.A. Crawford, J.A. John, W.J. Potts,
E.A. Schenectady, J.D. Brey, and C.M. Parker*, Tox. Res. Lab.,
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Exposure of male workers to 1,2-dibromo-3-chloropropane
(DBCP) has been associated with low sperm counts. The
effects of inhaled DBCP on spermatogenesis and fertility and
the possible reversibility of these effects was studied by
exposure of rabbits and rats to 0, 0.1, 1, or 10 ppm of
DBCP. Exposure to DBCP was for 14 consecutive weeks with
the exception of the 10 ppm rabbits which were exposed for
only 8 weeks. Results indicated a potential for inhaled
DBCP to interfere with spermatogenesis in rats and rabbits.
Rabbits had decreased sperm counts at 1 and 10 ppm between
the 6th-14th weeks of the study. All of the 10 ppm rabbits
appeared to be infertile when mated during the 14th week.
A significant dominant lethal effect was seen in rats at
10 ppm as evidenced by an increased incidence of resorptions
among unexposed females mated with exposed males. Exposure
has been completed and surviving animals are being monitored
for the reversibility of the effects of DBCP on sperm
counts in rabbits and fetal resorptions in rats.

274. COMPARATIVE ABSORPTION AND DISPOSITION OF CARBOFURAN, 3-
HYDROXYCARBOFURAN AND THE GLUCOSIDE OF 3-HYDROXYCARBOFURAN.
T.C. Marshall and H.W. Dorough, Grad. Cen. for Toxicology,
Univ. of Kentucky, Lexington, Kentucky.

Terminal carbamate insecticide plant residues to which
humans are exposed are primarily of a water soluble (conjugated)
and/or bound nature. The toxicological significance of
such residues is largely dependent on absorption from the
GI tract. An evaluation of the absorption and disposition of
radioactive labeled carbofuran (CF), 3-hydroxy-carbofuran (3-OH
CF), and 3-hydroxy-carbofuran glucoside was conducted. After
two days 20-30% of the dose of each compound was excreted
in bile, ~60% in urine, and 0-4% in feces. Intestinal
transit of these materials was determined following oral
doses to rats as well as absorption using in vivo isolated
GI segments. While CF and 3-OH CF were readily absorbed
from all sections of the GI tract, the glucoside conjugate
was directly translocated to the lower small intestine and
gastroc. At this point, cleavage of the glucosidic linkage
occurred and the freed 3-OH CF (a potent cholinesterase
inhibitor) was then absorbed. These investigations demon-
strate that conjugates of carbamate insecticides formed
in plants may provide a bioavailable source of toxic
exogenous compounds if consumed in the diet of man and other animals.
(Supported in part by EPA Grant R-805143020.)
275. FORMATION AND FATE OF NITROSAMIDES IN ANIMALS. Robert W. Rickard and H. Wyman Dorough. Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40506

Our previous studies showed that the guinea pig is a better model than the rat for nitroso studies since its low stomach pH is similar to that of man. Utilizing the guinea pig, studies were conducted to determine the extent of nitrosation of \(^{14}C\)-methylurea and two carbamate insecticides, \(^{14}C\)-carbofuran and \(^{14}C\)-carbaryl. Each compound was readily nitrosated in the presence of guinea pig stomach contents; reaction of 0.5 \(\mu\)mol of labeled compounds with 140 \(\mu\)mol of NaNO\(_2\) yielded approximately 25% nitrosation of each compound. In vivo yields of nitrosocarbofuran and nitrosocarbaryl (NC) in the stomach were <0.2% but was 4.5% for nitrosomethylurea. The greater in vitro yields suggested that much of the in vivo synthesized nitroso compounds may have been degraded prior to extraction. NC in solutions of different pH exhibited maximum stability between pH 3-5. At pH 1.5, the pH of the guinea pig stomach contents, NC was rapidly denitrosated to regenerate carbaryl. When NC was given orally to rats, 82% of the dose was excreted in the urine and 9% in the feces after 24 hr. This pattern of elimination is consistent with that obtained when carbaryl per se is administered to the rat. (Supported in part by EPA Grant R805143020.)

276. QUANTITATIVE ADMINISTRATION OF INSECTICIDE VAPORS TO RATS. W. A. Stubblefield and H. W. Dorough. Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40506

Pharmacokinetic studies in animals exposed via inhalation are hindered severely by difficulties encountered in quantitating the total dose inhaled and that portion which is retained. These were largely overcome in the present study by fitting rats with a facemask from which two tubes were extended. One tube was attached to the source of chemical vapors through which the animal inhaled. The other tube was attached to a polyurethane foam trap to collect chemicals in the expired air. Two one-way valves in the system prevented mixing of the inhaled and expired air. After exposure, the quantity trapped by the polyurethane foam represented expired chemical while the remainder of the dose was considered retained in the body. Studies with \(^{14}C\)-chlordane and heptachlor demonstrated that 77% of a dose inhaled over a 30 min period was retained in the body. The percentage of the calculated retained dose found in the urine, feces and body after 7 days was 103 ± 9 for chlordane and 100.2 ± 2 for heptachlor. Thus, the method for calculating the retained dose proved to be quite accurate. Patterns of distribution and excretion were similar to that in rats treated orally with the compounds.
277. COMPARATIVE CARBAMATE ESTER HYDROLYSIS IN FOUR MAMMALIAN SPECIES. W. H. Benson and H. W. Dorough. Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40506

Ester hydrolysis of carbamate insecticides destroys their anticholinesterase activity and is considered an important detoxification mechanism in mammals. In this study, the hydrolysis rates of carbfaril and Croneton were examined in four species. Respiratory 14CO2 resulting from the hydrolysis of orally administered 14C-carbonyl-labeled carbamates (0.2 mg/kg) was taken as a measure of in vivo hydrolysis rates. Ester hydrolysis was greater for Croneton than for carbfaril in all animals, although the relative hydrolysis rates among species was the same with both compounds. After 24 hr, gerbils had hydrolyzed 91% of the Croneton and 56% of the carbfaril. Guinea pigs hydrolyzed somewhat less of the compounds, 65 and 50%, but considerably more than rats and mice, 40 and 25%. Toxicity tests showed that gerbils and guinea pigs were more sensitive to carbamate poisoning than were rats and mice. These results do not necessarily minimize the role of ester hydrolysis in detoxification, but they do suggest that other important factors are involved in governing the inherent toxicity of carbamate insecticides to mammals. (Supported in part by EPA Grant R805143020.)

278. POSTPARTUM CPK AND LDH CARDIAC AND SERUM ISOZYMES AFTER 2,4,5-T, CARBARYL OR ANILINE TREATMENT. R.D. Courtney, Environmental Protection Agency, Research Triangle Park, N.C.

CPK and LDH isozyme patterns in serum and cardiac tissue were determined day 1 and 20 postpartum (pp) in CD-1 mice after treatment, IG, with 2,4,5-T (100 mg/kg), carbfaril (100 mg/kg), during gestation or with aniline (150 or 200 mg/kg) during gestation and lactation. The serum CPK isozyme pattern was markedly altered in all treatment groups on day 1 pp. By day 20 pp, the abnormal alteration in pattern persisted in the carbfaril group, while the aniline group showed another abnormal pattern with the appearance of an additional isozyme. In contrast, the 2,4,5-T serum CPK pattern was not significantly different from the control pattern. The CPK cardiac isozyme patterns on day 1 pp of the carbfaril and aniline groups showed minimal changes while the 2,4,5-T pattern was normal. The cardiac LDH isozyme pattern showed changes of lesser magnitude on day 1 and 20 pp. The serum LDH isozyme pattern did not differ significantly from control values on day 1 or 20 pp. Comparable changes were observed in the neonatal cardiac isozymes during development, with more changes in the CPK isozyme pattern than in the LDH isozyme pattern.
279. CARBOXYLAMIDASE INHIBITION BY DASANIT AND INTERACTION WITH ACETANILIDE IN THE RAT. H.E. Ouellette and S.B. Cohen, Dept. of Pharmacology and Toxicology, School of Pharmacy, University of Connecticut, Storrs, CT.

We previously reported that Dasanit potentiation of procaine toxicity in mice and rats was dependent upon Dasanit inhibition of liver esterase hydrolysis of procaine. The present studies demonstrate a relationship between Dasanit inhibition of liver amidase and alteration in acetanilide induced methemoglobinemia (MetHb) in male rats. At 1, 2, 4, 6 and 12 hours after acetanilide (400 mg/kg, ip) MetHb was 9, 10, 17, 13 and 7% of total Hb. When the same acetanilide dose was administered one hour after Dasanit (2.5 mg/kg, ip) the MetHb values were all between 2 and 4% of total Hb at the same time points after acetanilide. This Dasanit treatment caused 84% inhibition of liver acetanilide amidase.

One hour after 0.5 mg or 72 hours after 2.5 mg Dasanit/kg liver amidase was inhibited 58 and 20% respectively. Corresponding MetHb levels 4 hours after acetanilide were 6 and 14%. These results suggest that inhibition of tissue amidases by commonly used organophosphates may alter the toxicity of acetylcholinesterase type drugs.

(Supported by NIH Grant No. ES001093-02)


A continued search is under way for non-invasive techniques for monitoring pesticide exposures. Since saliva has been shown to contain a number of xenobiotics, it might provide a better tolerated sampling fluid than blood or urine. This investigation is to determine if a correlation exists between levels of pesticide and cholinesterase activity in plasma, erythrocytes and saliva. Anesthetized adult male Sprague-Dawley rats received 2, 4, or 8 mg/kg carbofuran or parathion by direct injection into the stomach using corn oil as the vehicle. The distribution of carbofuran or parathion and/or their metabolites in plasma, erythrocytes and saliva was monitored by GC. Cholinesterase activity was determined by the method Ellman. An apparent correlation exists between the cholinesterase activity in plasma, erythrocytes and saliva. (Supported in part by EPA grant number R804318010.)
281. ETHANOL POTENTIATION OF COCAINE-INDUCED HEPATIC NECROSIS.
Phenobarbital (PB) pretreatment of mice antagonizes the acute toxicity of cocaine (C), but produces a latent hepatotoxicity. This latent periportal necrosis is antagonized by pretreatment with SKF 525A. The purpose of this study was to determine the effect of ethanol, another inducer of mixed function oxidase activity, on C-induced hepatotoxicity. Mice were fed an ethanol containing liquid diet for 5 days. Pair-fed controls received a liquid diet in which the ethanol was isocalorically substituted with maltose-dextrins. Hepatic cytochrome P450 activity was determined after 5 days of ethanol pretreatment and there was approximately a 2-fold increase above controls. Groups of ethanol pretreated and control mice were treated with C (60 mg/kg i.p.) and sacrificed 30 hours later. Histological examination of the liver revealed a centrilobular necrosis in the ethanol pretreated mice. Similarly, SGPT activity was significantly elevated above controls. The shift in the site of the C-induced necrosis seen with PB or ethanol pretreatment may indicate the preferential induction of different enzyme systems capable of bioactivating cocaine. (Supported in part by USPHS Grants GM15431 and GM00058).

In two separate experiments the ability of isoniazid and ethanol to effect metabolism was investigated in male Hartley guinea pigs. In the first experiment ethanol (2 g/kg, orally) or saline was given simultaneously with an oral dose of $^{14}$C-isoniazid (20 mg/kg) and blood and urine collected up to 1 hr after dosing. Ethanol did not cause any change in the rate of $^{14}$C disappearance from blood although at 8-11 hr the level was above the control values. In both groups of guinea pigs 77% of the dose was recovered in urine after 1 hr; no significant changes were observed in the various metabolites. In the second experiment isoniazid (100 mg/kg, orally) was given 30 min prior to an oral dose of ethanol (1 g/kg). Blood ethanol was measured enzymatically up to 4.5 hr after dosing and it was found that the T$_1/2$ was 16.3 ± 5.6 min in the controls and 187.5 ± 10.5 min ($p < 0.01$) in the ethanol-treated animals. We conclude that ethanol has little or no effect on isoniazid but that isoniazid is a powerful inhibitor of ethanol elimination in the guinea pig.

The mechanism by which acute isoniazid (INH) overdose causes lactic acidosis is unclear. This study examines the role of convulsion in the development of lactic acidosis in dogs given po lethal doses of INH (75 mg/kg). Following INH, dogs did not develop acidosis until after they had convulsed (ca. 1 hr after INH). Acidosis became more pronounced with successive convulsions and was associated with marked increase of lactate, e.g., immediate post-convulsive level of 12.3 mEq/l as compared to control level of 1.83 mEq/l. Diazepam, 0.5 mg/kg, plus pyridoxine HCl, 150 mg/kg, injected iv immediately following INH, resulted in no convulsions and no change in pH and lactate. Administration of the antidotes after the second convulsion in INH toxicity prevented further convulsions and allowed recovery of blood pH and lactate to control levels. Finally, curarization of INH-treated dogs prevented motor seizures and marked increase of lactate, e.g., after second EEG convulsion lactate was 2.15 mEq/l as compared to control of 0.89 mEq/l. It is concluded that convulsion is the main cause of lactic acidosis in acute INH toxicity.


Halothane induces hepatic centrilobular necrosis in rats pretreated with phenobarbital and subjected to reduced oxygen tension (Lee, et al Pharmacologist 20: 258, 1978, Widger et al. Anesthesiology 44: 197, 1976). The purpose of the present study was to determine the effects of hyperthyroidism on the halothane-induced liver lesion. 12 male rats were treated with triiodothyronine (T3) (10 mg/kg orally for 5 days) prior to exposure to 1% halothane for 2 hours at normal oxygen tension while the control animals received the same exposure to halothane but no T3. Severe pericentral coagulative necrosis with extensive round cell infiltration developed in all of the livers from the T3 treated animals but no discernible changes were observed in the livers from the controls. Hyperthyroidism sensitizes the rat to halothane-induced hepatic necrosis. The mechanism for this sensitization may be an intracellular induced hypoxia thereby shunting halothane metabolism to the reductive pathway producing more reactive metabolites and subsequently hepatic damage. (Supported by USPHS Grant GM15431).
285. LETHAL SYNERGISM OF CHOLINESTERASE INHIBITORS BY LITHIUM.
N. Hatoun and W.M. Davis, Dept. of Pharmacology, School of
Pharmacy, Univ. of Mississippi, University, MS.

The report of a lethal interaction between sublethal doses
of lithium and physostigmine (Samples et al., Psychopharma-
cology 52:307, 1977) was confirmed and extended in several
regards. Male Sprague-Dawley rats were given an i.p. dose
(300 mg/kg) of lithium chloride 12 hr prior to a s.c. dose
of physostigmine sulfate (1.0 mg/kg). High mortality (91%)
resulted from the combination. To test generality of the
effect, Long-Evans rats were used under the same conditions
and identical results were obtained. Further study of Li
and physostigmine in S-D rats showed the combined effect to
be completely reversed by atropine sulfate (1.0 mg/kg, s.c.),
and slightly but not significantly reduced by the peripher-
ally-acting methylatropine Br (0.5 mg/kg, s.c.). A lesser,
but still significant, lethal synergism between Li and neo-
stigmine methylsulfate (0.3 mg/kg, s.c.) was found. While
the major component of the synergism for physostigmine
appears to be via the CNS, a peripheral contribution may also
be significant. These results have relevance to the proposed
clinical use of physostigmine for suppression of mania,
and to its widely-advocated use to reverse postoperative
somnolence. (Supported by the Research Institute of
Pharmaceutical Sciences, the University of Mississippi.)

286. POTENTIATION OF PHARMACOLOGIC RESPONSES TO BARBITURATES BY
DOPRAM® AND ITS PRESERVATIVE, L.K. Ho, B.A. Flint and
Jackson, MS 39216

The effect of DOPRAM®, a clinically used respiratory
stimulant, on responses to pentobarbital and hexobarbital
was evaluated. DOPRAM® was shown to significantly enhance
barbiturate induced narcosis and hypothermia in a dose-re-
lated manner. DOPRAM® significantly potentiated barbiturate
sleeping times when administered two hrs. prior to drug
administration. The half-life of pentobarbital in brain and
serum was shown to be significantly longer in animals receiv-
ing DOPRAM® 40 mg/kg, i.p. The waking brain and serum pen-
tobarbital concentrations were not different in either group.
The effect of the individual components of DOPRAM® (doxapram
HCl and chlorobutanol) on barbiturate narcosis and hypother-
mia was evaluated. Either doxapram HCl (20 and 40 mg/kg,
i.p.) or chlorobutanol (5 and 10 mg/kg, i.p.) potentiated
barbiturate narcosis and hypothermia. The hepatic drug meta-
bolizing enzymes were significantly decreased at 4 hrs. and
increased at 24 hrs. following an acute injection of chloro-
butanol, 200 mg/kg, i.p. These studies show that DOPRAM®
potentiates barbiturate effects. (Supported by Grant
DA 01403).
287. RAT LIVER MICROSONES METABOLIZE PARGYLE to PROPIOLALDEHYDE, A POTENT IRREVERSIBLE INHIBITOR OF ALDEHYDE DEHYDROGENASE (ALDH). F.N. Shirota, E.G. DeMaster and H.T. Nagasawa, Medical Research Laboratories, VA Hospital and Dept. of Medicinal Chemistry, University of Minnesota.

Pargylene when administered to rats caused an elevation in ethanol-derived blood acetaldehyde (AcH) similar to disulfiram. Unlike disulfiram, pargylene did not inhibit ALDH in vitro. SKF-525A pretreatment completely nullified this pargylene effect, while phenobarbital (PB) treatment enhanced the pargylene-induced rise in blood AcH by 65%, thereby implicating a metabolite of pargylene as the active inhibitor of ALDH. Indirect evidence (DeMaster & Nagasawa, Res. Comm. Chem. Path. Pharmacol., in press) suggested that the inhibitory metabolite of pargylene was propiolaldehyde (HC≡CCHO). PB-induced hepatic microsomes catalyzed the metabolism of pargylene to an aldehyde metabolite which was trapped as its semicarbazone (SC). The latter was extracted into EtOAc and identified by TLC as propiolaldehyde-SC. Acid treatment of this SC released propiolaldehyde which had a GC retention time identical to authentic (synthetic) prosiolaldehyde. N-Benzylpropargylamine similarly yielded propiolaldehyde. GC-MS analysis of pargylene-derived propiolaldehyde at 20 and 70 eV gave ions at m/e 54 (M+), 53 (M-1) and 26, also characteristic for synthetic propiolaldehyde.


We have previously reported that concurrent administration of aspirin protects mice from the toxicity of sublethal doses of retinoic acid (RA). In the present study, mice were treated daily for 21 days with the LD10 of RA (14 mg/kg) ip with or without a daily oral dose of a nonsteroidal anti-inflammatory (NSAI) drug. Mice were x-rayed on days 1, 8, 15, and the day after last treatment (day 22) for diagnosis of fractures. The order of potency of NSAI drugs for reduction of RA toxicity was indomethacin>fenoprofen>tolmetin sodium>ibuprofen>aspirin; this corresponds to their relative ability to inhibit prostaglandin synthesis. Indomethacin treatment resulted in 78% inhibition of RA-induced fractures. However, g-hydroxybenzoic acid, which does not inhibit prostaglandin synthesis, appears to have no effect on RA toxicity. These results suggest that prostaglandins may be involved in the expression of RA toxicity. RA was supplied by Hoffmann-LaRoche, Inc. Supported by Contract NO1-CP-22064, DCCP, NCI, NTH, DHQ. Eli Lilly and Co., McNeil Laboratories, Inc., and the Upjohn Company supplied NSAI drugs.
289. ABSTRACT WITHDRAWN


Autopsy and fixation of tissues immediately after death are important for the optimal interpretation of histopathologic material. Animals dying during unconventional hours, when surveillance may be reduced or lacking, may either be discarded or attempts may be made to minimize the effects of autolysis. These options have an important bearing on the outcome of toxicological investigations, particularly those involving histologic examination. In our experiments, one group of rats was kept at room temperature up to 18 hours with the carcass unopened. Three other groups were kept unopened at room temperature for 3 hours; one of these was opened thereafter for varying periods, another was refrigerated thereafter with the carcass opened, and the last refrigerated with the carcass unopened. The effects of autolysis were similar whether the carcass was opened or not, but refrigeration significantly arrested its progress in most organ systems, thus allowing a reasonable histopathologic assessment of the tissues for up to 8 hours after death. Surveillance, leading to prompt refrigeration of dead animals is therefore recommended.

291. CINOXACIN INDUCED ARTHROPATHY IN JUVENILE BEAGLE DOGS. L.C. Howard, D.C. VanSickle, K. Deshmukh, W.J. Griffing and N.V. Owen, Toxicology Division, Lilly Research Laboratories, Greenfield, IN and Department of Anatomy, Purdue University, West Lafayette, IN.

Cinoxacin (1-ethyl-1,4-dihydro-4-oxo[1,3]dioxol[4-5-g]cinnoline-3-carboxylic acid), a urinary tract antibacterial agent, was administered orally (250 mg/kg/day) to 2-3 month old beagle dogs. Lameness, soreness and swelling of weight bearing joints were observed after 2-7 doses. At necropsy, lesions on the humeral and femoral heads were focal in nature and characterized by blister formation, detachment of portions of superficial articular cartilage and shallow erosions. Cartilage samples had decreased amounts of collagen and degenerating chondrocytes. During the initial stages of the lesion, increased amounts of synovial fluid were observed. Elevated hexosamine and acid phosphatase were found in some synovial fluid samples. Concentrations of cinoxacin in synovial fluid did not differ between young and adult dogs. Susceptibility was related to age and dose. Gross lesions were not observed in dogs 8 months of age or older or in young dogs given 62.5 mg/kg/day for 7 days.
292. MARIHUANA SMOKE: CHRONIC TOXICITY EVALUATION OF MARIHUANA SMOKING IN THE CYCLOPUSUS MONKEY. W. B. Coate and R. W. Voelker, Hazleton Laboratories America, Inc. Vienna, VA
Sponsor: M. Steinberg

The organ toxicity potential of chronic marijuana smoking is in dispute. Cynomolgus monkeys were forced to inhale heavy doses of marijuana smoke daily for up to 12 months. Marihuana cigarettes, assayed at 1.2% $^{3}$THC (first 6 months) and 2.8% $^{9}$THC (last 6 months), and THC-extracted cigarettes were used. The cigarettes were puffed (35 ml in 2 seconds) every 60 sec. by A. D. Little, Mark II smoking machines and the boluses delivered to face masks. Marihuana smokers inhaled all the main-stream smoke from 1 or 4 (later 3) cigarettes/day while the THC-extracted smokers inhaled it from 4 (later 3) cigarettes/day. CORe and serum-THC levels averaged over 50% and over 500 ug/ml, respectively, in the high-level marihuana smokers. Heart rate and respiration rate showed daily transient decreases in the high-level marihuana smokers only, while rectal temperature was also reduced transiently in the THC-extracted smokers. No ophthalmological, hematological, clinical chemistry, organ weight, or histopathological changes were observed after 6 or 12 months except for lung changes seen in all smokers which were attributed to chronic inhalation of vegetable matter smoke. (Supported by Contract No. 271-75-306, with the National Institute on Drug Abuse).

293. SULPHONE METHEMOglobinemia. M. Schvartsman, Instituto da Crianca Hospital das Clinicas, School of Medicine, Sao Paulo, Brazil.

The study of 78 cases of ingestion of excessive doses by children less than 11 years old demonstrated that sulphone is a powerful methemoglobinizing agent. The symptoms and signs are mainly cutaneous (cyanosis) and neurological, including dizziness, incoordinated movements, nystagmus, stupor, coma and convulsions, and they are early, appearing 30 minutes after ingestion. Sulphonemies exceeding 10 mg% are potentially lethal. Methemoglobinemias exceeding 60% are associated with serious clinical pictures. There is inhibition of G6PD activity. The best management in serious cases is exchange transfusion with forced diuresis or peritoneal dialysis.
294. INHIBITION BY BRAN OF THE COLONIC COCARCINOGENICITY OF BILE SALTS IN RATS GIVEN DIMETHYLHYDRAZINE. T.A. Barbolt, J.B. Rodgers, and R. Abraham, Institute of Comparative and Human Toxicology and the Dept. of Medicine, Albany Medical College, Albany, NY

The role of bran and bile salts was investigated in colon carcinogenesis induced by dimethylhydrazine (DMH). Male Sprague-Dawley rats were used: Group I - Control, Group II - 0.5% bile salts, Group III - 30 mg/kg DMH (10 weekly doses), Group IV - bile salts & DMH, and Group V - bile salts, 20% bran & DMH. DMH was given orally, and bile salts and wheat bran were added to the basal diet. In Groups III, IV and V, the numbers of grossly observable colonic tumors/rat were 10.8, 8.4 and 11.6 respectively. Microscopic examination revealed a 100% increase in adenocarcinomas in Group IV when compared to Group III, whereas only a 40% increase was noted in Group V. These observations demonstrate that bile salts promote a significant transformation of adenomas to adenocarcinomas, and that this trend is reversed by bran. Supported by NIEHS Training Grant No. IT32-ES-07058-01.

295. DOSE-RESPONSE, SEX DIFFERENCES, AND THE EFFECT OF BRAN IN DIMETHYLHYDRAZINE-INDUCED INTESTINAL TUMORIGENESIS IN RATS. T.A. Barbolt and R. Abraham, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, NY

Dimethylhydrazine (DMH) is extensively utilized to induce colonic tumors. However, the possibility of a dose-response and sex difference occurring with this model have been overlooked. For this purpose, male and female weanling Sprague-Dawley rats were used: Group I - Control, Group II - 2% bran, Group III - 15 mg/kg DMH (10 weekly doses), Group IV - bran & 15 mg/kg DMH, Group V - 30 mg/kg DMH (10 weekly doses) and Group VI - bran & 30 mg/kg DMH. DMH was given orally and wheat bran was added to the basal diet. The number of grossly observable colonic tumors/rat in Groups III and V was 2.1 and 6.4 for males, and 0.2 and 0.6 for females. In Group V, 100% of the males but only 30% of the females developed colonic tumors. Male rats in Group IV had a significant reduction in the number of colonic tumors when compared to Group III. Thus, in the DMH model, consideration has to be given to the effects of a dose-response and sex differences with respect to intestinal tumorigenesis. Addition of bran to the diet inhibited the formation of colonic tumors. Supported by NIEHS Training Grant No. IT32-ES07058-01.

The objective of this study was to determine if nitrite alone had an oncogenic effect in rats. A total of 1381 Sprague-Dawley Charles River CD^R rats, about equally divided as to sex, were exposed to sodium nitrite in various vehicles and in concentrations ranging from 250-2000 ppm beginning from five days prior to birth throughout postnatal lifespan. Five hundred seventy three rats served as controls. There was a dose-response relationship for lymphoreticular tumors irrespective of vehicle-semipurified diet, water, natural product diet-and whether or not exposure started in utero or after weaning. The spectrum of non-lymphatic tumors was consistent with this strain of rat and it is concluded that nitrite has a tumor-promoting effect apart from a nitrosamine mechanism. Mechanisms for such an effect appear to involve in part, a depression and later a sensitization of lymphocytes resulting in proliferative response and neoplasia. (Supported in part by FDA).

297. CARCINOGENIC RISK ESTIMATION FOR CHLOROFORM: INCORPORATION OF PHARMACOKINETIC DATA. R.H. Reitz, P.J. Gehring, P.G. Watanabe, and C.N. Park, Toxicology Research Laboratory, Dow Chemical Company, Midland, MI 48640.

High doses of chloroform (CHCl_3) have been reported to induce tumors in rodents (National Cancer Institute, 1976). Using these data, the Environmental Protection Agency (EPA) has estimated the cancer risk to man from low levels of CHCl_3 in drinking water. However, failure to consider the role of metabolic activation has produced inconsistencies in these predictions. The two sets of data (rat and mouse) give different estimates of human risk. Furthermore, EPA's procedures incorrectly predict that rats will develop more tumors than mice from equivalent CHCl_3 exposures.

In contrast, after correction for metabolic activation, human risk estimations are consistent, the most sensitive species is correctly identified, and the magnitude of the risk estimate decreases more than 100X. Tumor induction by other chemicals (e.g. vinylidene chloride, 2-acetaminofluorene, and trichloroethylene) in various species also correlates well with the relative rate of metabolic activation. Consideration of pharmacokinetic parameters greatly increases the accuracy of interspecies extrapolations.
298. RELATIONSHIP BETWEEN METABOLISM OF METHYLAZOXY-METHANOL (MAM) ACETATE, INHIBITION OF MACROMOLECULE SYNTHESIS AND TUMOR INDUCTION. M.S. Zedeck and Q.H. Tan, Memorial Sloan-Kettering Cancer Center, New York, NY. Sponsor: H. Marquardt

Rat liver and colon are markedly sensitive to the acute and chronic effects of the carcinogen MAM acetate. This appears to be correlated with metabolism of MAM by NADH-dependent dehydrogenase enzymes in these organs and the active metabolite is probably the aldehydic form of MAM. Pyrazole, an inhibitor of alcohol dehydrogenase, prevents carcinogen-induced acute lethality and colon tumors. MAM acetate, 70 mg/kg, iv, inhibits DNA, RNA, and protein synthesis in liver and colon within 3 hr after treatment. Pyrazole, 180 mg/kg, ip, 2 hr prior to MAM acetate completely prevents inhibition of protein and RNA synthesis; inhibition of DNA synthesis is essentially unaffected. The results suggest that the aldehydic form of MAM inhibits RNA and protein synthesis and that inhibition of DNA synthesis is induced by another product of MAM, possibly the carbonium ion, such effect being unrelated to tumor induction.

299. CARCINOGENICITY AND METABOLISM OF 5-F-DMBA AND ITS 7-HYDROXY METHYL (5-F-7-HO-DMBA) AND 7-ACETOXYMETHYL (5-F-7-Ac-DMBA) DERIVATIVES. M. Chien and J.W. Flesher, Dept. Pharmacology, College of Medicine and the Graduate Center for Toxicology, Univ. of KY., Lexington, KY. 40506. Sponsor: M. Vore

5-F-DMBA and 5-F-7-Ac-DMBA were carcinogenic in male Sprague-Dawley rats by repeated s.c. injection, but were less active than DMBA, and 7-Ac-DMBA. Metabolism studies of these compounds with rat liver S-9 indicate that enzymatic hydrolysis of 5-F-7-Ac-DMBA to 5-F-7-HO-DMBA is a major pathway. The hydrolysis product, 5-F-7-HO-DMBA was conjugated with glucuronic acid. 5-F-7-HO-DMBA was ring hydroxylated to the extent of 16%. 5-F-DMBA was hydroxylated at the 7-methyl group to give 5-F-7-HO-DMBA (2-4%). Photo-oxides of all compounds were formed non-enzymatically. Non-enzymatic binding of 5-F-7-HO-DMBA was low, but was increased 5-fold in the presence of ATP. DNA binding of 5-F-7-Ac-DMBA was higher than 5-F-7-HO-DMBA. No significant enhancement in binding of 5-F-7-Ac-DMBA was found with ATP. DNA binding of the non-fluorinated compounds was greater than the corresponding fluorinated compounds. Reduced carcinogenicity and DNA binding of fluorinated compounds may be due to reduced formation and reactivity of ester(s). The data support the hypothesis that a reactive ester functions as an ultimate carcinogen. Grant #12-14-7001-1042 USDA.
300. COMPUTER MODELING OF CARCINOCENESIS MECHANISMS INCLUDING DOSE-RESPONSE RELATIONSHIPS - J. B. Reid, Chemical Hygiene Fellowship, CMIR, Pittsburgh, PA; E. Bingham, Univ. of Cincinnati, Cincinnati, OH and B. Chertow, Carnegie-Mellon Univ. Pittsburgh, PA.

A computer simulation program has been developed to model initiator, promoter and cocarcinogenesis-type experiments. The model includes a dosage parameter for the initiator and promotor or cocarcinogen application as relates to the probability of transformation and promotion events. The initiator dose randomly determines the number of transformational events and initiates growth of hypothetical tumors. These tumors are then subject to destruction by the immune parameter and hastened development by promotor action. At a critical size they escape the immune parameter and develop to a clinically observable tumor. Various distributions including normal, skewed, Weibull, etc., in the immune initiator and promotor functions have been tested to produce hypothetical experiments. Data is presented visually and analyzed by actuarial and Horton methods and are compared with actual laboratory data. Insight into possible detailed mechanisms of action of carcinogenesis with possible predictive capabilities is gained by using this model.

301. THE EFFECT OF DIETARY PROTEIN LEVEL ON THE SUBCELLULAR DISTRIBUTION AND BINDING OF AFLATOXIN IN RAT LIVER. K. D. Mainigi and T. C. Campbell, Div. of Nutr. Sci., Cornell University, Ithaca, N.Y.

The present work was undertaken to determine the effect of dietary protein level on the subcellular distribution of aflatoxin-macromolecular adducts. Male F-344 rats weighing 70-80 g were fed diets containing either 5% casein, 5% casein plus 2.5 ppm AFB1, 20% casein, or 20% casein plus 5 ppm AFB1. Rats from each dietary group were administered phenobarbital four days prior to sacrifice. After 21 days all rats were given i.g. dose of [3H] AFB1, at 12-45µCi/100 gbw, and killed at various times. Livers were processed into subcellular fractions which were assayed for both total radioactivity and binding of aflatoxin to lipid-free, acid insoluble macromolecules. Total radioactivity was always highest in the cytosol, while total specific activity was always greatest in the microsomal fractions. The feeding of low protein or aflatoxin in the diet significantly decreased the amount of radioactivity in all fractions, expressed both in terms of specific activity and total content, except for the cytosol, where total amount of radioactivity was increased. Pretreatment with phenobarbital significantly decreased the amount of total and and bound aflatoxin in all the subcellular fractions.
302. CO-MITOGENICITY OF TUMOR PROMOTING AGENTS. J.A. Fish, C.S. Baxter, and J.A. Bash, Depts. of Env. Hlth. and Microbiology Univ. of Cincinnati Col. of Med., Cincinnati, OH. Sponsor: P.B. Hammond

Evidence exists that the mechanism of action of tumor promoting agents may involve interaction with the immune response; hence the effects of these agents on the proliferative properties of mouse spleen T-lymphocytes was investigated. T-cells, normally in a resting (G0) state may be induced to proliferate in response to mitogens such as phytohemagglutinin (PHA). At low PHA concentrations (1:1620) the potent promoter TPA (12-O-tetradecanoyl phorbol-13-acetate, 1µg/ml) enhanced PHA-induced lymphocyte mitogenesis 6-fold, whereas at high concentrations of PHA (1:20) TPA inhibited the PHA-induced response by approximately 50%. Comitogenic activity was also shown by long chain alkane promoters, with tetradecane being the most potent, and fatty acid methyl esters, maximum activity (2-fold enhancement) being shown by methyl myristate. Possible correlations between in vivo tumor promotion and modulation of T-cell proliferation in vitro are at present being investigated, as well as whether the site of promoter interaction is the PHA-responding T-lymphocyte or another subpopulation which subsequently regulates T-cell proliferation.


TRIS was tested as a tumor initiator and a tumor promoter in a two-stage mouse skin tumorigenesis assay using a skin tumor-susceptible mouse strain (SENCAR) to increase the sensitivity of the mouse skin painting test system. Skin tumor initiating activity was tested by applying from 1 to 100 mg of material to the backs of mice followed by repeated application of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Skin tumor promoting activity was assessed by a single sub-carcinogenic application of DMBA followed by twice weekly applications of test material. Following eight weeks, the positive control group treated with DMBA as initiator and TPA as promoter produced 100% tumor-bearing animals. TRIS proved to be a potent tumor initiator over the dose range examined with no evidence of a strong dose dependence. Mice initiated with TRIS but not promoted did not exhibit skin tumors. However, if promotion with TPA commenced approximately one year after initiation, all mice had skin tumors within a six-week promotion period, demonstrating that the initiation phenomenon persists for up to one year. Tumor promoting activity of TRIS is currently being assessed.
304. THE PROBLEM OF ENVIRONMENTAL CARCINOGENS IN TROPICAL AFRICA.
E.A. Bababunmi, Dept. of Biochemistry, Univ. of Ibadan, Ibadan, NIGERIA.

The incidence of primary liver cancer in the countries of tropical Africa is the highest in the world. There is a growing belief that the relatively high prevalence of hepatocellular carcinoma in some West and East African states may have a multiple chemical factor etiology in such forms as food contaminants, herbal teas and environmental chemicals. Chemical carcinogens identified in the environment include aflatoxin, N-nitroso compounds, pyrrolizidine alkaloids, cyclamate, cycasin and DDT. Palmutoxin, a fungal contaminant of a local palm wine, can induce mutation in bacteria. There are other sources of potential carcinogens that are introduced by humans or occur naturally in such areas. Well known toxic or mutagenic chemicals include sapotoxin, capsaicin, prussic acid, oxalic acid, metronidazole, fluorooleic acid, quinine, hycanthone and mushroom toxin. With the arrival of various industries and foreign technology in African countries, and modernization of their big cities, atmospheric pollutants and narcotics present a new danger. There is a need to stimulate more research into the special toxicological problems that are peculiar to people living in the tropical areas of Africa.

305. DEPRESSION OF ATPase ACTIVITY IN HEPATOCHYCE SURFACE MEMBRANES OF RATS BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN TREATMENT.
B.V. Madhukar, F. Matsumura, K.H. Yang and R.E. Peterson, University of Wisconsin, Madison, WI.

$\text{Na}^+,\text{K}^+$ ATPase and $\text{Mg}^{2+}$ ATPase activities were determined in hepatocyte surface membranes (HSM) isolated from male rats killed 2,10,20 and 40 days after treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 10 or 25 $\mu$g/kg, po) or acetone/corn oil (control, po). In TCDD-treated animals a depression in the activity of both enzymes was detected 2 days after treatment. This depression became more pronounced at 10 and 20 days and was still present after 40 days. $\text{Na}^+,\text{K}^+$ ATPase was depressed to a similar extent by 10 and 25 $\mu$g/kg TCDD whereas $\text{Mg}^{2+}$ ATPase was reduced more by the high dose. The depression in both enzyme activities was subsequently shown not to occur secondary to decreased food and water intake and was reversed 10 days after TCDD administration (25 $\mu$g/kg) by treatment on days 6 to 9 with spironolactone (75 mg/kg/day) but not pregnenolone-16a-carbonitrile (75 mg/kg/day). Preincubation of control HSM with $10^{-13}$ to $10^{-3}$ M TCDD for 15 min failed to inhibit ATPase activity. Thus, in vivo treatment with TCDD produces a delayed onset, long term depression of HSM $\text{Na}^+,\text{K}^+$ ATPase and $\text{Mg}^{2+}$ ATPase but in vitro treatment has no effect. (Supported by NIH grants ES00857 and ES01332.)
306. EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON LIVER FUNCTION IN RATS, RABBITS AND GUINEA PIGS. M.D. Seefeld, K.H. Yang and R.E. Peterson, School of Pharmacy, University of Wisconsin, Madison, WI.

A single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was given to male rats (25 µg/kg, po), rabbits (25 µg/kg, ip) and guinea pigs (2 µg/kg, ip) and liver function assessed 10 days later. Control animals received acetone/corn oil. In TCDD-treated rats blood clearance of ouabain was decreased while indocyanine green (ICG) clearance was unaltered. The serum level of sorbitol dehydrogenase (SDH) was elevated but those of glutamic pyruvic transaminase (SGPT) and gamma glutamyl transpeptidase (GGTP) were not. Bile flow, bile salt excretion and biliary erythritol clearance were all reduced by TCDD and body weight was decreased 8% 10 days after treatment. In rabbits ouabain blood clearance was unaltered by TCDD treatment while clearance of ICG was greatly reduced. SDH and SGPT activities were elevated while GGTP activity remained unchanged. By day 10 body weight of TCDD-treated rabbits had dropped 2%. TCDD treatment in guinea pigs did not alter ICG clearance or SDH, SGPT and GGTP activities although a 35% reduction of body weight had occurred. Taken together these results show a variation between species in both the susceptibility to and in the nature of the hepatotoxic response to TCDD. (Supported by NIH grant ES01332.)

307. PHARMACOKINETICS OF 1,2,4-TRIBROMOBENZENE AND 1,2,4-TRICHLOROBENZENE. E.N. Smith and G.P. Carlson, Dept. of Pharmacology and Toxicology, Sch. of Pharmacy and Pharmaceutical Sciences, Purdue Univ., West Lafayette, IN.

Using 14C-labeled compounds the fate of 1,2,4-tribromobenzene (TBB) and 1,2,4-trichlorobenzene (TCB) in relation to the extent and duration of enzyme induction was investigated. The influence of starvation and phenobarbital in combination with the compounds was observed. Male rats received 1 mmol/kg/day of compound po for 7 days. p-Nitroanisole demethylation was elevated 16 days after the last dose of TBB but not consistently for TCB. Cytochrome P-450 and cytochrome c reductase were also induced. The highest induction levels observed were due to 4 days of starvation after 7 days of injection and 6 days of rest. Tissue analysis showed greater retention of TBB than TCB with highest levels in the fat. More TCB than TBB was excreted in the urine, and fecal excretion was only 5 to 10% of the urinary excretion. The results demonstrate that on an equal molar basis, TBB leads to higher levels of enzyme induction which are maintained for longer periods, is retained to a greater extent in fat and is more influenced by starvation than is TCB. (Supported by EPA Grant No. R805070).
308. THE EFFECT OF ALCOHOLS AND TOluene UPON METHYLENE CHLORIDE INDUCED CARBOXYHEMOGLOBIN IN THE RAT AND MONKEY. H.P. Ciucha, G.M. Savell and R.C. Spiker, Jr., Gillette Medical Evaluation Laboratories, Rockville, MD.

The effects of ethanol (EtOH), methanol (MeOH), isopropanol (IpoOH) and toluene upon methylene chloride (MeCl) induced carboxyhemoglobin (COHgb) levels were investigated in rats and monkeys. Inhalation studies in rats exposed to 50-5000 ppm MeCl produced a linear dose response and demonstrated that COHgb formation was weight dependent. Rats exposed for 1 hr to 5000 ppm MeCl and 8000 ppm EtOH and 5000 ppm MeCl and 1350 ppm IpoOH showed a significant inhibition of COHgb while similar exposure to MeCl and 11000 ppm MeOH did not alter the levels. The I.P. administration of MeOH to rats prior to a MeCl inhalation challenge caused a significant inhibition of COHgb. Monkeys exposed for 3 hr to 1000 ppm MeCl and 300 ppm MeOH had peak COHgb levels similar to MeCl controls. Monkeys exposed for 1 hr to 1800 ppm toluene, 4300 ppm MeOH and 4650 ppm MeCl showed COHgb inhibition as did rats when pretreated I.P. with toluene and challenged by inhalation with MeCl. All solvents tested produced inhibition of MeCl induced COHgb and neither the rat nor the monkey demonstrated the MeOH potentiation of COHgb that had been reported in the literature to occur in man.


The tissue distribution, metabolism, and excretion of 4,4'-dichlorobiphenyl (4,4'-PCB) were investigated in Beagles and Cynomolgus monkeys (M. Fascicularis). Following a single iv dose of 4,4'-PCB-14C (0.6 mg/kg) excretion, blood, bile, and tissues were collected at time intervals ranging from 15 min to 28 days. Samples were oxidized to 14CO2 and quantitated by scintillation counting. Selected tissues and fluids were extracted to separate parent from metabolites. Within 24 hrs the beagle excreted 50% of the dose as metabolites in the urine (7%) and feces (43%) with the remaining 14C present largely in fat, muscle, and skin. Bile and blood collected at 24 hrs consisted primarily of metabolites. By 5 days more than 90% was excreted. Unlike the dog, the monkey excreted <15% of the dose within 24 hrs with only 1% of this in feces. Blood and bile collected at 24 hrs consisted of metabolites, 75% and 100%, respectively. The remaining 4,4'-PCB was localized as parent primarily in fat (33%) with some in skin and muscle. By 28 days 59% of the dose had been excreted, primarily in the urine. Supported by N.I.E.H.S. # N01-ES-7-2111.

A comparison of the biological half-life of PCB's in several species indicate that the dog can eliminate highly chlorinated PCB's more rapidly than the mouse, rat, or monkey. To further define the mechanism for this rapid elimination, beagles (N=3) were administered an iv dose (0.6 mg/kg) of 14C-labeled 2,3,6,2',3',6'-hexa- (236-HECB), 2,4,5,2',4',5'-hexa- (245-HECB), 2,3,5,2',3',5'-hexachlorobiphenyl (235-HECB) or 2,4,5,2',4',5'-hexabromobiphenyl (245-HEBB). Daily excretion of 14C in the urine and feces was quantitated by oxidation to 14CO2 and/or direct scintillation counting. The 236-HECB was rapidly excreted with 70% recovered in 3 days. By day 15, 66% of 245-HECB was excreted. The 235-HECB was slowly excreted with only 14% recovered by day 20. Unlike 245-HECB, the 245-HEBB was excreted slowly with only 8% recovered by 25 days. In all cases, the major excretory route was via the feces. It is evident that the position of the chlorines governs the rate of elimination, probably by affecting the rate of metabolism. In addition, the specific halogen governs the degree of metabolism and subsequent excretion.

Supported by N.I.E.H.S. #NOl-ES-7-2111.

311. THE ROLE OF 2-BUTANONE METABOLISM IN THE POTENTIATION OF CC14-INDUCED HEPATOTOXICITY. F.K. Dietz, G.J. Traiger and V. Stella. Departments of Pharmacol. and Toxicol., and Pharm. Chem., Sch. of Pharmacy, Univ. of Kansas, Lawrence, KS.

Previous studies from this laboratory have shown that an acute pretreatment with 2-butanone (2-one) enhanced the hepatotoxicity of CC14 in rats. The purpose of this study was to determine the role of the metabolites of 2-one in this potentiation. GLC analysis of serial blood samples detected the presence of 2-butanol, acetoin and 2,3-butanediol (2,3BD) after the administration of 2-one (2.1 ml/kg, po). Pharmacokinetic studies using area under the blood concentration-time curves showed 28% of 2-one converted in vivo to 2,3BD. A 16-hr pretreatment with either 2-one (2.4 and 1.2 ml/kg, po) or 2,3BD (0.68 and 0.34 ml/kg, po) significantly enhanced the hepatotoxicity of CC14 (0.1 ml/kg, ip) as measured by serum glutamic-pyruvic transaminase (SGPT) activity, glucose 6-phosphatase (G6P) activity and triglyceride (TG) concentration.

The enhanced hepatotoxicity produced by 2-one as measured by SGPT and G6P was not significantly different from that produced by equivalent doses of 2,3BD. These results suggest that 2,3BD production appears to contribute to the potentiation of CC14 hepatotoxicity after 2-one pretreatment.

(Supported by NIH-DA-00890 and the University of Kansas).
312. POTENTIATION OF CHCL₃-INDUCED HEPATO- AND NEPHROTOXICITY BY n-HEXANE (H), METHYL n-BUTYL KETONE (MBK), and 2,5-HEXANEDIONE (HD). W.R. Hewitt, M.G. Côté, and G.L. Plaa. Dépt. de Pharmacol., Univ. de Montréal, Montréal, Canada.

Previous studies have suggested that administration of ketones or agents which are metabolized to ketones potentiates haloalkane toxicity in animals. To test this hypothesis, CHCl₃-induced hepato- and nephrotoxicity was evaluated in male Sprague-Dawley rats pretreated with 15 mmol/kg (po) of H, its ketonic metabolites MBK and HD, or acetone (A). After 18 hr a challenging dose of CHCl₃ (0.5 ml/kg, ip) was given. Liver and kidney damage were determined 24 hr later, using plasma GPT activity and renal cortical slice p-aminobenzoate (PAB) uptake. Neither H, MBK, HD, A or the CHCl₃ challenge dose produced marked liver or kidney injury when given alone. However H, MBK, HD, and A pretreatment potentiated CHCl₃-induced liver and kidney injury. The ability to potentiate CHCl₃-induced liver injury increased in the order of H < A < HD < MBK. Each agent had an approximately equal ability to potentiate CHCl₃-induced kidney damage. These results support the concept that ketones increase the susceptibility of animals to the toxic effects of haloalkanes. (Supported in part by Health and Welfare Canada and the Chemical Industry Institute of Toxicology).

313. ACUTE ALTERATION OF CHLOROFORM-INDUCED HEPATOTOXICITY BY MIREX AND KEPONE. D.J. Clanfline, W.R. Hewitt and G.L. Plaa, Dépt. de Pharmacol., Univ. de Montréal, Montréal, Canada.

A challenging dose of CHCl₃ produced marked hepatotoxicity in kepone (K) but not mirex (M) pretreated mice. This may have been due to an inability of the mirex treatment regimen to induce hepatic MFO activity. Male Swiss-Webster mice received a single oral dose of M (10, 50, or 250 mg/kg) or K (50 mg/kg). After 18 hr, hepatic microsomal aminopyrine N-demethylase and aniline hydroxylase activity and cyt. P-450 content were determined. Both M and K induced MFO enzyme activity to an approximately equivalent degree. Thus the inability of M to potentiate CHCl₃-induced hepatotoxicity cannot be explained by this mechanism. Subsequently, male Sprague-Dawley rats were given M or K (10 mg/kg, po) once daily for 3 days. CHCl₃ (0.5 ml/kg, po) was given 18 hr after the last dose of M or K. In contrast to mice, determination of liver damage 24 hr later showed both M & K potentiated CHCl₃-induced liver damage. However M elicited a smaller degree of potentiation than did K. These results suggest that K, possibly as a result of its ketonic structure, possesses a greater capacity to alter CHCl₃-induced liver damage than does M. (Supported by Health and Welfare Canada and the Chemical Industry Institute of Toxicology).

Subacute ingestion of low concentrations of the flame retardant Firemaster BP-6 (polybrominated biphenyls, PBB) does not cause functional damage in adult rats. Because of their lipophilicity and resistance to metabolism, PBBs are concentrated in milk and may present a hazard to nursing young. To assess such hazards Sprague-Dawley female rats were fed 0 or 100 ppm PBB from day 8 of gestation until the pups were weaned at 28 days. Half of the male pups from PBB-fed dams were put on 0 ppm PB5 diet and half on 100 ppm PBB diet for an additional 26 days. Rats receiving PBBs through the dam only had lower body weight than controls, liver enlargement and increased sensitivity to CHCl3 and CCl4. Rats receiving PBBs through the dam and post-weaning had lower body weight than controls, enlargement of the liver and kidney, dilation of the renal pelvis and increased sensitivity to CHCl3 and CCl4. Thus, developing animals may be more sensitive to the toxicity of PBBs. (Supported by USPHS grant ES00560 and a grant from the Mich. Dept. of Agric.)


Controversy exists as to whether impurities or pure halogenated hydrocarbons are responsible for the induction of hepatic microsomal hemoprotein(s) P-450. To investigate this we analyzed purified and crude HB using GC-MS and compared their inductive effects on hepatic monooxygenase activity. Male and female Sprague-Dawley rats (c 100 g) and rainbow trout (c 100 g) were injected i.p. with 0.3 mmol/kg of purified or crude HB in corn oil (1 ml/kg). Rats were sacrificed after 4 days, fish after 5 days and microsomes prepared. Microsomal ethoxycoumarin- and ethoxyresorufin-O-deethylations (EROD and ECOD) from pretreated rats were elevated 4- and 9-fold respectively over control values in both sexes. HB pretreatments resulted in hypochromic shifts of λmax values for carboxyferrocytochrome P-450. No changes in benzphetamine-N-demethylation (BeND) were noted. Microsomes from pretreated fish demonstrated no changes in EROD, EROD, BeND or Soret maxima. The data suggest that purified HB is a potent inducer of hepatic microsomal monooxygenase activity in rats but not rainbow trout and impurities in crude HB apparently do not affect the inductive properties of HB.
316. METHANOL POTENTIATION OF CARBON TETRACHLORIDE (CCl₄)-INDUCED HEPATOTOXICITY IN RATS. L.R. Cantilena, Jr., S.Z. Cagen, and C.D. Klaassen. Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS 66103

Plasma levels of alanine aminotransferase (ALT) were measured 24 hrs after CCl₄ was administered (0.1 ml/kg ip) to rats pretreated with a single oral dose of methanol (7.0 ml/kg). Maximum ALT (100 times greater than saline control) occurred when methanol was given 48 hrs prior to CCl₄. The relative magnitude of methanol potentiation of CCl₄ hepatotoxicity in rats was compared to that observed with equitoxic doses of isopropanol (2.5 ml/kg) and ethanol (6 ml/kg) using levels of hepatic glucose-6-phosphatase, hepatic accumulation of triglycerides, and ALT as indices of liver damage. Methanol was found to potentiate CCl₄ toxicity to approximately the same degree as isopropanol and much greater than pretreatment with ethanol. Methanol pretreatment did not significantly potentiate CCl₄-induced lipid peroxidation in vivo, as measured by the presence of conjugated dienes. Both methanol and isopropanol pretreatment significantly increased the rate of irreversible binding of ¹⁴C CCl₄ to microsomal protein in vitro. The results show methanol and isopropanol are equally effective in potentiating CCl₄ hepatotoxicity and suggest that the mechanism of potentiation is probably the same. (Supported by USPHS GM-30996).

317. Assessment of Tumor Initiating and Promoting Activity of a Mixture of Polybrominated Biphenyls (Firemaster BP-6), and certain Purified Isomers of PBB. Richard K. Haroz and Steven D. Aust. Battelle Research Center, Geneva, Switzerland, and Michigan State University, East Lansing, Michigan.

A mixture of polybrominated biphenyls as well as purified isomers from this mixture were tested as tumor initiators and tumor promoters in a two-stage mouse skin tumorigenesis assay using a skin tumor-susceptible mouse strain (SENCAR). Skin tumor activity was tested by applying material to the backs of mice followed by repeated application of the tumor promoter 12-O-tetradecanoylphorhol-13-acetate (TPA). Skin tumor promoting activity was assessed by a single subcarcinogenic application of DMBA followed by twice weekly applications of test material. Following eight weeks, the positive control group treated with DMBA as initiator and TPA as promoter produced 100% tumor-bearing animals. After fourteen weeks of treatment neither the PBB mixture nor the purified major component 2,2', 4,4', 5,5'-hexabromobiphenyl exhibited tumor initiating or promoting activity. A second congener 2,4,5,3', 4', 5'-hexabromobiphenyl which gives a 3-methylcholanthrene type induction is presently under test. Results to date indicate a lack of both tumor initiating and promoting activity for polybrominated biphenyls.

Previous work showed that DMSO markedly increased the lethality of CCl₄ in mice and rats but paradoxically protected against the hepatotoxic effects of CCl₄. To elucidate possible mechanisms by which DMSO might exert these contrasting effects the MDA-generating capacity (MDA-GC) of liver and brain was evaluated in mice at 3, 6, and 12 hrs after ip administration of DMSO (5 ml/kg), CCl₄ (0.05 ml/kg) and the combination of DMSO with CCl₄. DMSO and CCl₄ each produced generally opposite effects on the MDA-GC of brain and liver, and the effects of DMSO also opposed those of CCl₄ in each tissue. However, when DMSO was given with CCl₄, both brain and liver displayed increased MDA-GC at each time period. It is possible that the increased MDA-GC seen in brain is related to the increased lethality of DMSO given with CCl₄; however, it is not clear how the increased MDA-GC in liver explains the mechanism by which DMSO protects against the hepatotoxicity of CCl₄.


Rats were given single oral doses of mirex and dechlorinated derivatives in oil and relative liver, adrenal and gonad weights as well as selected liver chemistry parameters were determined. Mirex and some dechlorinated derivatives caused an increase in relative liver and adrenal size, a decrease in relative gonad size, a deposition of lipid in the liver and gave no evidence of liver damage. No outstanding difference in pharmacological effects among the compounds was noted. With doses up to 600 mg/kg for up to 2 weeks, relative (organ weight/body weight) liver and adrenal weights increased up to 100%, while relative gonad weight decreased up to 30%. Female rats which died from a given dose had greater relative adrenal weights than did survivors. Increases in relative liver weight demonstrated a dose-response relationship, whereas ovary weight decreases and adrenal weight increases did not. At 4 days following doses of up to 150 mg/kg, glutamic pyruvic transaminase (GPT) and DNA in liver homogenates were lower in treated animals; protein, dry weight and RNA were not altered, and lipid was increased 15-60%. Serum GPT appeared unchanged.
320. TIME COURSE OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN (TCDD) EFFECTS ON LIVER FUNCTION IN RHESUS MONKEYS. M.D. Seefeld and R.E. Peterson, University of Wisconsin, Madison, WI.

Blood clearance of indocyanine green (ICG) and serum levels of sorbitol dehydrogenase (SDH), gamma glutamyl transpeptidase (GGT), and glutamic pyruvic transaminase (SGPT) were assessed at weekly intervals in male monkeys treated with a single oral dose of TCDD: 75 µg/kg (n=1), 25 µg/kg (n=2) and 5 µg/kg (n=1); control animals received acetone/corn oil. The monkeys given 75 or 25 µg/kg exhibited periorbital edema, loss of body and facial hair, acneform eruptions, and a substantial weight loss with a moribund state occurring 4 to 6 weeks after treatment. In these animals ICG clearance increased during the first few weeks following treatment but then declined steadily until death. SDH and SGPT were elevated in all debilitated animals while GGT was variable. The animal given 5 µg/kg showed some hair loss and a moderate decrease in weight which returned to normal after 13 weeks. ICG clearance also changed biweekly but the magnitude of change was less. All serum enzymes were elevated at some time during intoxication. Thus, acute TCDD treatment first increases and then decreases ICG clearance and elevates serum enzymes to varying degrees at different times after treatment. (Supported by NIH grant ES01332.)

321. SPECIES DIFFERENCES IN THE EXCRETION OF MERCAPTURIC ACIDS BETWEEN RATS AND CHIMPANZEES DOSED WITH NAPHTHALENE AND DIETHYLMALEATE. K.H. Summer, K. Rozman, F. Coulston and H. Greim, Abt. f. Toxikologie der GSF, D-8042 Neuherberg, ICMES Albany Medical College, Holloman AFB, NM.

Seutter-Berlage et al. (Int. Arch. Occup. Environ. Hlth 39: 45, 1977) proposed increased levels of mercapturic acids (MA) in the urine of man as an indicator of exposure to environmental chemicals. To evaluate this, a dose-effect relationship was established in the chimpanzee: 1. The "endogenous" thiocyclers in the urine of chimpanzees and rats are 18.0 ± 1.1 and 94.4 ± 2.8 µmol/h/kg, resp.; the values for chimpanzees are similar to man. 2. After naphthalene (30, 75, 200 mg/kg) MA rose to 92, 186 and 408 µmol/24 h/day in rats. In chimpanzees the treatment failed to increase urinary MA. 3. Diethylmaleate (30, 75, 200 mg/kg) showed a dose-dependent increase in MA in both species; the increase in rats was about twice of chimpanzees. These results suggest that the chimpanzee is a relevant model for man to study the urinary excretion of MA derived from electrophilic compounds, whereas the rat is not. Experiments with 14C-naphthalene indicate that the species differences observed are due to differences in glutathione conjugation. However, differences in the activities of activating enzymes or in the GSH-S-transferases cannot be excluded.

The local toxification and detoxification of xenobiotics by the lung is likely to be of considerable importance in understanding the pulmonary damage and toxicity induced by chemicals. We have investigated the detoxification ability of the lung by studying the conjugation of α-naphthol in the isolated perfused lung. Perfusate containing 14C-α-naphthol (initial concn. = 10.3 μM) was recirculated through the rabbit lung at a flow-rate of approximately 130 ml/min. Lungs were perfused for periods up to 60 min and aliquots of perfusate removed at specific times. Perfusate samples were analyzed for unchanged naphthol and conjugated metabolites using thin-layer chromatography (butanol:acetic acid:water, 4:1:1, v/v). Naphthol in the perfusate declined monoexponentially with respect to time, resulting in a clearance of naphthol by the lung of about 8 ml/min. Appearance of metabolites in the perfusate was approximately linear over 30 min; 50-60% of the original naphthol was metabolized. The majority of the metabolism was due to the formation of sulfate conjugation with only minor amounts (<5%) due to glucuronide conjugation.


A general analytical scheme for the isolation and identification of urinary metabolites of vinyl-type compounds has been applied to the metabolites of acrylonitrile and acrylamide in rats. Metabolites were isolated from urine by ion-exclusion chromatography and methylated prior to analysis by GC-MS or GC-IR. In urine of rats administered acrylamide seventy percent of the radioactivity was present as N-acetyl-S-(3-amino-3-oxopropyl) cysteine. In urine from rats dosed with acrylonitrile, 25% of the radioactivity was present as N-acetyl-S-(2-cyanoethyl) cysteine and another 25% as a compound tentatively identified as 4-acetyl-3-carboxy-5-tetrahydro-1,2-2H-thiazine. These metabolites support conjugation with glutathione as a significant metabolic pathway for vinyl-type compounds. The latter acrylonitrile metabolite suggests the formation of an epoxide intermediate.
324. The In Vivo Biotransformation of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in the Rat. J. C. Ramsey, J. G. Hefner, R. J. Karbowksi, W. H. Braun and P. J. Gehring, Toxicology Research Laboratory, The Dow Chemical Co., Midland, MI 48640

This study presents evidence for the in vivo biotransformation of TCDD in the rat. Three male rats were implanted with indwelling bile loop cannulas. They were then given daily oral doses of 15 mcg 14C-TCDD per kg. After 2, 4, or 6 doses, the bile loop of one rat was opened and bile was collected for 24 hr. Biliary 14C was excreted at a rate similar to the excretion of 14C in the feces of rats fed 14C-TCDD in a previous study. Selective solvent extraction revealed that the biliary 14C activity was due to compounds more polar than TCDD itself. Incubation of bile with β-glucuronidase resulted in the extraction of more 14C activity, implying the presence of glucuronide conjugates of 14C-TCDD metabolites. Liquid chromatography of bile has revealed the presence of at least 5 distinct radioactive peaks, none of which was due to 14C-TCDD. The data did not indicate extensive enterohepatic circulation of radioactivity derived from 14C-TCDD. These results, in conjunction with the results of previous studies, indicate that TCDD is slowly metabolized in the liver to a variety of metabolites, which are then excreted in the bile.


The distribution and excretion of 14C-labeled TCDD in guinea pigs was studied to determine if differences in these parameters might explain reported differences in species sensitivity to TCDD. 14C-labeled TCDD was administered by gavage. The guinea pigs were sacrificed in groups of 3-5 animals each at 1, 2, 6, 12 and 22 days after dosing and samples of the major tissues, bile, plasma, urine and feces were analyzed for radioactivity. Only half of the dose was absorbed. Twenty-two days after dosing, the fat, liver, adrenals and thymus contained (±SD) 0.75±0.17, 0.40±0.14, 0.33±0.08, and 0.72±0.08 % dose/g, respectively. The remaining tissues, plasma, urine and bile did not consistently contain measurable amounts of radioactivity. Based on the clearance of radioactivity from the fat, liver and adrenals, the half life for the elimination of TCDD in guinea pigs was estimated to be between 22 and 43 days. The data suggest that differences in tissue distribution may contribute to the reported differences in species sensitivity to TCDD.

The anticoagulant drugs warfarin and phenprocoumon are metabolized by cytochrome P-450 dependent liver microsomal mixed function oxidases to 4'-, 6-, 7- and 8-hydroxylated products. Microsomes were prepared from male rats (130-170 g) pretreated with saline, phenobarbital (PB) or 3-methylcholanthrene (MC). (-)-Warfarin (-W), (+)-warfarin (+W), (-)-phenprocoumon (-P) and (+)-phenprocoumon (+P) were labeled with $^{14}$C in the coumarin ring and incubated with microsomes and an NADPH generating system. Metabolites were separated by tlc. PB pretreatment uniformly increased +W metabolism (x3) and altered metabolite profiles for -W and +P, increasing 7- and 8-hydroxylation of -W and 6- and 8-hydroxylation of +P preferentially. MC pretreatment greatly increased 6- and 8-hydroxylation of all substrates (60-100 fold for -P). The metabolism of -P is unaffected by PB pretreatment and strongly enhanced by MC pretreatment, whereas 7-hydroxylation of -W is induced by PB (x4) and only slightly induced by MC (x1.5), making these useful substrates for classifying inducers of P-450.


Species selectivity of RH-787 (N-3-Pyridimethyl-N'-p-nitrophenyl urea) seems to depend on biotransformation. These studies compared fates of Py- and NP-$^{14}$C-787 in rats and dogs. Human VACOR metabolites were examined in poisoning cases. Although single oral 30 mg/kg doses of 787 were lethal to rats but not dogs, they produced higher blood levels in dogs. However, most $^{14}$C in rat blood was due to 787, while dog blood contained primarily p-nitrophenyl urea. Rat urine contained large amounts of 787 while dog urine had <1%. Dog urine contained 40-60% unidentified polar material, but rat urine only 5-20%; this material from rats, but not dogs, was partially hydrolyzed by $\beta$-glucuronidase to 787. Other identified metabolites include amino-787, acetamido-787, p-aminophenyl urea, p-acetamidopenyl urea, p-nitroaniline, p-phenylenediamine, p-acetamidophenylurea, nicotinic acid, nicotinuric acid and nicotinamide. Most were found in all three species, but concentrations varied. The presence of parent compound in rat and human urine suggests that they may be more sensitive to VACOR because of inefficient metabolism. Tolerance in dogs is probably due to rapid detoxication.
328. A GENERAL METHOD FOR THE IN VITRO PRODUCTION AND IDENTIFICATION OF N-DEALKYLATED METABOLITES. J. Jernigan, D. Taber, and R.D. Harbison, Department of Pharmacology, Vanderbilt Medical Center, Nashville, TN.

The in vivo dealkylation of tertiary amines is often inefficient; metabolites are recovered only with great difficulty or are not recoverable from biological fluids. The purpose of this study was to develop a general method for the in vitro N-dealkylation of tertiary amines. The prototype substrate used was N-acetyl proclainamide which is N-dealkylated in vivo to N'-desethyl N-acetyl procainamide. The in vitro system uses potassium ferricyanide as the reagent. About 10 mole equivalents of 0.03 M solution of potassium ferricyanide in 0.5 M phosphate buffer (pH 7.0) is needed for optimum conversion of 1 equivalent of N-acetyl procainamide to N'-desethyl N-acetyl procainamide. The yield of N'-desethyl N-acetyl procainamide recovered was 40% of the theoretical. The metabolite was identified and separated by high pressure liquid chromatography. As this method can conveniently be run on a multi-milligram scale and it is specific for N-dealkylation of tertiary amines, a workable supply of the authentic N-dealkylated metabolite of a tertiary amine is readily available for analytical and biological investigations. (Supported by USPHS Grants GM15431 and ES00782).


The kinetics of TAA in male Fischer 344 rats, CD-1 mice and Beagle dogs following single or repeated 6 hr exposures were determined. Concentrations of TAA in rat plasma immediately following 6 hr exposures to 150, 500 and 1500 ppm were 10, 75 and 350 µg/ml, respectively. The clearance of TAA from the plasma of rats exposed to 150 ppm was apparently first order (t½ = 100 min) but exhibited saturation kinetics following 500 and 1500 ppm single exposures. Upon repeated exposure to 50 ppm, TAA was cleared by rats (t½ = 47 min) and dogs (t½ = 69 min) in an apparent first order manner. End exposure plasma concentrations after repeated exposure to 1000 ppm were ≈110 µg/ml in rats and mice and 480 µg/ml in dogs. Following repeated exposure to 1000 ppm the plasma clearance of TAA was apparently first order (t½ = 29 min) in mice but exhibited Michaelis-Menten kinetics in rats and dogs. The data demonstrate that the kinetics of TAA are highly species and concentration dependent. This suggests that toxicity may increase disproportionately at high exposure concentrations and that valid extrapolations will depend on adequate knowledge of the kinetics.

In order to gain greater insight into pulmonary absorption, distribution and excretion of 1-((2-methyl-4-([2-methyl-phenyl]azol)phenyl)azo)-2-naphthalenol (C.I. Solvent Red 24), the colorant was synthesized having a $^{14}$C label on the methyl group attached to the middle aromatic ring and administered to rats via intra-tracheal intubation. Pulmonary absorption after 96 hours amounted to 60% and 98% of that absorbed radioactivity was excreted as metabolites in urine and feces. The latter was the major excretory route. Tissue distribution of the remaining radioactivity showed fractional percentages in blood, liver, skin, adipose tissue and elsewhere. It is clear from this study that the colorant is readily absorbed through the pulmonary system, rapidly metabolized and, for the most part, excreted as metabolites, which include a conjugate of 4-amino-2',3(14C)dimethylazobenzene.

331. EFFECTS OF CADMIUM ON HEPATIC MICROSMAL CYTOCHROME P-450 CONTENT AND SUBSTRATE SPECTRAL BINDING IN MALE AND FEMALE RATS. D.H. Pence and R.C. Schnell, Hazleton Labs America, Vienna, VA and Dept. PCOL/TOX, School of Pharmacy, Purdue University, W. Lafayette, IN 47907.

Previous studies have shown a sex-related difference exists in the ability of cadmium to inhibit hepatic microsomal drug metabolism in rats. Three days following in vivo cadmium treatment, a significantly decreased level of cytochrome P-450 (CYT P-450), as well as a significant reduction in the magnitude of spectral binding of hexobarbital (HX), ethylmorphine (EM) and aniline (AN) was observed in hepatic microsomes isolated from male rats, but not female rats. In vitro addition of Cd$^{2+}$ ($10^{-6}$ - $10^{-3}$ M) to hepatic microsomes isolated from naïve rats produced a significant concentration-dependent reduction in microsomal CYT P-450 content of male rats, but only high ($5 \times 10^{-4}$ M) Cd$^{2+}$ concentrations caused a significant reduction in female microsomes. Binding spectra were reduced in a concentration-related, substrate dependent manner in both sexes following in vitro addition of Cd$^{2+}$. In male microsomes, cadmium significantly reduced AN and HX binding, but did not significantly reduce EM binding in male-, or HX, EM or AN binding in female derived microsomes. (Supported by NIEHS ES-00921 and NIEHS ES-0071).
332. PHARMACOKINETICS OF EPICHLOROHYDIN (EPI) ADMINISTERED TO RATS BY GAVAGE OR INHALATION. F.A. Smith, P.W. Langvardt and J.D. Young, Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, MI. 48640 Sponsor: W.H. Braun

Single oral doses of 100 or 1 mg/kg Epi-1,3-14C were given to groups of 4 male Fischer 344 rats each, and two additional groups were exposed for 6 hours to 100 or 1 ppm vapor. By 72 hours, about 90% of the dose had been excreted in the urine (46 to 54%) and in the breath as 14CO2 (25 to 42%) regardless of the dose level or route. Urinary metabolites from orally dosed rats showed 7 peaks by ion-exclusion chromatography. Only 6 peaks were resolved in urine from rats exposed by inhalation. As none of the urinary metabolites or radioactivity excreted in the breath was parent compound, essentially 100% of the elimination from the body is accounted for by the metabolism of Epi. Comparison of Epi-3-14C and Epi-1,3-14C excretion data indicates if any Epi carbon-to-carbon bond is broken the molecule is metabolized completely to CO2. A 4 to 5 times greater concentration was seen in the target organ for toxicity (nasal turbinate) than in the lung following inhalation but not oral dosage. This route dependent difference in distribution may be manifest as a route related difference in the toxicity of Epi. (Supported by the Manufacturing Chemists Association).

333. IN VIVO METABOLISM AND COVALENT BINDING OF BENZENE IN BONE MARROW. R.D. Irons, J.G. Dent, T.S. Edgar, and D.E. Rickert, Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Sponsor: J.E. Gibson

Benzene metabolites have been isolated from rat bone marrow following subcutaneous administration and inhalation exposure. However, since the liver is the major site of benzene metabolism, it is not known whether benzene can be metabolized directly in bone marrow, the principal target organ for benzene toxicity. Using isolated hind limb perfusion, in which the venous return was diverted and collected for HPLC analysis, the metabolism of benzene was studied in male F344 rats. 1-2 mCi of 14C-benzene was introduced directly into the femur marrow cavity. Radioactive compounds recovered from blood achieved peak levels at 10 min. (phenol, 4540 ± 773 pmoles; catechol, 222 ± 94 pmoles; hydroquinone, 92 ± 73 pmoles). The same radioactive metabolites were recovered from bone marrow where phenol concentrations averaged 64 pmoles/mg wet tissue. Following exhaustive extraction, radioactivity bound in bone marrow represented 1.47% of the total metabolites recovered from blood and bone marrow. No metabolism was detected in preparations in which marrow was aspirated before introducing benzene. We conclude that benzene is directly metabolized and covalently binds in bone marrow in the absence of liver metabolism.
334. DISPOSITION OF n-Hexane IN RATS AFTER SINGLE AND REPEATED INHALATION EXPOSURE. J.S. Bus, E.L. White, and C.S. Barrow, Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Sponsor: J.E. Gibson

n-Hexane (HX) produced neuropathy may be mediated through the metabolites methyl n-butyl ketone (MBK) and 2,5-hexanediol (HD). The purpose of this study was to examine the disposition of HX in nerve tissue compared to other tissues after inhalation exposure. Male, F-344 rats received either a single or repeated (5 days) 6 hr/day 1000 ppm HX exposure. At various times after exposure, samples of blood, liver, kidney, brain, and sciatic nerve were analyzed by gas chromatography-mass spectrometry for HX, MBK and HD. HX and MBK were rapidly eliminated from all tissues after single or repeated exposure, and were undetectable 4-8 hr after exposure. Less than 0.05 µg HD/ml or g wet weight was detected in blood, brain, liver, and kidney 24 hr after treatment. Sciatic nerve HD levels, however, declined to 0.63 and 0.56 µg/g wet weight by 12 hr after the respective exposures, and declined slightly further to 0.48 and 0.46 µg/g wet weight, respectively, 24 hr after exposure. These results indicated 1) the disposition of HX was similar after single or repeated inhalation exposure, and 2) that HD, a suggested neurotoxic metabolite, was selectively retained in sciatic nerve tissue.

335. IN VITRO INTERACTIONS OF 1,2-ALKENE OXIDES WITH EPOXIDE HYDRATASE. J.G. Dent and S. Schnell, Chem. Ind. Inst. of Tox., Res. Tri. Fk., NC. Sponsor: J.E. Gibson

Alkene and arene oxides have been implicated as carcinogens. The low molecular weight alkene oxides represent a group of industrially important chemicals but little is known about their metabolism and toxicity in mammals. We investigated the inhibitory effects of 1,2-alkene oxides on rat liver microsomal epoxide hydratase in vitro using benz(a)pyrene 4,5 oxide (180µM) as the substrate. All the compounds studied were inhibitors of epoxide hydratase, the IC₅₀ values determined were: 2.6x10⁻⁶, 1.4x10⁻⁴, 2.4x10⁻³, 4.1x10⁻², 2.7x10⁻², 3.5x10⁻², 1.5x10⁻², and 1.2x10⁻²M for the 1,2-oxides of ethylene, propylene, butene, pentene, hexene, heptene, octene, and decene, respectively. Furthermore, the inhibition was determined to be competitive indicating these compounds are all substrates for the hydratase. Although the weak inhibitory effects of ethylene, propylene and butene indicate these compounds are very poor substrates for the enzyme, at low concentrations of these three oxides (<1x10⁻²M) an increased hydratase activity was observed (ca. 120% control). We conclude, epoxide hydratase is unlikely to play a major role in detoxifying the smaller molecular weight oxides.
336. HIGH PRESSURE LIQUID CHROMATOGRAPHY OF 2,6- AND 2,4-
TOLUENEDIAMINE (TDA) AND ITS APPLICATION TO THE ANALYSIS OF
2,4 TDA IN URINE AND PLASMA. P.D. Unger and M.A. Friedman,
Dept. Biochemical Toxicology, Corporate Medical Affairs,
Allied Chemical Corporation, Morristown, NJ.

Toluenediamine isomers are suspect carcinogens in wide-
spread use in the isocyanate industry and dye industry. No
satisfactory biomonitoring procedures are currently avail-
able. In the present study, 2,6- and 2,4-toluenediamine
(TDA) were resolved as sharp peaks in the order 2,6-TDA,
2,4-TDA by normal-phase high-pressure liquid chromatography
on a small particle (10 μm) column in 3 min. by an aceto-
nitrile-water saturated chloroform elution solvent (8:2,
V/V) with detection by ultraviolet absorbance at 250 nm.
The relationship between peak height and quantity injected
was linear over a range of 0.025-2.0 μg for both compounds.
Retention time and peak height were highly reproducible,
with coefficients of variation [[(x-μ)/σ]100] of 0.58% and
1.52% for retention time and 1.003% and 1.18% for peak
height for 2,6- and 2,4-TDA, respectively. Detection was
very sensitive, allowing detection of 3-5 ng 2,6-TDA and
5-10 ng 2,4-TDA. Quantitative recovery of 2,4-TDA from
spiked urine and plasma samples by extraction with methylene
chloride was obtained over a range of 10-200 μg/ml.

337. METABOLISM OF 2,4-DINITROTOLUENE IN ADULT RAT PRIMARY HEPATOYCTE CULTURES. G.M. Decad, J.C. Dent, and D.E. Rickert,
Chemical Industry Institute of Toxicology, Research Triangle
Park, NC. Sponsor: J.E. Gibson

Dinitrotoluene is a potent hepatocarcinogen in the rat.
Cultured rat hepatocytes were used to investigate the meta-
bolism of 2,4-dinitrotoluene, the major component of tech-
nical grade dinitrotoluene. Hepatocytes were prepared from
adult male, Fischer-344 rats and cultured on collagen-coated
culture dishes in Waymouth 752/1 medium with fatty acids,
BSA, and hormones. After 20 hr in culture, medium contain-
ing [U-14C]2,4-DNT (0.53 μCi, 78 μg) was added to each dish.
After 8 hr at 37°C (95% air-5% CO2) the medium was analyzed
by reverse-phase HPLC. Thirty-five percent of 2,4-DNT was
metabolized. Polar metabolites A (6.4%), B (6.0%), C (1.2%)
may be conjugates. A major metabolite, 18.9%, cochromato-
graphed with authentic 2,4-diaminotoluene. 2,4-Diamino-
toluene has previously been shown to be mutagenic and carcino-
genic. These results suggest that cultured hepatocytes are
able of reductive metabolism of foreign compounds under
conditions of normal oxygen tensions, and that this system
may be useful in correlating the metabolism of dinitroto-
luene with its carcinogenicity.
338. "PARTIAL PURIFICATION OF HEPATIC GLUTATHIONE S-TRANSFERASES
OF THE LITTLE SKATE, Raja erinacea: ALKENE AN ARENE OXIDE
METABOLISM. Gary L. Fourman and John R. Bend, Lab. Pharm.,
NIH/NIH, Research Triangle Park, NC and Mt. Desert Island

Specific glutathione S-transferase (GS-T) activities of little skate hepatic cytosol differ markedly from those in rat or rabbit with respect to substrate specificity (Bend et al., Ann. N. Y. Acad. Sci., 298: 509-521, 1977). Microsomal supernatant fractions pooled from male little skate livers were eluted from a DEAE-cellulose column with a 0-0.3 M KCl gradient in 0.01 M potassium phosphate buffer, pH 8.0. Five separate peaks of enzyme activity (with 1-chloro-2,4-nitro-
benzene [DNBC]) were obtained. Each peak was concentrated by ultracentrifugation and applied to a column of epoxy-activated
Sepharose 6B which had been reacted with glutathione (GSH).
After removal of the GS-T activity from this column by elution with 5 mM GSH in 50 mM Tris, each peak was again concen-
trated and electrophoresed (SDS, PAGE). Each peak yielded one band near 25,000 daltons. Enzymic analysis showed 85% of the activity with styrene 7,8-oxide was present in peaks 1 and 3 while 85% of the activity with benzo(a)pyrene 4,5-
oxide was present in peaks 2 and 4. At least 5 enzymes, all differing markedly in substrate specificity, appear to be responsible for the GS-T activity in little skate liver.

339. EFFECTS OF METABOLISM ON THE MUTAGENICITY OF THE NITRO-
NAPHTHALENES IN SALMONELLA TYPHIMURIUM. C. Benkendorf,
D. Johnson, and E. Cornish, Dept. of Ind. Env. Hlth., Sch.
of Pub. Hlth., Univ. of Michigan, Ann Arbor, MI.

The mutagenic response of 1- and 2-nitronapthalene in the
Salmonella/mammalian microsome test due to metabolism by induced and uninduced rat liver was evaluated by subtract-
ing the number of mutants prior to enzymatic activation from the number of mutants present after activation. The results suggest that the two nitronapthalenes are meta-
bolized differently. High levels of ring-hydroxylating enzyme inactivate 1-nitronapthalene in the presence of air at the dose levels used. If ring-hydroxylation of 1-NW is blocked by nitrogen, the nitroreductive pathway is predomi-
nant and mutagenicity is increased. 2-Nitronapthalene appears to follow a reductive pathway even in the presence of air, and may owe its increased mutagenic activity with induced liver to an induction of conjugating enzymes. In uninduced liver, nitroreductase activity may be competitive with hydroxylase activity and, even in the presence of air, a portion of the 1-nitronapthalene is reduced. In this case, the presence of oxygen may allow the creation of an oxidation-reduction cycle involving nitroso and hydroyl-
amine intermediates of 1-nitronapthalene.
340. EFFECT OF COBALTOUS CHLORIDE TREATMENT ON 2-METHYLNAPHTHALENE DISPOSITION AND HEPATIC CYTOCHROME P-450 CONTENT IN CARP. P. D. Guiney, M. Dickins and R. E. Peterson, University of Wisconsin, Madison, WI.

The effect of cobaltous chloride (CoCl₂) treatment in carp (125-550g) on the disposition of 2-methyl [8-¹⁴C]naphthalene (MeNap)-derived radioactivity was studied. Fish were treated with CoCl₂ (60 mg/kg/day, 2 days, ip) or saline (control) and administered ¹⁴C MeNap (5 mg/kg, ip) 12 hr after the last treatment. Compared to control, the content of MeNap equivalents in CoCl₂-treated fish was lower in gall bladder bile (p<0.05), higher in liver (p<0.05), and similar in skeletal muscle 12, 24 and 48 hr after MeNap administration. However, by 72 hr after MeNap there were virtually no differences between the two groups in bile, liver and skeletal muscle content of MeNap-derived ¹⁴C. Solvent extraction of bile and liver indicated that control fish contained a greater percentage of polar MeNap equivalents initially but not after 72 hr. Hepatic microsomal cytochrome P-450 content and 7-ethoxycoumarin-O-deethylase activity were similar in CoCl₂-treated and control fish throughout the study. Thus, CoCl₂ treatment alters the disposition of MeNap in carp by a mechanism that does not involve a decrease of hepatic cytochrome P-450 content. (Supported by Sea Grant).

341. THE UPTAKE, ELIMINATION AND METABOLISM OF 1,2,4-TRICHLOROBENZENE IN RAINBOW TROUT. M. J. Melancon Jr., D. R. Branson and J. J. Lech, Dept. of Pharmacology, Med. Col. of Wisconsin, Milwaukee, WI and Dow Chemical USA, Midland, MI.

The uptake and elimination kinetics and biotransformation of trichlorobenzene were studied in rainbow trout. Trout were exposed to aqueous trichlorobenzene in static systems for short term exposures and continuous flow delivery systems for longer exposures. The trichlorobenzene derived material in selected tissues was measured at various times during uptake and depuration. Trichlorobenzene derived material in bile and tissue extracts was examined by solvent partitioning and thin layer chromatography for the presence of biotransformation products. When evaluated by a computer program (BIOFAC), muscle trichlorobenzene levels from an 8 hr exposure at .012 μg/ml and a 35 day exposure at .02 μg/ml gave bioconcentration factors of approximately 100 while those from 4 day exposures at .001 and .01 μg/ml gave bioconcentration factors of approximately 400. Bile from trout which had been exposed to .25 μg/ml trichlorobenzene for 24 hr contained 5 μg/ml of trichlorobenzene and 10 μg/ml of biotransformation products. Muscle and liver extracts contained 0.8% and 3.7% of biotransformation products, respectively. (Supported by NIEHS, USEPA, Wisconsin Sea Grant College Program and Dow Chemical Company).

Studies have shown that hepatic microsomal monooxygenation may be induced in rainbow trout by polycyclic aromatic hydrocarbons and crude PCBs, but not by phenobarbital-like inducers. To further investigate this we have studied the inducing properties of 2,2',4,4',5,5'-hexachlorobiphenyl (I), 2,2',4,4',6-(II) and 3,3',4,4'-(III) tetrachlorobiphenyl. Rainbow trout were injected i.p. with 0.3 mmol/kg of each isomer or Arochlor 1242 dissolved in corn oil (1 ml/kg). Fish were sacrificed 5 days later and microsomes prepared. Ethoxy- coumarin-0-deethylation (ECOD) activity in control microsomes was 0.023 ± 0.003 nmol/min/mg. Only A1242 (0.186 ± 0.053) and III (0.246 ± 0.015) stimulated this activity. Ethoxyresorufin-0-deethylation (EROD) was induced only by Arochlor 1242 and III (control, 0.025 ± 0.009; A1242, 0.808 ± 0.379; III, 0.519 ± 0.101). The noncoplanar isomers (I and II) elicited no increase in EROD or ECOD. Benzphetamine-N-demethylation was unaffected by any of the pretreatments. SDS-PAGE demonstrated a novel protein band at 57,000 mol. wt. in microsomes of fish treated with A1242 or III, but not with I or II. These experiments further demonstrate the refractive nature of rainbow trout to induction of hepatic microsomal cytochrome(c) P-450 (phenobarbital-type).

343. METABOLISM OF 2-NAPHTHYLAMINE SULPHONIC ACIDS. P.L. Batten, Imperial Chemical Industries Ltd., Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, UK. Sponsor: Dr. I.F.H. Purchase

The metabolic fate of Tobias acid (2-naphthylamine-1-sulphonic acid) and Brönner acid (2-naphthylamine-6-sulphonic acid) was studied in dogs with particular reference to the possible carcinogenicity of these commercially important dye-stuffs intermediates. After oral administration of either acid (5mg/kg) the major route of elimination is the kidneys (>80% within 24h). Analysis of the 24h urine by a combination of high pressure liquid chromatography followed by gas chromatography indicated very small amounts of 2-naphthylamine (<0.1% of the dose), thus demonstrating that the extent of desulphonation of either acid was small. The possibility that these acids may be oxidized to hydroxylamines analogous to those which are believed to be carcinogenic metabolites of 2-naphthylamine was investigated by thin layer chromatography analysis of the urine of dogs given Tobias acid. None of the N-oxidation product 2-naphthylhydroxylamino-1-sulphonic acid was detected. Tobias and Brönner acids do not appear to be potentially carcinogenic as a result of biotransformation either by desulphonation to 2-naphthylamine or by oxidation to reactive species structurally similar to those known to be produced from 2-naphthylamine.

A new HPLC method was developed to provide quantitation of DES metabolites in blood and urine. Samples were collected from adult monkeys after they received an intravenous dose of radiolabeled DES. Following the addition of alternately labeled DES, the samples were treated with an equal volume of MeOH/EtOH (1:1) to precipitate salts and proteins. The pellet resulting from centrifugation was washed 2x with MeOH/EtOH and the supernatants combined. After evaporation, the samples were dissolved in MeOH/H2O (1:1) and injected onto a Lichrosorb C18 column (10.2 x 250 mm). The radioactivity was eluted with a 50 min linear gradient from 10 to 100% MeOH in H2O (0.01 M NH4C2H3COOH). The 1.0 ml fractions revealed 5 metabolites. From among these, mass spectrometric analysis identified E and Z DES, E and Z-DES-monogluconide, and Z-DES-monogluconide. The recovery of alternately labeled DES was >90%. This HPLC method provides for fast, efficient quantitation of DES metabolites from biological fluids (Z = cis, E = trans).


Studies of microsomal mixed function oxidase activity in bovines have been conducted. In dairy cattle, cytochrome P-450 concentration is 1) equivalent to concentrations in the rat, 2) active at birth, 3) sensitive to high dietary iodine and 4) by organ, liver > kidney > lung > mammary; NADPH-cytochrome P-450 reductase activity is 1) slightly less compared to rat liver but equivalent to rat lung 2) active at birth, 3) not sensitive to high dietary iodine and 4) by organ, liver > lung > kidney > mammary; Ethylmorphine N-demethylease activity is 1) much less compared to rat, 2) not very active at birth, 3) sensitive to high dietary iodine and 4) by organ, liver > mammary > kidney = lung. Ethoxyxoumarin O-deethylose activity is 1) much greater in both liver and lung compared to rat, 3), moderately active at birth 3) not affected by high dietary iodine, and 4) by organ, liver > lung > kidney = mammary; Hexobarbital hydroxylase activity is 1) much less in liver compared to rat but much greater in lung compared to rat, 2) low at birth, 3) not sensitive to high dietary iodine and 4) by organ, lung > liver > kidney > mammary. A sex difference was observed only with N-demethylease which was more active in males. Castration of males had no affect on activity.
346. MUTAGENIC ACTIVITY IN AGRICULTURAL WASTE SMOKE CONDENSATE. H.E. Olsen, M.Y. Fukayama, C.I. Wei, D.P.H. Hsieh. Dept. of Environmental Toxicology, Univ. of Ca., Davis, CA.

Each fall in the Central Valley of California, a large air pollution problem is created due to the burning of an estimated 2 million tons of waste rice straw. Since previous studies demonstrate mutagenic activity in airborne particulates from various sources, the mutagenicity of rice straw smoke condensate was investigated using the Ames Mutagen Assay. Under simulated conditions, smoke condensate from rice straw burnings was collected on glass fiber filters using a high volume air sampler. The organics were extracted from the filter and fractionated into weakly acidic, strongly acidic, basic, neutral and amphoteric compounds. Mutagenic activity to strains TA 98 and TA 100 was observed in the weakly acidic, basic, neutral and amphoteric fractions as well as the crude smoke condensate. Relative potencies of the fractions are of the order: bases > neutrals > amphoteric > weak acids. Dose response curves demonstrate significant activity in microgram quantities per plate in the most active fractions. These results suggest that rice straw smoke may be a source of potentially carcinogenic air pollutants which warrant further toxicological characterization.


The toxicity of 1,3-dinitrobenzene (1,3-DNB) tested in three test systems is reported. The responses of a cell culture test system, an activity test and a 16-week classical animal toxicity test system to 1,3-DNB were determined and compared with one another. The tests were carried out in the same laboratory in an attempt to determine whether faster and less expensive toxicity tests could yield results that compare with classical methods. The 1,3-DNB was presented in an aqueous solution in each test. A concentration of 8 ppm caused increased spleen weight of rats in the 16-week in vivo study. The effective 50% concentration in the tissue culture assay was 10 ppm. Activity measured on the activity wheel was also altered in animals given drinking water containing 8 ppm 1,3-DNB. This is an example of a toxic chemical in which each of the test systems gave a comparable toxic response.
348. METHYLMERCURY EXCRETION DURING PREGNANCY AND LACTATION.
M.R. Greenwood, T.W. Clarkson, R.A. Doherty, L. Magos and
P.N. Bennett, Environ. Hlth. Sci. Ctr., Univ. of Rochester,
Rochester, NY; Med. Res. Council TCOX Unit, Carshalton, U.K.
and Univ. of Bath, Bath, U.K.

The objective was to determine if pregnancy was a factor
influencing the excretion of methylmercury and if there was
a corresponding change in tissue levels. Previous reports
have shown an increased rate of excretion during lactation.
Pregnant CBA/J mice injected with the methylmercury Hg\(^{203}\)
isotope at 2 mg/Kg body weight have a blood half-time of
4.3 days whereas control mice have a blood half-time of 5.6
days, similar to that seen during lactation. Whole body
half-times are reduced from 10.7 to 8.3 days. Brain, liver,
kidney and other tissue levels in animals killed 39 days
after injection (17-18 days of pregnancy and 22-23 days of
lactation) are 50% lower than in control animals. Mice
killed at day 16 or 17 of pregnancy (12 days after injec-
tion) have tissue levels 20% lower than control animals.
Pregnant and lactating mice have shorter half-times and
tissue levels of methylmercury are substantially lower than
in control animals given the same dose of methylmercury.

349. KEPONE: CELLULAR SITES OF ACTION. E.L.Carmines, R.A.Carchman
Richmond, VA.

Kepone (10\(^{-8}\)M to 10\(^{-4}\)M) inhibited a number of basic
cellular and biochemical responses. Using the P388D1 cell
line, an in vitro cell system was developed utilizing a
mosaic of parameters (i.e. cell proliferation, oxygen con-
sumption, phagocytosis, and calcium metabolism) as indi-
cators of cytotoxicity. This cell line has been shown to
possess many morphological and functional characteristics
of macrophages. Kepone (1x10\(^{-8}\)M to 1x10\(^{-4}\)M) inhibited
cellular proliferation (90 to 20%) and stimulated oxygen
consumption (260 to 97%). The ability of the P388D1, cells
to phagocytize opsonized sheep red blood cells was initially
enhanced followed by a depression over a four hour treatment
period. Kepone produced a significant decrease in the size
of the mitochondrial exchangeable calcium pool while
increasing the efflux rate constant of that pool. The
effects of Kepone on energy metabolism and calcium distri-
bution may be facets of cellular events related to the
observed in vivo toxicity. (Supported in part by EPA grant
number R-804701010.)

An animal model developed from Sprague-Dawley breeding stock is being used to investigate experimental hypertension. It consists of two genetically selected lines of rats; one susceptible (S) and the other resistant (R) to salt-induced elevations in blood pressure. To determine whether exposure to SO₂ alters the development of salt-induced hypertension, a study was conducted in which separate groups of S or R rats on high or low salt diets were exposed to either air or SO₂. Exposures to SO₂ were for 6 hr/day, 5 days/week for 31 weeks at a concentration of 50 ppm. Weight gain of all groups of animals was similar. In SO₂ exposed rats, blood pressure in R animals on both high and low salt diets and S rats on a low salt diet were slightly, but consistently lower than those of air exposed rats. In SO₂ exposed S rats on a high salt diet blood pressures were consistently higher than those of their air exposed counterparts. To date, there are no differences in mortality between air and SO₂ exposed groups. We conclude that exposure to SO₂ significantly increases blood pressure in S rats on high salt diets, however, the increase is small and may not be biologically significant. (Supported by U.S.D.O.E.)


Citizens in the state of Michigan were exposed to food containing PBB's in 1973-74 following widespread contamination of farm animals with Firemaster, a polybrominated biphenyl fire retardant. To determine whether PBB exposure has caused human illness, a prospective study of 4,500 persons was undertaken. The average age of the group was 28 years with an equal number of males and females. Serum PBB levels were found to be satisfactory for verifying exposure ranged from 0 to 1.9 ppm, and corresponded to the risk of exposure. Males and young children had a significantly greater proportion of the higher values. No associations were found between serum PBB levels and symptom prevalence rates for 16 conditions possibly related to PBB toxicity.

PBB was shown to cross the placenta and concentrate in body tissues proportionate to the adipose content of such tissue. PBB levels appear stable in humans sampled over a 3 year period. Biochemical test abnormalities have not shown any association with serum PBB levels. Periodic testing and medical review will continue in order to define any delayed illnesses which may occur more frequently in the exposed group.
352. EXHALED MERCURY AS AN INDEX OF INORGANIC Hg BODY BURDEN
J.D. Dunn, T.W. Clarkson and L. Magos, Univ. Rochester, Sch. of Med. and Dent., NY, and Toxicology Unit, MRC, Carshalton, CR

Exhalation of mercury is a minor route of elimination in mammals exposed to inorganic Hg. This process was investigated as a function of time and initial dose in CBA/J mice given subtoxic doses of \(^{203}\) HgCl\(_2\) ranging from 17 to 500 \(\mu g/kg\). Radioactivity exhaled per unit time increased rapidly in all dose groups in the 24 hrs following the single ip injection. During the subsequent two-week period when no further treatments were administered, a significant correlation (r=0.97) was established between the amount exhaled in a standard time interval and the mercury remaining in the body. Moreover, the pulmonary output of Hg averaged \(2.3\times10^{-2}\) percent of the simultaneous body burden regardless of initial dose. Prior to sacrifice at fifteen days after mercury, a 2 gm/kg ethanol dose elicited approximately a six-fold increase in the Hg exhalation rates over control values in all groups. All major organs and tissues may have contributed to the exhaled pool of mercury. The persistent and dose-independent rate of mercury body burden exhalation under these experimental conditions supports breath sampling as a possible non-invasive indicator of human inorganic Hg exposure.

353. THE TOXICOLOGICAL EVALUATION OF METHYL METHACRYLATE (MMA).
M H Litchfield PhD, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire, SK10 4JJ, UK. Sponsor: Dr. J Smith

The monomer, methyl methacrylate (MMA), has been the subject of an extensive toxicological programme supported on an intercompany basis over the past three years. Published data from other sources has appeared also during this period. This paper reviews the toxicological information now available on the compound & assesses the implications for human exposure. MMA is rapidly & extensively metabolised & the reaction products & the possible mode of breakdown have been identified. The probable lack of toxicological activity implied by the metabolic profile is borne out by the lack of effect in subchronic or chronic studies with high dose levels of MMA & involving three species. The compound has been subjected to teratogenic evaluation in rats & mice by inhalation & assessed for mutagenic activity by bacterial & animal tests. The carcinogenic potential was assessed initially by in vitro predictive short term tests & subsequently by the evaluation of long term inhalation studies in rats and hamsters. Limited surveys in man support the findings from the toxicological programme that MMA is a compound of low toxicity & presents little hazard to man exposed within the limits of the present TLV of 100 ppm.
ABSTRACTS: EIGHTEENTH ANNUAL MEETING


The fate of octane(0) and hexadecane(H) was determined in the Florida lobster to assess the potential for bioconcentration and persistence of crude oil constituents in a human food source. Following a single 10 mg/kg iv injection of [14C]O or [14C]H animals were sacrificed at various times from 6 hr to 56 days. Tissues were dissected, weighed, and analyzed for radioactivity. A flow-limited anatomical compartmental model was postulated for the pharmacokinetics of O and H in the lobster. The model simulates the tissue distribution and disposition kinetics observed. Whole body elimination half-life of O and H was 12 and 32 days respectively. Hepatopancreas accounted for 65 - 88% of the radioactivity recovered for either compound at all times and 95% of the radioactive material in this organ 3 days after H dosing was parent compound. The simulation results suggest that blood flow-limited physiological compartmental modeling concepts previously applied to closed vascular systems are applicable to the open circulatory systems of crustaceans as well.


The herbicide paraquat (methyl-viologen) has been shown to produce severe lung fibrosis in both laboratory animals and human subjects. Its effect on the kidney has been much less studied. A method has been developed in this laboratory to produce "acellular kidneys" which depends on exhaustive extraction with detergents. The superstructure composed of basement membrane remains intact in these preparations. Using this method, changes in kidney basement membrane obtained 9 days after a single dose of paraquat (I.P. 25 mg/Kg) were studied. Electron microscopy showed numerous "spiky" protrusions in the luminal side of tubular basement membranes of the affected kidneys, while glomerular basement membrane remained intact. Chemical analysis of the damaged basement membrane showed increased levels of hydroxylysine, 3-hydroxyproline and cysteine, possibly indicating increased basement membrane synthesis. Parallel to this finding, the synthesis of collagenous proteins of the kidney was increased which was reflected by both elevated prolyl hydroxylase levels and incorporation of proline into extracellular matrix. (Supported by a grant from ONR N00014-77-C-0506.)

The Omaha study of 1238 blood leads (Pb B) from 831 children at air lead (Pb A) concentrations of .02 - 1.69 µg/M³, 1971-1977, predicts, at ages 6-18 years, an increase of Pb B of 0.6 µg/dl as Pb A increases from 1 to 2 µg/M³ if soil (Pb S) and house dust (Pb HD) are constant. The multiple regression, Pb B = 8.1 Pb A - 0.03Pb S - 0.10Pb S - 0.10 Pb HD - 0.07, closely approximates the observed changes in four major community studies. In this model, Pb A, Pb S and Pb HD account for 21% of the variance of Pb B. At the high background lead of the Omaha population, the equation requires an air quality standard of 1.0 µg/M³ or below to achieve an acceptable mean blood lead of 19.5. A new environmental paradigm is emerging in which the equilibrium between increments in atmospheric lead and the residual soil and dust lead is the critical factor in lead exposure.


In order to evaluate the level of exposure of the workers of two battery plants and to help select the most appropriate parameters of biological monitoring for our prevention program, an EDTA chelation test was done on exposed workers for whom blood lead values varied between 40 and 140 µg/100 ml. The maximum urinary lead concentration found in one worker was 13,200 µg/l after chelation with EDTA 1 g I.V. In one plant, both erythrocyte free protoporphyrin (FEP) and urinary aminolevulinic acid (U-ALA) were measured simultaneously. Significant correlation was found between PbU-EDTA and FEP, but not between PbU-EDTA and U-ALA. In the other plant, only FEP was measured beside blood and urinary lead. Significant correlation was again found between PbU-EDTA and FEP. The relative usefulness of the various biological parameters for lead exposure will be discussed.
358. EVALUATION OF POPULATION EXPOSURE TO ARSENIC, LEAD, CADMIUM AND MERCURY IN THE VICINITY OF A COPPER SMELTER.


As part of a study of the effects of a copper smelter upon the environment, the concentration of several toxic metals (lead, cadmium, arsenic and mercury) were measured in the hair of the local population and of two outside control populations located in other cities of the same geographical area. Mercury, lead, ALA-D activity and F.E.P. were also determined in blood. A total of 500 persons (300 in affected area) selected using epidemiological criteria, were investigated. A total of 6000 analyses were performed on collected blood and hair samples. Concentrations of lead, cadmium and arsenic in hair tended to increase simultaneously and with increasing proximity to the smelter. Blood ALA-D activity inhibition followed the same pattern and was age-dependent, children being the most affected.

359. TOXICOLOGICAL RESPONSES OF NONHUMAN PRIMATES TO 2,3,4,6,7,8-HEXACHLORODIBENZOP-DIOXIN (HCDD). J.R. Allen, J.P. Van Miller, D.A. Barsotti, and L.A. Carstens, Univ. of Wisconsin Medical School, Dept. of Path. & Regional Primate Res. Center, Madison, WI 53706.

Male juvenile rhesus monkeys were fed a diet containing 5 ppb of 2,3,4,6,7,8-HCDD for six months. During this period the animals consumed approximately 13 μg of HCDD. By the third month of exposure there was congestion and swelling of the eyelids and loss of eyelashes that became more severe with time. By the sixth month, the animals had difficulty seeing due to the swelling of the eyelids and marked ocular discharge. There was also loss of hair which was obvious by the fourth month, and extended over the entire body by the sixth month. Intermittent periods of vomiting and diarrhea occurred from the fourth to the sixth month. Anemia and leukopenia were apparent in the animals by the sixth month. Similar signs and lesions have been reported to occur in monkeys fed 500 ppt 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Allen et al. Fed. Cosmet. Toxicol. 15:401, 1977). Indications are that TCDD is approximately 10 times more toxic than HCDD to rhesus monkeys when the exposure occurs over an extended period. Supported in part by ES01339 and ES01839.
360. TOXICOLOGICAL EFFECTS PRODUCED IN NONHUMAN PRIMATES CHRONICALLY EXPOSED TO FIFTY PARTS PER TRILLION 2,3,7,8-TETRACHLORO-DIENZO-p-DIOXIN (TCDD). S.L. Schantz, D.A. Barsotti and J.R. Allen. Univ. of Wisconsin Medical School, Dept. of Path. & Regional Primate Res. Center, Madison, WI 53706.

Adult female rhesus monkeys were fed a diet containing 50 ppt TCDD for 20 months. Prior to TCDD exposure the animals had normal hemograms, serum chemistry and serum estradiol and progesterone. After 7 months on the TCDD diet (total TCDD intake 0.35 µg/kg body wt.) attempts were made to breed the females to control rhesus males. There were 4 abortions and 1 stillbirth. Two of the animals were bred on repeated occasions and did not conceive. The remaining 2 animals were able to carry their infants to term. All of the 8 control animals conceived and gave birth to normal infants.

During the 20 months of exposure to TCDD (total intake of 0.9 mg/kg body wt.) the animals have experienced hair loss, hyperkeratosis particularly of the arms, and weight loss. There has also been a decrease in serum cholesterol, hematocrits and white blood cells and an increase in serum glutamic pyruvic transaminase. The above changes were similar to but not as severe as those experienced by rhesus monkeys fed a diet containing 500 ppt TCDD (Allen et al., Food Cosmet. Toxicol. 15:419, 1977). (NIH grants ES01839 & ES01339)

361. TCDD CONTAMINATION IN ITALY: THE RISK ASSESSMENT OF LOW LEVEL EXPOSURE, CUMULATIVE EFFECT AND LONG-TERM CONSEQUENCES. G. Reggiani, Medical Research Board, F. Hoffmann-La Roche & Co. Ltd., Basle, CH.

July 10, 1976, a runaway reaction in a plant producing trichlorophenol caused the contamination of 726 acres of culture land and populated area at a level of TCDD of 15-2000 µg/m² for about 115 acres and of 1-15 µg/m² for the rest of the area. The acceptable levels of exposure for the population were established at 0.075 µg/m² for the indoor walls of the houses, at 0.1 µg/m² for the outdoor walls and at 5 µg/m² for the open ground. The incidence of chloracne remained between 0.6 and 1.5 % in the surveyed population with a clear trend to rapid and spontaneous healing. Other toxic effects observed in occasion of acute exposure did not occur or remained at the subclinical level. Assessment of the risk estimation of continuous low level exposure and possible cumulative effect has been made using the threshold limit values calculated for TCDD at the working place for women and the mathematical procedure of Mantel and Bryan for cancer incidence rates.

The metabolism of exogenous chemicals is associated with their detoxification and excretion, however this process is also associated with cellular toxicosis and chemical carcinogenesis. This study describes the comparative in vitro microsomal-mediated metabolism of biphenyl and the halogenated biphenyls using 4-chloro, 4-bromo, 4-fluoro and 4-iodobiphenyl as model substrates. The metabolism was monitored by determining the formation of lipophilic hydroxylated products, low molecular weight trichloroacetic acid (TCA) soluble conjugates and irreversibly bound substrate-macromolecular adducts using both non-induced and Aroclor 1254 induced rat hepatic microsomes. The order of substrate metabolic reactivity for the non-induced microsomes was 4-fluorobiphenyl > 4-iodobiphenyl > 4-chlorobiphenyl > biphenyl > 4-bromobiphenyl and for the induced microsomes the 4-chloro and 4-fluorobiphenyl substrates were interchanged. The metabolic rate data obtained from both the induced and non-induced microsomes did not show a correlation between substrate lipophilicity and size or the substituent electronic effects.

363. HEPATIC ULTRASTRUCTURAL CHANGES OF RATS EXPOSED TO POLYCHLORINATED BIPHENYL ISOMERS: HEXACHLOROBIPHENYL-INDUCED NEOPLASTIC NODULES, R.H. Weltman and D.H. Norback, Wm. S. Middleton VA Hosp. and Univ. of Wis., Madison, WI.

Sequential hepatic alterations of rats receiving diets containing 100 ppm of 2,2',5,5'-tetrachlorobiphenyl (TCB) or 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) were evaluated over a two year period. TCB-induced changes consisted of central cell hypertrophy, pigmented Kupffer cells, and vacuolated, pigmented hepatocytes. HCB evoked pigmentation, central and midzonal cell hypertrophy, lobular-sized areas of hyperplasia, and neoplastic nodules, in sequence. Neoplastic nodules comprised the surrounding parenchyma and lacked a lobular architecture. Nodule cells were characterized by eosinophilic ground glass appearance, extensive interdigitations of the plasmalemma, proliferated smooth endoplasmic reticulum (ER), disorganized rough ER, many polyribosomes, numerous large lucent mitochondria, pigmented lysosomes, and convoluted nuclei with multiple nucleoli. Other HCB-induced changes included cysts lined by ductal epithelium, and blood cells. Thus, the lower chlorinated TCB was less toxic than the HCB, which induced neoplastic nodules. (Supported by NIH grants CA22140 and ES 00958)
364. **TRANSFORMATION IN VITRO INDUCED BY AROCLOR 1254: ULTRA-STRUCTURE OF TRANSFORMED CELLS**, D.H. Norback and G.J. Swedo, Wm.S. Middleton Memorial VA Hospital and Univ. of Wis., Madison, WI.

Mouse embryo C3H/10T1/2 Cl8 fibroblasts were morphologically transformed by 6 week exposure to 10 μg/ml Aroclor 1254, which contains a mixture of polychlorinated biphenyls, in cell culture media. Control cells did not undergo transformation. Transformed foci consisted of 10-40 layers of cells admixed with necrotic debris, arranged in both parallel and criss-crossed patterns at the perimeter. By transmission or scanning electron microscopy, transformed cells were rounded and irregular with surface projections of blebs, microvilli, and ruffles; whereas control cells were elongated with smooth surfaces. Deep involutions of the nuclear envelope appeared as pseudonuclear inclusions. Nuclei possessed prominent nucleoli, predominately composed of granular elements. Chromatin was dispersed whereas control cells contained more heterochromatin. The cytoplasm contained well-developed dilated Golgi vesicles and smooth endoplasmic reticulum, concentric membrane arrays, decreased rough endoplasmic reticulum, and increased numbers of lysosomes, myelin figures, and lipid droplets. Microfilaments and microtubules were decreased and displayed a disordered arrangement. Intercellular junctions were decreased. (Supported in part by NIH Grant CA 22140)

365. **INDUCTION OF HEPATIC MFO ENZYMES IN TROUT FED PCB, SOME PROPOSED PCB REPLACEMENTS, AND RELATED COMPOUNDS.**
R. F. Addison¹ and F.C.P. Law²; ¹Marine Ecology Lab., P.O. Box 1006, Dartmouth, N.S., Canada, ²Coll. Pharmacy, Dalhousie Univ., Halifax, N.S., Canada. Sponsor: D. J. Ecobichon.

Brook trout (Salvelinus fontinalis) were fed PCB (Aroclor 1254), proposed PCB replacements based on chlorodiphenyl ethers (XFS-4169L: Dow) or isopropyl chlorobiphenyls (Chloralkylene 12: Prodelec) or related compounds over a two or three week period and the induction of hepatic MFO enzymes was studied.

Aroclor 1254 and 3-methylcholanthrene (3-MC) induced microsomal protein and Cytochrome P-450 concentrations, and activities of ethoxyxoumarin 0-de-ethylase, aniline hydroxylase, and aldrin epoxidase; 3-MC was the more powerful inducer. Neither XFS-4169L nor Chloralkylene 12 induced components of the MFO system. None of the three major components of XFS-4169L (4-chlorodiphenyl ether, 2-sec-butyl-4'-chlorodiphenyl ether and 4-sec-butyl-4'-chlorodiphenyl ether) induced trout hepatic MFOs. A chlorinated terphenyl (Aroclor 3460) induced the activity of ethoxyxoumarin-0-de-ethylase only.

Polybrominated biphenyls (PB2) Firemaster FF1 (lot No. 7042) which was accidentally mixed into cattle feed in Michigan is poorly eliminated from mammalian systems. Feces are the major excretory route. Four groups of 10 adult male Sherman strain rats were given 0, or a single dose of 500 mg PB2 in corn oil/kg body weight (b.w.) by stomach tube. Three weeks after dosing PB2 treated rats and rats given corn oil in groups of 10 received either a 20% synthetic fiber diet, a 4% synthetic fiber diet, Purina chow or Purina chow and 2% mineral oil/kg b.w. 3 times a week for 3 months. PB2 levels were determined in blood, liver and adipose tissue by electron capture gas chromatography. Lipids were determined in all livers. All rats given PB2s had enlarged livers. Based on wet weight, PB2 concentrations were lowest in livers of rats fed Purina chow. These livers contained the lowest concentration of lipids. The lowest PB2 blood levels were observed in the rats given the 4% fiber diet. Morphological changes in the liver were more severe in rats on synthetic diets with PB2s.


Although photomirex and mirex are known environmental contaminants, little is known about their toxic effects on animals. Hence, Sprague-Dawley weanling rats were fed rations containing 0, 0.05, 0.5, 5 or 50 ppm photomirex or 5 or 50 ppm mirex for 28 days. Liver and testis samples from two animals of each group were chosen and processed for electron microscopy. In animals fed 5 or 50 ppm photomirex or 50 ppm mirex many hepatocytes exhibited a proliferation of smooth endoplasmic reticulum, and a concomitant reduction of glycogen. The myoid cells of the testicular limiting membrane at 0.05 ppm photomirex depicted disorganized architecture. The occurrence of gaps among cells of seminiferous epithelium was common in rats receiving photomirex or 50 ppm mirex. Nuclear inclusions were seen in differentiating spermatogenic cells of photomirex-fed rats. Sertoli cells of treated animals were frequently vacuolated. Photomirex is, therefore, more toxic to rats than mirex.

Supported by NWH Canada contract 082-77-00058.
368. THE COMPARATIVE TOXICITY OF TECHNICAL AND ANALYTICAL PENTA-
CHLOROPHENOL IN CATTLE. E.E. McConnel, C.E. Parker, B.N.
Gupta, M.I. Luster and J.A. Moore, National Institute of
Environmental Health Sciences, Research Triangle Park, NC.

The objective of this study was to compare the effects of
technical(t) vs analytical(a) grade PCP in cattle. Four
groups of 3 female yearling Holstein calves were exposed
for 160 days via feed to aPCP or tPCP and mixtures thereof.
A fifth group served as unexposed controls. All treated
animals received the same amount of PCP; 20 mg/kg/day for
42 days followed by 15 mg/kg/day. Fat biopsies for chemical
analyses were obtained before, during (70 days) and at the
end of the study. Liver samples were collected at the lat-
ter two times. Major findings included a dose related de-
crease in body weight gain, decreased feed efficiency and
progressive anemia in tPCP cattle while those exposed to
aPCP were comparable to the untreated controls. Liver and
lung weights were increased while the thymus was decreased.
Marked villous hyperplasia of the urinary bladder was found
in 2 of 3 animals exposed to the highest level of tPCP.
There were minimal hepatic lesions. Immunological studies
suggested a progressive dose related alteration in cell me-
diated immunity but no effect on humoral immunity. This
study indicates that the toxicity of PCP in cattle is primar-
ily attributable to its contamination with toxic impurities.

369. DISPOSITION OF TRIS-(1,3-DICHLORO-2-PROPYL)-PHOSPHATE IN THE
RAT. H.B. Matthews and M.W. Anderson, Laboratory of Pharma-
co- kinesics, NIEHS, Research Triangle Park, N.C. 27709

Tris-(1,3-dichloro-2-propyl)-phosphate (Fyrol FR-2) is
used as a flame retardant in children's sleepwear. The dis-
position of carbon-14 Fyrol FR-2 has been studied in the rat
as part of a comparative study of this and another halogenat-
ed alkyl phosphate flame retardant, tris-(2,3-dibromopropyl)
phosphate. At least 90% of an oral dose of Fyrol FR-2 was
absorbed from the gastrointestinal tract. Following either
gastrointestinal absorption or IV injection Fyrol FR-2 was
rapidly distributed throughout the body, the highest being
observed in kidney, liver and lung. Fyrol FR-2 was subject
to rapid and extensive metabolic degradation and its
metabolites were eliminated in bile, feces and urine as well
des degraded to CO₂ and expired. Elimination of Fyrol FR-2
derived radioactivity in bile exceeded excretion in feces,
indicating that a portion of the material in bile was subject
to enterohepatic recirculation. The total body burden of
Fyrol FR-2 decreased relatively rapidly, greater than 80%
of the dose was excreted within the first 24 hr. following
exposure; however, traces of Fyrol FR-2 derived radioactivity
were detected in most tissues 10 days after exposure.

Sprague-Dawley rats and Golden Syrian hamsters have been exposed by inhalation to 3500, 1500, 500 or 0 ppm of methylene chloride, 6 hrs/day, 5 days/wk for two years. Interim sacrifices with clinical and pathologic studies were carried out at 6, 12, 15 (rats only) and 18 months of the study with final sacrifice at 24 months. Animals dying during the study or sacrificed in moribund condition were examined pathologically. Significant findings in rats were: increased number of benign mammary tumors in female rats at all exposure levels; increased incidence of benign mammary tumors in male rats at the highest exposure level; non-life-shortening treatment related changes, similar to those seen in aging animals, in the livers of male and female rats at all exposure levels. Pathologic examination of hamsters is not complete, but no evidence of oncogenic effect has been found based on gross pathologic examinations.


Biliary permeability was assessed in pentobarbital anesthetized rats by the Intrabiliary Pressure (IBP) technique. IBP is the maximum differential pressure (measured by pressure transducer) generated within the biliary tree during an intrabiliary infusion of 150 μl of saline at 2.27 μl/sec. The bile flow rate and IBP were measured in each rat prior to (control), during and following a 30 min infusion with TLC (0.46 μmol/min, i.v.). Along with the TLC induced cholestasis, TLC treatment increased the control IBP of 20.9 ± 0.3 to 26.4 ± 1.3 mm Hg (mean ± S.E.). Similar results were found when rats were treated with a combined infusion of TLC and dehydrocholate (0.61 μmol/min). In contrast to these results, the combined infusion of TLC and taurocholate or glycocholate (0.61 μmol/min) resulted in no change in bile flow or IBP. The increase in IBP by TLC is consistent with an increase in hepatobiliary resistance or a decrease in permeability. This effect can be antagonized by taurocholate and glycocholate. (Supported by NIH grants GM 16503 and ES 07043).

Measurement of the transmembrane potential of the liver cell may be an indicator of the functional integrity of the liver. The hepatocyte transmembrane potential was measured in pentobarbital Na (45 mg/kg, i.p.) anesthetized, artificially ventilated rats. A 24 hr pretreatment of rats with CCl₄ produced a depolarization from the control potential of -47.5 ± 1.2 to -39.4 ± 2.9, -27.1 ± 3.4 and -25.1 ± 5.2 (mean ± SE) mV at doses of 0.33, 0.75 and 1.0 ml/kg, p.o., respectively. In a paired experiment, the control potential of -47.6 ± 1.6 mV was significantly changed to -39.9 ± 3.0 following treatment with taurocholate Na (100 μM/kg, i.v.). In contrast to this finding, treatment with tauroliothocholate (0.46 μM/min) was shown to produce a hyperpolarization of the hepatocyte transmembrane potential. The detergent (micelle forming) activity of taurocholate and the insoluble nature of tauroliothocholate could be responsible for the observed changes. The changes with these bile salts correlate somewhat with their effects on alteration of biliary tree permeability. (Supported by NIH grants GM 16503 and ES 07043).

373. BILIARY EXCRETORY DYSFUNCTION IN THE RAT FOLLOWING EXPOSURE TO PHOTOMIREX. L. R. Curtis and H. N. Mehendale, Dept. of Pharmacol. & Toxicol., Univ. MS Med. Ctr., Jackson, MS

Exposure to 50 and 150 ppm of photomirex (PM), in the diet of male Sprague-Dawley rats for 15 days resulted in impaired biliary excretion of polar metabolites of imipramine (PM-MM) and phenolphthalein glucuronide (PG) in intact animals. Biliary excretion of PM-MM and PG was reduced 13% and 21% respectively at 50 ppm PM; and 20% and 28% respectively at 150 ppm PM. Biliary excretion of PG was also examined in 50 ppm PM rats 24 hrs after an ip dose of 0.1 ml CCl₄/kg. In these animals biliary excretion of PG was reduced 40%, while CCl₄ alone reduced it by 25%. SGPT and SCOT were unaltered by either PM dose or CCl₄, while the 50 ppm PM-CCl₄ combination resulted in 8 and 7 fold increases in the respective enzymes. Hypertrophy of the liver was observed with 50 ppm PM (39% increase in liver wt), 150 ppm PM (102% increase), and 50 ppm PM-CCl₄ (100% increase), while CCl₄ alone did not alter this parameter. Histologically, no necrosis but liposis was observed in the livers of rats receiving PM. Liposis and slight centrilobular necrosis was seen after CCl₄ and PM-CCl₄ resulted in considerable centrilobular necrosis. (Supported by BRSR 5 807 RR 05386 and RSD-07045-01).
ABSTRACTS: EIGHTEENTH ANNUAL MEETING

374. ALTERATIONS IN HEPATIC TRANSPORT SYSTEMS IN ISOLATED RAT HEPATOCLYES AFTER TREATMENT WITH MICROSOMAL ENZYME INDUCERS. D. L. Eaton and C. G. Klaassen, Dept. Pharmacology, Univ. Kansas Medical Center, Kansas City, KS 66103.

The effects of phenobarbital (PB), 3-methylcholanthrene (3-MC), pregnenolone-16α-carbonitrile (PCN), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and kepone on the hepatic uptake of ouabain and procaine amide ethobromide (PAEB) were examined in isolated rat hepatocytes. The initial velocity of uptake (Vo) for ouabain was increased to 150 and 200% of control after PB and PCN, respectively. TCDD decreased the Vo of ouabain by 50%. The Vo of PAEB was not affected by any of these compounds. Cellular concentrations of ouabain and PAEB approached steady-state levels after about 45 min. Steady-state levels of ouabain were significantly increased after treatment with PCN and 3-MC, and steady-state levels of PAEB and the bile acid taurocholate were also increased by 3-MC. TCDD and kepone significantly depressed the steady-state levels of ouabain. Phenobarbital was the most effective inducer of cytochrome P-450 and microsomal protein and PCN was the least effective. These data demonstrate that there is no apparent relationship between the ability of xenobiotics to increase hepatic cytochrome P-450 and their ability to alter hepatic carrier-mediated transport systems. (Supported by USPHS Grant AM-14513).


Acetaminophen (APAP) at low pH such as found in mammalian stomach, reacts with nitrite to form 3'-nitro-4'-hydroxyacetanilide (NHA) and other minor products. The effect of oral co-administration of sodium nitrite and APAP on hepatotoxicity in fasted mice was investigated by measuring serum GPT and GOT activity 23 hours following treatment. Administration of 300 mg/kg of APAP to fasted mice resulted in marked elevations of SGPT and SGOT. The co-administration of 100 mg/kg of sodium nitrite resulted in about an 80% block in these elevations. Oral administration of 389 mg/kg of NHA (equivolular to 300 mg/kg APAP) to fasted mice did not result in elevated SGPT or SGOT activities. These results were consistent with the hypothesis that in part, nitrite prevented APAP hepatotoxicity by converting APAP in the stomach to a non-hepatotoxic compound. An additional mechanism(s) appears to be involved since i.p. administration of 100 mg/kg of sodium nitrite also blocked the elevations in SGPT and SGOT elicited by orally administered APAP. It appears unlikely that nitrite protected against APAP hepatotoxicity by inhibiting the microsomal P450 system in that i.p. administered nitrite was not capable of preventing bromobenzene or carbon tetrachloride induced hepatotoxicity.
376. COMPARISONS IN HEPATIC RESPONSE TO MIREX WITH CD-1 AND B6C3F1 MICE. B.A. MAYES AND R. ABRAHAM, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, N.Y. 12208.

Previous studies have shown that mirex causes significant changes in tetraploid and octaploid but not diploid hepatocytes in CD-1 mice. B6C3F1 hybrid strain mice are extensively used for carcinogenic studies and it was, therefore, of interest to compare the effects of mirex in this strain. Comparisons after 2, 4 and 8 weeks of dietary exposure to 15 ppm mirex indicated a greater susceptibility of the hybrid strain with increased mortality and necrosis as early as 4 weeks. Coulter counter analysis of the hepatic nuclei revealed that B6C3F1 mice had consistently increased tetraploid and octaploid volumes with decreased tetraploid frequency, while CD-1 mice changed very little. DNA syntheses increased 6-fold in hybrid and 4-fold in CD-1 mice and was most prominent in the tetraploid and octaploid cells as detected autoradiographically. These changes indicate an increased susceptibility of B6C3F1 polyploid hepatocytes to mirex and may also explain the resistance of rats and monkeys to these chemicals since the latter have predominantly diploid cells. Supported by NEHS Training Grant #LT32-ES-07058-01.


Specimens obtained by percutaneous liver biopsy from 125 patients were assayed for HPHA. Patients with only alcohol related liver disease had significant elevations of HPHA in all stages of liver disease. Active alcoholic patients taking drugs known to be hepatotoxic (chlorpromazine, isoniazid) had significant elevations of HPHA over active alcoholic patients with identical hepatic histopathology. Non-alcoholic patients on methotrexate with steatosis had significantly greater elevations of HPHA compared to alcoholic patients with steatosis. Alcoholic patients with gastrointestinal malignancy without hepatic metastases or biopsy had HPHA that was consistent with that observed in patients with only alcoholic liver disease. However, large elevations in HPHA were observed in a group of non-alcoholic patients with gastrointestinal malignancy but with normal hepatic morphology. These data indicate that the use of hepatotoxic drugs will elevate HPHA beyond the 95% confidence level for alcoholic patients with similar hepatic histopathology. Further, the presence of malignant disease of the GIT will result in large elevations in HPHA in non-alcoholic patients with normal hepatic morphology. These elevations in HPHA precede microscopically observable accumulation of fibrous tissue.

Pregnant hamsters were exposed by intubation to a single oral dose of hydrazine hydrate (260 mg/kg) or 1,2-dimethylhydrazine dihydrochloride (150 mg/kg) as a neutralized solution on the twelfth day of gestation (4 days before birth). Comparable doses of methylazoxymethanol acetate were lethal to the dams within a 24 to 48-hour period. Groups of animals (N=3) were sacrificed 1 or 2 days before birth and 3 or 4 days, 10 days, 15 days, 24 days and 53 or 60 days after birth to evaluate the development of intestinal brush border enzymes, lactase, sucrase and alkaline phosphatase, as compared to the development of these enzymes in control animals. The results indicate that hydrazine had a stronger diminishing effect on lactase and sucrase development than did 1,2-dimethylhydrazine. A screen for teratogenic effects revealed no incidence of cleft palate formation in the offspring. While there are no apparent teratogenic effects, biochemical alterations reveal more subtle effects of these environmental toxins on this developing organ system.


A decrease in the hepatic excretory capacity for bromosulfophthalein (BSP) occurs in some women receiving oral contraceptives or during pregnancy. Treatment of rats with estrone, estradiol-17-β or ethinyl estradiol for 5-7 days decreases the biliary excretion (BE) of BSP and decreases bile flow (BF) 40-50%. Female rats (250±20g) were anesthetized, the femoral artery, vein and bile duct cannulated and temperatures maintained at 37°. 3H-estradiol glucuronide (3H-EG) at a dose of 4,6,8 or 10 mg/kg iv decreased BF 33,58,82 or 100% respectively within 15-30 min; BF returned to 75-100% of control values within 3 hr. Administration of 5.2 mg/kg (11 μmole/kg) iv of 3H-EG, the calculated ED50, decreased BF 64% and the BE (μmole/min/kg) of bile acids 69%. Estradiol-17-β (11 μmole/kg iv) increased BF 30% within 15 min; BF returned to control values within 3 hr. Estrogen-induced cholestasis may therefore be due to accumulation of a metabolite(s) which inhibits bile acid and organic anion transport. Supported by Basil O'Connell Starter Grant, National Foundation March of Dimes.
380. STUDIES OF ACUTE HEPATOTOXICITY OF STYRENE OXIDE IN RATS.
Saroj Chakrabarti and Jules Brodeur, Département de médecine du travail et d'hygiène du milieu, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada.

Since epoxide intermediates are known to be implicated in hepatotoxicity, we have studied the metabolism and hepatic injury caused by i.p. administration (375 mg/kg, in corn oil) of styrene oxide (SO) in adult male rats. Liver glutathione was significantly reduced at 2 h, but returned close to normal at 6 h. Serum glutamic-oxaloacetic transaminase activities were elevated during the entire time period (24 h) of study, but those of serum glutamic-pyruvic transaminase and serum alkaline phosphatase increased at 24 and 2 h respectively. Serum protein concentration remained decreased till 24 h. An increase in both prothrombin time and urinary urobilinogen concentrations were observed only at 6 h and 2 h respectively. A decrease in body weight and an increase in liver weight were observed at 24 h. Urinary (but not fecal) nonprotein sulfhydryl concentrations were increased at 2 and 6 h, while urinary mandelic and phenylglyoxylic acids continuously increased during the entire time period. These data confirm that SO could produce acute hepatic injury in rats. (Supported by MRC, MA-6159).

381. HEPATOTOXIC INJURY INDUCED BY STYRENE IN ADULT MALE RATS.
Saroj Chakrabarti and Jules Brodeur, Département de médecine du travail et d'hygiène du milieu, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada.

Administration of a single high dose (400-1200 mg/kg, i.p. in corn oil) of styrene failed to produce any biochemical lesion due to liver injury in rats, although urinary metabolites were increased with dose of styrene. However, when rats were given styrene (700 mg/kg, i.p.) for three consecutive days and sacrificed after 24h, the following biochemical parameters were significantly changed: (1) increase in the activities of serum glutamic-oxaloacetic transaminase (30%), glutamic-pyruvic transaminase (39%) and γ-glutamyl transpeptidase (107%); (2) decrease in serum albumin concentration. Urinary metabolites e.g. hippuric acid, mandelic acid, phenylglyoxylic acid and nonprotein sulfhydryl contents were all increased. Administration of styrene (700 mg/kg) for three days to rats pretreated with phenobarbital (75 mg/kg, i.p.) for five days produced the same pattern of changes in the biochemical parameters as well as in the metabolites (except non-protein sulfhydryl groups) as above. (Supported by MRC, MA-6159).

Many xenobiotics are excreted by both liver and kidneys. The purpose of the present studies was to examine alterations of excretion after damage to either system. Rats were pretreated with CCl₄ (1 ml/kg, 1 d) or K₂Cr₂O₇ (10 or 20 mg/kg, 1 or 3 d). The common bile duct, both ureters, a femoral artery and vein were cannulated. ³H-Digoxin (DIG) was given as an iv bolus (3 mg/kg). After CCl₄ the biliary excretion and hepatic concentration of DIG were decreased by 30%; the bile flow rate and plasma concentration of DIG were unchanged, while the urinary excretion was slightly increased. One day after 10 mg/kg K₂Cr₂O₇ plasma concentration and urinary excretion of DIG tended to be decreased while the biliary excretion tended to be increased. After 3 d, biliary excretion was still slightly elevated. Animals which received 20 mg/kg K₂Cr₂O₇ were anuric at 1 d, after 3 d urinary excretion was decreased by 80%, biliary excretion was increased by 45% and the plasma concentration was increased. Thus, hepatic and renal excretory systems can compensate for impaired function of the other in the excretion of digoxin. (Supported by ESD-07045-01 and BRSG 5507 RR 05386.)

383. PHOTOMIREX: A NINETY DAY TOXICITY STUDY IN RATS.
Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, and Bow Valley Holdings Ltd. (V.E.V.), Guelph, Ontario.

Photomirex (8-monohyromirex) is a demonstrated environmental contaminant and was observed in previous short-term studies to produce lesions in the liver, thyroid and testes. The present study was undertaken to confirm those observations and to determine the effects after a longer period of exposure. Male rats were fed photomirex for 12 weeks at levels of 0.20, 1.0, 5.0, 25 and 125 ppm in the diet. Deaths were observed in animals receiving the highest dose. Decreased body weight gain and food intake were also observed in that group. Liver weights were increased at 5.0 ppm photomirex and higher. Photomirex caused changes in several biochemical parameters including SDH and microsomal mixed function oxidase activity. Dose related histological abnormalities were observed in the thyroid and liver starting at the lowest dose level. Lesions of the testes were only observed at the highest dose level. These results confirm earlier findings and show that photomirex is a potent hepato- and thyrotoxin.

As part of a programme designed to investigate specific differences in toxicity between rodent species it was decided to assess the susceptibility of the hamster to the acute toxicity of chlorinated hydrocarbons. Acute LD50 toxicity studies were conducted with 13 pesticides. The chemicals were administered orally by intubation to \( \hat{\gamma} \) and \( \delta \), 6 weeks old, Syrian golden hamsters. In select cases behavioural tests (open field) were conducted in groups of survivors before killing the animals 2 weeks after starting. The LD50 was calculated by the method of Weil (Biometrics 8, 249, 1952). The following LD50 values (mg/kg body wt) were found: Atrazine=1000; Chlorgabenzilate=700; 2,4-D=500; Dieldrin=100; Endrin=17 in \( \hat{\gamma} \) and 12 in \( \delta \); Heptachlor=160; Hexachlorobenzene \( > 1600 \); Hexachlorobutadiene=960 in \( \hat{\gamma} \) and 1920 in \( \delta \); Methoxychlor \( > 2000 \); Mirex=125 in \( \hat{\gamma} \) and 250 in \( \delta \); PCNB \( > 4000 \); Pentachlorophenol=168 and Toxaphene=200. When comparing the present results with data previously reported in rats it can be concluded that the hamster is as sensitive as the rat to the acute toxicity of most of the compounds studied.


As part of a program to assess the toxicity of the poisonous weed, tansy ragwort (Senecio jacobea), male rats were fed diets containing 0 and 5% ground, dried tansy plant for periods up to 8 weeks. Rats were sacrificed after 1, 2, 4, 6, and 8 weeks on diet. Reduced growth and food consumption were observed after 1 week of feeding the tansy diet. Time-dependent decreases in liver and kidney weights, and increases in lung and spleen weights occurred in the treated animals. Hematological examination revealed marked leukocytosis and reduction in hematocrit and RBC counts, the former occurring as early as 1 week after treatment. Total serum protein and serum albumin levels fell after 4 weeks of treatment. Treatment-related increases in iron content of liver, spleen, tibia and serum, and copper content of liver and serum were observed. However, liver zinc levels were reduced. The significant pathological changes were megalocytosis of hepatocytes, splenomegaly, and ascites, particularly in 6- to 8-week treated rats. (Supported by NIH grants No. CA-22524 and ES-00210).

Permethrin, a pyrethroid insecticide has been shown to be considerably more lethal to fish than to mammals. The in vitro metabolism of permethrin in both rainbow trout and carp was investigated to determine whether differences in biotransformation processes might play a role in the selective toxicity. Cis and trans (14C) permethrin were incubated with RB trout and carp liver microsomes. Both carp and RB trout microsomes readily hydrolyzed the ester of the trans isomer of permethrin. The cis isomer was considerably more resistant to esterase attack and was primarily metabolized via oxidative pathways. Oxidative metabolism also occurred on the trans isomer but the action of the oxidases on this isomer seemed secondary to the hydrolytic activity of the esterases. Preliminary in vivo metabolism studies on RB trout exposed to (14C) permethrin showed little parent compound remaining in the bile, the majority of 14C derived permethrin being polar metabolites. The data indicate that fish are capable of metabolizing permethrin both in vivo and in vitro and the pathways appear qualitatively similar to those seen in mammals. (Supported by NIH Grant ES 01080 and the University of Wisconsin Sea Grant Program).

387. TREATMENT OF MERCURY POISONING USING DIMERCAPROL AND DIALYSIS. D. W. Teat, E. J. Stramoski, M. D. Ellis, Texas State Poison Center, Univ. of Texas Medical Branch, Galveston, TX., B. A. Bartholomew, R. Pietila, and G. Stanbaugh, Texas Tech School of Medicine, Lubbock, TX.

A 23 year old male patient was successfully treated with a combination of dimercaprol, peritoneal and renal dialysis, after ingesting a commercial product containing mercuric chloride. The patient presented for treatment in acute renal failure as characterized by no measurable urine output, BUN of 19, and serum creatinine of 2.2. Peritoneal dialysis was initiated upon admission and continued for 3 days. On the fourth hospital day, the patient's condition worsened, as evidenced by continued anuria, BUN of 100, and serum creatinine of 8.4. Dimercaprol was then administered in dosages of 500 mg. deep I.M. The dialysate mercury level before administration of dimercaprol was 2500 ug/L, as opposed to 17,500 ug/L, following combination therapy with dimercaprol and peritoneal dialysis. The patient's urine output began to improve gradually, and renal dialysis was begun on the eighth hospital day. The remaining hospital course was characterized by dramatic improvement in renal function. Total urine output on day of discharge, 21 days after admission, was 2100 cc. It is concluded that massive overdoses of mercury may be managed with dimercaprol and dialysis.
388. A TWO-YEAR CHRONIC TOXICITY STUDY OF TRICYCLAZOLE IN MICE. L.C. Howard, N.V. Owen, S.S. Young, D.M. Morton and L. Golberg, Toxicology Division, Lilly Research Laboratories, Greenfield, IN and Chemical Industry Institute of Toxicology, Research Triangle Park, NC

Tricyclazole is a fungicide effective in controlling rice blast disease. In a 3 month subchronic toxicity study, 400 ppm increased liver weights in female mice. Higher doses (1000-3600 ppm) produced mortality, increased liver weight and SGPT, decreased body weight and caused proliferation of small bile ducts. Subsequently, a chronic toxicity-oncogenic study was initiated. ICR mice were maintained for 2 years on diets containing 50, 140 or 400 ppm of tricyclazole (3.5-45.9 mg/kg/day). Treatment-related effects on survival, body weight, water consumption, organ weights, hematology, clinical chemistry and gross and histopathologic measurements were not observed. No variations, of biological importance, in the incidence of neoplasms were observed. A no effect level of 400 ppm was supported by the results of this study.


The influence of physicochemical parameters eg. redox potential (E1/2), Taft's polar inductive effect constant (σ*), and Taft's steric factor (Eσ) on paraquat and its homologs (viologens) as determinants of herbicidal potency were quantitatively examined. Duckweed is a sensitive indicator of bipyridylium herbicidal activity. Ten mature duckweed plants were exposed to four concentrations of paraquat homologs bracketing the effective chlorotic dose in 100 ml of water. Exposure time was 48 hr with continuous illumination. For comparison, the homologs were also administered subcutaneously to female Sprague-Dawley rats at concentrations bracketing the mammalian lethal dose (Toxicol. Appl. Pharmacol. 45, 233, 1978). Herbicidally effective concentrations were 2.7, 11.6, 236, 71, 31, 51, 13 and 43 mM for methyl (paraquat), propyl, iso-propyl, butyl, methyl-pentyl, hexyl, octyl and benzyl viologen, respectively. The lowest lethal doses in rats for the same compounds were 105, 351, > 185, 125, 55, 37, 281 and 18 μmole/kg. Side chains bulkier than propyl resulted in greatly decreased herbicidal potency. (Supported in part by NIH-ES 00125).
390. DELAYED NEUROTOXICITY OF O-ETHYL 0-2,4-DICHLOROPHENYL PHENYLPHOSPHONOTHIOATE: EFFECTS OF A SINGLE ORAL DOSE ON HENS. M.B. Abou-Donia, D.G. Graham, and A.A. Komeil. Departments of Pharmacology and Pathology, Duke University Medical Center, Durham, North Carolina 27710.

Delayed neurotoxicity was produced in hens, after the administration of a single oral dose of technical (96.43%) O-ethyl 0-2,4-dichlorophenyl phenylphosphonothioate (S-Seven) in gelatin capsule. Doses ranged from 800 to 5,000 mg/kg. Hens given 5,000 mg/kg also received atropine sulfate to protect them against acute toxicity. S-Seven caused ataxia which progressed in some hens to paralysis and death, and caused loss of appetite and weight. TOCP-treated hens developed delayed neurotoxicity while those given parathion showed leg weakness, but with subsequent recovery. Atropine sulfate and gelatin capsule controls remained normal. Degeneration of axons and myelin in the spinal cord was the most consistent histologic change and was identical to that found in TOCP control hens. Only one hen showed sciatic nerve degeneration. Three groups of hens which were given a single oral dose of 100, 200 or 400 mg/kg of S-Seven showed no neurologic signs of delayed neurotoxicity and their nerve tissues were not damaged. (Supported in part by EPA Contract No. 68-02-2452).

391. DELAYED NEUROTOXICITY OF S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE: DIFFERENCES DUE TO ROUTE OF ADMINISTRATION. M.B. Abou-Donia, D.G. Graham, B.L. Reichert, and P. Timmons. Departments of Pharmacology and Pathology, Duke University Medical Center Durham, North Carolina 27710

Oral administration of single oral doses of S,S,S-tributyl phosphorotriothioate (DEF) ranging between 100 and 1,000 mg/kg caused ataxia in hens. The dose 100 mg/kg was the "threshold single oral dose". Daily oral administration of small doses (0.5 to 20 mg/kg) of DEF produced ataxia in hens. The daily dose of 0.1 mg/kg showed no effect. The only unequivocal histopathologic change was seen in the sciatic nerve of one of the hens given daily 20 mg/kg of DEF. On the other hand, topical administration of a single 1,000 mg/kg or daily doses of 40 mg/kg of DEF caused ataxia, which progressed to paralysis and death in some hens. Most of topically treated hens showed axons and myelin degeneration of nerve tissues. It is postulated that orally administered DEF may be rapidly metabolized in the gastrointestinal tract of the hen. The results indicate the necessity of evaluating the delayed neurotoxic effect further by topical application. (Supported in part by EPA Contract No. 68-02-2452, NIEHS Grant No. ES01186 and NIEHS Training Grant in Toxicology No. ES07002).
392. INHIBITION OF AXONAL TRANSPORT BY DELAYED NEUROTOXIC ORGANOPHOSPHORUS ESTERS: A POSSIBLE MODE OF ACTION. B.L. Reichert and M.B. Abou-Donia. Department of Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

Several delayed neurotoxic insecticides were examined for antitransport activity upon fast axoplasmic transport of proteins in the rat optic nerve. These chemicals were: O-methyl 0-4-bromo-2,5-dichlorophenyl phenylphosphonothioate (leptophos); O-methyl 0-2,5-dichlorophenyl phenylphosphonothioate (DBL); O-ethyl 0-4-nitrophenyl phenylphosphonothioate (EPN); O-ethyl 0-4-cyanophenyl phenylphosphonothioate (cyanoephosph) and (O-ethyl 0-2,4-dichlorophenyl phenylphosphonothioate (Seven). TOCP and parathion were used respectively as positive and negative controls. [3H]-Proline and the compound were injected into one eye and a similar solution lacking the drug was introduced into the contralateral eye. Marked inhibition of transport was observed by all insecticides and TOCP. By contrast no antitransport activity was observed with parathion. Differential antitransport activities for delayed neurotoxic compounds and for parathion, are consistent with the hypothesis that these esters, act through inhibition of axonal transport. (Supported in part by NIEHS Grant No. ES01186 and NIEHS Training Grant in Toxicology No. ES07002)


We have previously shown that mice tolerant to the lethal effects of morphine sulfate (MS) were not tolerant to the lethal effects of etorphine. The purpose of the present study was to see whether this phenomenon of non-cross tolerance in morphine tolerant animals also occurred for the lethal activity of heroin-HCl (H) and other narcotic agonists. An intravenous infusion was used to quantitate the mean lethal dose (LD) of narcotic drugs. Drugs were infused into the mouse tail vein at various rates and drug doses until the animals expired. The LD for MS was 497.5 ± 18.2 mg/kg. This increased to 614.7 ± 36.0 mg/kg in 72 hr morphine pelleted (MF) animals. Removal of the MF resulted in a gradual decrease in LD to 468.3 ± 18.0 mg/kg after 72 hours. Similarly the LD for H was increased from 55.2 ± 2.0 mg/kg in control animals to 65.5 ± 2.4 mg/kg in non-withdrawn MF animals. As with MS the LD for H returned to control values 72 hrs after MF removal. Similar protection to methadone-HCl or nialoxone-HCl was not conferred by MF implantation. Obviously, MF induced tolerance does not uniformly confer cross-tolerance to the lethal effects of all narcotic agents. (Supported by NIH grants DA 00451 and ES 07043).
394. MUSCMOL ANTAGONIZES METHYLHYDRAZINE INDUCED CONVULSIONS,
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6570th Aerospace Medical Research Laboratory, Wright-
Patterson Air Force Base, OH 45433

Exposure to methylhydrazine (MMH) can cause convulsions
leading to death. MMH inhibits the synthesis of gamma-
aminobutyric acid (GABA), a putative inhibitory transmitter
in the brain. MMH induced convulsions may be caused by the
resulting loss of GABA's inhibitory action in the brain.
This study was done to determine how muscimol, a potent GABA
agonist, affects MMH induced convulsions. Groups of male
ICR mice were given injections of 1 mg/kg ip muscimol or
saline. Fifteen minutes later both groups were injected
with MMH in doses ranging from 18 to 90 mg/kg ip. Pretreat-
ment with muscimol increased the time to first convulsion
and death at all MMH doses tested; the mean percent increases
were 30% and 115%, respectively. Muscimol also shifted the
dose-response curves for the convulsigenic and lethal
effects of MMH to the right. The LD50 for the convulsigenic
effect of MMH was 22.2 mg/kg versus 25.5 mg/kg with muscimol
pretreatment while the LD50's were 25.5 mg/kg and 28.2 mg/kg.
These results support the mechanism of MMH induced con-
volusions discussed above and also suggest that muscimol might
be useful to treat MMH intoxication.

395. EVALUATION OF DELAYED NEUROTOXIC POTENTIAL OF CHRONICALLY
ADMINISTERED DYFONATE® IN ADULT HENS. J. L. Miller, L.
Staubfer Chemical Company, Richmond, CA., and Dept. of Vet.
Extension, Univ. California, Davis, CA.

Acute administration of Dyfonate® (Fonofos, O-ethyl S-phenyl
ethylphosphonodithioate) produced mild inhibition (19%,
P<0.05) of hen brain neurotoxic esterase (NTE). Because
inhibition of NTE activity has been related to the develop-
ment of delayed neurotoxicity in hens, the neurotoxic poten-
tial of chronically administered Dyfonate® was studied in
this species. Separate groups of 10 adult hens received
either no treatment, or 90 daily oral doses of corn oil (1
ml/kg), tri-o-tolyl phosphate (TOTP, 60 mg/kg), or Technical
Dyfonate® (2, 4, or 8 mg/kg). Dyfonate® produced signs of
acetylcholinesterase inhibition which usually subsided with-
in 4-5 hours after treatment. TOTP produced signs of de-
layed neurotoxicity, (e.g., paralysis) that appeared by the
tenth day and progressed to death or cachexia within 31 days.
Histologic examination revealed retrograde axonal degener-
ation in sciatic nerves and spinal cords of TOTP-treated hens.
There was no treatment-related neuropathy in the Dyfonate®
treated hens, indicating that chronic Dyfonate® treatment
did not produce delayed neurotoxicity.
396. ORGANOPHOSPHATE NEUROTOXICITY: NEUROTOXIC ESTERASE IN HUMAN NERVOUS TISSUE. M. Lotti and M.K. Johnson, MRC Toxicology Unit, Carshalton, Surrey U.K. Sponsor: M. S. Rose

Neurotoxic esterase (NTE) has been recognized as the target enzyme for organophosphorous compounds causing delayed neurotoxicity in hens. We have now characterized NTE of human nervous tissue. The activity in whole brain is similar to that for hens. The distribution of NTE among many defined areas of human brain differs from that of acetylcholinesterase. AChE activity was greatest in nucleus caudatus. NTE activity was greatest in all the cortical areas, less in cerebellum (one half) and in spinal cord (one third). With improved assay procedures NTE was clearly detectable (ca. 50 nmoles/min/g wet wt) in human femoral nerve. This agrees with previous observations on sheep sciatic nerve.


Neurotoxic esterase (NTE) in brain and spinal cord of adult hens is selectively inhibited by neurotoxic organophosphorus compounds. The recent finding in our laboratory of NTE in lymphocytes presented the possibility of utilizing these cells as an accessible monitor of NTE inhibition in nervous tissue after exposure to neurotoxic organophosphorus compounds. Dose-response curves for inhibition of NTE in peripheral blood lymphocytes and brain were determined 24 hr after administration of 0.5,15,35,65,100, and 200 mg/kg of TOTP to adult hens. Mean % inhibition in lymphocytes was 0.0 5.7,37.9,51.4,67.6,64.7, and 92.1, and in brain was 0.0,14.9, 25.5,57.6,80.4,80.1, and 88.3, respectively. These data, combined with values obtained using the neurotoxic compounds EPN,Leptophos, and DFP, yielded a correlation coefficient of 0.88 for a plot of brain vs lymphocyte NTE inhibition. These results suggest that NTE inhibition in lymphocytes could be used to measure exposure to neurotoxic organophosphorus compounds and to predict potential neurotoxic hazard.
398. EFFECTS OF DDT AND PARATHION ON GOLDFISH RETINA/PIGMENT EPITHELIUM/CHOROID CONCENTRATIONS OF SEROTONIN, DOPAMINE AND NORADRENALINE. T.O. McDonald and M. Fingersman, Department of Biology, Tulane University, New Orleans, LA 70118

5-HT, DA and NE were assayed spectrofluorometrically. Fish were maintained at 15°C in a closed environmental chamber and a 12 hour light/12 hour dark cycle. Treated fish were sacrificed by decapitation 5 hours into the light cycle. 5-HT, DA and NE levels showed a circadian rhythmicity with one major peak and one secondary peak each day. Peak values were 2.35 and 2.15 µg/mg wet tissue at 8 hours light and 11 hours dark, respectively, for NE. For DA, they were 22 and 12 µg/mg wet tissue at 5 hours light and 8 hours dark, respectively. 5-HT concentrations were 8.25 and 5.70 µg/mg wet tissue at 11 hours light and 11 hours dark, respectively. Twenty four hours after IP injection DDT (50 mg/kg) elevated 5-HT (200%), NE (300%) and DA (20%) whereas parathion (100 mg/kg) had no effect on 5-HT and DA but it reduced NE 40%. DDT and parathion produce disoriented behavioral changes in fish which may be related to their effects on these neurotransmitters in the retina/pigment epithelium/choroid as well as to their induced changes in brain neurotransmitters.

399. BEHAVIORAL EFFECTS OF EMBRYONIC X-IRRADIATION OF RATS. B.F. Schneider and S. Norton, Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, KS 66103

Rats were exposed on gestational day 15 to 125r x-irradiation, a treatment known to severely alter CNS morphology. At 4–6 weeks of age the behavior of these animals was studied using two different methods. The circadian locomotor activity of irradiated rats was similar to that of controls tested individually in a residential maze. When tested in groups of 4 rats the irradiated group was hyperactive compared with controls, during the nocturnal period. After morphine sulfate, 2mg/kg s.c., the increase in locomotor activity was greater for irradiated than control rats. Successive frames of photographic film were analyzed to determine the frequency, duration and sequencing of 11 behavior acts. Morphine sulfate, 2mg/kg s.c., increased patterning of behavior acts in both groups. After morphine, irradiated rats showed a greater increase than controls in frequency of behavior acts. These data suggest that latent behavioral effects of brain damage may become manifest, dependent on the testing situation.

(Supported by USPHS Grants MH17279, MH43860 and GM07044)
400. NEUROBEHAVIORAL SEQUELAE OF NEONATAL LEAD EXPOSURE IN RATS. S. Overmann and D. Woolley, Univ. of California, Davis, CA.

Symptomatic pediatric plumbism is known to result in neuro-behavioral sequelae. However, deficits in overtly asymptomatic populations are less well documented. A previous study of asymptomatic rats poisoned only during lactation described persistent behavioral alterations evidenced on postweaning evaluation (Overmann, Tox. Appl. Pharm., 41, 439, 1977). The current study examined pre-weaning development of rats poisoned via provision of lactating dams with lead-adulterated drinking water (0, 0.02 or 0.2% lead acetate). All rats were overtly free of poisoning symptoms, though small decreases in dam fluid intake and pup body weight were observed at the 0.2% exposure level. Latency of turning on an inclined plane (negative geotaxis) was decreased by the 0.02 but not 0.2% lead regimen. Duration of forelimb suspension and appearance of auditory startle were not affected by lead exposure but maturation of mid-air righting and eye opening were delayed. Lead exposure increased weight of adrenals and kidneys and decreased hematocrit but did not affect brain or cerebellum weight. The results indicate that subtle alterations in behavioral and physiological ontogeny follow low-level developmental lead exposure. (Supported by ES05057 and ES01503).


Golden Hamsters (Mesocricetus auratus) were intubated with endrin (0.75 and 1.5 mg/kg) on day 5-14 of gestation. The reactive locomotor activity and rearing behaviors of the dams were observed for 5 min. in an open-field throughout gestation and at weaning. Endrin-treated dams were significantly less active (p<.01) than were controls during gestation but not at weaning.

The offspring of these dams were tested in the open-field (5 min.) at 15, 20, 27, 34 and 44 days of age. Fifteen day old pups at the 1.5 mg/kg dose were approximately 90% more active than were controls. This difference disappeared by 34 days of age. The results of the present study demonstrate that prenatal endrin exposure has potent behavioral effects in dams and their offspring.
402. TERATOGENIC EFFECTS OF ENDRIN IN THE GOLDEN HAMSTER.
N. Chernoff, R.J. Kavlock, L.E. Gray and R.C. Hanisch.
Health Effects Research Laboratory, US EPA, Research
Triangle Park, NC 27711  Sponsor: R.W. Chadwick

Endrin is a cyclodiene insecticide used for the control of
cotton pests. Previous work indicated that a single oral
dose of endrin administered on day 8 of gestation induced
fetal toxicity in hamsters (Ottolenghi et al., Teratology,
1974, 9:11). The experiments presently reported compared
the effects of a single oral dose (maximum 10.0 mg/kg)
administered on d8, with effects of multiple dosing on d5-14
(maximum 3.5 mg/kg/day). The single dose experiments pro-
duced no changes in maternal lethality or weight gain. No
compound-related differences were noted in % fetal death or
fetal weight.

Significant incidences of fused ribs and meningoenceph-
aloceles were seen at doses of 5 mg/kg/day or greater. The
multiple dose regimen elicited different responses.
Maternal lethality and weight loss occurred at doses of 1.5
mg/kg/day or greater. Significant dose-related increases in
fetal mortality and decreases in fetal weight were noted at
these dose-levels. The occurrence of defects was lower and
the few meningoencephaloceles seen occurred only in the 1.5
mg/kg/day dose group.

403. BIOCHEMICAL and BEHAVIORAL EFFECTS of RESERPINE in the HORSE
C.G. White, W. E. Woods and T. Tobin, Grad. Center for Tox-
icology and Dept. Vet. Science, Univ. of KY, Lexington, KY.
Sponsor: H.W. Dorough.

The tranquilizer reserpine is reportedly used to calm
hyperexcitable race, trotting and show horses. To monitor
the biochemical effects of reserpine, horses were dosed IV
with reserpine and the subsequent inhibition of serotonin
uptake by platelets from these horses was studied in vitro.
Reserpine at 0.1 μg/kg in vivo had no effect on platelet
uptake of serotonin in vitro, while 0.2 μg/kg or greater
doses produced the maximal (>50%) inhibition observed. At
low doses (0.2 μg/kg) inhibition lasted <24 hours while at
high doses (up to 22 μg/kg) it lasted >48 hours. At 50 μg/kg
of reserpine spontaneous motor activity in these horses was
depressed for 5 days, while at 0.2 μg/kg no behavioral effects
were observed. Inhibition of platelet serotonin uptake in
vitro is thus a highly sensitive indicator of reserpinization
in vivo in horses and is a more sensitive indicator of re-
serpinization than behavioral effects.

Supported by grants from the Kentucky Equine Drug
Research Fund.

The narcotic analgesic fentanyl (Sublimaze) is reportedly used to stimulate racing horses but no data is available on its pharmacokinetics or behavioral effects in the horse. The locomotor response to fentanyl was quantitated by isolating horses in a shielded box stall and injecting saline or increasing doses of fentanyl. The response was measured by counting the number of steps the horse took with its left foreleg in each 2-min. period. Horses injected with saline averaged 4 steps per 2-min. Following injection with .02 mg/kg fentanyl, their motor activity peaked at about 100 steps/2-min by 4 min after dosing and had returned to baseline by 60 min.

After rapid IV injection of fentanyl (.02 mg/kg) the decline in plasma levels measured by RIA was best described by a 2 compartment system, with half-lives of 5, 39 and 180 minutes respectively. Fentanyl is a potent locomotor stimulant in the horse and this effect was well correlated with plasma levels of the drug. Supported by a grant from the Kentucky Equine Research Fund.

405. TOXICITY OF TOPICALLY APPLIED ACETYLBIS(ETHYL TETRAMETHYL TETRALIN (AETT) IN RATS. B.H. Arthur, W.D. Johnson, W.J. Griffing, and J.L. Emmerson, Toxicology Division, Lilly Research Laboratories, Greenfield, IN.

AETT has been a common component of cosmetic fragrances until recent studies in rats revealed neurotoxicity. Fischer 344 rats were used to evaluate the effects of 30 daily dermal applications of 50 or 100 mg/kg of AETT. Body weight gain was significantly decreased at both dose levels in females but not in males. Clinical signs of toxicity, more pronounced in females, included blue discoloration of integument, abnormal gait, ataxia, and mild tremors. Serum bilirubin was increased in both sexes. In high dose females, blood urea nitrogen was elevated and erythrocyte and leukocyte counts were depressed. Teased fiber studies of sciatic nerves revealed swelling and accumulation of osmiophilic debris in paranodal areas. These lesions were more pronounced in females. Electron microscopy revealed abnormal myelin profiles within the myelin sheaths. These data indicate that the initial lesion was in the myelin sheath with secondary axonal damage. Results of this experiment indicate that topical applications of AETT to rats produce a neurologic dysfunction and lesions in the peripheral myelin sheath.
406. PATHOLOGY OF ETHANOL ADDICTION BY INHALATION IN MICE.
Darcy G. Perkins and Robert B. Forney, Department of
Pharmacology and Toxicology, Indiana University School
of Medicine, Indianapolis, IN.

Because of the mode of administration of ethanol in the
inhalation model of addiction, it was necessary to deter-
mine if severe respiratory complications developed during
exposure. Mice were exposed to increasing doses of ethanol
for 10 days, ranging from 12 mg/l to 25 mg/l. Groups of
mice were sacrificed on days 1, 4, 7, and 10 of exposure.
Blood ethanol concentrations were determined at time of
sacrifice. Lung, liver, brain, kidney, and spleen tissues
were removed for study. Weights of specific organs were
recorded and organ/body weight ratios were calculated.
Microscopic pathological examinations were conducted at the
termination of the experiment. Varying degrees of fatty
liver were seen in the experimental group with no such
changes occurring in the control group. A low incidence of
chronic lung inflammation was seen. Due to the type of
inflammation response and its appearance in the control
group, this type of inflammation was not attributable to
the inhalation of ethanol. In summary, this inhalation
model does not cause severe respiratory complications.
(Supported in part by USPHS Grant T32 GM 7097.)

407. EFFECTS OF HEAT AND CARBON MONOXIDE ON CONTINUOUS AVOIDANCE
PERFORMANCE. W.F. Sette and Z. Anmeu, Dept. of Env. Hlth.
Sci., Johns Hopkins Univ., Sch. of Hgy. & Pub. Hlth., Balti-
more, MD 21205 Sponsor: R. Rubin

We used a continuous (Sidman) avoidance schedule to study
the effects of heat and CO, the 2 major products of any com-
bustion process, on integrative CNS function.

Four adult male Long-Evans rats were trained to press a
lever to postpone foot shocks (0.5 sec, 2 mA). Each response
postponed the next shock by 30 sec; otherwise shocks occurred
every 5 sec. Rat were exposed to 1660ppm CO for 30 min at
24°C, 32°C and 36°C. The exposure order was mixed, but tem-
perature exposures preceded CO-temperature combinations.
Chamber temperatures were kept constant (41°C) for the 75 min
session; 30 min CO exposures began 15 min into the session.

All subjects showed an increase in shock rate and decreases
in response rate following CO at 24°C. Only one rat showed
an effect at 32°C and a enhanced response to CO at 32°C. 36°C
produced increased shock rates and decreased response rates
throughout the session in all subjects; CO at this tempera-
ture disrupted the performance earlier and for a longer per-
iod than at 24°C; the magnitude of the combined effects were
additive rather than synergistic. These differences were not
due to differences in carboxyhemoglobin levels after CO at 24°C
and 36°C. (Supported in part by NBS Grant H.18.5009).
408. A SENSITIVE RADIOENZYMATIC ASSAY FOR THE SIMULTANEOUS DETERMINATION OF DOPAMINE AND SALSOILINOL. R. A. Dean, D. P. Henry, R. R. Bowsher and R. B. Forney. Department of Pharmacology and Toxicology, Indiana University School of Medicine and Lilly Research Laboratories, Indianapolis, IN.

Salsoinol, a condensation product of dopamine and acetaldehyde, has been proposed to occur in vivo with ethanol exposure. It has not been detected after the administration of ethanol alone. We have developed a radioenzymatic assay for the simultaneous determination of dopamine and salsoinol. Dopamine and salsoinol are radiolabelled by O-methylation using the enzyme catechol-O-methyl transferase and its cosubstrate, $^{3}H$-S-adenosylmethionine, as the methyl donor. Specificity is achieved by selective solvent extraction, thin layer chromatography and ion pair solvent extraction with bis-diethylhexyl hydrogen phosphate. The assay is linear over a 1000 fold concentration range. Separate assay of standard samples has a coefficient of variation of 5%. Sensitivities of 2 and 3 pg are obtained for dopamine and salsoinol, respectively. Sample sizes may vary from 1 mg to 1 g by use of alumina adsorption. We are presently assessing the in vitro and in vivo formation of salsoinol in the rat following exposure to acetaldehyde or ethanol. (Supported in part by USPHS Grant T32 GM 7097.)

409. THE EFFICACY OF COMPLEXING THERAPY ADMINISTERED AT VARIOUS TIMES AFTER EXPOSURE TO METHYL MERCURY. L.J. Zimmer and D.E. Carter, Toxicology Program, Univ. of Arizona, Tucson, AZ. Sponsor: L.G. Sipes

Complexing treatment begun at various times after methyl mercury (MM) exposure was used to estimate both when a lesion becomes irreversible and the clinical effectiveness of therapy. MM hydroxide was given po to female rats at 13.3 mg/kg x 3 days. Therapy with 2,3-dimercaptopropanol (BAL), 60 mg/kg x 5 days sc, or D-penicillamine (DPA), 1200 mg/kg x 5 days sc, was begun 3, 7, or 14 days after the first MM dose. Weight and the appearance of neurotoxic signs were recorded. Tissue levels of Hg were determined using $^{203}HgCl_2$. Beta-glucuronidase activity in nervous tissue was used to establish the presence of degeneration. Both BAL and DPA prevented signs, reversed weight loss, and reduced tissue Hg when begun on day 3 but only DPA was effective when begun on day 7 as signs began to appear. DPA begun on day 14 removed Hg but did not effect signs or weight. Complexing therapy can be effective after peak tissue levels are reached but not after signs or nerve degeneration occurs.

The distribution of methyl mercury was studied by macroscopic autoradiography in five macaca monkeys, weighing 2.3 to 3.5 kg. They have received 25 mg/kg and 1.100 µCi/kg of 203Hg methylmercuric chloride. One of them was killed three hours after venous injection and the four others, 4, 24, 48 hours and 6 days after oral administration. Macroscopic autoradiography was performed using Ulberg's technique, with a L.K.B.-PMV 450 microtome on the whole body of the animals. Moreover, serial slices of the brains were collected.

From the autoradiograms obtained, several informations can be drawn: the mercury is rapidly absorbed; it is distributed in all the body and mainly localized in liver, kidney, myocardium and well definite areas of the central nervous system (central grey nucleus, cerebellum...). These specific localizations could be correlated with human toxic clinical observations.


Methyl n-propyl ketone (2-pentanone, MPK) is structurally similar to the neurotoxin, methyl n-butyl ketone (MnBK) except that MPK contains 5 carbon atoms rather than 6. This structural similarity prompted a comparison of the chronic toxicity, particularly the neurotoxic potential, of MPK to that of MnBK and hexane. MPK and MnBK were administered to male rats at concentrations of 0.25, 0.5 and 1.0% in the drinking water for 10-13 months. Hexane was administered as a saturated solution. Methyl propyl ketone (1.0%) and hexane produced a slight decrease in body weight, but no significant toxicologic effects were seen at any dose used. Methyl n-butyl ketone produced a significant body weight reduction at all levels tested. Animals ingesting 0.5 and 1.0% MnBK exhibited clinical signs of neurotoxicity. Morphologic changes similar to those previously described for this compound were seen in all MnBK treated groups. The results of this study indicate that methyl n-propyl ketone did not produce neurotoxicity indicating a neurotoxic potential considerably less than that of methyl n-butyl ketone.
412. DELAYED NEUROTOXICITY OF LEPTOPHOS: EFFECT OF PURE, TECHNICAL GRADE AND DEGRADATION PRODUCTS ON THE HEN.
M.B. Abou-Donia, D.G. Graham, A.A. Komeil and P. Timmons,
Departments of Pharmacology and Pathology, Duke University Medical Center, Durham, N.C. 27710.

Pure and technical grade leptophos (O-methyl O-4-bromo-2,5-dichlorophenyl phenylphosphonothioate) and some of its degradation products were screened for delayed neurotoxicity following daily oral administration of 10 mg/kg dose to hens. The chemicals were: O-methyl O-2,5-dichlorophenyl phenylphosphonothioate (DBL); O-methyl O-4-bromo-2,5-dichlorophenyl phenylphosphonate (Teptophos oxon); phenylphosphonic acid (PPA) and 4-bromo-2,5-dichlorophenol (BCP).

All hens given pure and technical grade leptophos, DBL and Teptophos oxon developed ataxia which progressed to paralysis and death in some hens. Histologic examination showed in most hens marked axon and myelin degeneration in the sciatic nerve and spinal cord. By contrast no delayed neurotoxicity was produced in hens given PPA or BCP. TOCP-treated hens developed delayed neurotoxicity while those given parathion showed leg weakness, but with subsequent recovery. Gelatin capsule controls remained normal.

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