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The Lilly bacterial mutagen screening method employing concentration gradient plates has recently been described (Res. Commun. Chem. Pathol. Pharmacol. 16, 523, 1977). The method allows the assessment of the mutagenic potential of test chemicals in eight histidine auxotrophs (Salmonella typhimurium) and two tryptophan auxotrophs (Escherichia coli) over a 10,000-fold concentration range. Both unactivated and activated (9000g fraction from Aroclor-induced rat liver homogenate) plates are used. Of 414 compounds tested, 108 (26%) were positive in one or more strains. Of these positives, 82 did not require activation. Among aromatic nitro compounds, 48 of 63 (76%) were active. Of 273 "unknowns" screened, 10% were active. As expected, more compounds were active in TA100 or TA98 than in the others. However, 94% of the compounds active in one of these plasmid-containing strains were active in one or more other strains, suggesting that positive responses in TA100 or in TA98 do not represent "artificial" or "unreal" results. Surprisingly, more "hits" were observed in the repair competent strains, G46 and C3076, than in their uvr-, LPS- counterparts, TA1535 and TA1537. The method allows classification of mutagens as to type (frameshift vs base-pair substitution) and gives some suggestion of potency. In the course of our studies several new types of chemical mutagens have been found.

2. Dominant Lethal Studies in Rats of Five Hair Dye Components: 2-Nitro-p-Phenylenediamine, 4-Nitro-o-Phenylenediamine, m-Phenylenediamine, 2,4-Diaminoanisole Sulfate, and 2,5-Diaminoanisole Sulfate. C.W. Sheu and S. Green, Food and Drug Administration, Washington D.C.

The mutagenicity of hair dye components has been demonstrated in bacteria, yeast, Drosophila, and cultured mammalian cells, but not in mammals. Therefore, male rats exposed to maximally tolerated doses of five hair dye components were studied by a dominant lethal test. Each component was tested at three dosage levels with 15 random-bred male rats per level. The highest dose, selected on the basis of subacute toxicity testing, generally reduced weight gains without being lethal. Freshly prepared solutions were injected ip at 1 ml/kg three times a week for 10 weeks. Rats injected with dimethylsulfoxide and triethylenediamine served as solvent and positive controls, respectively. A majority of rats survived the treatment at the levels tested, and were mated to two virgin females each per week for 2 weeks. The females were sacrificed at midterm of pregnancy, and analyzed for live and dead implants. Dominant lethality was evaluated on the basis of four criteria: dead implants per pregnant female, dead implants per total implants, proportion of females with one or more dead implants, and proportion of females with two or more dead implants. 2-Nitro-p-phenylenediamine, 2,4-diaminoanisole sulfate, and 2,5-diaminoanisole sulfate produced negative responses whereas m-phenylenediamine and 4-nitro-o-phenylenediamine induced weak dominant lethality in the first trial. On retesting the weakly positive components, m-phenylenediamine produced negative response, and work on 4-nitro-o-phenylenediamine is in progress.

Unscheduled DNA synthesis in the germ cells of male mice has been demonstrated (G. A. Segal et al.) after administration of methyl, ethyl, and isopropyl methanesulfonate, cyclophosphamide, and mitomycin. An attempt was made to evaluate the effectiveness of this response as an in vito mutagenic assay. Compounds to be tested were injected ip at a variety of doses up to approximately 50% of their respective LD50's. Thirty minutes after administration of test substances the mice were given intratesticular injections of 30 μC of tritiated thymidine (1HdT) under anesthesia. Sixteen days after injection the sperm was removed from the caudal epididymis, the sperm heads were isolated and counted, and the incorporation of 1HdT/106 sperm heads was measured. Ethyl methanesulfonate (EMS), cyclophosphamide, 6-chloropurine, safrole, 2-acetylaminofluorene, dimethylnitrosamine, 6-mercaptopurine, aflatoxin B1, ethionine, acriflavine, mitomycin C, and triethylenemelamine were tested by this protocol. Only EMS and cyclophosphamide, as previously published, and mitomycin C gave positive results. The method used as an in vito mutagenic assay does not appear to offer any advantage over currently employed methodologies, i.e., dominant lethal or heritable translocation tests.

4. *Mutagen(s) in Human Feces: Reduction of Mutagenic Capacity by Fecal Components.*
   James E. ASwell, Marion Ehrlin, and Tracy D. Wilkins, Anaerobic Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. (S. D. Cohen)

Previous studies in our and other laboratories have shown that certain individuals (21% of North Americans) excrete in their feces an ether-extractable substance(s) which induces the reversion of the histidine requirement of Salmonella typhimurium test strain TA-100. Fecal samples from individuals identified as M+ (mutagen positive) were freeze-dried, extracted with cold ether, and dried. The extracted mutagen was then incubated with an equivalent amount (w/w) of the individual's own wet feces or with the wet feces from an M- donor. Incubation was at 37° C for 15 min, followed by an additional ether extraction and assay with the TA-100 test strain. Exposing results as mutagenic ratios (MR = histidine revertant colonies per plate/average spontaneous revertant colonies per plate), the MR of an ether extract from an M+ fecal sample was reduced from 10.1 ± 0.9 (mean ± SD, N = 4; 1412 ± 122 colonies/plate) to 1.2 ± 0.4 (168 ± 53 colonies/plate) following incubation with an M- fecal sample. The ether extract from another M+ donor with an MR of 3.4 ± 0.5 (N = 6; 244 ± 39 colonies/plate) was reduced to 1.0 ± 0.1. This inhibition of mutagenic potential occurred within 15 min and was not increased by incubation at 37° C for up to 8 hr. A reduction in mutagenic capacity was also noted at 4° C, but the effect was not as pronounced. In addition, incubation of M+ fecal extracts with autoclaved fecal specimens from M- donors resulted in a reduction in mutagenic potential. Bran fiber did not, however, cause a similar reduction. Therefore, the capacity of an ether-extractable mutagen(s) from human feces to induce reversion of histidine-requiring S. typhimurium TA-100 can be significantly reduced by interaction with certain components in the feces of individuals lacking any detectable fecal mutagen. (Supported by a grant from the Abercrombie Foundation and by NCI Contract NO1-CP-55685.)

5. *Triethylenemelamine-Induced Gene Mutations at Biochemical Loci in the Mouse.* E. R. Soares, Genetic Toxicology Department, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina. (Leon Golberg)

Chemically induced single gene mutations at biochemical loci of the mouse have heretofore not been observed. Lesions of this kind could be of considerable importance in that such mutations would allow for detailed biochemical and molecular analysis of mutant gene products (i.e., aberrant enzymes). In an effort to induce mutations at specific enzyme loci, individual male mice of the strain DBA/2J were treated by ip injection with Hanks' balanced salt solution or triethylenemelamine (TEM: 0.25 or 0.30 mg/kg). Each injected male was then paired for 7
nights with two strain C57BL/6J females, at the end of which time the females were replaced with two additional females for a second 7 nights. Kidney and blood were surgically removed from each of the F_1 offspring from these crosses and analyzed for altered enzymes using standard starch gel electrophoretic techniques. The zymograms prepared from these gels were examined for evidence of TEM-induced mutations at the following loci: Es-1, Es-3, Dip-1, Gpi-1, Gpd-1, Hbb, Id-1, Ldh-1, Mod-1, and Pgm-1. Throughout this study tissues from 6381 F_1 animals (3782 treated, 2599 controls) were analyzed for induced variability at the above loci. To date 12 putative mutations have been detected at the following loci: (1) three null mutations at Es-1; (2) three null mutations at Es-3; (3) one null mutation at Gpi-1; (4) two mobility variants at Ldh-1; (5) one mobility variant at Pgm-1; and (6) two null mutations at Pgm-1. Of these mutations, four have been confirmed as heritable: (1) a null at Es-1; (2) a mobility variant at Ldh-1; and (3, 4) the two nulls at Pgm-1. Crosses are presently underway to determine if the remaining mutations are heritable. These data clearly indicate TEM induction of mutations at biochemical loci. The observation that several of these mutations are heritable suggests that these techniques may be used in the study of the biochemical and genetic basis for such mutations.


Although there are many in vitro tests for potential carcinogenicity, each in various stages of validation, they are not all equally effective in predicting potential carcinogenicity for all classes of chemicals. Therefore, when studying the structural prerequisites for carcinogenicity within a given class of compounds it is important to use the most appropriate in vitro tests. This is in contrast to using the individually most favored test or a battery of different tests. A structure-activity study of the flame retardant tris(2,3-dibromopropyl) phosphate using the Ames assay reveals that chemically unrelated esters of 2,3-dibromopropanol have carcinogenic potential. Similarly, carcinogenic potential can be defined for a derivative of indoline using the cell transformation assay of Styles. Results obtained using both tests for a series of compounds related to the carcinogen hexamethylphosphoramide will be presented. The dependence of all in vitro test results for nondirecting acting compounds upon the microsomal system employed is discussed with examples.

7. Determination of Differential DNA Damage Induced by Several Classes of Chemical Mutagens in Modified Selye's Granuloma Pouch Cells. I. P. LEE and G. ZBINDEN, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, and ETH and University of Zurich, Schwerzenbach, Switzerland.

Although useful, current in vitro short-term tests for mutagens and carcinogens lack in vivo physiological and biochemical complexities necessary for threshold determinations as well as extrapolation of test data to whole animals. As a result of our search for short-term in vivo test systems, the modified Selye's granuloma pouch system was found to be useful in studying DNA–carcinogen interaction and cellular events such as the cell transformation and their subsequent tumorigenicity. The dorsal surface of Zurich albino rats (200–250 g) was shaved and 30 ml of air was injected into the underlying subcutaneous space. Subsequently, 0.25% croton oil in tricarpryllin was injected directly into the pouch to induce fibroblast proliferation. Twenty-four hours later, the pouch fibroblast DNA was prelabeled with ^3Hthymidine (0.5 µCi/g body wt: sp act 46 Ci/mmole). ^3Hthymidine was injected four times (every 3 hr) directly into the pouch. Eighteen hours after the last ^3Hthymidine injection, the rats received one of three classes of test chemicals by intraperitoneal route (monofunctional, polyfunctional alkylating agents and DNA intercalating agents); 24 hr after the administration of test chemicals, animals were sacrificed to isolate fibroblasts from the pouch and DNA was analyzed by the alkaline elution technique. These studies demonstrated that single strand DNA breaks were primarily induced by monofunctional alkylating agents while inter- and intrastrand crosslinking of DNA was primarily associated with polyfunctional alkylating agents. Thus, it appears that the
combination of alkaline elution analysis with the modified Selye's granuloma pouch system might be a useful in vivo indicator of chemical mutagens and carcinogens.


Enhancement of mutagenesis, induced with a chemical carcinogen, by a tumor-promoting agent has been previously noted in cultured V79 Chinese hamster cells. To examine further the potential of the above system, linear alkanes of specific even chain length, an aryl derivative of dodecane, and a phorbol diester were examined for relative ability to enhance mutagenesis induced by the carcinogen methyloxazymethanol (MAM) at the ouabain-resistance locus. At concentrations of 0.12 mM, decane, dodecane, and tetradecane gave average enhancements of mutagenesis of 24, 18, and 30%, respectively, above control, whereas under the same conditions hexane and hexadecane showed no or only slight activity. These data demonstrate a remarkably good correlation between the relative activities of these compounds and those found previously in in vivo experiments. Two of the most potent in vivo promoting agents, tetradecanoyl phorbol acetate (TPA) and phenyldecanate at concentrations of 0.16 μg and 0.7 μg, respectively, both enhanced MAM-induced mutagenesis by 30%. No promoter showed mutagenic activity per se. These findings suggest that the above system has considerable potential in detecting environmental tumor-promoting agents and are consistent with the postulate that in vivo such agents act via derepression of latent carcinogen-induced damage to the genome. (Supported by a Chemical Industry Institute of Toxicology Fellowship, and by NIH Grants ES 00159 and Es 00127.)


A mammalian cell transformation assay, using colony formation in semisolid agar as the criterion for transformation, has been validated as a short-term test for carcinogenic organic chemicals. The test is able to distinguish between carcinogenic and noncarcinogenic chemicals with about 90% accuracy. BHK-21 CI 13-derived cells in volumes of 1 ml containing 10⁶ cells were incubated at 37°C for 4 hr on an orbital shaker (120 cycles/min) with five concentrations of benz[a]pyrene, benzo[a]pyrene, benzo[a]pyrene, and 2-acetylaminofluorene and Aroclor-induced rat postmitochondrial supernatant in serum-free medium (Eagle's minimal essential medium without calcium and buffered with Hepes). After incubation, the cells were centrifuged and resuspended in 10 ml of growth medium (Dulbecco's modification of Eagle's MEM containing antibiotics and 10% calf serum). Cell survival was estimated by plating 0.05 ml of cell suspension into growth medium and counting colonies after 6 days of incubation. Molten agar solution (0.625 ml; 5% w/v) in water at 80°C was added to the remainder of each suspension to give a solution containing 0.3% agar. After pouring the suspension, the cultures were incubated for 21 days at 37°C and colonies of cells in agar counted. Results were expressed as transformation frequencies per 10⁶ survivors and a fivefold increase at the LC₅₀ over spontaneous frequency was taken as indicating a positive result. The reproducibility of the transformation frequency dose–response curves is better than that for survival or incidence of transformed colonies. The reasons for this variability are not known, but perhaps differences in batches of S-9 mix are responsible since this factor is the one likely to vary the most in the assay. Thus, it is important to use transformation frequency rather than incidence to express results. This argument could be extended to bacterial mutation assays for carcinogens where mutation frequencies are usually not calculated and only a very crude estimate of survival is used. The use of an equitoxic dose (LC₅₀) to assess the results of the assay ensures that variations in toxicity of a compound due to S-9 mix or other factors, or when different compounds are tested, can be minimized and the results expressed in terms of a standard biological end point.

Because many of our major local foodstuffs have been found to be heavily contaminated by some toxigenic fungi such as *Aspergillus flavus*, in the markets, we decided to screen several of the metabolites of *A. flavus* for mutagenicity. The results were compared with those of aflatoxin B<sub>1</sub>, the well-known mutagenic/carcinogenic metabolite of *A. flavus*. This paper reports the mutagenic properties of palmotoxins B<sub>9</sub> and G<sub>9</sub>, which are metabolites of *A. flavus* which is cultured on palm sap, a very popular alcoholic beverage in Nigeria. The present results indicate that palmotxin B<sub>9</sub> is only one-eight as mutagenic as aflatoxin B<sub>1</sub> with the Ames *Salmonella typhimurium* test system, although these two toxins have comparable toxicity profiles. However, in accordance with the relatively lower toxicity of palmotxin G<sub>9</sub> (Bassir and Adekunle, *FEBS Lett.* 2, 23, 1968) the number of revertants induced by this compound is consistently less than that caused by palmotxin B<sub>9</sub>. Although recent reports (Uwaifo et al., *J. Agric. Fd. Chem.*, in press) suggest that the structures of palmotoxins B<sub>9</sub> and G<sub>9</sub> are similar to the heterocyclic nature of the aflatoxins, the palmotoxins, unlike the aflatoxins, do not seem to be frameshift mutagens. The palmotoxins revert preferentially the bacterium TA 1535, a tester strain which is specific for the base-pair substitution mutagens (McCann et al., *Proc. Natl. Acad. Sci. USA* 72, 979, 1975). The bacterium TA 100 was also used to confirm the type of mutation induced by these toxins. It seems to us, therefore, that Nigerian foodstuffs contain mutagens other than the aflatoxins and N-nitrosé compounds.

11. *The Fate of Phenol, o-Phenylphenol, and Disphenol in Rats*. TIMOTHY A. Gbodi and FREDERICK W. Oehme, Comparative Toxicology Laboratory, Kansas State University, Manhattan, Kansas.

The metabolism, influence of pentobarbital on metabolism, biliary excretion, enterohepatic circulation, urinary excretion, and tissue distribution of phenol (P), disphenol (DNP), and o-phenylphenol (OPP) were studied in male rats using 14C-labeled compounds. P and OPP rapidly disappeared from blood, but DNP disappearance was slow and constant throughout the 5-hr experimental period. Disappearance of P, DNP, and OPP from blood was faster in pentobarbital-anesthetized rats than in unanesthetized rats. OPP exhibited the highest biliary excretion followed by P and DNP. All major P, DNP, and OPP biliary metabolites were glucuronide conjugates. Enterohepatic circulation was detected for P and OPP and their metabolites, but none was found for DNP. Renal excretion of P and OPP was 90% completed 48 hr after iv administration, but only 47% of the DNP was recovered in urine in 4 days. Glucuronide and sulfate conjugates were urinary metabolites of P; the major urinary metabolite of OPP and DNP was the glucuronide conjugate. Significant amounts of unchanged OPP and DNP occurred in urine. Highest tissue residues after 2 hr were found in kidney and liver of P- and OPP-treated rats and in muscle and blood of DNP-treated rats. It was concluded that: (1) When compared to P and OPP, the excretion of DNP was slowest; (2) of the three phenolics, repeated administration of DNP is most likely to result in increased body burden; and (3) physicochemical properties other than molecular weight play significant roles in a chemical's biliary excretion and metabolism.


Metabolic activation of benz[a]pyrene (BP) and 7,8-dihydroxy-7,8-dihydrobenz[a]pyrene (BP 7,8-dihydридридиол) to intermediates showing high binding affinity for nucleic acids was demonstrated in microsomes and highly purified rat hepatic monooxygenase systems.
Conditions were determined for optimum measurement of binding using a rapid, sensitive microfiltration assay. BP 7,8-dihydriodiol exhibited up to fivefold higher binding affinity in all systems tested when compared with BP. Polyguanilic acid was the best acceptor for both activated BP and BP 7,8-dihydriodiol. Binding of BP was prevented by the addition of highly purified epoxide hydrase, while binding of the dihydriodiol was unaffected. Induction of the monooxygenase system was shown to give rise to parallel increases in binding for both compounds.


Methyl n-butyl ketone (MnBK) has produced peripheral neuropathy in experimental animals and is implicated in an occupationally produced neuropathy. Since occupational exposure to MnBK occurs by inhalation or skin contact, both the absorption and elimination of MnBK vapor and its absorption through skin were investigated. Studies were carried out first with male beagle dogs and subsequently with human volunteers. Humans exposed for 7.5 hr to 10 or 50 ppm or for 4 hr to 100 ppm of MnBK vapor absorbed between 75 and 92% of the inhaled vapor. Unchanged MnBK was not eliminated extensively in the postexposure breath or in urine. 2,5-Hexanenedione, a metabolite of MnBK known to be neurotoxic in rats, was found in the serum of humans exposed to either 50 or 100 ppm of MnBK. The absorption and elimination of MnBK in dogs was similar to that observed in humans. The skin absorption of 11-14ClMnBK or a 9/1 (w/v) mixture of Mek/11-14C|MnBK was determined by excretion analysis. Two volunteers exposed by skin contact to 11-14C|MnBK absorbed 4.8 and 8.0 μg min⁻¹ cm⁻², respectively. The skin exposure to the Mek/11-14C|MnBK mixture resulted in the respective absorption of 4.2 and 5.6 μg min⁻¹ cm⁻² by two individuals. Two volunteers given an oral dose of 11-14C|MnBK (2 μCi; 0.1 mg/kg) excreted 49.9 and 29.0% of the dose, respectively, as respiratory 14CO₂ over 3 to 5 days and 27.6 and 26.0% of the dose, respectively, in urine over 8 days. Both 11-14C|MnBK and Mek/11-14C|MnBK were absorbed through the skin of dogs. These findings show that MnBK is readily absorbed by the lungs, the gastrointestinal tract, and through the skin. It is not eliminated extensively unchanged in breath or urine, and is metabolized to CO₂ and 2,5-hexanenedione. Radioactivity derived from 11-14C|MnBK was excreted slowly by man, thus suggesting that repeated daily exposure to high concentrations of MnBK may lead to a prolonged exposure to neurotoxic metabolites.


The fate of ethylene dibromide (EDB) was studied in rats following inhalation exposure to determine if a change in the disposition of EDB in the body occurred with increasing exposure concentrations. Male rats were exposed to 7, 25, or 75 ppm of 14C-labeled EDB for 6 hr and the routes and rates of elimination of 14C activity were followed for 48 hr after termination of exposure. The percentages of recovered radioactivity excreted by the various routes were similar for all exposure levels. The urinary excretion of radioactivity was the major route of elimination, accounting for 80% of the recovered radioactivity. The rates of excretion of urinary 14C activity were similar for all exposure levels with a half-life (t₁/₂) of 5.1 to 5.6 hr. Urinary metabolites of EDB following inhalation exposure appear to be derived from GSH conjugation. The metabolism of EDB appears to constitute a detoxification mechanism and the data suggest that EDB is detoxified more efficiently at a low exposure (6–7 ppm) compared to higher exposures (25, 75 ppm). Thus, the fate of inhaled EDB in rats is dependent on the exposure concentration and it may be inappropriate to predict the effects in rats of low-level exposure (<6–7 ppm) from the results of exposure to concentrations greater than 6 to 7 ppm of EDB.

The toxicity of many chemicals results from biotransformation products formed from the chemical rather than from the chemical per se. In such cases, the incremental response may become diminishingly smaller with increasing dose or exposure because activation of the chemical to the toxic form follows apparent Michaelis–Menten rather than apparent first-order kinetics. To illustrate this concept, rats were exposed to concentrations ranging from 1.4 to 4600 ppm of vinyl chloride for 6 hr and the total amount metabolized was determined. The amount metabolized followed apparent Michaelis–Menten kinetics. For rats, the logarithmic probability incidence of angiosarcoma versus the amount of vinyl chloride metabolized (rather than the exposure concentration of vinyl chloride) is linear. Assuming no threshold (in spite of evidence to the contrary), extrapolation of the data below the range of doses causing experimentally observable responses predicted an incidence of 0.01% hepatic angiosarcoma in rats exposed to 4.6 ppm of vinyl chloride. Theoretical extension of the extrapolation to humans exposed daily for 8 hr to 1 ppm suggests an incidence of 1.5 per 100,000,000. This theoretical incidence is less than that expected to occur spontaneously. It is concluded that pharmacokinetic parameters must be elucidated before designing toxicological experiments or before interpreting the results therefrom.

16. *Studies of the in Vitro Metabolism of Thioacetamide and Thioacetamide Sulfinic.*

William R. Porter and Robert A. Neal, Center in Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

Quantitative radiotracer methods, utilizing either thin-layer or high-pressure liquid chromatography, have been developed for the study of the metabolism of thioacetamide and its sulfinic metabolite, thioacetamide-S-oxide. Thioacetamide is oxidized to thioacetamide-S-oxide by rat liver microsomes and by a reconstituted mixed-function oxidase system comprised of purified cytochrome P-450, purified NADPH-cytochrome P-450 reductase, and phospholipid. The reaction requires NADPH (which requirement can be only partially met by NADH) and is inhibited by carbon monoxide, SKF 525A, and an antibody to rat cytochrome P-450. Thioacetamide-S-oxide is oxidized by rat liver microsomes to acetamide and unidentified polar products; some of the substrate becomes irreversibly bound to the microsomes during the course of the reaction. The reaction has the same cofactor requirements and generally responds to inhibitors in a manner similar to that observed for thioacetamide. Michaelis–Menten kinetic parameters were determined for the metabolism of thioacetamide and thioacetamide-S-oxide by microsomes from untreated and from both phenobarbital- and 3-methylcholanthrene-pretreated rats. The *Km*’s for formation of thioacetamide-S-oxide from thioacetamide were 0.06, 0.11, and 0.09 mM, respectively. The *Km* for the formation of acetamide from thioacetamide-S-oxide and for irreversible binding to microsomes did not differ significantly; a value of about 0.4 mM was obtained in all cases. Phenobarbital pretreatment increased metabolism of both thioacetamide and thioacetamide-S-oxide. The results indicate that thioacetamide-S-oxide is an obligatory intermediate in the two-step metabolic conversion of thioacetamide to acetamide, polar products, and microsome-bound material and that both steps require, at least in part, the cytochrome P-450 mixed-function oxidase system.


Ethynlenethiourea (ETU) is a teratogen in the rat, producing a range of deformities. The cat is currently under investigation to determine the response of this species to teratogenic agents. In the present study, two female cats were dosed iv with 4 mk/kg of 14C-labeled ETU, a known
contaminant and metabolite of the ethylenebisdithiocarbamate class of fungicides. From the
decline of radioactivity in the blood, the half-life of total radioactivity was calculated to be 3.5 hr,
a value similar to that found in the rat. Urinary excretion (48 hr) accounted for 88% of the total
dose. Analysis of 24-hr urine indicated two major radiolabeled compounds which were identified
as unchanged ETU and S-methyl ETU. Small amounts of at least two other minor metabolites
were also present. Previous work has shown that urinary radioactivity from ETU-dosed rats is
predominantly unchanged ETU. The present results indicate that the cat readily converts ETU
to S-methyl ETU. The toxicological significance of this metabolic conversion is under
investigation.

18. Disposition of Zinc Pyrithione in the Rat. J. H. Weidig, R. Wentworth, M. Gallo, and J.
Babish, Olin Corp., New Haven, Connecticut; Cornell University, Ithaca, New York; and

Zinc pyrithione (ZpT), an antimicrobial agent, is used as an antiseborrheic agent in
shampoos. The pharmacokinetic profile of [2,6-14C]ZpT was defined in male and female rats
following a single orally intubated dose of 0.5, 1.25, or 12.5 mg/kg in corn oil. Kinetic constants
obtained were utilized to predict 14C plasma concentrations at steady state to correlate with
findings of skeletal muscle weakness observed in long-term feeding studies and mechanism
studies for this effect. [14C]ZpT activity was determined in plasma, urine, and tissues over the
240 hr study period. A two-compartment open model was used to describe the disposition of
ZpT. Some parameters measured for the same dose indicated sex-related differences: plasma
elimination, renal clearance, time of peak blood concentrations, and rate of oral absorption. The
terminal plasma t1/2 (β phase) was 0.005 ± 0.002 hr⁻¹ (mean ± SD) and the renal excretion t1/2
(β phase) was 0.020 ± 0.007 hr⁻¹. The red blood cell to plasma binding ratio was 5 to 1. In the
urine and feces, respectively, 66 to 77 and 13 to 21% (range) of the dose was recovered. Total
recovery of 14C was 95 ± 5% following radioassay of tissues, organs, and carcass. Elimination
of 14C by expired air was <0.1% of the dose. Plasma protein binding was found to be ≤12%.
Analysis of urine by tlc (two systems) suggested that the major metabolite was a glucuronide
conjugate. Calculation of steady-state 14C plasma concentrations (max and min) indicated 1.18–
0.86, 2.62–1.49, and 24.81–19.11 μg/ml for the 0.5, 1.25, and 12.5 mg/kg doses, respectively.

19. Alterations in Rat Liver Mitochondrial Urea Cycle Enzymes following in Vivo Treatment
with Aroclor 1254. K. V. Ebner and Daniel Couri, The Ohio State University College of
Medicine, Department of Pharmacology, Columbus, Ohio.

Fatty acids and their metabolic derivatives, and Aroclor 1254 pretreatment inhibit the mito-
chondrial urea cycle enzymes (Pharmacologist 19, 199, 1977). Kinetic studies of carbamyl
phosphate synthetase (CPSI) and ornithine transcarbamylase (OTC) were made following
Aroclor treatment of male Wistar rats (100 mg/kg/24 hr) for 1, 2, and 4 days. The Kₘ and Vₐₘₕ
for ammonium increased with CPSI after treatment. The Kₘ for bicarbonate was unaltered but
there was an increase in the Vₐₘₕ. Bicarbonate stimulated CPSI when its concentration was
higher than ammonium. OTC exhibited substrate inhibition with ornithine but not carbamyl
phosphate in control animals and was variable for ornithine after treatment. When these rate-
limiting concentrations were compared to lower, equimolar concentrations (2, 5, and 10 mM) to
evaluate enzymatic activities, ammonium and ornithine at 15 mM inhibited CPSI and OTC
activities, respectively. These effects were similar for both control and treated rats, but more
pronounced in the treated animals. During the Aroclor treatment, liver size and serum
ammonium concentrations increased, while ADP/O and respiratory control ratios and food and
water intake were similar for treated and control animals. These studies show that CPSI and
OTC are inhibited by their own substrates at high concentrations following Aroclor 1254
treatment and coincides with triglyceride accumulation and elevation of serum ammonium
levels.
20. The Effects of Bromochlorodifluoromethane and Mn$^{2+}$ on Rate-Dependent Changes in Contractility of Isolated Atria. R. A. Davis and K. C. Back, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

The mechanism by which bromochlorodifluoromethane (BCF) and other halogenated alkanes produce negative inotropy in cardiac muscle is unknown. Treppe, poststimulation potentiation, and the force–frequency relationship are changes in contractility which occur when stimulation rates are altered. The mechanism of these rate-dependent changes is thought to be an alteration of calcium (Ca$^{2+}$) conductance or utilization. The effects of BCF on rate-dependent changes were studied in isolated guinea pig left atria. Using this model, BCF was compared with Mn$^{2+}$, a known blocker of Ca$^{2+}$ conductance, in order to obtain new information relevant to the mechanism of negative inotropy produced by BCF. The bath concentration of BCF (approximately $2.9 \times 10^{-4}$ M) and Mn$^{2+}$ ($2.5 \times 10^{-4}$ M) used for these studies decreased isometric developed tension by 50% at the stimulation frequency of 2 Hz. BCF did not affect the percentage increase of developed tension during treppe but greatly decreased poststimulation potentiation. Mn$^{2+}$ depressed treppe and had no effect on poststimulation potentiation. The force–frequency relationship was depressed 50% by BCF at all stimulation rates studied, whereas Mn$^{2+}$ produced greater depression at frequencies above 0.5 Hz. A comparison of the dose–response curves for the negative inotropic effect of BCF at stimulation rates of 0.25 and 1 Hz shows that increasing the stimulation rate does not competitively antagonize this effect. The results of this study suggest that BCF has no effect on Ca$^{2+}$ conductance but does affect cellular Ca$^{2+}$ utilization.

21. The Effects of Paraquat and Diquat on Rat Kidney. Edward A. Lock and John Ishmael.

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The herbicide paraquat (N',N'-dimethyl-4,4'-bipyridinium) is structurally and chemically related to diquat (N,N'-ethylene-2,2'-bipyridinium). In man acute renal failure is frequently observed following accidental or intentional ingestion of the concentrated products. The aim of the present study was to establish whether paraquat and diquat produce acute renal damage in the rat, similar to that in man, and if the rat is a suitable model to elucidate the mechanism whereby damage occurs. A single administration of paraquat (680 μmol/kg body wt po or 108 μmol/kg sc) or diquat (680 μmol/kg po) to male rats produces marked diuresis, proteinuria, and glucosuria 6 to 24 hr after dosing. An increased excretion of the urinary enzymes, N-acetyl-β-D-glucosaminidase and alkaline phosphatase, is seen after oral paraquat but not diquat. Histopathological examination of the kidneys showed mild hydropic change in the proximal convoluted tubules. Renal clearance of inulin, p-aminobenzoic acid (PAH), and N'-methyl nicotinamide (NMN) is markedly reduced 2 hr after a single oral dose of paraquat or diquat, but not after a single subcutaneous injection of paraquat. These changes indicate that renal impairment occurs in the rat as in man. Studies in vitro with renal cortical slices have shown that both paraquat and diquat depress NMN but not PAH accumulation, indicating competition for the base transport system. However, rats treated with oral paraquat or diquat for 24 hr showed no depression of NMN or PAH accumulation by slices of renal cortex in vitro. Both paraquat and diquat cause hemococoncentration. Measurement of plasma volume with $^{125}$I-labeled albumin and red cell volume with $^{51}$Cr-tagged cells showed both compounds reduced the plasma volume with no change in red cell volume. This reduction in plasma volume is likely to alter renal hemodynamics and may explain the reduced renal clearance seen following oral administration of these bipyridyls. The reduction in plasma volume after diquat and, to a lesser extent, paraquat is as a result of water loss from the tissues into the lumen of the gastrointestinal tract.
22. **Chemically Induced Modification of Chlorinated Hydrocarbon Solvent Nephrotoxicity.**

W. M. Klute and J. B. Hook, Department of Pharmacology, Michigan State University, East Lansing, Michigan.

Renal toxicity of several chlorinated hydrocarbon solvents depends upon biotransformation of the parent compound to a nephrotoxic metabolite. Since many environmental contaminants are potent inducers of the microsomal enzymes responsible for generation of these toxic metabolites it was of interest to evaluate the effects of chronic dietary exposure to certain environmental contaminants on the nephrotoxicity of chlorinated hydrocarbon solvents. ICR male mice were fed a standard diet supplemented with 100 ppm of polybrominated biphenyls (PBBs) for 14 to 21 days or 200 ppm of polychlorinated biphenyls (PCBs) for 28 to 36 days. The mice were challenged 24 hr prior to sacrifice with an ip injection of several doses of trichloroethylene (TCE) or 1,1,2-trichloroethane (TCE). Sprague-Dawley male rats were fed a standard diet containing 100 ppm of PBB or 200 ppm of PCB for 20 days, or fed control diet and given 30 mg/kg of hexachlorobenzene (HCB) in corn oil orally every 72 hr over a 20-day period. The rats were then challenged with various doses of CCl$_4$, administered orally 48 hr prior to sacrifice. The renal toxicity of TRI was enhanced by PBBs and PCBs as evidenced by greater reduction in renal $p$-aminophenol accumulation (PAH S/M) in treated animals than in controls following 1.0 ml/kg of TRI. PAH S/M after 0.15 ml/kg of TCE was reduced in PBB but not PCB-treated animals. PBB, PCB, and HCB all increased CCl$_4$ nephrotoxicity, as shown by a significant decrease in PAH S/M in treated but not control tissue after 0.03 ml/kg of CCl$_4$. The kidney weight to body weight ratio after 0.25 and 2.0 ml/kg of CCl$_4$ was greater in HCB-treated and PCB-treated animals than in controls. Thus, environmental contaminants that induce microsomal enzymes can increase the toxic potential of chemicals metabolized to nephrotoxins by microsomal enzymes.

23. **Postnatal Renal Function in Rats Exposed Prenatally to Environmental Contaminants.**

K. M. McCormack and J. B. Hook, Department of Pharmacology, Michigan State University, East Lansing, Michigan.

Methylmercury is a widespread environmental pollutant that is toxic to the central nervous system and kidney. Treatment with high concentrations of methylmercury causes glomerular and proximal tubular degeneration and changes in renal cytoplasmic and mitochondrial enzyme systems. Methylmercury crosses the placenta and doses which produce no maternal toxicity have been reported to result in histopathological alterations in kidneys of offsping. The purpose of this investigation was to determine if functional changes correlate with structural alterations in kidney following in utero exposure to methylmercury. Rats were treated, intraperitoneally, with a single dose of 4 mg/kg of methylmercury on the eighth day of pregnancy. Renal function of the newborn rats was assessed 1, 7, and 14 days postnatally. Organic ion transport capacity was ascertained by incubating thin renal cortical slices in oxygenated media containing a representative anion, $p$-aminophenol (PAH), or cation, $N$-methyl-nicotinamide (NMN). The ability of the kidney to produce glucose and ammonia was also determined using renal slices. Methylmercury had no consistent effect on the accumulation of PAH or NMN or on gluconeogenesis and ammoniagenesis. However, kidney to body weight ratios were increased 7 and 14 days postnatally and pup weight gain was retarded at 14 days after birth. These results indicate that methylmercury affects kidney and body weight but no specific renal functional lesion was identified.

24. **A Comparison of the Toxicity of Four Inotropic Catecholamines Given by Continuous Intravenous Infusion to Dogs.**


A comparative study of the toxicity of isoproterenol hydrochloride, 1-norepinephrine bitartrate, dopamine hydrochloride, and dobutamine hydrochloride was made in dogs. The catecholamines were continuously infused over a period of 96 hr via the external jugular vein into ambulatory beagles equipped with a self-contained infusion unit. For comparative purposes the dosage levels of the individual compounds were based on their inotropic potency and the infusion
rates (µg/kg/min) represented various multiples of the maximum myocardial contractile tension dose. The parameters used to evaluate the comparative toxicity included mortalities, side effects, tachycardia and ventricular arrhythmias, SGOT and CPK values, and macroscopic and microscopic examination of the myocardium. The findings indicated that dobutamine hydrochloride given to dogs by continuous infusion has a much greater margin of safety than isoproterenol hydrochloride, which was far better tolerated than norepinephrine bitartrate or dopamine hydrochloride.

25. Role of Renal Metabolism in the Pathogenesis of Renal Cortical Necrosis Produced by 4-Ipomeanol in the Mouse. M. R. BOYD and J. S. DUTCHER, Clinical Pharmacology Branch, National Cancer Institute, Bethesda, Maryland. (J. J. McPhillips)

Administration of the furan derivative, 4-ipomeanol (IPO), to mice resulted in the preferential alkylation of the kidneys by an IPO metabolite. Correspondingly, unlike other species we have studied, the mouse responded to IPO with a striking renal cortical necrosis, in addition to pulmonary bronchiolar necrosis. Autoradiographic studies demonstrated that the covalently bound IPO metabolite was highly localized in those renal cortical tubules which became necrotic. In vitro studies demonstrated that IPO was converted to an alkyling metabolite via cytochrome P-450-dependent mixed-function oxidase activity present in mouse liver, kidney, and lung microsomes. Formation of an alkyling metabolite of IPO was greatly increased in liver microsomes, but not renal microsomes, from C57BL/6J mice pretreated with 3-methylcholanthrene (MC). In vivo hepatic alkylation by an IPO metabolite was markedly elevated in the induced mice, while renal alkylation was markedly decreased. IPO produced striking centrilobular hepatic necrosis in the induced mice, but much less intense renal necrosis, as compared to noninduced controls. In contrast, MC pretreatment had no significant effect on target organ alkylation and toxicity by IPO in “noninduceable” DBA/2J mice. These results support the view that the highly reactive metabolite of IPO that causes renal necrosis in mice is actually formed within the target tissue, and not transported to the kidney after formation in the liver.


A series of preliminary experiments have been conducted with Colorado oil shale and spent shale obtained from the 10-ton experimental retort at the Laramie Energy Research Center. The experiments were initiated to determine: (1) the bioavailability of an organic residue marker added to shale; and (2) the pulmonary disposition and the response in the lung to these particulates. In rats repeatedly dosed intratracheally (IT) with 6 to 20 mg of oil shale and spent shale dust suspensions (0.9 µMMD), the dusts had little effect on specific activity of pulmonary polycyclic hydrocarbon biotransformation enzymes. Pulmonary disposition of 10 mg of IT spent shale labeled with a marker for the organic residue, 6 ppm of [3H]benzo(a)pyrene ([3H]Bzp), was investigated. Most of the [3H]Bzp remained attached to the spent shale particles, and its interaction with subcellular macromolecules was minimal. Pulmonary retention of particulates was biphasic (t½ of 4 and 18 weeks) with accumulations of particles and radioactivity in lymph nodes. In another ongoing experiment, rats received three weekly 10-mg IT injections of oil shale or spent shale dusts and are being sacrificed over an 8-month period. An initial acute inflammatory response occurred with increases in lung weight, soluble non-collagenous protein fragments, and acid phosphatase activity. Proline hydroxylase was initially elevated, indicating an increased potential for collagen synthesis. At later sacrifices, lung weights were further elevated with concurrent increases in hydroxyproline (collagen) content. Microscopically, lungs examined to date have so far (0 to 6 months postexposure) contained particulates in macrophages. There was also a mild granulomatous response, and accumulations of proteinaceous material similar to those observed in alveolar lipoproteinosis. Fibrotic response was minimal. (Supported by Energy Research and Development Administration.)
27. Effect of the Intravenous Administration of the Solubilized Plasticizer, Di(2-ethylhexyl)phthalate on the Lung and on Survival of Transfused Rats. R. J. RUBIN and J. C. F. CHANG, Division of Toxicology, Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland.

Di(2-ethylhexyl)phthalate (DEHP), the plasticizer used in vinyl plastic storage bags, has been shown to migrate into stored blood or blood products used for human transfusions. Previously this laboratory (Toxicol. Appl. Pharmacol. 33, 514, 1975) showed that iv administration of DEHP, solubilized in a nonionic detergent to male rats, resulted in a respiratory distress syndrome that rapidly progressed to death from respiratory failure. The LD₅₀ was approximately 250 to 300 mg/kg. A subsequent report (Toxicol. Appl. Pharmacol. 37, 154, 1976) established that the pulmonary pathology was characterized by an inflammatory state commonly referred to as "shock lung." The present report deals with the effect of the acute iv administration to rats of DEHP solubilized directly in donor rat blood without added detergent. DEHP was sonicated into donor rats at concentrations up to 10 mg/ml. DEHP-containing plasma was then added back to the original volume of packed cells to reconstitute the normal hematocrit with resulting concentrations of DEHP in whole blood ranging up to 5 mg/ml. DEHP-free blood for control experiments was prepared in an identical manner. Ultrasound centrifugal analysis of DEHP solubilized in this manner revealed no significant differences in the distribution of DEHP among various lipoprotein and protein fractions from that seen when DEHP migrates from a plastic surface into stored blood. In a series of exchange transfusions, DEHP-containing blood was infused into the femoral vein of anesthetized rats while blood was simultaneously being withdrawn from the contralateral femoral artery until either 40 or 80 ml/kg was exchanged. This allowed doses as high as 400 mg/kg to be administered. During the exchange, blood pressure was not significantly altered. All nine rats transfused with control blood survived. The lung weight/body weight ratio was not significantly different from non-transfused rats and all lungs looked grossly normal. However, there was a DEHP dose-related increase in lung edema and in lethality with an LD₅₀ of approximately 200 mg/kg. At 400 mg/kg (given as 5 mg/ml × 80 ml/kg) six of six rats died, all with severe lung hemorrhage and edema. In replacement transfusion experiments anesthetized rats were bled until their blood pressure fell to 50 mm Hg, which was held for 30 min. After 30 min of shock, donor blood (1.25 mg of DEHP/ml) was reinfused at a volume equal to that originally shed. Five of five control animals survived the procedure and although the lung weights were significantly elevated, none were grossly hemorrhagic. Two of six rats transfused with 7.7 to 13 mg/kg of DEHP died within 90 min of the transfusion. Their lungs were grossly hemorrhagic, as were the lungs of the four survivors. These results confirm the pulmonary toxicity of DEHP in rats in a system where the DEHP is solubilized directly in plasma without the addition of a detergent. These results also reveal a marked increase in sensitivity to the pulmonary toxicity of DEHP in animals whose blood pressure is held at shock levels prior to transfusion.


(John Doull)

There is a great need for a quantitative basis for rapidly estimating the risk to human health of a variety of mixtures of environmental pollutants. The enzymatic profile of airway fluid offers one means of monitoring the lung's response to inhaled toxicants. Syrian hamsters were exposed either by bronchopulmonary lavage or by inhalation to various levels of pollutants. The enzymatic activity in the airways was sampled by lavage at various times after exposure. The cellular content of the lavage fluid was separated by centrifugation and a differential cell count was made. The cell-free supernatant was assayed for lactic dehydrogenase, as a measure of cell death; and for acid phosphatase, β-glucuronidase, and proteases, as a measure of released lysosomal activity. Sialic acid and total protein content of the lavage fluid were determined as a measure of mucous secretion and vascular leakage, respectively. Tissue levels of the same
enzymes were measured and, in addition, glucose 6-phosphate dehydrogenase was assayed as a marker enzyme of the pentose shunt pathway. Histopathological studies were made to correlate the enzymatic changes with the morphological changes. From this study, we found that the contents of the alveolar fluid as well as the enzymatic profile of lung tissue, can be used to quickly assess the acute toxic level of a pollutant and thereby to select those materials which require further in-depth toxicological studies. (Research performed under USERDA Contract No. EY-76-C-04-1013.)

29. Proliferative Response of Lung to Triton X-100 Lavage. N. A. HACKETT and R. F. HENDERSON, Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico. (Roger O. McClellan)

Triton X-100 (a polymer of p-isocetylpolyoxyethylenephenol) is a surfactant agent which is commonly used in pressurized aerosol consumer products. Lavage administration distributes this agent uniformly throughout the lungs. Triton X-100 (0.05%) causes injury to the lung as indicated by release of lactic acid dehydrogenase (LDH) from tissue into the alveolar fluid. Cytokinetic techniques were employed to determine if disturbance of natural lung surfactant could initiate lung cell proliferation. Syrian hamsters were lavaged with 80% lung volume of 0.9% saline containing 0.05% Triton X-100. Lung cell [3H]thymidine uptake was evaluated in animals which received a 2-hr pulse of label prior to sacrifice at 2, 18, 24, 48, and 72 hr after lavage. Assay of LDH released into alveolar fluid during lavage indicated immediate injury. Whole lung tissue uptake of [3H]thymidine was significantly increased after Triton X-100 as compared to saline lavaged control. Triton X-100 lavage did not alter the population distribution of Type I1 cells or alveolar macrophages at 18 hr after lavage although [3H]thymidine uptake into alveolar macrophages was greater in Triton X-100 lavaged lungs (35%) vs saline lavaged controls (20%). Exposure of lungs to Triton X-100 (0.05%) causes an increased uptake of [3H]thymidine which is not attributed to Type I, Type II, or endothelial cells but to increased incorporation of label into alveolar macrophages and injured ciliated airways. (Research performed under USERDA Contract No. EY-76-C-04-1013.)


The direct effects of carbon monoxide and nitrogen were studied in the isolated spontaneously contracting rat heart. Hearts removed from male white rats were perfused via the aorta with oxygenated (95% O2 - 5% CO2) Krebs-Henseleit solution while heart rate, pulse pressure, and lactate production were recorded. After 30 min the hearts were challenged with 95% N2 - 5% CO2 (N2) or 95% CO - 5% CO2 (CO) for 10 min. The preparation was then reoxygenated and allowed to recover for 10 min. Heart rate at the end of 3 min of stress declined to 56% (272 ± 9 to 157 ± 12 beats/minute) and 36% (269 ± 7 to 98 ± 10) in N2 and CO, respectively. After 2 and 9 min of reoxygenation, heart rate recovered to 31 and 84% of its control value in the N2-challenged hearts, and to 54% and 100% in the CO-challenged hearts. At the end of 2 min of stress, pulse pressure had declined to 85% (69.8 ± 1.7 to 59.6 ± 4.3 mm Hg) and 66% (69.5 ± 0.7 to 45.8 ± 3.4) in N2 and CO, respectively. Recovery of pulse pressure after 2 min of reoxygenation was 12% of the control value in N2 and 43% in CO. By the end of 6 min of stress, the rate of lactate production was significantly greater in the N2 hearts (1.41 ± 0.06 vs 0.77 ± 0.12 mg/g dry wt/min). At the end of 10 min of reoxygenation, lactate production remained significantly higher in the N2 hearts (0.14 ± 0.05) compared to the CO hearts, which had returned to control values (0.03 ± 0.01). These results indicate that CO- and N2-induced anoxia may have different mechanisms of action on the heart.
31. Studies on the Inhalation Toxicity of Two Phosphoramidothioate Insecticides to Rodents and Quail. P. E. Berteau and R. E. Chiles, University of California, School of Public Health, Naval Biosciences Laboratory, Naval Supply Center, Oakland, California. (W. F. Durham).

Acephate (O,S-dimethyl acetylatedphoramidothioate), an insecticide of low mammalian oral toxicity, is considered to owe its insecticidal activity to deacetylation in insects to the more toxic methamidophos (O,S-dimethyl phosphoramidothioate). Because little information is available on the inhalation toxicity of these two pesticides, we considered the possibility that conversion of acephate to methamidophos may take place when certain vertebrate species inhaled aerosols of acephate. We exposed groups of female mice, rats, or quail to respirable aerosols of aqueous formulations of methamidophos or acephate. Doses were adjusted by varying the exposure time to a duration of up to 5 hr. Plasma cholinesterase depression was determined potentiometrically and recuperaion time was ascertained. Aerosol concentrations of active ingredients were about 0.65 mg/liter for methamidophos, and about 2.2 mg/liter for acephate. No rats died after 5 hr of inhalation exposure to the 2.2 mg/liter concentration of acephate but three of eight mice died after 5 hr exposure to the same concentration. Quail were more susceptible to acephate than mammals; three of six died when exposed for 100 min. Based upon the aerosol concentrations, duration of exposure, minute volumes, and information on whole body deposition, inhalation LD50 values of 18.7 mg/kg to mice and about 9.0 mg/kg to rats were estimated for methamidophos. Clinical signs were limited to mild tremors. No evidence of conversion of acephate to methamidophos during atomization was detected. The toxicity of the two insecticides is not appreciably different via the inhalation route than the oral route. Extent of plasma cholinesterase depression was compatible with mortality incidence; recovery was rapid with methamidophos but slow with acephate. (Supported by the Office of Naval Research.)


The major emphasis of current in vitro toxicity tests is to provide a rapid, sensitive method to determine the possible adverse effects of environmental agents on all classes of the human population. A series of experiments were conducted to evaluate the applicability of a cell culture screen based on toxicant effects on cellular metabolic processes. The relative toxicity of aldrin, parathion, and paraaxon was measured using five cell lines (BALB/c 3T3, WI-38, HeLa, rat lung, and human foreskin fibroblasts). Cytotoxicological responses were determined using the prescreen confluency assay and the clonal toxicity assay. Pesticide effects on metabolic processes related to cell growth were examined by monitoring radioprecursor incorporation into cellular protein and RNA. Aldrin was clearly the most inhibitory to cell growth in all cell lines. All the pesticides exhibited at least a minimal dose-dependent inhibitory effect on synthesis of cellular protein and RNA in most cell lines. Cytotoxicological effects on the different cell lines were similar. Metabolic dysfunction appeared to be cell line dependent. In general, aldrin appeared to inhibit protein synthesis specifically while parathion and paraaxon were more inhibitory to RNA synthesis. The inhibitory effects of the pesticides on protein synthesis correlated better with comparative cytotoxicological responses than did effects on RNA synthesis. Exceptions to this correlation probably indicated different toxic mechanisms. Results from this study indicate that specific biochemical or metabolic indicators might be used to provide a rapid, sensitive cell culture screen for pesticides. (Supported by NIEHS Grant ES-00865 and by Battelle, Columbus Laboratories.)

33. Effects of Selected Organophosphorus Compounds on Protein Synthesis in a Serially Cultured Neuroblastoma Cell Line. Michael J. Harvey and Raghbir P. Sharma, Toxicology Program, Utah State University, Logan, Utah.

Diisopropylfluorophosphate (DFP) has been reported to cause an early inhibition of protein synthesis in the spinal ganglia of cats. If organophosphorus compounds can cause a
partial blockage of protein synthesis, normal turnover of neuronal proteins could establish conditions similar to those which occur in Wallerian degeneration. In this study neuroblastoma 2-A cells, a homogeneous cell system exhibiting many of the functional properties of normal neurons, was used to establish dose–response relationships of three organophosphorus compounds, tri-o-toly1 phosphate (TOTP), DFP, and Bidrin. The incorporation of 14C from glucose was used as an indicator of metabolic activity and the incorporation of L-[14C]leucine as an indicator of protein synthesis. Neuroblastoma cells were grown in tissue culture dishes in minimum essential media (MEM) with 5% fetal calf serum and 0.1 μCi/ml of one of the precursors, D-[6-14C]-glucose (sp act 51.2 μCi/mmoll or L-[U-14C]leucine (sp act 309 μCi/mmol). The cells were incubated at 37°C in a humidified atmosphere of CO2: air (5:95%) for 24 hr. After this period, the excess label was chased out, the cells were suspended in 5 ml of trypsin–versine solution, counted in a hemocytometer, and collected on 2.1-cm glass fiber filter disks, and the radioactivity was counted. The experiments were repeated with the neuroblastoma cells growing in media with no serum. The cells were also observed under phase contrast microscopy. The organophosphorus compounds caused the neurites to have a shrunken, rough, and irregular appearance. Swellings along the length of the axon were observed, especially with DFP-treated cells. All three compounds caused a dose-related reduction of 14C incorporation from glucose. DFP and TOTP caused an inhibition of the uptake of 14Cleucine, while Bidrin did not. Further screening may reveal selective changes in protein synthesis resulting from different types of organophosphates.


Maneb, a dithiocarbamate, will produce a peripheral neuropathy although the mode of action is different from that of the organophosphates. Prior investigations have not yielded information concerning the reversibility of the neuropathy nor its selective toxicity to other organ systems as a consequence of age, sex, and dose. Two experiments were conducted in which male and female outbred albino rats (CD strain) were used. In the first study, dosing began on Day 21 postpartum and continued through Day 30. For the second investigation, dosing began on Day 33 postpartum and terminated at Day 90. Apart from controls, the dose levels were 75 (first experiment only), 150, 300, and 600 mg/kg/day. All animals were dosed by gavage using a corn oil suspension of maneb. Controls received only the corn oil. During the course of each study, all animals were observed for clinical signs of neuropathy. Rats that became ataxic (with indications of paralysis) were removed from treatment and allowed to recover. At the end of dosing, all animals were sacrificed, and trunk blood was collected for hematology and serum chemistry. Whole body as well as individual organ weights also were obtained. In the first experiment, there were no clinical indications of peripheral neuropathies in either males or females. In the second study, females on the 300 and 600 mg/kg/day regimens became ataxic with subsequent paralysis at an earlier age than males. Moreover, the number of females exhibiting peripheral neuropathies was fivefold higher than male rats. In both experiments not only were decreases in organ weights, particularly the thymus, observed (males and females) but statistical differences also were found in various serum chemical and hematological parameters. These changes were more predominant in older females (second experiment). The data suggest that maneb toxicity can be related to age and sex. It would also appear that although the peripheral neuropathy can be demonstrated, it tends to be reversible once treatment has ceased irrespective of whether the animal has become paralyzed or not.

35. Toxicity of Paraquat and Other Selected Viologens in Rats. John H. Ross and Robert J. Krieger, Department of Environmental Toxicology, University of California, Davis, California.

Paraquat (methyl viologen, 1,1'-dimethyl-4,4'-bipyridylum dichloride) is a widely used, non-selective herbicide the ingestion of which causes severe lung lesions in many animals
including man. The compound is reported to be taken up in the lung by an active transport mechanism which concentrates it severalfold over plasma levels. Investigations of structure-activity relationships of homologs in blocking paraquat uptake into rat lung slices (Toxicol. Appl. Pharmacol. 41, 134, 1977) included examination of their gross toxicological effects. Methyl, propyl, hexyl, and benzyl viologen were dissolved in distilled water and administered subcutaneously at geometrically increasing levels (dosage factor = 1.5) to female Sprague-Dawley rats. Two rats received a single dose of one of these compounds at each dosage level. The lowest dosage required to kill both rats and mean time to death are as follows: methyl viologen (72 hr, 105 μmol/kg), propyl viologen (96 hr, 351 μmol/kg), hexyl viologen (96 hr, 37 μmol/kg), and benzyl viologen (16 hr, 18 μmol/kg). Symptomatology included piloerection, weight loss, swelling around the site of injection, and dyspnea. Some rats also developed chromodacryorrhea, blood around the nares, and hair loss on the dorsal portion of the head extending to the scapulae. A focal necrosis developed at the site of injection regardless of the homolog administered. Animals that died had extensively hemorrhaged lungs which did not sink in water. The pleural cavities of rats treated with hexyl or benzyl viologen were filled with a clear yellow, serous fluid (1.5 to 3.5 ml) which contained 22.5 ± 3.5 mg/ml of protein. Stomachs of all animals that died were full (sometimes distended) with food while the intestines were empty except for gaseous bloating. Adrenals of all treated animals were significantly heavier than controls (44.2 ± 6.7 vs 24.9 ± 2.2 mg), respectively. (Supported in part by NIH-ES00125.)

36. Development of an Animal Model for Prediction of Agricultural Field Reentry Hazard. CLINT SKINNER and WENDELL KILGORE, Department of Environmental Toxicology, University of California, Davis, California.

This paper describes a modification of the reentry model of Guthrie et al. (Arch. Environ. Contam. Toxicol. 2, 3, 1974) and some initial characterization and evaluation. Early studies using a compound of high oral, low dermal toxicity (guthion) produced 30 and 65% greater cholinesterase depression in nonmuzzled vs muzzled animals exposed overnight to peach leaves treated with 210 and 410 ppm of guthion formulation, respectively. The need for a model reflecting the predominantly dermal, subacute, intermittent exposure of the farm worker led to the following protocol: In each group five 35-g male Swiss Webster mice were muzzled and exposed to a 144-sq in. cage containing 50 g of laboratory dosed field pesticide treated leaves for 10 hr nightly for 4 to 7 days. Food consumption, body weight, red blood cell AChE, plasma ChE, and neurological signs were monitored throughout preexposure, exposure, and recovery periods. Blood samples of 50 μl were taken from the tail and individual duplicate ChE and pooled AChE values were determined at 24- or 48-hr intervals. Studies using dosage progressions of formulated parathion (95, 165, 361 ppm), methyl parathion (70, 107, 207 ppm), and phosdrin (35, 75, 350 ppm), on orange leaves and guthion (262, 374, 817 ppm), and parathion (90, 202, 313 ppm) on peach leaves indicate that a group consistent dose response can be obtained by this method of self-exposure. The X ChE sp. SD for all studies to date is 22% of the mean. The dose vs ChE and AChE depression curve for parathion on orange leaves was consistently steeper than for methyl parathion. The X ChE depression per ppm of compound was 0.24, 0.38, and 0.40% for parathion and 0.17, 0.25, and 0.30% for methyl parathion on the 1st, 2nd, and 4th days of exposure. Parathion-treated animals reached plateau ChE depressions at 2 days while methyl parathion animals were decreasing at 4 days. These results agree with our acute dermal (hind feet) LD₅₀ in rate of onset and potency—phosdrin > parathion > methyl > parathion > guthion. Formulated phosdrin-treated leaves, however, caused no deviations from control values. High volatility and instability of this compound make acute toxicity data less relevant to field hazard. On peach leaves, guthion showed no deviations from muzzled controls while parathion gave X ChE depressions per ppm of 0.28 and 0.23% on Days 2 and 4 of exposure. Body weight, food consumption, and neurological data correlated well with ChE and AChE. (Supported by NIEHS Training Grant ES00125-10.)
37. **Immunosuppression in Mice Administered Methyl Parathion and Carbofuran by Diet.** A. FAN, J. C. STREET, and R. M. NELSON, Toxicology Program, Department of Animal, Dairy, and Veterinary Science, Utah State University, Logan, Utah.

Methyl parathion (O,O-dimethyl-O-4-nitrophenylthiophosphate, MP) and carbofuran (2,2-dimethyl-2,3-dihydrobenzofuronyl-7-N-methylcarbamate, CF) have been reported both to delay and suppress the development of active immunity in the rabbit. The present research was directed toward quantifying the dosage relationships of these pesticides to host resistance and acquired immunity in the mouse. Various parameters of host defense against microbial infection were investigated. Groups of Swiss (ICR) mice were fed Purina laboratory chow diet providing 0.08, 0.7, 3.0 mg of MP/kg/day or 0.1, 0.6, 1.0 mg of CF/kg/day along with untreated controls. To evaluate passive host resistance, a single LD50 challenge dose of *S. typhimurium* C57 cells was given ip after 4 weeks of diet treatment. Active immunity was induced in other groups by weekly injection of vaccine (acetone-killed *S. typhimurium*) during the period of diet treatment. Dosage-related increases in mortality were seen in unvaccinated mice under both chemical treatments, and protection by immunization was decreased. Pesticide treatment extending beyond 2 weeks was required to obtain significant increases in mortality. Increased mortality was associated with increased numbers of viable bacteria in blood, decreased total γ-globulins and specific immunoglobulins in serum, and reduced splenic blast transformation in response to mitogens. Serum opsonin activity of CF-treated animals was slightly reduced. However, cellular hypersensitivity to DNFB was not affected by these pesticide treatments. These results support those of other investigations indicating effects of environmental toxicants upon the resistance and immune competency of experimental animals.

39. **The Metabolism and Excretion of Toxaphene and Selected Toxaphene Fractions.** GERALD A. POLLOCK and WENDELL W. KILGORE, Department of Environmental Toxicology, University of California, Davis, California.

Seven-day excretion studies using rats treated with [14C] toxaphene or [14C] toxaphene fractions ("nonpolar" or "polar" fraction) were conducted. Small differences in the total excretion (urine and feces) of the groups were found. The amount of excretion for toxaphene was 58.2%; "nonpolar" fraction, 69.2%; and "polar" fraction, 56.0%. The activity excreted in the urine for all groups was more polar than the starting material and, therefore, had been metabolized. Toxaphene fat residues from treated rats were different than residues from spiked controls. The major difference was an increase in the amount of polar activity in the residues from treated animals. Similarly, metabolites were present in fat samples from rats treated with the toxaphene fractions. The residue from rats treated with a nonpolar fraction was predominantly parent material (89%) and 11% more polar metabolites. The residue from rats treated with the polar fraction was also primarily parent material, but 11% of the activity was less polar. Apparently, the metabolism of this fraction resulted in the formation and storage of less polar metabolites.


Dinoseb (4,6-dinitro-2-sec-butylphenol; DNBP), a substituted dinitrophenol, is registered with the EPA for use in agriculture as a herbicide. Studies with DNBP were initiated due to its consideration as a substitute for canceled herbicides. A combination subchronic feeding and single generation reproduction protocol was utilized. Eight groups of 35- to 38-day Sherman strain rats (14 rats of each sex/group) were fed a diet fortified nominally with 0, 50, 100, 150, 200, 300, 400, and 500 ppm of Technical DNBP (80%) for 60 days and bred, and the parents and offspring continued on study for a total exposure of the parents of 153 days. Bioeffect parameters on the parents included lethality, growth, food consumption, clinical chemistry, tissue residue, behavior, microsomal enzymes, and pathology. Reproductive parameters included
fertility, fecundity, mortality, viability, lactation, and growth. The 300, 400, and 500 ppm groups were terminated at 21 days due to mortality. 14, 100, and 100%, respectively. Growth in the remaining groups was depressed monotonically at 200, 150, 100, and 50 ppm. Organ weight (liver, spleen, heart, lung, brain) was decreased while the organ weight/body weight ratios increased. Blood alkaline phosphatase, alanine aminotransferase, potassium, and BUN were significantly increased while LDH and cholinesterase were depressed. Residue levels were dose dependent with blood > feces > urine > adipose > brain > liver. Aminopyrine N-demethylase activity was increased. Discrimination learning was not affected while locomotor activity was increased at 200 ppm. A significant pathologic change was diffuse tubular atrophy of the testes, particularly at 200 ppm. Fertility, fecundity, neonate survival, weight gain, viability, and lactation were all depressed. In conclusion, effects of DNPB appear to be dose related and warrant further study.

40. Tests for Possible Carcinogenicity of 20 Pesticides in Osborne–Mendel Rats and B6C3F1 Mice. JANE F. ROBENS, Tracer Jitco, Inc. Rockville, Maryland.

Twenty pesticides (i.e., the chlorinated hydrocarbon insecticides, chlordane, heptachlor, aldrin, dieldrin, photodiethyl, endrin, kepone, toxaphene, and lindane; the organophosphorus insecticides, tetrachlorvinphos, dimethoate, dichlorvos, malathion, phosphamidon, parathion, and azinphosmethyl; the fungicides, captan and chlorothalonil; and the herbicides chloramben and picloram) were tested for possible carcinogenicity in Osborne–Mendel rats and B6C3F1 mice. The chemicals were administered in feed at one of two concentrations for 80 weeks to both species; the rats were observed for an additional 30 weeks and the mice for 10 weeks before termination of the studies. The highest concentration tested for each chemical was the maximum compatible with survival for the period of the studies. The second concentration tested was half of this. Repeats of specific groups and lowering of the concentrations during some studies was necessary to achieve adequate survival. Following gross necropsy, 24 tissues from each treated and control animal were examined microscopically. Signs of toxicity, dose-related decreases in survival, and/or decreased mean body weight were observed in all studies, indicating that maximum tolerated doses were used. In rats, with several of the compounds there were small increased incidences of proliferative lesions of the endocrine organs, usually in one dose group of one sex. Because these increases were small and not consistent and because of variable background incidences, they were not considered to indicate that the compounds were carcinogenic. These included increases of proliferative cells of the thyroid in animals treated with chlordane, heptachlor, photodiethyl, aldrin, tetrachlorvinphos, picloram, and captan and of adrenal cortical adrenomas in animals treated with captan, aldrin, dieldrin, and tetrachlorvinphos. In mice, with several of the compounds there was an increased incidence of hepatocellular carcinoma and/or adenoma in treated groups compared with that of control animals; these incidences were statistically significant and were considered to indicate that the compounds heptachlor, chlordane, kepone, aldrin, and tetrachlorvinphos were carcinogenic. The only other tumor observed in a significant incidence in mice was polyloid carcinoma of the duodenum in captan.

41. Excretion of Carbaryl into the Saliva of the Rat. MICHAEL L. ZIMMERMAN, RICHARD G. MAY, and JOSEPH F. BORZELLICA. Division of Toxicology, Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Because carbaryl (1-naphthyl N-methyl carbamate, Sevin) is a widely used insecticide, the potential for intoxication is high. Since saliva samples are better tolerated by the working public than blood or urine samples, a positive correlation between blood and salivary pesticide levels would suggest the use of saliva as an indicator of exposure. (1) In vivo: Anesthetized adult male Sprague–Dawley rats received a single dose of 100, 200, or 400 mg of carbaryl/kg body wt by injection directly into the stomach. Emulphor (polyoxyethylated vegetable oil) was the vehicle used. Carbaryl and α-naphthol, a major metabolite, were detected using appropriate gas chromatographic methods. The concentration of carbaryl and α-naphthol in plasma and saliva
peaked at 45 to 60 min following injection. This was followed by a gradual decline to control concentrations by 48 hr postinjection. The data suggest a relationship between concentrations in plasma and saliva. Concentrations in tears and urine increased during the initial 6-hr observation period. This was followed by a gradual decline during the subsequent 42 hr. (II) In vitro: The uptake and efflux of [14C]carbaryl (10⁻³, 10⁻⁴, or 10⁻⁵ M) were studied in slice preparations from rat submaxillary gland under various incubation and washout conditions. Incubation with sodium cyanide (10⁻³ M) or dihydrogenol (10⁻¹ M), or aeration with N₂ at 37°C or incubation at 5°C did not significantly alter the uptake or efflux of [14C]carbaryl. Carbaryl (10⁻¹ M) did not affect O₂ consumption by these slices. These data suggest that the uptake and efflux of carbaryl within the submaxillary gland are not energy dependent. (Supported in part by EPA Grant R-804318010.)

42. Relative Sensitivities of Various Biochemical, Toxicological, and Pathological Techniques in Demonstrating Sublethal Lesions in the Rat following Oral Administration of Low Levels of Methoxychlor. J. Michael Morgan and John P. Hickenbottom, Department of Pharmacology, University of Mississippi, University, Mississippi. (W. Marvin Davis).

Since most insecticides are capable of producing toxicological damage acutely or on a chronic basis when encountered at low levels via the oral route, we have attempted to identify several methods which would indicate sublethal changes caused by acute low-level dosing of a relatively nontoxic insecticide in the rat. Male Holtzman rats (200–250 g), seven per test group, received pure corn oil or 10, 40, 160, or 640 mg of methoxychlor (MC)/kg in corn oil by oral intubation, and were sacrificed 24 hr after treatment. All assays were performed on liver sections and blood samples taken at this time. While SGOT showed an increase at the highest dose, SGPT showed no increase at all. Microscopic examination of liver tissue with hematoxylin and eosin as well as Sudan black B (fat stain) yielded no observable fatty accumulation. Liver glycogen levels showed a steady decrease from the lowest to the highest dose of MC, and glycogen phosphorylase activity decreased at the two highest doses, while glycogen synthetase levels showed no significant change. Glucose 6-phosphatase activity increased at the highest dose of MC. Liver pyruvate levels and lactate:pyruvate ratios showed no significant change, although lactate levels decreased at all but the lowest dose. It appears that relatively low doses of MC (compared to the oral LD₅₀ in rats, 6–7 g/kg) promote the utilization of liver glycogen, and that changes in glycogen concentration, glycogen phosphorylase activity, and lactate concentration could be detected at an oral MC dose of 160 mg/kg; serum transaminases and histological evaluation of liver samples were less sensitive. (Supported by The University of Mississippi Research Institute of Pharmaceutical Sciences.)

43. Comparison of the Metabolism of Biphenyl, 4-Chlorobiphenyl and 4-Fluorobiphenyl. K. Halpaap, E. C. Horning, and M. G. Horning, Baylor College of Medicine, Houston, Texas.

In earlier studies in our laboratory a dihydrodiol was identified as a metabolite of biphenyl, demonstrating that biphenyl was metabolized by way of an epoxide intermediate. The metabolism of 4-chloro-, 4-fluoro-, and 4,4'-dichlorobiphenyl was investigated to ascertain if the corresponding dihydrodiols were urinary metabolites. Following intraperitoneal administration of the three halogenated biphenyls to male Sprague-Dawley rats (30 mg/kg), 24-hr urine samples were collected and the metabolites were isolated and characterized by GC-MS procedures. In addition to the previously identified metabolites of 4-chlorobiphenyl, a dihydrodiol, two hydroxylhydrodiols, a triol, and a tetrod were isolated from urine. The metabolism of 4-fluorobiphenyl was similar to that of 4-chlorobiphenyl. The major metabolites were 4-hydroxy-4'-fluorobiphenyl and 3,4-dihydroxy-4'-fluorobiphenyl. 3-Methoxy-4-hydroxy-4'-fluorobiphenyl, a dihydrodiol, a hydroxyhydrodiol, and a trihydroxy derivative of 4-fluorobiphenyl were characterized by GC-MS. Larger amounts of dihydrodiol were found in urine after administration of 4-chloro- and 4-fluorobiphenyl that after administration of biphenyl. The
dihydriodols were excreted as neutral (unconjugated) metabolites and as acidic conjugates. A dihydriodiol metabolite of 4,4′-dichlorobiphenyl was not detected. From these results we conclude that 4-chloro- and 4-fluorobiphenyl are metabolized by the epoxide-diol pathway, and epoxidation occurs in the unsubstituted phenyl ring. Substitution of chlorine in the 4,4′ positions apparently blocks epoxide formation. (Supported by Robert A. Welch Foundation Grant Q-125.)


Nitrofurantoin, a widely used urinary tract antiseptic, is a potent mutagen in S. typhimurium TA100 and causes DNA damage in mammalian cell cultures. Polyneuropathy and pulmonary and hepatotoxicities have been observed in humans. Formation of toxic metabolites of nitrofurantoin may be responsible for the observed toxicities; however, metabolites of nitrofurantoin have not been previously identified. As determined from high-pressure liquid chromatography (hplc), three metabolites were formed by the anaerobic incubation of nitrofurantoin with rat liver 9000g supernatant and in the isolated perfused rat liver. The major metabolite (M-1) was also identified in the urine of a patient on nitrofurantoin. M-1 was purified by preparative hplc using a reverse phase μBondapak C18 column and yielded a white powder with an ultraviolet λmax(H2O) at 278 nm. M-1 was identified as 1-[(3-cyano-1-oxopropyl)methylene]amino]-2,4-imidazolidinedione by chemical ionization mass spectral analysis (MH+ = 209) and Fourier transform nuclear resonance (δ ppm = 2.76 triplet, 3.35 triplet, 4.20 singlet, 7.10 singlet). Confirmation of the structural data for M-1 was obtained by catalytic reduction of nitrofurantoin to a compound with ultraviolet, mass spectral, and hplc data identical to those of the metabolically produced M-1. Further structural confirmation was obtained from infrared analysis (cyano band at 2250 cm⁻¹) and elemental analysis (C, 45.95; H, 4.58; N, 26.07). (Supported by Grants USPHS CA-18435 and UCASC-36.)

45. Mechanism of the Metabolic Activation of Chloroform. L. R. Pohl, B. Bhosnian, and G. Krishna, National Institutes of Health, Bethesda, Maryland. (L. Dolberg)

We recently reported (Fed. Proc. 36, 396, 1977) that cysteine blocks the in vitro covalent binding to microsomal protein of a metabolite of [14C]chloroform (COCl₂) as 2-oxothiazolidine-4-carboxylic acid (OTC). To characterize further this activation process, we incubated [14C]CHCl₃ with liver microsomes from phenobarbital-pretreated rats either in the presence of SKF 525-A, or in atmospheres of N₂, CO:O₂, or [14O]O₂. The formation of COCl₂ was inhibited by SKF 525-A, and atmospheres of N₂ or CO:O₂. The phosgene that was trapped from the reaction conducted in an atmosphere of [14O]O₂ contained nearly 100% [14O]O in the 2-oxo group. Moreover, when [14C]CHCl₃ was incubated with liver microsomes there was no covalent binding and [3H]HCl was not incorporated into OTC. These findings suggest that the C–H bond of CHCl₃ is oxidized by a cytochrome P-450 monoxygenase to produce trichloromethanol. This intermediate would spontaneously dehydriodchlorinate to yield the toxic agent phosgene, which could bind covalently to protein. To determine if this mechanism of metabolic activation could be responsible for the hepatotoxicity produced by CHCl₃, we compared the hepatotoxicity of CHCl₃ with deuterium-labeled chloroform (CDCl₃) in phenobarbital-pretreated rats. Twenty-four hours after the administration of CHCl₃ (0.2 ml/kg ip in sesame oil) the rats showed extensive centrilobular necrosis of the liver and markedly elevated levels of serum transaminase (SGPT). In contrast, the CDCl₃-treated rats had nearly normal livers and SGPT values which did not differ from the control animals. When the dosage was increased to 0.4 ml/kg, all of the rats administered CHCl₃ died within 24 hr, while all of the rats administered CDCl₃ survived. This deuterium isotope effect on toxicity supports the in vitro mechanism of activation by establishing that in vivo the C–H bond of CHCl₃ is involved in its metabolic activation and hepatotoxicity.

Chlorinated s-triazine trione compounds are used in swimming pool disinfection, bleaches, sanitizers, and detergent applications. In aqueous systems chlorinated s-triazine trione compounds exist in equilibrium with cyanuric acid and hypochlorous acid. After the available chlorine has been liberated, only neutral salts of cyanuric acid (s-triazine trione) remain. Previously reported studies show low acute and chronic toxicity for cyanuric acid as well as lack of any teratogenic or carcinogenic effects. Radiocarbon-labeled cyanuric acid was prepared and administered orally to rats held in metabolism cages. Dose level was 5 μCi (0.44 mg/kg). $^{14}C$ assay showed in rats 50–90% in urine, 2–3% in feces, and 1–5% in respired air. All animals were sacrificed at 96 hr and samples of brain, gonads, heart, kidney, spleen, liver, muscle, skin, fat, blood, blood plasma, and intestinal contents were combusted and $^{14}C$ was determined by scintillation counting. Only traces of $^{14}C$ were found in any tissue. Cyanuric acid is readily absorbed after oral dosing of rats and is rapidly eliminated with no accumulation in any tissue.

47. Continual Assessment of Liver Enzyme Induction following Low-Level Feeding of DDT to Rhesus Monkeys. S. J. Gee, C. R. Clark, R. I. Krieger, and J. L. Miller, Department of Environmental Toxicology, University of California, Davis, California.

Liver enzyme induction has been studied in rhesus monkeys, Macaca mulatta, using protocols which do not require sacrifice of the animals and which are sensitive to changes in oxidative metabolic capacity. Three male monkeys have been fed low levels of inducer (5–500 ppm of DDT) during a 3-year period. In an initial test, 10, 100, and 500 ppm of DDT fed during consecutive 30-day periods increased oxidase activity in liver biopsy homogenates and significantly decreased antipyrine plasma half-life (APH). Termination of DDT feeding resulted in return of metabolic functions to control levels and a slower decline of fat, blood, and urine chlorohydrocarbon levels. In the second study using the same monkeys, five doses (5, 50, 25, 50, and 100 ppm) were used and the duration of feeding periods was sufficient to establish stable blood levels. APHs, oxidations of model substrates in needle biopsy homogenates, and chlorohydrocarbon levels in blood, fat, and urine were monitored. No significant increases in oxidase activities were seen until well into the 100-ppm feeding period. At the end of the 100-ppm feeding period (216 days), APHs had decreased 22, 25, and 23%. Rates of oxidation of aldrin, dihydroisodrin, p-nitroanisole, and benzaldehyde increased; only aldrin and dihydroisodrin were statistically significant ($p < 0.05$). Aldrin epoxidase increased 217, 175, and 200%; dihydroisodrin hydroxylase, 182, 147, and 204%; p-nitroanisole O-demethylase, 138, 133, and 147%; and benzaldehyde hydroxylase, 108, 140, and 228% over controls. Methods used to measure changes in hepatic oxidases had no effects on animals. They remained in good health throughout the study, with no changes in clinical chemistry. (Research supported in part by NIH ES 00054 and 00125 and a Shell Foundation Fellowship.)


XPS-4169L is a mixture of monochlorinated diphenyl oxides being developed as a dielectric fluid. The fate of ring-labeled p-chlorophenyl oxide and its mono- and dibutylated derivatives was studied in the rat. In addition, the fate of a mixture of these components was compared in rats and a monkey. The three components were absorbed extensively (90%) after oral administration. Addition of butyl groups led to a progressive increase in the tissue/plasma $^{14}C$ ratio. Elimination of p-chlorophenyl oxide was monophasic ($t_{1/2} = 38$ hr) with equal amounts of $^{14}C$ recovered in the urine and feces. Elimination of the butylated components was biphasic with over 75% of the $^{14}C$ recovered in the feces. Greater than 80% of the dose was excreted
within 3 days and the remaining mono- and dibutylated components were eliminated with estimated half-lives of 65 and 71 hr, respectively. The monkey eliminated more of the mixture in the urine than the rats did; however, the overall rate of elimination was similar in the two species. Consistent with the concentration of these components found in the tissues during the sub-chronic rat toxicity study, the data indicate that the potential of the dibutylated component to accumulate upon repeated administration exceeds that of the two other components and that the concentration of these components in the tissues plateaued during the subchronic toxicity study. The data further indicate that the potential of these components to accumulate in rats and monkeys should be similar.


As part of our effort to select animal species rationally for chronic toxicity studies, comparative metabolism of $^{14}$C ethylenediamine ($^{14}$C EDA) was conducted in several strains of rats and mice. Hilltop Wistar rats were intubated with $^{14}$C EDA in distilled water at 1.2, 5, and 10 mg/kg, and their urinary metabolic profiles from AG 50W-X8 cation exchange columns were compared. Based on this comparison, it appeared that the dosage level of 5 mg/kg was approaching the saturation level for the metabolic capacity of the rat. Material balance studies for 48 hr following oral dosing of 5 mg/kg to the rat revealed that urinary and fecal excretion of radiochemicals amounted to approximately 61 and 3%, respectively, of the administered dose; 9% of the radioactivity excreted was $^{14}$CO$_3$, indicating extensive metabolism of a major portion of administered $^{14}$C EDA. The major organs (liver, kidney, lungs, and brain) contained less than 1% of the radioactivity at the end of a 48-hr experimental period. Comparable results were obtained from endotracheally treated rats with the exception of a higher fecal excretion rate. Further studies in rats indicated no obvious differences between sexes and among several strains. When similar experiments were conducted with orally dosed (5 mg/kg) Hilltop Swiss Webster mice, urinary and fecal excretion and $^{14}$CO$_2$ production amounted to approximately 70, 5, and 12% respectively, of the administered dose. The amount of radioactivity in the major organs was also low. The above information and that gathered from experiments in progress concerning in vivo and in vitro comparative metabolism of $^{14}$C EDA in various species will be compared with human tissue studies.


The metabolism and excretion of ethylbenzene (EB), ethylcyclohexane (ECH), and methyl-ethylbenzene (MEB) isomers in rats and dogs were investigated. Three Harlan-Wistar rats (100–120 g) were placed in a 6-liter glass metabolism cage, and the chamber air was recirculated at 1 liter/min. For a 6-hr exposure period, the chamber was charged with $^{14}$C-labeled EB intermittently to hold a chamber concentration of approximately 1 mg of EB/liter. A major portion, i.e., 80 to 90% of the absorbed $^{14}$C-labeled EB, was excreted in urine and about 10% was exhaled in breath. The radioactivity in 13 organs accounted for less than 1% of the absorbed dose. Similarly, the remaining routes of excretion, CO$_2$, or feces accounted for 1% of the absorbed dose. Similar excretion studies were conducted for ECH and MEB in rats. Based on the chromatographic profiles, six metabolites of E8 and eight metabolites of ECH and MEB were found in rat urine. Metabolic pathways of these three hydrocarbons in the dog were similar to those of the rat. Using an in vitro organ-maintenance technique, the metabolic capacity of liver, lung, and kidney from the rat and dog was evaluated. The liver explants of the rat and the dog semiquantitatively produced the in vivo urinary metabolites of EB, ECH, and MEB. The metabolic activities from these selected organs, when compared to total urinary metabolites, clearly show that the in vivo metabolites produced are not limited to the liver but represent a composite of metabolites formed from all organs.
51. The Metabolism and Distribution of 2,4,5-Trichlorophenoxyacetic Acid in Pregnant Mice. R. P. KOSKAI, M. A. AHMED, R. D. HARRISON, and M. T. BUSH, Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, Tennessee.

Quantitative study is limited on the distribution, metabolism, and elimination of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in the pregnant mouse. The present study was undertaken to: (1) measure the distribution of 2,4,5-T in maternal blood, embryos, placentas, and yolk sacs at Day 12 of gestation, (2) identify and quantitate major metabolites in the above tissues, and (3) identify andquantitate major metabolites in urine and feces. Twelve mice (Swiss Webster) were treated with a single oral dose of [carboxy-14]Cl2,4,5-T (100 mg/kg; 1.22 μCi/mg) on Day 12 of gestation and sacrificed (three per group) after 0.25, 0.5, 2, and 24 hr. Maternal blood, embryos, placentas, and yolk sacs were analyzed by solvent extraction, tlc, and countercurrent distribution. Expressed as a percentage of the administered dose per gram of tissue, the unchanged 2,4,5-T found in maternal blood, placentas, yolk sacs, and embryos was 3, 0.5, 0.5, and 0.2%, respectively, after 0.25 hr. and 4, 2, 2, and 0.6%, respectively, after 24 hr. No major metabolites other than 2,4,5-T were detected. To look further at the metabolic fate of 2,4,5-T, urine and feces were collected and analyzed from mice of the same strain given daily 2,4,5-T (100 mg/kg) on Day 12 of pregnancy. Analyses showed that the radioactivity was largely eliminated in the urine, 69 to 78% of the administered dose in 7 days. Feces contained 5 to 9% of the dose. In the urine unchanged 2,4,5-T accounted for 35 to 44% of the dose, and 22 to 33% as very polar material. Unchanged 2,4,5-T in the feces was 3 to 6% and 1 to 2% as polar material. These data indicate that 2,4,5-T administered to pregnant mice is largely distributed and eliminated as 2,4,5-T and very polar material. (Supported by USPHS Grants ES0027 and ES00782.)

52. The Protective Role of Methionine and Glutathione Biosynthesis during Drug Metabolism by Isolated Hepatocytes. D. J. REED and S. ORRENius, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon, and Department of Forensic Medicine, Karolinska Institutet, Stockholm, Sweden.

The protective role of glutathione during thiol depletion by metabolic reactive intermediates was investigated with freshly isolated rat hepatocytes. Glutathione, after conversion to the diethylphenyl derivative of the corresponding sulfonic acid, was isolated and quantitated and its radiospecific activity was determined by reverse phase ion-exchange hplc. The uptake and utilization of exogenous [35S]cysteine, [35S]methionine, or [U-14C]serine during glutathione resynthesis after in vivo glutathione depletion with diethylmaleate demonstrated a high rate of glutathione synthesis from these precursors by hepatocytes (11 to 22 nmol/hr/10^6 cells). The rapid utilization of [35S]methionine and [U-14C]serine for glutathione synthesis provided strong evidence for extensive participation of the cystathionine pathway. The kinetics of glutathione depletion during bromobenzene (0.6 mM) metabolism was established with hepatocytes isolated from rats after prior administration of phenobarbital and/or diethylmaleate. Methionine provided a much greater protection of cell viability against bromobenzene reactive intermediates than either cysteine or cysteamine. The evidence continues to support the concept that glutathione has a major protective role against intracellular reactive intermediates generated by cytochrome P-450-catalyzed reactions. (Supported by an American Cancer Society Eleanor Roosevelt-International Cancer Fellowship (D.J.R.) and the Swedish Medical Research Council, Grant No. 03X-2471.)


Calcitriol (1α,25-dihydroxycholecalciferol) is a potent metabolite that appears to be the biologically active form of vitamin D3. It is 1α-hydroxylated in the kidney, and therefore, is critical to patients on renal dialysis and may also be an important drug for other clinical
conditions related to the physiological role of vitamin D. The administration of calcitriol to rats by intubation of the stomach for 6 months at dose levels of 0.02, 0.08, and 0.3 \( \mu \text{g/kg/day} \) was associated with dose-related changes at all dose levels. Reduction in body weight and food consumption, increased serum calcium, as well as slight changes in other clinical laboratory values and organ weights were primarily noted in the high- and mid-dose groups; these changes were either absent or less extensive in the low-dose group. Histologic examination of tissues from rats of all groups given calcitriol revealed dose-related changes consisting of calcification in kidney tubules and cardiac myofibers as well as bone changes; in the low-dose group of rats the changes consisted only of focal mild evidence of calcification and mild bone changes of limited significance. The administration of calcitriol as a solution in Neobee oil in capsules to beagle dogs at dose levels of 0.08 \( \mu \text{g/kg/day} \) for 26 weeks or 0.3 \( \mu \text{g/kg/day} \) for 39 days resulted in marked anorexia, severe weight loss, deterioration of physical condition, increased serum calcium and urea nitrogen, as well as other changes in clinical laboratory measurements, calcification of soft tissues, bone changes, and death. Two of six dogs died at the 0.08 \( \mu \text{g/kg/day} \) dose level during the six months of treatment. Two of six dogs were sacrificed at the 0.3 \( \mu \text{g/kg/day} \) dose level after receiving 39 daily doses and the remaining four dogs were observed for approximately 21 weeks; there was recovery of body weight loss and general condition, but some alterations in clinical laboratory measurements did not completely regress and some calcification of soft tissues persisted. The administration at 0.02 \( \mu \text{g/kg/day} \) to dogs for 26 weeks caused only inconsistent alterations in clinical laboratory measurements and pathological changes of limited extent.


The principal biologically active metabolite of Vitamin \( D_3 \) is \( 1\alpha,25\)-dihydroxycholecalciferol (calcitriol) (DeLuca, Fed. Proc. 33, 11, 1974). This compound is of interest for use in bone disease resulting from inadequate or absent hydroxylation of vitamin \( D_3 \), such as in chronic renal failure. Reproduction and teratology studies were performed in rats and rabbits using dosages of 0.02, 0.08, and 0.3 \( \mu \text{g/kg/day} \) of calcitriol administered by oral intubation as a solution in Neobee oil. Rabbits treated from Day 7 through 18 of gestation with 0.3 \( \mu \text{g/kg/day} \) exhibited mortality in 3 of 15 rabbits, a marked maternal weight loss, an increased resorption rate, and an increase in neonatal mortality during the first 24 hr after birth. Lower dosage groups were apparently normal. Two litters at 0.3 \( \mu \text{g/kg/day} \) and one litter at 0.08 \( \mu \text{g/kg/day} \) contained fetuses with multiple abnormalities. In rat teratology studies no substantial differences were noted between controls and animals treated with calcitriol from Days 7 through 15 of gestation with respect to litter sizes, resorption rates, pup weights, or external, visceral, and skeletal abnormalities. No adverse effects on either fertility or the usual parameters of neonatal development were noted in rat reproduction studies in which males were pretreated for 63 days and females were pretreated 2 weeks prior to mating through sacrifice on Day 13 of gestation or through Day 21 of lactation. No adverse effects on perinatal development were noted in litters from females treated from Day 15 of gestation through Day 21 of lactation. Hypercalcemia and hypophosphatemia were observed in treated female rats at sacrifice on Day 21 of lactation at dosages of 0.08 and 0.3 \( \mu \text{g/kg/day} \) and increased serum urea nitrogen was observed at 0.3 \( \mu \text{g/kg/day} \). Hypercalcemia was noted in pups from dams receiving 0.08 and 0.3 \( \mu \text{g/kg/day} \) of calcitriol. There was no decrease in the percentage bone ash as determined on Day 21 of lactation in either treated females or their pups when compared to controls at the dosages studied. The studies demonstrate that in pregnant animals the effects of calcitriol are similar to those reported for vitamin \( D_3 \). In rabbits, dosages of 0.3 \( \mu \text{g/kg/day} \) produce maternal and fetal toxicity. In rats, except for changes in serum chemistry values, no adverse effects on reproduction or pup development were noted at dosages up to 0.3 \( \mu \text{g/kg/day} \) of calcitriol.

The synthetic pyrethroid insecticide, resmethrin, was evaluated to assess the risks associated with its proposed use as an impregnant on military fabrics. Acute and repeated applications of the material to rabbit skin failed to produce irritant or aeneform reactions. Cotton sateen cloth impregnated with resmethrin produced only a slight irritant reaction in a 24-day wear test with rabbits. No teratologic effects were found in a study in which up to lethal doses of chemical were added to ground feed. In vitro mutagenic (Ames) testing did not indicate mutagenic activity. Daily ingestion of 1500 mg/kg was needed to kill Sprague-Dawley or Long-Evans rats in 14- and 90-day feeding studies. Daily intravenous doses of up to 25 mg/kg for 15 days in beagle dogs produced no toxic signs, gross toxic effects, or compound-related changes in major enzyme systems. Based on these tests, a safe impregnation level, for use under predefined conditions, was recommended.

56. The Pathogenesis in Guinea Pigs of Cecal and Colonic Ulceration Induced by Two Sulfated Polysaccharides. T. Barbolt, G. Estel, R. Abraham, and F. Coulston, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

A comparative study of the ulcerogenic process in the large bowel was undertaken using two sulfated polysaccharides; a degraded carrageenan (C16) which is stored indefinitely in lysosomes without degradation, and dextran sulfate, which is hydrolyzed by lysosomal enzymes. Male Hartley strain guinea pigs were used: Group I, control; Group II, 5% C16; and Group III, 0.2% dextran sulfate in 5% sucrose. Both materials were given in ad libitum, the C16 for 2 weeks and the dextran sulfate for 11 days. The ulcerogenic process induced by C16 is initiated by uptake of the macromolecule into lysosomes of macrophages in the lamina propria which stimulates the release of lysosomal enzymes (acid phosphatase and β-glucuronidase), subsequently damaging the surrounding tissue. Early lesions with dextran sulfate were characterized by focal loss of glands, sloughing of surface epithelium, and degeneration of crypts. There was some storage of dextran sulfate in lysosomes of macrophages in the lamina propria, but not to the extent seen with C16. Later, extensive loss of surface epithelium and glands was noted, but the thickness of the lamina propria was normal. Mesenchymal cells were prominent in the lamina propria, and a diffuse acute inflammatory response was present. These findings suggest that the process of ulcerogenesis is different when induced by an indigestible macromolecule compared to one that is degraded. C16 induces ulceration by storage in macrophage lysosomes which appears to play a major role, whereas dextran sulfate probably causes direct damage to the epithelium. (Supported in part by NIEHS Research Grant 2-P01-ES00226-10, NIH Training Grant 3-T01-ES00103-10, and Research Career Development Award 1-K04-ES70608-05.)

57. Lysosomal Mechanisms in Experimental Hepatic Porphyria in Rats. R. Abraham and N. Ringwood, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

A variety of xenobiotics produce hepatic porphyria in animals that closely parallel the situation in man. To alleviate porphyria cutanea tarda, patients are usually administered a single therapeutic dose of chloroquine. In the present investigation an experimental animal model was used to elucidate the events involved in the therapeutic response of hepatic porphyria (porphyria cutanea tarda, PCT) to chloroquine. Male Sprague-Dawley albino rats (150–200 g) were divided into four groups: Group I, control; Group II, a single oral dose of 100 mg/kg of chloroquine; Group III, 300 mg/kg of 3,5-dicarboxethoxy-1,4-dihydro-2,4,6-trimethyl pyrine (DDC) given orally once a day for 5 days; Group IV, DDC given for 5 days and then given a single dose of chloroquine. Rats from Groups II and IV were killed at 2, 6, and 24 hr after treatment with chloroquine; and those from Groups I and III after 5 days. Ultrastructural examination of
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livers from rats given DDC revealed many spicule-, tubular-, or fibril-shaped crystals found in lysosomes or free in the cytoplasm of hepatocytes and Kupffer cells, and occasionally in bile ducts. The crystalline deposits exhibited a characteristic cross birefringence when viewed under polarized light. Livers from animals given chloroquine subsequent to DDC-induced porphyria exhibited a marked decrease in liver porphyrin storage as early as 2 hr after chloroquine treatment. Twenty-four hours later no observable crystalline formations were noted in liver cells. "Sunburst"-like arrangements of crystals were seen more frequently in bile canaliculi of livers. These findings suggest that: (a) porphyrins are stored in lysosomes and (b) that chloroquine relieves this condition by labilizing lysosomes and initiating an autophagic response bringing about fusion between "porphyrin-laden lysosomes" and chloroquine-induced lysosomes, leading to their eventual exocytosis into the bile canaliculi. (Supported in part by NIEHS Research Grant 2-P01-ES00226-10, NIH Training Grant 3-T01-ES00103-10, and Research Career Development Award 1-K04-ES07068-04.)


Liver growth may be due to either hypertrophy and/or hyperplasia. Both processes share some biochemical events, including DNA synthesis. The factors determining whether a cell will divide (hyperplasia) or remain blocked at G1 in the cell cycle (hypertrophy) are generally unknown. An understanding of these events would greatly improve our knowledge of the factors that control the proliferation of cells by xenobiotics. Previous studies suggested that the cell cycles of tetraploid and octoploid hepatocytes in mouse liver were influenced by PCB (Aroclor 1254) and Mirex. Mirex appeared to block liver cell replication at the G2 phase, producing hepatocytes of higher ploidy, whereas a combination of PCB and Mirex triggered the hepatocytes into mitosis from the G1 block and decreased the number of tetraploids and octaploids. To evaluate further the mechanisms involved in these processes two immunosuppressants were used: cortisone acetate, which stimulates cells into dividing, and azothioprine, which blocks cell division. Adult male CD-1 mice were used: Group I, corn oil; Group II, cortisone acetate (50 mg/kg); Group III, Mirex (4.5 mg/kg); Group IV, Mirex + cortisone acetate; Group V, azothioprine (40 mg/kg); Group VI, PCB (25 mg/kg) + Mirex; Group VII, PCB + Mirex + azothioprine. All chemicals were administered po except cortisone acetate, which was given im. Groups II and IV had reduced nuclear volumes of diploids and tetraploids, whereas Group III (as previously reported) had increased tetraploid and octaploid volumes. As reported earlier, Group VI had decreased diploid and increased octaploid volumes; this effect was not altered by azothioprine. Autoradiographic analysis with [3H]lthymidine showed increased hepatocyte labeling in the midzonal and central regions of Groups III, VI, and VII, but not in Groups II and IV. Cortisone acetate appeared to overcome the G1 block induced by Mirex whereas azothioprine failed to block cell division in Group VI. (Supported in part by NIEHS Research Grant 2-P01-ES00226-10, NIH Training Grant 3-T01-ES00103-10, and Research Career Development Award 1-K04-ES07068-04.)

59. Results of Two-Year Toxicological Studies in Rats of Vinylidene Chloride Incorporated in the Drinking Water or Administered by Repeated Inhalation. L. W. Rampy, J. F. Quast, C. G. Humiston, M. F. Balmer, and B. A. Schweitz, Toxicology Research Laboratory, Health & Environmental Research, Dow Chemical Company, Midland, Michigan.

Male and female Sprague–Dawley rats were exposed to vinylidene chloride (VDC) orally or by inhalation in 2-year toxicological studies. VDC was given in the drinking water at concentrations (mean ± SD) of 0, 68 ± 13, 106 ± 22, and 220 ± 35 ppm which produced dosage levels of 0, 5.9 ± 0.6, 10.0 ± 1.2, and 19.3 ± 2.7 mg/kg for male rats and 0, 7.5 ± 0.4, 12.6 ± 1.1, and 25.6 ± 2.4 mg/kg for female rats. Forty-eight rats/sex/VDC level and 80 rats/sex in the control group were used in the 2-year study with an interim kill of an additional 10 rats/sex/level at 90 days. In the inhalation study, rats were exposed to 0, 10, or 40 ppm of VDC vapor 6 hr/day, 5 days/week for 5 weeks, after which the exposure levels were changed to
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0, 25, and 75 ppm of VDC. Exposure continued for a total of 18 months and the rats were held for observation an additional 6 months. Interim kills occurred at 1, 6, and 12 months. A separate 90-day study using 20 rats/sex/level was conducted at 0, 25, and 75 ppm of VDC vapor. There were 86 rats/sex/level in the 2-year portion of the study. The parameters monitored were: body weight, food and water consumption (drinking water study only), hematology, clinical chemistries, cytochemistry of bone marrow cells (inhalation study only), mortality, terminal organ weights and gross, and histopathology. In the drinking water study, there was increased cytoplasmic vacuolation of hepatocytes in the livers of male rats of the highest dose level and females of all three dose levels. Based on gross and histopathologic evaluation, tumor incidence in rats given VDC in drinking water was not greater than in control rats. Increased cytoplasmic vacuolation was also seen in the livers of male and female rats exposed by inhalation to either level of VDC and sacrificed after 3, 6, and 12 months of exposure. Based on gross autopsy data, tumor incidence in rats exposed to VDC vapor was not greater than in control rats. Microscopic examination is being conducted on the tissues collected at the termination of the inhalation study.


Butylated monochlorinated diphenyl oxide is a dielectric fluid for use by the electrical industry in power capacitors. A subchronic toxicity study in which rats were administered the dielectric fluid in their diet at levels providing 0, 5, 15, 45, and 90 mg/kg/day for up to 156 days has indicated a low order of toxicity. Gross and histopathologic examinations showed reversible changes in the livers and kidneys of the animals on the two highest levels of treatment; the livers and kidneys of animals on the two lowest levels of treatment were similar to the controls. Treatment-related decreases in body weight gain were observed in both male and female rats. All other parameters evaluated were comparable between treatment and control groups. The concentration of the dielectric fluid components in tissues (in order from lowest to highest, plasma, liver, brain, muscle, and kidney) from rats receiving 90 mg/kg/day were low, from the detection limit (<0.05 µg/g by GC–MS) to 4 µg/g, compared to fat which was 160 to 180 µg/g. The fat from rats receiving 5 mg/kg/day, the no-adverse-effect dose level in this study, contained about 9 µg/g of the dielectric fluid components at the apparent plateau, which was reached in 30 to 60 days. The apparent plateau was in agreement with the results of a single-dose pharmacokinetic study.

61. Chronic Toxicity of 2,4-Dinitrotoluene in the Rat. H. V. Ellis, III, C. B. Hong, J. C. Dacre, and C. C. Lee, Midwest Research Institute, Kansas City, Missouri, and U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, Maryland.

Four groups of 38 male and 38 female Charles River CD albino rats were fed diets containing 0% (control), 0.0015% (low), 0.01% (middle), and 0.07% (high) 2,4-dinitrotoluene (2,4-DNT). Most rats were fed 24 months, then necropsied. There were intermediate necropsies after 12 months and 1-month recovery studies after both necropsies. Intake of 2,4-DNT by males and females in the low-dosage group averaged 0.58 and 0.71 mg/kg/day; in the middle-dosage group, 3.9 and 5.0 mg/kg/day; in the high-dosage group, 35.0 and 46.0 mg/kg/day, respectively. From the start of the study, the rats fed the high dose had decreased feed consumption and weight gain, leveling off after 9 months, near 480 and 260 for males and females versus 700 and 400 for the control rats. Periodic hematological examinations revealed that high-dose rats had mild anemia with compensatory reticulocytosis. Characteristic lesions found in high dose rats at 12- and 13-month necropsies included testicular atrophy with aspermatogenesis; hyperplastic foci, nodules, and hepatoma in the liver; splenic hemosiderosis and/or extramedullary hematopoiesis. Partial recovery of the testicular and splenic lesions was found after withdrawal
of 2,4-DNT. Unscheduled deaths occurred starting the 13th month in the high-dose rats and the 17th month in the other groups; half the high group had died by Month 20. Most high-dose rats had an increased incidence of tumors in various organs; those surviving longer had hepatic masses, presumably hepatomas. Histopathology in progress will clarify, but the high dose seems toxic, the middle dose equivocal, the low dose nontoxic. (Supported by U.S. Army Medical Research and Development Command under Contract No. DAMD-17-74-C-4073.)

62. Inhalation of Sulfate Particulates. I: Effects on Growth, Pulmonary Function, and Locomotor Activity. J. P. Lewkowski, L. Hastings, A. Vinegar, J. Leng, and G. P. Cooper, Department of Environmental Health, University of Cincinnati College of Medicine, and Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

The toxicity of H₂SO₄, SO₂, Al₃(SO₄)₃, and K₂SO₄ was studied in rats and guinea pigs in five different experimental exposures 7 to 14 weeks in duration. Each exposure consisted of two different test atmospheres plus a control atmosphere of clean air. The effects of these exposures on food and water consumption, growth, forced activity, spontaneous activity (SLA), and pulmonary function were assessed. Exposure to Al₃(SO₄)₃ (2.0–2.6 mg/m³) was the most detrimental. Pulmonary function studies indicated that Al₃(SO₄)₃ having a mass median aerodynamic diameter (MMAD) of 2.0 μm increased the static deflation volumes of juvenile rats, decreased the static deflation volume of guinea pigs, and caused an increased pulmonary resistance and respiratory rate in adult rats. However, exposure to Al₃(SO₄)₃ having an MMAD of 1.4 μm caused primarily an increased respiratory rate and decreased lung compliance in adult rats. SLA during exposure and treadmill performance for up to 80 days postexposure was significantly decreased by exposure to Al₃(SO₄)₃ having an MMAD of 1.4 μm. Exposure to 4 mg/m³ H₂SO₄ (MMAD of 0.5 μm) increased pulmonary resistance and respiratory rates in adult rats. However, at lower concentrations (2.0–2.5 mg/m³) little or no effects were observed except for a depression in SLA during exposure. K₂SO₄ (1.3 μm; 2.0 mg/m³) and SO₂ (1 mg/m³) had little or no effect. In none of these studies were there significant effects on growth or food and water consumption. We conclude that the Al cation is more important in determining toxicity than acidity or the sulfate anion, and that most of the effects observed are probably attributable to pulmonary insult. (Supported by EPA Contract 68-03-0492 and NIEHS Grant ES-00159.)

63. Inhalation of Sulfate Particulates. II: Pulmonary Biochemical Effects. V. N. Fineilli, S. D. Lee, R. M. Danner, J. Boiannon, L. McMillan, and G. P. Cooper, Department of Environmental Health, University of Cincinnati College of Medicine and Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

A series of experiments on inhalation toxicity of sulfuric acid, aluminum sulfate, and potassium sulfate were conducted in adult and newborn rats to discern whether the effects of sulfates are due to the SO₄²⁻ anion or to the related cations. The parameters measured in this study were primarily those involving pulmonary alveolar macrophage (PAM), extracellular lysozyme of the lung lavage, and the permeability of the alveolar wall as measured by the leakage of intravenously injected ¹³¹I-labeled albumin into the alveolar fluids. Aluminum sulfate showed much greater toxicity than sulfuric acid and potassium sulfate. A significant increase of PAM was observed in adult and young rats exposed to either aluminum sulfate or to sulfuric acid, while a decrease was seen in the potassium sulfate-exposed animals. However, the morphology of the macrophages from the rats exposed to aluminum sulfate showed abnormalities such as swelling and granulation while these cells appeared normal in the animals exposed to the other compounds. A dramatic increase of extracellular lysozyme and ¹³¹I-labeled albumin in the lung lavage of the aluminum sulfate-exposed rats, but not in the remaining animals, indicates that the toxicity is due to Al³⁺ and not to the SO₄²⁻ ion. Regression analysis between the extracellular lysozyme and the ¹³¹I radioactivity in the lung lavage yielded a highly significant positive correlation (r = 0.829, n = 125). (Supported by EPA contracts 68-03-0492 and 68-03-2011 and NIEHS Grant ES-00159.)
64. *Maturation of Sulfite Oxidase Activity in the Rat and Rabbit.* R. E. Gregory and A. F. Gunnison, New York University Medical Center, Institute of Environmental Medicine, New York, New York. (E. D. Palmes)

Sulfite oxidase (EC 1.8.3.1), a mitochondrial enzyme, catalyzes the oxidation of sulfite to sulfate; its deficiency in human infants has been reported to cause fatal neurological damage. Therefore, the maturation of this enzyme is an important factor in the protection of the fetus and neonate from sulfite intoxication. Others have shown by an in vitro assay technique that sulfite oxidase activity in rat liver increases slowly from birth, requiring 10 to 15 days to attain 50% of the adult complement of enzyme. Using a similar assay method we have confirmed in Wistar rat liver the general developmental pattern observed by these workers and, furthermore, have shown a similar pattern in rat kidney. Asymptotic regression functions fitted to our data indicate good agreement between data and model. Sulfite oxidase activity in New Zealand white rabbit liver and kidney showed developmental patterns which differed markedly from each other as well as from the same tissues in the rat. Rabbit kidney showed no substantial change in specific activity from birth to adulthood. In contrast, however, the specific activity in rabbit liver increased from approximately 50% of the maternal levels at birth to a level two to three times greater than maternal levels at approximately 3 weeks of age. This pattern of maturation in rabbit liver resembles that exhibited by some microsomal enzymes. (Supported by NIEHS Grants ES00617, ES00005, and ES00260.)


Inhaled sulfur dioxide and ingested or injected sulfite salts have previously been shown to split reactive disulfide bonds in the plasma proteins of several mammals producing plasma S-sulfonate compounds. A study designed to investigate the possible presence of similarly reactive disulfide bonds in other mammalian tissues showed that highly elastic tissues such as blood vessels, lung, and trachea contained measurable quantities of protein S-sulfonates following in vivo treatment with sulfite. First-order rate constants for the formation of protein S-sulfonates in these tissues in the rabbit have been determined as has their biological stability. All available evidence suggests that the reactive disulfides in these tissues are located in the microfibrillar protein associated with elastin. (Supported by NIEHS Grants ES00617, ES00005, and ES00260.)


Chemically defined diets were fed to mice to produce lung lipids having either high or low unsaturated fatty acid composition. Vitamin E was supplemented at 0, 10.5, or 105.0 mg/kg of diet as a-tocopheryl acetate. Mice fed unsaturated fat diets had a significantly higher peroxidizability index (PI), reflecting the higher unsaturated fatty acid composition of their lung lipids. While vitamin E supplementation did not alter the composition of the lung lipids of air-exposed mice, high vitamin E (105.0 mg/kg) increased the LT50 to 45 days compared to 31 days for mice receiving either 0 or 10.5 mg/kg of vitamin E and exposed continuously to 1 ppm of ozone. High vitamin E and unsaturated fat diets delayed the onset of mortality and prolonged the LT50. Pulmonary edema was evidenced by increases in the lung wet weights of mice exposed to 0.64 ppm of ozone for 5 weeks. Mice fed saturated fat diets and low vitamin E (10.5 mg/kg) had lungs of a mean weight of 365.5 ± 13.5 mg, while mice fed unsaturated fats and high vitamin E (105.0 mg/kg) had a mean weight of 221.7 ± 6.1 mg. Mice fed no or low vitamin E and either unsaturated or saturated fat diets had a mean of 292.5 ± 17.7 mg. The lung weights of all of the exposed animals were significantly greater (about 50%) than the comparable air exposed groups. The apparent protection afforded by high vitamin E levels in combination with unsaturated fat
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diets was statistically significant as was the apparent deleterious effect of saturated fat diets and low vitamin E (p < 0.01). Ozone exposure caused alterations in the composition of lung lipid fatty acids depressing palmitic, palmitoleic, and arachidonic acid levels and elevating the stearic and oleic acid levels. Mice fed high levels of vitamin E had intermediate values between those of the air-exposed animals and the ozone exposed animals fed either no or low vitamin E. These data suggest that the dietary intake of vitamin E is the major contributing factor to the morbidity of ozone. The relative PI, a proposed index of lability to oxidant attack, had little or no relationship to morbidity. The onset of mortality did appear dependent on the dietary fat composition. The loss of arachidonic acid suggests peroxidation in vivo of tissue unsaturated fatty acids, but the relationships between tissue vitamin E and unsaturated lipids during oxidant stress are more complex than previously proposed from in vitro models. Higher dietary levels of vitamin E may be required regardless of dietary fat intake for those individuals exposed to oxidizing air pollutants. (Supported by NIH Grants 5 R01 ES00798 and 5 T32 ES07002 and by the EPA.)

67. A Rapid and Specific Gas Chromatographic Analysis for Cysteine-Sulfonate in Hydrolyzates of Sulfonated Proteins. J. D. De Bethisy and J. C. Street, Toxicology Program, Utah State University, Logan, Utah.

It has been shown in previous studies that when SO₂ is absorbed by rabbits via either inhalation or oral exposure, the hydrated form, bisulfite, interacts with plasma proteins where it is suspected to be in the form of cysteine-S-sulfonate residues. A rapid and specific gas chromatographic analysis procedure for cysteine-S-sulfonate has been developed to better study the distribution of sulfite in biological systems. Sulfonated proteins are enzymatically hydrolyzed to ensure stability of the labile S-sulfonate bond. The hydrolyzate is then applied to a 7-cm cation-exchange column and eluted with 0.01 N HCl which elutes the acidic amino acids, including the cysteine-S-sulfonate, with the void volume of the column, leaving behind any remaining cysteine. The silylated derivatives of the column effluent are prepared using BSA. These derivatives are injected into a gas chromatograph equipped with a flame-photometric detector and sulfur filter, 1.95% OV-17 on a Gas-Chrom Q 1/16-in. glass column, operating at an oven temperature of 125°C. A ratio of cysteine-S-sulfonate to total cysteine in the sulfonated protein is obtained by derivatizing an aliquot from the hydrolyzate before and after the ion-exchange chromatography. The presence of cysteine-S-sulfonate has been substantiated by amino acid analysis on a Beckman automatic analyzer using a Durrum single column system. The method has been applied to analysis of plasma proteins from animals exposed to SO₂.

68. The Effects of Long-Term Exposure to Concentrated Recycled Water. N. Gruener, Gulf South Research Institute, New Orleans, Louisiana.

A study was made to determine the feasibility of reusing treated effluents for drinking purposes. Some 400,000 liters of finished water prepared from domestic sewage from the Washington, D.C. area was concentrated by reverse osmosis to a volume of 200 liters. The concentrate contained 700 mg/liter of total organic carbon (TOC), approximately one-third of the original organic content. Five hundred mice (B6C3F1), half male and half female, were distributed among five groups (one control and four experimental) and exposed to the concentrate for 90 days. The mice were exposed to TOC levels 50 to 5000 times the predicted daily human intake. Another 300 mice were exposed throughout gestation—lactation and then for an additional 90 days. General toxicological parameters, such as body weight and food consumption, as well as more specific assays (hematological, blood chemistry, behavioral assessment, measurement of mixed-function oxidase activity in the liver, and mutagenic assays) were carried out. The toxicological and public health implications will be analyzed.

69. Fate of Bis(2-chloroethyl) ether in Rats after Acute Oral Administration. R. D. Ligg, W. H. Kaylor, J. W. Glass, S. M. Pyle, and R. G. Tardiff, Environmental Protection Agency, Cincinnati, Ohio.

Bis(2-chloroethyl) ether (BCEE) has been identified as a contaminant in several U.S. municipal drinking water supplies and has been reported by Innes et al. (J. Nat. Cancer Inst. 42,
1101–1114, 1969) to have carcinogenic properties. Consequently, studies were undertaken to determine the fate of this compound upon ingestion in the rat. Bis([2-14C]chloroethyl) ether (40 mg/kg) was administered to male Sprague–Dawley (CD) rats by intubation. Animals were placed in glass metabolism cages equipped with traps for urine, feces, and expired air. At 24 hr after exposure the animals were sacrificed, and samples of all major organs and G.I. contents were taken for 14C assay. Preliminary results show that virtually all BCEE is excreted transformed and that more than 60% of the compound is excreted within 24 hr as urinary metabolites. The major metabolites of [14C]BCEE were separated and identified by gas chromatography–mass spectrometry. Metabolic profiles in urine of control and treated rats were compared, and those peaks unique to the dosed sample were identified. One major metabolite was thiodiglycolic acid (TDGA). Lesser metabolite was identified as 2-chloroethanol-β-D-glucuronic acid. The presence of these two metabolites demonstrates that cleavage of the ether linkage is a major step in biotransformation of BCEE. The products of this cleavage then conjugate with the nonprotein free sulphydryl groups or with glucuronic acid. Quantitative analysis of TDGA shows that the former is the major route of conjugation by the rat.

70. Results of a Ninety-Day Inhalation Toxicity Study of 1,2,3-Trichloropropene in Laboratory Animals. M. J. Mckenna, J. F. Quast, and G. A. Stevens, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

Male and female Sprague–Dawley rats, Golden Syrian hamsters, and male beagle dogs were exposed to 0.3, 10, or 25 ppm of 1,2,3-trichloropropene (TCP) for 6 hr daily, 5 days/week, for a total of 65 to 67 exposures in 92 to 94 days. Body weights were monitored throughout the study and all animals were observed daily for signs of toxicity. Selected hematology, urinalysis, and clinical chemistry parameters were evaluated. Hepatic nonprotein sulphydryl (NPSH) assays were conducted on separate groups of rats and hamsters following single and repeated (66 or 67) exposures to the test material. Gross and microscopic pathological examinations were conducted at the termination of the experiment. Weights of specified organs were recorded and organ/body weight ratios were calculated. Eye irritation was observed in dogs exposed to 10 and 25 ppm of TCP. Other adverse effects were limited to animals of the 25-ppm exposure group and included: eye and upper respiratory tract irritation in rats and hamsters, respectively, decreased body weights in male hamsters and rats of both sexes, elevated SGPT activity in rats of both sexes, and decreased liver cell size in livers of male hamsters. Single inhalation exposures to TCP produced a dose-related decrease of hepatic NPSH concentrations in both rats and hamsters which was not observed following repeated exposures. The decrease in liver NPSH concentrations suggests that liver glutathione may play a major role in the metabolism and detoxification of TCP. Recovery of NPSH concentrations to normal following repeated exposure to TCP indicates the presence of a compensatory mechanism responsive to the maintenance of sufficient glutathione to detoxify the test material.

71. The Toxicity of Butadiene to Rats by Inhalation. C. N. Crouch and D. H. Pullinger, Hazleton Laboratories Europe Ltd. (R. Scala)

Five groups of Sprague–Dawley rats were exposed to 1,3-butadiene gas at concentrations of 0, 1000, 2000, 4000, and 8000 ppm, respectively, 6 hr/day, 5 days/week for 13 weeks. Forty male and forty female animals were used in each group. Exposure took place in five chambers, each of 8 m³ capacity, in an area prepared specifically for the handling of a potentially explosive gas at concentrations just below the lower explosive limit. Determination of the distribution of butadiene in each chamber and regular monitoring of the concentration of butadiene, 4-vinyl-1-cyclohexene, t-butyl catechol, and other impurities in the chamber during exposure ensured controlled treatment conditions. During the last 6 to 8 weeks of exposure, moderately increased salivation was observed, particularly at higher concentrations of butadiene, but no other clinical signs of note were recorded. No adverse effects on growth rate or food consumption were seen. Haematological and blood biochemical investigations and urine analysis after 2, 6, and 13 weeks
exposure showed no treatment-related effects. Investigations using a modified rotating rod test showed no treatment-related impairment of neuromuscular function after 13 weeks of exposure. Macroscopic and histopathological examination after 2, 6, and 13 weeks exposure showed no untoward changes related to exposure to butadiene gas. (Sponsored by the International Institute of Synthetic Rubber Producers.)


Krypton-85, a long-lived radioactive gas, is a major effluent released to the environment during the dissolution of nuclear fuel elements during reprocessing. These investigations were undertaken to provide quantitative data for determining whether 85Kr exposure represents a particular hazard to fetuses, which are known to be more radiosensitive than are adults. Fourteen gravid ewes, 73 to 123 days of gestation, were prepared 1 to 2 days before exposure by cannulation of maternal and fetal arteries and veins. Blood concentrations were monitored during and after a 1.5 to 2.0-hr exposure to air containing approximately 50 μCi/liter of 85Kr. Equilibrium was reached less rapidly in the fetus than in the ewe, but samples from all vessels attained steady-state levels of approximately 13.5 nCi/liter by 1 hr of exposure. Mean radioactivity in maternal arterial blood dropped to 10% of the maximum level by 9 min post-exposure; 32 and 60 min were required for the maternal venous and fetal blood, respectively, to reach this value. Most ewes were reexposed 1 to 2 days later and killed after attaining equilibrium to provide tissue samples for radionuclide analysis. The mean concentrations in adult tissues decreased in the order: lung > lymphoid > adrenal > liver = diaphragm = skeletal muscle = kidney = ovary > small intestine > uterus > large intestine. Most tissues from the 16 lambs followed a similar rank order and concentrations in all fetal tissues were similar to or less than those for the corresponding adult tissue. The 85Kr concentrations in the placenta were significantly higher than in any of the fetal tissues. These data provide a basis for developing a model for estimating the likelihood of producing deleterious effects on the conceptus by inhaled krypton. (Supported by U.S. Energy Research and Development Administration Contract EY-76-C-06-1830.)


Previous work on the metabolism of di-2-ethylhexylphthalate (DEHP) (Lake et al., Toxicol. Appl. Pharmacol. 32, 355, 1975) suggested that the toxic effects of this compound were due to its monooester analog mono-2-ethylhexylphthalate (MEHP). Since very little information was available on the toxicity of MEHP, the present studies were initiated as part of a general program to assess the potential hazard of this compound. The acute oral LD50 values of MEHP in male and female rats were found to be 1800 and 1340 mg/kg, respectively. In a subacute toxicity study male weanling rats (10 per group) were fed diets containing 0, 25, 100, 400, 1600, or 6400 ppm of MEHP for 28 days. The results showed decreased body weight gains at the highest dose, increased liver weights, at the two highest doses, increased heart weights at 1600 ppm, and no effect on food consumption. No gross or microscopic changes were observed on pathological examination. Hepatic microsomal enzyme activity was not affected by MEHP. Serum sorbitol dehydrogenase activity decreased in the 400-, 1600-, and 6400-ppm groups. Hemoglobin and hematocrit levels were decreased in the 25-, 100-, 1600-, and 6400-ppm groups. White blood cell concentration decreased at 100-, 1600-, and 6400-ppm MEHP levels. In a separate teratology experiment female rats were dosed orally with 0, 50, 100, 200, 225, 450, and 900 mg/kg of MEHP daily on Days 6 to 15 of gestation. Doses above 200 mg/kg resulted in maternal toxicity. No fetal anomalies were observed in either the skeleton or viscera above those noticed in the control group. The results presented here indicate that MEHP is more acutely
toxic than DEHP but is not teratogenic in the rat. Longer feeding studies are planned to
determine the significance of changes found in the 28-day study.

74. Effects of Low-Level Exposure to Acrylamide in Water on Spontaneous Locomotor
Activity. J. P. Lewkowski, Y. Y. Yang, J. G. Orthoefer, D. A. Fox, and R. G. Tardiff,
Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati,
Ohio.

In this study, activity has been used as a test to assess acrylamide toxicity. Long–Evans rats
30 to 33 days old were housed in standard 35-cm-diameter activity wheels and were exposed to
acrylamide in water at three different levels, 0, 25, and 100 ppm for 13 weeks. Analysis of
variance indicated a significantly decreased weekly night activity (F = (2, 27) = 11.2, p = 0.0003)
as well as decreased weekly day activity (F (2, 27) = 8.5, p = 0.0014) as a result of this
exposure. Duncan’s test indicated that the night activity of the 100-ppm group was significantly
different from the control and the 25-ppm groups at the 0.05 probability level but that there was
no significant difference evident between the control and 25-ppm group. A subsequent
experiment was undertaken using Long–Evans rats 42 to 44 days old and exposure levels of 0,
50, and 100 ppm. Once again, the analysis of variance indicated that treatment effects were
evident in night activity (F (2, 24) = 6.0, p = 0.0079) but not in day activity (F (2, 24) = 3.2, p =
0.0570). Further analysis was done using the ANOVA by week; if significant results were
obtained, then Duncan’s multiple range tests were performed. This analysis indicated that the
100-ppm group became significantly less active than the 50-ppm and control groups at Week 5
and thereafter. Furthermore, the 50-ppm group did show a significant difference from the control
group during Week 9. The decrease in night activity in the 100-ppm group was evident several
weeks prior to the development of obvious hindlimb neuropathy; such neuropathy was not
observed in the 50-ppm group during the exposure period. In general, no differences were
observed in food and water consumption and body weight gain. Preliminary observations on
pathological specimens indicate focal regions of myelin degeneration present in the sciatic nerve
of the 50-ppm animals after 14 weeks of treatment. A similar change occurs after 4 weeks of
treatment in the 100-ppm group. The relative sensitivity of the behavioral indices are compared
to the histopathologic evaluations.

75. The Relative Neurotoxicity of Methyl n-Butyl Ketone and Its Metabolites. W. J.
Krasavage, J. L. O’Donoghue, and C. J. Terhaar, Toxicology Section, Health, Safety,
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Methyl n-butyl ketone (MnBK) has been associated with peripheral polynuropathy in
humans. Studies in experimental animals have shown MnBK to be neurotoxic and revealed that
MnBK and n-hexane have common metabolites. In addition, studies in animals have shown that
some of these metabolites cause a similar peripheral neuropathy. This communication presents
data from a number of studies in an attempt to compare the relative neurotoxicity of MnBK and
its metabolites. All compounds were administered orally to male rats in the drinking water or by
gavage. The concentration in the water ranged from 0.25 to 1.0% and the doses given by gavage
were from 200 to 1200 mg/kg. Appropriate controls were included. Body weights and water
consumption were recorded and the animals were observed for clinical and morphologic signs of
neuropathy. Neurotoxic effects were seen after the administration of the parent compound
MnBK and with the metabolites 2,5-hexanediol, 2,5-hexanediol, and 5-hydroxy-2-hexanone.
Other metabolites, γ-valerolactone and 2,5-dimethylfuran, were negative.

76. The Oral and Dermal Uptake of Radiolabeled Tris(2,3-dibromopropyl) Phosphate by Rats
Safety Commission, Washington, D.C.

Recent evidence has indicated that the flame retardant tris(2,3-dibromopropyl)phosphate
(TRIS) is both a mutagen and carcinogen. The present experiments were designed to determine
the potential of TRIS for dermal and gastrointestinal absorption. New Zealand white rabbits (2–3 kg) and Osborne–Mendel rats (200–250 g) of each sex were used. For dermal absorption studies, a single application of \(^{14}C\)TRIS (either 0.9 or 0.05 ml/kg) was made to the clipped backs of the animals. For the oral uptake studies, a single dose of 25 mg/kg of \(^{14}C\)TRIS in propylene glycol was administered by gastric intubation. \(^{14}C\)TRIS (0.75 mg/kg) was also administered iv. Urine and feces were collected at 6, 24, 48, 72, and 96 hr. Animals were then sacrificed and samples of blood, brain, liver, kidney, fat, muscle, and gonads were taken. Recovery of isotope for dermally exposed rabbits and rats at both exposure levels ranged from 89.4 to 103.3% with the major route of excretion being the urine. Radiolabel appeared in the urine within 6 hr from the time of application. Rabbits absorbed approximately twice as much radiolabel as rats at both exposure levels (3.73% of the 0.9 ml/kg dose and 14.3% of the 0.05 ml/kg dose). The kidney and liver were the organs of highest specific radioactivity in both species, followed by fat, muscle, gonads, blood, and brain. For both rats and rabbits following either oral or iv exposure, most of the radiolabel appeared in the urine (50–75% within 24 hr and 1–22% within 4 hr). The initial rapid excretion of radiolabel was followed by a slower rate of excretion. Rabbits excreted 74.6 and 77.5% of the oral and iv doses, respectively, in 96 hr. For rats, the corresponding figures were 60.2 and 65.5%. The data show that TRIS is absorbed at a moderate rate from the skin and rapidly from the G.I. tract. Rabbits and rats both retain substantial amounts of the radiolabel up to 4 days following oral or iv exposure.

77. Behavioral Toxicology in Rats of a Mixture of Solvents Containing Substances Subject to Inhalation Abuse by Humans. G. T. PRYOR, L. R. BINGHAM, and R. A. HOWD, Life Sciences Division, SRI International, Menlo Park, California. (Gordon W. Newell)

There are reports of voluntary inhalation of various volatile substances by young members of our society, presumably for their psychoactive effects. Most of the abused substances are mixtures and very little is known about the behavioral and/or toxic effects of such mixtures. We are examining the behavioral and toxic effects of a composite mixture that contains some of the reportedly abused substances. 25% methylene chloride, 5% methanol, 43% heptane, 23% toluene, 2% xylenes, and 1% other hydrocarbons, in young male Fischer rats. Sparging this mixture with air generates an atmosphere containing methylene chloride, methanol, heptane, and toluene in the proportions 80:9:9:2 as measured by gas chromatography. The 30-min LC50 for static exposure of groups of four rats in a 131-liter chamber was 173 mg/liter (total volatiles). Exposure for 10 min (60 to 226 mg/liter) caused a systematic concentration-dependent behavioral syndrome that was correlated with blood concentrations of the four substances. The syndrome observed during the exposure was characterized by a decrease in rearing and grooming, the appearance of ataxia, abnormal scratching, hindlimb flaccid paralysis, and, finally, unconsciousness. The number of symptoms in the sequence increased with increasing chamber concentrations and their onset was earlier. Four intermittent exposures for 10 min with 15 min between exposures caused cumulative effects, whereas recovery after each exposure was nearly complete when the interval was 40 min. Experiments are in progress to determine which of the four components are mainly responsible for these effects and whether or not they interact. (Supported by Contract No. 271-77-3402, National Institute on Drug Abuse.)


Using death as an end point, tetrachloroethylene (TCE) has been shown to produce a greater-than-additive effect when given orally in combination with several other compounds. We have attempted to determine more sensitive indicators which would respond in a synergistic fashion when animals are exposed by inhalation to combinations of organic solvents. Male rats were exposed for 4 hr to various concentrations of TCE, dioxane, butyl ether (BE), acetonitrile (ACN), trichloroethylene (TCP), and dichloropropane (DCP). The serum enzymes, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), glucose 6-phosphatase (G-6-Pase), and ornithine carbamyl transferase (OCT) were measured in rats prior
to exposure, immediately after exposure, and at 24 and 48 hr postexposure. The enzymes, SGOT, SGPT, and OCT, were markedly elevated as a result of exposure to the above compounds whereas G-6-Pase was only occasionally altered. Neither TCE in combination with dioxane, BE, or ACN, nor TCP with DCP resulted in a greater-than-additive effect. On the contrary, in many instances the effects were significantly less-than-additive when tested for interaction.

79. Application of Flow Cytometry for the Evaluation of Myelotoxicity in Experimental Animals. R. D. Irons and P. K. Horan, Department of Pathology, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina, and Department of Pathology, University of Rochester, School of Medicine and Dentistry, Rochester, New York. (James E. Gibson)

The purpose of this study was to investigate the applicability of using laser-based flow cytometry to study the effects of myelotoxic agents on rabbit bone marrow. Discontinuous ficoll hypaque gradients of rabbit bone marrow were used to separate immature blood cell precursors from mature blood elements. An optimum single-step gradient was found using a density of 1.077. Heterophil and band form granulocytes as well as red blood cells were found to pellet out, whereas lymphocytes and blast cell precursors were isolated in the buoyant fraction. Using flow cytometry, cells in the buoyant fraction were shown to have a proliferative index of between 15 and 30%, indicating that 15 to 30% of the precursor cells are in the S, G2, and M phases of the cell cycle. This is compared to a proliferative index of 15 to 25% in humans. This information was then correlated with conventional labeling index studies in the same animals and used to evaluate the effects of multiple bone marrow aspirations on proliferative index and labeling index and the effects of myelosuppressive agents on labeling index, proliferative index, and bone marrow differential cell counts. The combination of these techniques has resulted in the development of a successful rabbit model system for quantitative assessment of bone marrow cytotoxicity.

80. A Subacute Study in the Rat on Graded Levels of Restricted Food Consumption and Its Effect on Tissue to Body Weight Ratios, Serum Clinical Chemistry, and Hematological Parameters. W. Dairman, Department of Toxicology, Hoffmann–La Roche, Inc., Nutley, New Jersey. (E. A. Pfister)

It is often difficult to differentiate between direct toxic effects of a test compound on tissue to body weight ratios, clinical chemistry, and hematological values and those secondary to decreased food consumption. The effect of 14 days of restricted food consumption on these parameters was investigated in the growing rat. Groups of 10 female CD rats (initial mean body weight, 138–139 g) were fed ad lib. (controls) or restricted to 83% (group A), 53% (group B), or 30% (group C) of the control intake for 14 days. At sacrifice (Day 14) the mean body weights (+SE) were 178 ± 3.2, 158 ± 1.7, 119 ± 2.2, and 80 ± 1.3 g for the controls and groups A, B, and C, respectively. Increased tissue to body weight ratios (as a percentage of control) were observed as follows: brain—A, 112%; B, 143%; C, 202%; stomach—A, 118%; B, 140%; C, 157%; lung—C, 134%, kidney—B, 108%; C, 122%; submaxillary gland—B, 116%; C, 115%; thymus—B, 126%; and thyroid—C, 133%. Tissues exhibiting a decrease (as a percentage of control) were: thymus—C, 19%; spleen—C, 51%; liver—A, 90%; B, 78%; C, 64%; uterus—B, 76%; C, 67%; and large intestine—91%. Those which were unaffected by food intake were the heart, small intestine, adrenal, and ovaries. A similar study in male CD rats resulted in food intake restrictions of 62% (group D), 42% (group E), and 25% (group F) of control. Initial mean body weights were 162 to 163 g and final body weights (+SE) were 276 ± 4.2, 204 ± 1.9, 161 ± 1.6, and 104 ± 2.0 for the controls and groups D, E, and F, respectively. Increased tissue to body weight ratios were found for the following: brain—D, 130%; E, 158%; F, 230%; testes—D, 136%; E, 159%; F, 191%; stomach—D, 162%; E, 162%; F, 188%; thyroid—F, 129%; and submaxillary gland—D, 123%; E, 126%; F, 143%. Tissues exhibiting a decrease
were: thymus—E, 82%; F, 25%; seminal vesicles—E, 72%; F, 32%; spleen—E, 80%; F, 54%; liver—D, 75%; E, 68%; F, 60%; and dorsal prostate—F, 67%. Tissues unaffected were: heart, lung, adrenal, kidney, and small and large intestine. At sacrifice group F serum GOT, GPT, and BUN values were elevated by 533, 393, and 215% of the controls, respectively. Serum alkaline phosphate was decreased to 70, 64, and 52% of control in groups D, E, and F, respectively. Serum BUN values were depressed in groups D and E to 54 and 71% of control and SGPT was decreased in group D to 62% of the control. Hemoglobin concentration and hematocrit exhibited a trend toward higher values with increasing food deprivation. Total WBC were depressed to 63, 55, and 53% of the control in groups D, E, and F, respectively.


Studies have shown the commonly used diethylhexylphthalate (DEHP) to have lung toxicity after intravenous injection (Schulz et al., Toxicol. Appl. Pharmacol. 33, 514, 1975) and liver toxicity after repeated oral administration (Lake et al., Toxicol. Appl. Pharmacol. 32, 355, 1975). The toxicity of one of the most acutely toxic phthalate esters, diallylphthalate (DAP), was studied because it showed liver, lung, and intestinal lesions after a single oral dose. Rats were orally dosed with 14C-labeled and unlabeled DAP and were examined after single and repeated dosing regimens. The acute oral LD50 was determined to be 970 mg/kg and necropsy showed liver congestion, lung edema, and peritoneal fluid accumulation with hemorrhage in the lung, liver, and intestinal tract. Most animals died 4 to 6 hr after dosing and had labored breathing before death. Blood, liver, lung, and kidney levels as measured by 14C-labeled on the C-7 of the phthalate portion of the ester peaked at 4 hr, with the kidney showing the highest distintegration per minute per milligram. The liver and lung contained about one-fourth that found in the kidney and were approximately equal. No unchanged DAP could be extracted from the tissues examined. Repeated dosing at 250 and 300 mg/kg showed decreases in liver ethyl morphine demethylase, P-450, and microsomal protein content and increases in the liver/body weight ratio that reached a maximum value after 4 days of treatment. This increase in liver/body weight ratio occurred simultaneously with an increase in the liver wet weight/dry weight ratio, showing the liver weight increase was due to fluid accumulation. The lung was also found to have an increased fluid content. The oral toxicity of DAP appears to be different from that found for DEHP based on gross observations, LD50 values, and liver and lung effects.


Human exposure to sleepwear containing the flame retardant Tris(2,3-dibromopropyl) phosphate (TRIS) may occur for many years. Subchronic 90-day studies were begun in rats to determine if such exposures could produce toxic effects when either TRIS or a primary metabolite 2,3-dibromopropanol (DBPOH) was given. Groups of up to 48 young adult Osborne-Mendel rats received daily gavages of TRIS or DBPOH at doses of 25, 100, or 250 mg/kg in propylene glycol for 13 weeks. Control groups received either vehicle, normal saline, or no treatment. Variable responses investigated included organ to body weight ratios, growth rates, hematological profiles, urine analyses, and morphological changes in liver, kidney, spleen, lungs, brain, adrenal, stomach, bladder, and gonads. Weight gains for males were 34 to 50% less in the test groups and for females were 40% less in the test and vehicle groups in comparison to control values. Liver–body weight ratios were lower for both sexes in the low TRIS group but was higher only in females in the highest dose TRIS group when compared to control. Kidney ratios were 18% lower than controls for both sexes and TRIS groups. Testicular ratios in the test groups were 25% lower than control values. A prominent lesion was present in the kidney of all TRIS-treated rats. There was an increased incidence and severity of chronic nephritis with associated regenerative epithelium, hypertrophy, and dysplasia of renal tubular epithelial cell
nuclei. The complex of changes was more severe with the high dose and among males. With the exception of kidney, no meaningful histopathological alterations were detected in other tissues which could be attributed to TRIS. Changes induced by DBPOH are being analyzed.


The primary rabbit dermal irritation assay as described in the Federal Hazardous Substances Act (FHSA) is used by the Consumer Products Safety Commission (CPSC) to determine the labeling requirements of household products. One of its obvious deficiencies is the descriptive imprecision for preparing the form in which solid materials are applied. Experimental findings show that the form, concentration of test material, and the degree of patch occlusivity are prime factors influencing the degree of skin change. Six commercially available detergents were tested. The effects produced by four anionic products were similar at all concentrations tested. The effects of two others were comparable and were less irritating than the four. In the dry powder form with 4 hr of exposure all the detergents were nonirritating. The effects on the skin were also time related. It was observed that the greater the occlusivity the more severe the skin irritation elicited by all the products. This study demonstrated that concentration, exposure duration, and occlusivity of the patch contributed to the net effect of granular detergents upon the skin of rabbits.


Various methods exist for assessing the irritancy potential of compounds, from the Draize eye and skin tests, which estimate tissue damage, to the more objective, such as the mouse writhing test, which assess pain (sting/burn) response. In order to establish a toxicologic profile on surfactants of interest, including possible ranking as far as degree of irritancy and development of a predictive procedure as far as extrapolation of effects to humans, a study was undertaken employing several of the current methods. The mouse writhing test, mice were injected ip with 0.2 ml of the test surfactant and classical writhing, as well as other symptoms, were indicative of a positive response. Surfactants were ranked from least to most irritating, as follows: sodium lauryl polyether sulfate (SLES), Miranol MHT (M-MHT), Miranol C2M (M-C2M), triethanolamine lauryl sulfate (TEALS), sorbitan monopalmitate (SMP), stearalkonium chloride (SCL), sodium lauryl sulfate (SLS), ammonium lauryl sulfate (ALS), sodium coco methyl tauride (SCMT), benzethonium chloride (BCL), and stearyl dimethyl benzyl ammonium chloride (SDMBAC). Additionally, irritancy (sting/burn) was characterized by the upper respiratory tract irritancy test. RD50 values (concentration producing a 50% inhibition of respiratory rate) were determined in mice via the inhalation route. Surfactants tested were ranked as follows, from least to most irritating: SLES, M-C2M, M-MHT, SCMT, TEALS, ALS, and SLS. A third method, the blepharospasm test, was used to evaluate surface irritancy by instilling a 0.01 ml volume into rabbit eyes and observing blepharospasm as indicative of a positive response. BD50 values (dose that was needed to produce blepharospasm in 50% of the animals) were determined and the following ranking was made, from least to most irritating: M-MHT, SLS, SCMT, ALS, TEALS, SLES, and M-C2M. Rankings with this test were at variance from those of previous tests, perhaps due to the influence of eye anesthesia which was elicited by the surfactants. Standard Draize eye and skin tests yielded the following rankings based on tissue damage, from least to most irritating: TEALS, SCMT, ALS, and SLS. Interestingly, with both tests, no difference in irritancy could be established between surfactants in the undiluted form or the highly diluted solutions. Ranking could only be made with intermediate dilutions but was inconsistent.
85. *The Pharmacokinetics and Metabolism of Aniline Hydrochloride in Fischer 344 Rats.*

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The purpose of this study was to determine the absorption, distribution, elimination, and metabolism of aniline hydrochloride in Fischer rats. In pharmacokinetic studies, male rats were administered [¹⁴C]aniline HCl by oral gavage at three separate doses: 10, 30, and 100 mg/kg. At selected times after dosing, the rats were sacrificed and the following tissues were analyzed for radioactivity: brain, lung, kidney, liver, spleen, heart, and plasma. Urine and feces were collected at 12-hr intervals for 48 hr after treatment. Peak plasma radioactivity was observed at 0.5, 1.0, and 2.0 hr for the 10, 30, and 100 mg/kg doses, respectively, and by 24 hr after dosing plasma radioactivity concentrations had decreased to less than 2% of peak concentrations for all doses. Radioactivity was distributed to all tissues, with highest peak levels observed in kidney, followed by liver, plasma, lung, heart, spleen, and brain for all doses. By 48 hr after dosing, less than 0.1% of total administered radioactivity of any of the three doses remained in any of the tissues examined. Recovery of administered radioactivity excreted in the urine 48 hr after dosing was 96, 91, and 77% for the 10, 30, and 100 mg/kg doses, respectively. Approximately 0.1% of urinary radioactivity was chloroform extractable from pH 8 urine. In other experiments, rats were gavaged with 100 mg/kg of nonradioactive aniline HCl and urine collected for 8 hr. Acid hydrolysis of the urine yielded 42.3% recovery of the dose as *p*-aminophenol, while hydrolysis with β-glucuronidase/arylsulfatase yielded 16.2% recovery as *p*-aminophenol. These results indicate that aniline HCl is rapidly eliminated from the rat in the urine, particularly as an acid-hydrolyzable metabolite of *p*-aminophenol.

86. *Subacute Oral Toxicity of TNT and a TNT/RDX Mixture to Dogs and Rodents.* James V. Dilley, Charles A. Tyson, Daniel P. Sasmore, Ronald J. Spanggord, Gordon W. Newell, and Jack C. Dacre, SRI International, Menlo Park, California, and U.S. Army Medical Bioengineering Research and Development Laboratory, Fort Detrick, Frederick, Maryland.

The objective of this study was to evaluate the toxicity of munitions compounds that find their way into wastewaters at Army ammunition plants. Rats and mice were fed TNT (2,4,6-trinitrotoluene) or TNT/RDX (TNT/1,3,5-trinitrohexahydro-1,3,5-triazine) 1.6/1 mixtures in their diet for 90 days. Dogs were dosed daily by capsule with TNT or the TNT/RDX mixture for 90 days. The TNT/RDX 1.6/1 mixture is representative of untreated wastewaters from Army ammunition plants conducting load, assemble, and pack operations. During the treatment period, the highest dose rats and mice (0.25 and 0.125% TNT in the diet, respectively, and 0.5% TNT/RDX for both species) exhibited weight loss, reduced food intake, and red urine. Dogs on the highest dose (20 mg/kg/day of TNT or 50 mg/kg/day of TNT/RDX mixture) exhibited weight loss, ataxia, nystagmus, and other neurological signs, as well as an orange urine. Hematological changes in all three species (high dose only) included a reduced red cell count, reduced hemoglobin, hematocrit, and an increased red cell volume with increased numbers of reticulocytes. Clinical chemistry changes included increased cholesterol and decreased SGPT activity in rats and dogs. Histopathological findings included hemosiderosis of the spleen, and sometimes the liver, in all three species receiving the highest doses. Testicular atrophy with focal interstitial cell hyperplasia was seen in the rats receiving 0.25% TNT diets. This was also seen in rats receiving 0.5% TNT/RDX and dogs receiving 50 mg/kg/day of TNT/RDX. Female rats receiving 0.5% TNT/RDX were found to have a hypoplasia of the uterus. No-effect levels of TNT in the diets of rats and mice were 0.01 and 0.005%; for TNT/RDX, these values were 0.003 and 0.005%, respectively. In dogs, the no-effect level was 0.2 mg/kg/day for TNT and 0.5 mg/kg/day for TNT/RDX. In general, the TNT content of the mixture was dominant in producing toxicity. (Supported by the U.S. Army Medical Research and Development Command, under Contract No. DAMD 17-76-C-6050.)

Previous studies in our laboratory have indicated that increased covalent binding of $[^{14}C]$vinylidene chloride (VDC) metabolites in hepatic tissue is associated with VDC-induced hepatotoxicity. This study was conducted to elucidate the relationship between glutathione (GSH) depletion and hepatic macromolecular binding of $^{14}C$ activity following inhalation exposure to $^{14}C$VDC. Male rats were exposed to constant concentrations of $[^{14}C]$VDC (range, 5–200 ppm) for 6 h. Immediately after exposure the animals were sacrificed and hepatic GSH content and covalent binding of $^{14}C$ activity to hepatic tissue were determined. Hepatic GSH concentrations declined with increasing VDC exposure concentrations. However, appreciable covalent binding of $^{14}C$ activity in the liver was found only after VDC exposures which depleted GSH levels by 30% or greater. The relative distribution of hepatic $^{14}C$ activity in lipid, protein, and nucleic acid fractions was unchanged by the VDC exposure concentration. Preliminary data for mice exposed to $[^{14}C]$VDC indicate an increased ability for production of the reactive metabolite of VDC over that observed in the rat. These studies indicate that VDC undergoes an initial biotransformation in vitro to a reactive metabolite, the detoxification of which is dependent upon the availability of hepatic GSH. Alkylation of hepatic tissue increases markedly when VDC exposures are sufficiently high to produce enough reactive metabolite to exceed the availability of GSH for detoxification. Thus, covalent binding of VDC metabolites to hepatic tissue macromolecules represents a biochemical event which precedes the development of VDC-induced hepatotoxicity.

88. Effect of Hypoxia on the Toxicity of 1,1-Dichloroethylene or Acrylonitrile. Rudolph J. Jaeger, Harvard School of Public Health, Boston, Massachusetts.

1,1-Dichloroethylene (1,1-DCE), a halogenated derivative of ethylene, is acutely hepatotoxic in fasted but not fed rats at 150 to 200 ppm. Acrylonitrile (ACN), a cyano derivative of ethylene, is not hepatotoxic but is acutely lethal to fasted but not fed rats at 100 to 250 ppm. Both compounds decreased the concentration of hepatic nonprotein sulfhydryl groups (expressed as glutathione (GSH)). Both compounds are detoxified in the liver by a GSH-dependent pathway. Are they activated by the mixed-function oxidase system (MFOS) prior to their deactivation, or are they directly reactive with GSH? We had previously shown that Aroclor 1254 (PCB) pretreatment protected the fasted rat from injury following 1,1-DCE exposure. We used hypoxia (7% O$_2$) to inhibit the activity of the MFOS and determined the effect such inhibition had on the toxicity of 1,1-DCE or ACN. In fasted rats exposed to 1,1-DCE (300–400 ppm), serum sorbitol dehydrogenase (SDH) activity increased 572-fold, while liver GSH fell to 60% of control. With hypoxia and 1,1-DCE exposure, SDH rose only 7-fold, and GSH changed little. A second experiment, with similar exposure concentrations but less apparent injury, gave similar results. Blood GSH concentrations were not significantly affected by exposure in either case. However, lung GSH decreased significantly but less in hypoxia plus 1,1-DCE. ACN did not bind to cytochrome P-450 in vitro. After 300 to 350 ppm of ACN in fed rats, PCB did not increase lethality, but hypoxia did. Liver GSH decreased to 48% of control (air plus ACN). In hypoxic rats given ACN, GSH concentrations fell to 25% of control. Like 1,1-DCE, blood GSH was not affected by ACN but lung GSH fell dramatically (70 and 36% of control for air and 7% O$_2$, respectively). These results suggest that while the role of the MFOS differs in the toxicity of these two ethylene derivatives, they are not directly reactive with GSH in vivo. (Supported by NIEHS Center Grant ES-00002.)
89. Increased Biliary Tree Permeability Produced by Bile Salt Treatment of Rats. James R. Olson and James M. Fujimoto, Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin.

Biliary permeability was assessed in pentobarbital-anesthetized Sprague-Dawley male rats by the segmented retrograde intrabiliary injection (SRII) and intrabiliary pressure (IB-P) techniques. The SRII into the bile duct cannula consists of an initial 40 μl of a [3H]sucrose solution washed in with 110 μl of saline. Since the distended biliary tree capacity is near 40 μl, the 110 μl of saline causes the marker compound ([3H]sucrose) to be filtered by the biliary epithelium. Five seconds following the SRII, bile flow was restarted and the content [3H]sucrose in individual drops of recollected bile was determined. The percentage recovery of [3H]sucrose following SRII represented the sucrose retained in the biliary system. IB-P is the maximum pressure (measured by pressure transducer) generated within the biliary tree during an SRII. In each rat, bile salts (100 μM/kg) were given iv in a femoral cannula following control SRII and IB-P measurements. Control [3H]sucrose recovery of 34.4 ± 3.7% (mean ± SE) and IB-P of 20.0 ± 1.0 mm Hg were reduced to 13.9 ± 3.9% and 15.0 ± 0.7 mm Hg, respectively, following treatment with taurocholate Na. Similar results (46.7 ± 4.8%, 19.8 ± 0.9 mm Hg vs 11.9 ± 4.0%, 12.8 ± 0.6 mm Hg) were seen with glyccholate Na. The findings of reduced sucrose retention and IB-P are consistent with an increase in biliary permeability following treatment with these bile salts. In contrast, treatment with cholate Na showed no significant differences in sucrose recovery (35.5 ± 2.9 vs 32.5 ± 4.4%) and IB-P (19.3 ± 0.7 vs 19.1 ± 2.4 mm Hg), indicating no change in biliary permeability. An intermediate change in permeability was seen with dehydrocholate Na treatment. All treatments produced a choleretic effect but the change in biliary permeability was not directly associated with the degree of choleraxis. (Supported by USPHS Grant GM16305.)


Toxic effects of primaquine in man include abdominal cramps and diarrhea. In a study of the effects of primaquine on the G.I. tract we have observed an antimuscarinic action of the compound. Using an in vitro model, the change in pressure in the isolated guinea pig gall bladder, it was shown that primaquine was a competitive antagonist of acetylcholine. The pA2 of both atropine and primaquine was calculated (pA2 atropine = 8.57, pA2 primaquine = 4.57). The pA2 values indicate that primaquine is much less potent than atropine. An in vivo model showed that the effects of the two agents are synergistic when they are administered simultaneously. Primaquine in large doses decreases the transport of a carbon meal in the G.I. tract in the mouse. At dose levels of 52.5 and 60 mg/kg, primaquine is approximately equipotent to 10 mg/kg of dextroamphetamine in this respect. Atropine (1 mg/kg) does not affect the transport of a carbon meal but this dose when combined with 60 mg/kg of primaquine slows transport more effectively than when the antimalarial alone is administered. These data indicate that primaquine is a competitive antagonist of acetylcholine on the guinea pig gall bladder and although its antimuscarinic effect is weak, it is synergistic with atropine on the intestinal musculature in vivo. (Supported by U.S. Army Research and Development Command Contract No. DADA 17-67-C-7136.)

91. Effect of Thioacetamide Pretreatment on the Hepatic Transport of Organic Anions. A. Hunter, Department of Pharmacology, East Carolina University School of Medicine, Greenville, North Carolina. (J. P. Davanzo)

The effect of thioacetamide pretreatment on the hepatic transport of the organic anions, dibromophenolsulfonphthalein (DBPS) and sulfobromphthalein (BSP), was studied in anesthetized rats with a cannulated bile duct. Each rat received an ip injection of thioacetamide
(4 mmol/kg) dissolved in saline and at times ranging from 24 to 72 hr after injection, (DBPS) (50 mg/kg) or BSP (82 mg/kg) was administered via the femoral vein. Control rats were pretreated with saline (5 ml/kg). The plasma concentration, liver concentration, and biliary excretion of DBPS or BSP were determined 30 min after their administration. At 24, 48, and 72 hr following the administration of thioacetamide the excretion of DBPS into the bile was significantly depressed. At 24 hr (0.15 mg/min/kg), at 48 hr (0.19 mg/min/kg) and at 72 hr (0.28 mg/min/kg) DBPS was excreted into the bile, showing a marked decrease in relation to the controls (0.52 mg/min/kg). The 30-min plasma concentration of DBPS was significantly increased over the controls (0.12 mg/ml) at 24, 48, and 72 hr after administration of thioacetamide (0.28, 0.24, and 0.21 mg/ml, respectively). The hepatic concentration of DBPS was lower than the controls 48 and 72 hr after thioacetamide pretreatment. In the study using BSP the biliary excretion of BSP and BSP metabolites was significantly decreased from the controls at all time intervals by thioacetamide pretreatment. The 30-min plasma concentration of BSP in the thioacetamide pretreated rats was 1.8 to 2 times higher than the plasma concentration in the control animals. At 48 and 72 hr the BSP liver concentration was lower in the thioacetamide pretreated rats than in the control rats. The data from this study demonstrate that thioacetamide pretreatment decreases the hepatic transport of organic anions.


There is an accumulation of fluid in the lumen of rat stomach over the first 24 hr following an oral LD50 dose of diquat (N,N'-ethylene-2,2'-bipyridilium). Cellular changes in rat stomach have been examined 24 hr after oral administration of diquat (LD50 dose), and the relationship of these changes to fluid accumulation has been explored. Following oral diquat, damage is most marked in the antrum of the stomach, with erosion of the surface mucous-secreting layers of cells and some of the underlying glandular mucosa. Minor erosions are also observed in the fundus together with a selective vacuolation of parietal cells. Ultrastructural examinations indicate that the vacuoles in these cells are fluid-filled. Edema fluid is also present in the interstitium of the fundus, particularly at the tips of villi and at the junction of glandular with nonglandular stomach. No abnormalities are seen in the vasculature of the stomach. It is concluded that fluid accumulation in the lumen of the stomach 24 hr after oral diquat is not due to vascular damage. Erosion of the protective mucous-secreting layer following oral dosing may have a significant effect on fluid balance in the stomach, and our results also suggest an effect on fluid balance in the parietal cells.


Following oral administration of an LD50 dose of diquat (N,N'-ethylene-2,2'-bipyridilium) there is a rapid, biphasic accumulation of fluid in the lumen of the stomach. Following subcutaneous diquat (LD50 dose) a significant increase in fluid is observed but is delayed and less than that following oral dosing. Both oral and subcutaneously administered diquat inhibit the rate of gastric emptying. Onset of inhibition following both routes of administration is very rapid and following oral dosing effects are maintained for up to 18 hr. After subcutaneous dosing, the rate of gastric emptying begins to return to normal between 4 and 7.5 hr. This is probably a consequence of falling plasma concentrations of diquat following the subcutaneous route of administration. Fluid accumulation was detected in anesthetized rats over the first 4 hr following pyloric ligation and subsequent administration of an oral LD50 dose of diquat. No fluid
accumulation was detected following subcutaneous injection in this experimental system. It is concluded that the early phase of fluid accumulation following oral dosing is not a consequence of inhibition of gastric emptying. Data are not available to assess the significance of prolonged inhibition of gastric emptying to the later phase of fluid accumulation following oral and subcutaneous diguat.

94. Covalent Binding to Colon Tissue and Its Inhibition by Fecal Material. Marion Ehrich, Roger L. Van Tassel, and Tracy D. Wilkins, Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. (S. D. Cohen).

The present study was undertaken to develop a short-term in vitro test for measuring the direct interaction of exogenous and endogenous chemicals with colon tissue under conditions reasonably similar to the in vivo environment. $^{14}C$Xenobiotic (50 µl, 1 µCi, 2 µmol) was incubated with segments of whole rat colon at 37°C under N₂ in a 2-ml total volume for 30 min. The incubation was done in the presence and absence of whole human fecal suspension (100 mg dry wt.). Binding was stopped by precipitating the colon protein with 10% trichloroacetic acid (TCA), after which the colon tissue was serially extracted with chloroform : methanol (2 : 1), 10% TCA, 5% TCA, 95% ethanol, ethanol : ether : chloroform (2 : 2 : 1), and ether. The quantity of covalent binding was indicated by the radioactivity that remained following the extractions. Expressed as nanomoles bound per gram dry weight of extracted tissue, covalent binding of 3-methylicholanthrene (3MC), dieldrin, dimethylhydrazine (DMH), cholesterol, and valine was 469 ± 118, 262 ± 59, 67 ± 4, 177 ± 76, and 27 ± 10 (mean ± SD, N = 3–6), respectively. Binding of the known carcinogens, 3MC and dieldrin, was quantitatively larger than that of cholesterol, valine, or DMH. In an incubation system including fecal suspension with the chemical and colon tissue, covalent binding of 3MC, dieldrin, and cholesterol was reduced by 95, 92, and 84%, respectively. This inhibitory effect on binding was only 41% with the colon carcinogen DMH. These results indicated that this method may be used to examine parameters affecting colon tissue–xenobiotic interactions. (Supported by Grant IN 117 from the American Cancer Society and a grant from the Abercrombie Foundation.)

95. Interaction of Carrageenan with Some Dietary Nutrients in Relation to Their Absorption from the Gastrointestinal Tract of Rats. M. Rhee, E. P. Pittz, R. Abraham, D. Rourke, and F. Coulston, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The effect of CG on the absorption and binding of some dietary constituents essential to the rat was studied in vivo and in vitro in order to assess the ability of CG to modify their rate of absorption from the GI tract. The nutrients considered were protein (determined as total nitrogen), $[^{1}H]$histidine, and $[^{14}C]$choline. In vivo studies were carried out to determine differences in absorption of $[^{1}H]$histidine and total nitrogen (ileal vs $[^{1}H]$histidine and duodenal nitrogen) between control rats (males, 300 g) and rats fed 5, 10, and 15% CG. Diets were calorically balanced with alphacel to compensate for CG bulk. Nitrogen was determined by the Kjeldahl method and the other nutrients by scintillation counting procedures. The results of these studies indicate that CG only slightly decreases nitrogen and $[^{1}H]$histidine absorption, with no dependence on CG concentration between 5 and 15% CG. In vitro studies measuring the rate of dialysis across Visking dialysis tubing of nutrients in the presence and absence of CG indicate that: (a) CG slows the rate of dialysis and/or digestion by pepsin of bovine serum albumin; (b) CG decreases the rate of dialysis of $[^{14}C]$choline up to 2.7 hr, after which equilibrium is rapidly attained (by 4 hr). At equilibrium, $[^{14}C]$choline is bound to CG to the extent of 6.1 mg of choline/g of CG (at a ratio of 35 mg of choline/g of CG initially in the dialysis bag). The results are interpreted in terms of the possible significant interactions that CG can have with dietary
components in terms of their G.I. tract absorption. (Supported by NIEHS Grants 2-P01-ES00226-10 and 2T01-ES00703-10.)


To test the effects of various diets on 2-AAF-induced carcinogenic liver and bladder lesions, weaning female BALB/c mice were continuously fed diets containing combinations of 12 or 24% protein, 4 or 24% corn oil, and 0, 50, 100, or 500 mg/kg of 2-AAF for approximately 78 weeks. At 65 and 78 weeks on test, groups of mice were sacrificed from each treatment group and the livers and brains were removed, frozen in liquid nitrogen and subsequently homogenized and assayed for 19 different liver and 10 different brain enzymes. Feeding 2-AAF increased the activities of 13 liver and 6 brain enzymes and decreased the activities of 2 liver enzymes. The activities of 7 liver enzymes were increased and the activities of 6 liver and 7 brain enzymes were decreased by high-fat diets. Feeding a high-protein diet increased the activities of 7 liver and 3 brain enzymes and decreased the activities of 6 liver enzymes. These data indicate that the effects of diet in BALB/c mice are substantial in magnitude and significantly impact upon toxicological responses which may be expressed through change in the activity of a number of common liver and brain enzymes.

97. *Induction of Mixed-Function Oxidase in Primary Cultures of Mouse Hepatocytes.* K. K. Dougherty and J. L. Byard, Department of Environmental Toxicology, University of California, Davis, California.

Methods for the isolation and culture of primary mouse hepatocytes have been developed. However, the usefulness of these cultures for studying the metabolism and mechanism of action of foreign chemicals is limited by the rapid decline of mixed-function oxidase (MFO) of hepatocytes in primary culture. This decline of MFO activity in culture is probably due to the absence of the humoral factors known to maintain the differentiated state and MFO activity in *vivo*. In the experiments reported, humoral factors thought to maintain MFO activity in *vivo* were added to primary cultures of mouse hepatocytes to determine if these factors could maintain *in vitro* levels of MFO. Maintaining *in vitro* levels of MFO was considered essential before using this model to study induction of MFO by chlorinated hydrocarbons. Hepatocytes were prepared from adult male Charles River CD-1 mice. The hepatocytes were cultured on plastic petri dishes coated with rodent tail collagen in Waymouth's 752/1 medium supplemented with albumin, amino acids, fatty acids, and hormones (serum-free). Cytochrome P-450 was determined spectrophotometrically as the reduced CO complex in freshly isolated hepatocytes and in those cultured for 24 hr. MFO activity was measured in cultured hepatocytes as the *N*-demethylation of *para*-chlooro-*N*-methylalanine (PCMA). All cultures contained insulin. Additional hormone supplements were added to the medium in 1 µl of propylene glycol/ml of culture medium. The same concentration of propylene glycol was present in control cultures. A mixture of testosterone (10^-6 M), estradiol (10^-6 M), corticosterone (10^-5 M), D-thyroxine (10^-5 M), glucagon (5 x 10^-8 M), 5-α-dihydrotestosterone (10^-6 M), and vitamin E (5 µg/liter) was found to increase the cytochrome P-450 concentration 200% above that in hepatocytes cultured in the absence of added hormones. However, this hormone mixture was only able to maintain cytochrome P-450 at 51% of the level found in freshly isolated hepatocytes. *N*-Demethylation of PCMA was stimulated 160% by the addition of testosterone (10^-6 M), D-thyroxine (10^-5 M), and glucagon (5 x 10^-8 M) to the culture medium. In summary, added hormones maintained higher levels of cytochrome P-450 and MFO activity in primary cultures of mouse hepatocytes, although *in vitro* levels have not yet been achieved.
98. **The Effect of Polymer Pyrolysis Fumes on the Activity of Rat Hepatic Cytochrome c Oxidase.** W. C. Thomas, Jr., E. J. O’Flaherty, R. H. Bell, and K. L. Stremmer, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio. (Paul B. Hammond)

Hydrogen cyanide, which inhibits utilization of oxygen *in vivo* by combining reversibly with cytochrome c oxidase (EC.1.9.3.1), is included among the thermal degradation products of polymeric materials which contain nitrogen. A model flame-retarded rigid polyurethane foam was degraded in air at the rate of 1 g/min at 500°C. Adult male Sprague–Dawley rats were exposed to the pyrolysis products for 5 to 8 min. Surviving animals were sacrificed at variable time intervals from 0 to 30 min after termination of exposure. Control rats were exposed to a flow of heated air and sacrificed at the same time intervals. Cytochrome c oxidase activity was assayed by measuring the ability of hepatic mitochondrial preparations to oxidized reduced cytochrome c. Surviving experimental rats showed signs of moderate to severe respiratory distress during and shortly after exposure but had recovered by 30 min. There was no significant inhibition of cytochrome c oxidase activity either in rats dying as the result of exposure or in surviving experimental rats at any time up to 30 min postexposure.

99. **Lymphatic Absorption of Benzo(a)Pyrene from the Intestinal Tract of the Unanesthetized Rat.** J. B. Reid and E. Bingham, Carnegie–Mellon Institute of Research, Pittsburgh, Pennsylvania and University of Cincinnati, Cincinnati, Ohio.

Benzo(a)pyrene (BaP) is a pervasive environmental contaminant and a known carcinogen for animals. Human exposure by the oral route is likely via food intake or by transfer by mucociliary clearance. Lymphatic absorption of xenobiotics has received little attention. Experiments were run to elucidate the role of the lymphatics in transportation of BaP from the intestinal tract utilizing two vehicles. With olive oil, almost one-fifth of the absorbed dose, given intraduodenally, is transported in the chylomicron phase of the lymph. This material is predominantly in the unmetabolized form. The initial organ to receive this dose is the lung due to the anatomy of the system. Tissues and fluids contained predominantly metabolites. The relationship between biliary excretion and lymphatic uptake was investigated. In addition, chylomicrons containing BaP were harvested from a donor and injected, iv, into a test animal. The blood was rapidly cleared (1 to 2 hr) by biliary excretion. It is important to consider the ramifications of tissue contact with a potential carcinogen in the parent form since the ultimate carcinogen may be formed at the target tissue.

100. **The Use of Primary Liver Cell Cultures to Study Hepatotoxic Agents.** D. Acosta, D. Anuforo, and R. V. Smith, Department of Pharmacology and Drug Dynamics Institute, College of Pharmacy, University of Texas, Austin, Texas. (James V. Bruckner)

A system for growing primary monolayer cultures of neonatal rat liver cells has been developed in our laboratory. By growing the cultures in arginine-deficient medium, fibroblastic overgrowth was inhibited and relatively pure cultures of parenchymal hepatocytes were obtained. This cell culture system was used to study the cytotoxicity of agents known to be hepatotoxic: carbon tetrachloride, alcohol, norethindrone, tetracycline, and nitrofurantoin. Caffeine and sodium salicylate were evaluated as agents thought to be relatively nontoxic to the liver. Cytotoxicity was evaluated by microscopic changes in cellular morphology and by measurement of leakage of cytoplasmic enzymes into the culture medium: argininosuccinate lyase (ASAL), lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and acid phosphatase (AP). The cultures were treated with each of the agents in concentrations ranging from $5 \times 10^{-6}$ to $1 \times 10^{-3}$ M and for durations from 1 to 24 hr. We found ASAL to be the most sensitive in predicting early cell injury and AP the least sensitive; while the other three enzymes were equally sensitive in evaluating cytotoxicity. Of the agents tested, carbon tetrachloride was the most toxic and nitrofurantoin the least toxic. For example, treatment of the cultures with carbon tetrachloride (20 μL/ml) for 6 hr resulted in ASAL
activity that was 500% of control values; tetracycline (5 x 10^{-4} M) for 6 hr, 400% of controls; and norethindrone (5 x 10^{-4} M) for 6 hr, 250% of controls. The hepatotoxic agents demonstrated a dose- and time-dependence order of cytotoxicity in the cultures.

101. Effect of a Cholestatic Agent on Isolated Rat Hepatocyte Function. J. LAVIGNE, B. G. PRIESTLY, and G. I. PLAA, Département de pharmacologie, Faculté de médecine, Université de Montréal, Montréal, Canada.

Acute administration of α-naphthylisothiocyanate (ANIT) to several animal species results in cholestasis, i.e., cessation of bile flow. ANIT cholestasis is accompanied by increased plasma concentrations of bile acids and bilirubin. The effect of this cholestatic agent on the release of bile acids by isolated parenchymal liver cells was investigated. Hepatocytes were obtained from rat liver by a collagenase perfusion technique. This method gives a high yield of cells (~2-3 x 10^8 cells/250 g rat). Viability, as measured by the trypan blue exclusion test, was over 95% at the beginning of the incubation. Total bile acids in the supernatant were determined enzymatically with hydroxysteroid dehydrogenase. Bile acid increased linearly for 3 hr of incubation when hepatocytes were isolated from normal rats. When the rats were pretreated with ANIT (300 mg/kg, po) 16 to 18 hr before isolation of the hepatocytes, in vitro bile acid increase remained normal. Previous studies demonstrated that this was the critical in vivo period during which the bile flow diminished until cholestasis was complete. Similar results were obtained when hepatocytes were isolated 24 hr after treatment, a time when cholestasis was observed in 100% of the animals in vivo. The effect of ANIT in vitro was also studied. The addition of different concentrations up to 10^{-3} M did not change the concentration of bile acids in the supernatant from control values. The release of GPT by the cells and the percentage of viability remained the same after 2 hr of incubation. Therefore, the appearance of bile acid in the supernatant is not modified when ANIT is added directly to isolated hepatocytes, nor when hepatocytes are isolated from ANIT-pretreated rats at a time when intrahepatic cholestasis appears in vivo (16 hr after ANIT treatment) or is complete in vivo (24 hr after ANIT treatment). (Supported by the Medical Research Council of Canada.)


Previous studies from our laboratory have shown that both Δ⁸- and Δ⁹-tetrahydrocannabinol (THC) stimulated basal murine hepatic tyrosine aminotransferase (TAT) activity, but inhibited the steroid-mediated induction of the enzyme by hydrocortisone in vivo. The purpose of the present investigation is to elucidate further the site of action for the cannabinoid effects on the TAT system by studying the hepatic murine RNA polymerase systems. Male ICR mice were used in all these studies. Both RNA polymerase A (Mg^{2+}-dependent) and polymerase B (Mn^{2+}-dependent) were assayed by the method of Gelboin et al. (1966) from a purified nuclear pellet from treated mice. Δ⁸-THC and Δ⁹-THC (200 mg/kg ip) increased both polymerase A basal activity by 30 and 42%, respectively, and polymerase B activity by 42 and 27%, respectively, as compared to controls. Pretreatment of mice 12 hr before sacrifice with Δ⁸-THC (200 mg/kg ip) inhibited the peak steroid induction produced by 150 mg/kg of hydrocortisone ip given 9 hr before sacrifice by 51% in the polymerase A system and by 34% in the polymerase B system. In contrast, Δ⁹-THC (200 mg/kg ip) given 12 hr before sacrifice stimulated the peak steroid induction with maximal effects seen at 37.5 mg/kg of hydrocortisone ip—a 63% increase in polymerase A induction and a 46% increase in polymerase B induction as compared to respective steroid controls. These results indicate that although Δ⁸- and Δ⁹-THC both stimulated basal RNA polymerase activity, as seen with the TAT enzyme system, these compounds seem to be acting at different sites in the cell to affect the inhibition of TAT induction by steroid. (Supported by HEW Grants DA01248, T32DA07027, and DA00490.)
103. **Decreasing Oral Toxicity of an Essential Oil from Myrtle by Adaptive Liver Stimulation.**
H. UEHLEKE and M. BRINKSCHULTE-FREITAS, Department of Pharmacology, University of Tübingen, D-74 Tübingen, West Germany.

Essential oil of myrtle (M) is used in pharmaceutical preparations, toilet goods, air refreshers, etc. The 160–180°C fraction of the ether extracts of Myrtle leaves contains 90% eucalyptol and dipentene (d,l-limonene), 5% camphene, 2% α-pinene, 1% β-pinene, and 1 to 2% unidentified compounds. The acute oral toxicity in male NMRI mice of this fraction (M) diluted 1:1 with peanut oil was found to be 2.3 ml of M/kg; in male Wistar rats 3.8 ml/kg. The animals died by depression of the central nervous system. Daily application of M to rats for 3 weeks at doses higher than 1.5 ml/kg produced initial loss of weight which was regained after a few days. Consecutive daily doses of M were more toxic than a single dose given only during the initial 3 days. Later, the rats developed tolerance to high doses of M. After 3 weeks of treatment with 2 ml/kg daily the mean lethal dose of M in rats increased to approximately 6.6 ml/kg. This was explained by adaptive mechanisms. Daily oral administration of 1 and 2 ml/kg for 12 days increased the relative liver weight of rats by 18 and 28%. A threshold appeared between 0.2 and 0.5 ml/kg. The protein of the isolated hepatic microsomal fraction increased by 24 and 33%, and liver cytochrome P-450 was elevated by 40 and 65%. Also hepatic cytochrome b1 increased. These alterations in the liver were fully reversible within 8 days after the last application of M.

104. **Percutaneous Absorption of Cyanide from Aqueous Sodium Cyanide.** P. H. DUGARD and S. J. MAWDSLEY, Central Toxicology Laboratory, Imperial Chemical Industries Ltd., Alderley Park, Near Macclesfield, Cheshire, England. (A. A. B. Swan)

Diffusion cell measurements of the absorption of cyanide across human epidermis were made to assess the hazard resulting from clothing wetted by liquor during NaCN manufacture. The time course of absorption of 14CN through 1.8 cm² of tissue at 30°C was followed from aqueous NaCN solutions similar to those encountered in manufacture (1, 10, 40% w/v) and from tracer Na14CN alone (0.0025%) in 0.2 M glycine buffer. Stratum corneum (SC): vehicle partition coefficients were also measured and the apparent diffusion constants for CN⁻ and HCN were calculated. The CN absorption rates at steady state for unbuffered solutions (pH 11.2–11.4) and from tracer alone buffered to pH 11.2 were proportional to concentration (thus obeying Fick’s law of diffusion). The maximum steady absorption rate was not achieved for approx 90 min. Absorption rates showed a strong pH dependence (pH 9.0–12.0) and the permeability constant for HCN (pKₐ 9.3) was calculated to be 25 times greater than that of CN⁻. No pH dependence was apparent for SC: vehicle partition coefficients and thus the SC diffusion constant for HCN is 25 times greater than that of CN⁻. Since the initial build up of absorption rate depends on the diffusion constant, HCN is absorbed with very little lag whereas CN⁻ rate increases for about 90 min. These results permitted estimations of the early time courses of absorption (by combination of CN⁻ and HCN contributions) for different exposures and the resulting absorption patterns were compared with the detoxification rates for CN and the body burden producing toxic effects. The calculations showed, for example, that a large area contact with 10% NaCN at pH 11.4 leads to symptoms within 25 min and death in about 1 hr. Even if clothing is removed, washing may not extract a toxic amount of CN dissolved in the stratum corneum. Observations in a small number of industrial accidents available for comparison are in agreement with the calculations. CN absorbed by the skin from clothing wetted with a NaCN solution may lead to toxic effects in certain cases.


Many forms of chemical-induced toxicity or carcinogenesis are preceded by covalent binding of reactive intermediary metabolites to cellular macromolecules (Jollow, Kocsis, Snyder, and Vainio, Eds., "Biological Reactive Intermediates," Plenum Press, New York, 1977). We have
previously suggested that benzene toxicity is due to a toxic metabolite of benzene (Andrews et al., Biochem. Pharmacol. 26, 293, 1977). We now report covalent binding of a $[^3]$H$\text{H}$benzene metabolite in liver and bone marrow. $[^3]$H$\text{H}$Benzene (0.5 ml/kg) was given twice daily for up to 10 days to study metabolism and distribution of $[^3]$H$\text{H}$benzene and its metabolites and their covalent binding. Labeled metabolites in urine increased during the first 4 days of treatment, plateaued from the 4th to 6th days, and then decreased until the experiment was terminated on Day 10, a time at which severe toxicity was apparent. In contrast, concentrations of both $[^3]$H$\text{H}$benzene and its metabolites increased in blood, liver, fat, and bone marrow during the entire 10-day period. Metabolite concentrations in each organ were approximately 10-fold greater than those of free benzene. Bone marrow, the site of toxic action of benzene, displayed the highest metabolite concentrations, analysis showed the metabolites were phenolic conjugates of glucuronic acid and sulfate. To determine irreversibly bound radioactivity tissue samples of liver and bone marrow were washed exhaustively with methanol:ether (3:1) until no more radioactivity could be extracted and then burned in a tissue oxidizer before counting. Although soluble metabolite concentrations in bone marrow were greater than those in liver, liver covalent binding exceeded that found in bone marrow. A progressive increase in binding occurred in both organs as treatment continued and toxicity increased. Since both soluble metabolites and covalent binding increased in bone marrow in parallel with increasing toxicity, it remains to be determined which is of greater significance in the production of benzene toxicity. (Supported by NIEHS Grant ES00322.)

106. The Toxicity of Inhaled Benzene. Carroll A. Snyder, Bernard D. Goldstein, Arthur Sellakumar, Roy E. Albert, and Sidney Laskin, Institute of Environmental Medicine, New York University Medical Center, Tuxedo, New York.

Exposure to benzene vapor has been implicated in the etiology of a variety of blood dyscrasias, including pancytopenia, aplastic anemia, and leukemia. In order to develop animal models for benzene-induced blood dyscrasias we have been engaged in a series of investigations using AKR, C-57 B1, and Charles River CD-1 mice and Sprague-Dawley rats. Animals were exposed for 6 hr/day, 5 days/week to either 300 or 100 ppm of benzene vapor in 1.3-m$^3$ exposure chambers. Matched controls were exposed to filtered, conditioned air in identical chambers. Peripheral blood cell counts were determined biweekly from test and control animals using venous tail blood. At 300 ppm all three mouse strains exhibited severe lymphocytopenia and anemia while the rats showed moderate lymphocytopenia and only a trend to anemia. All 300-ppm-treated animals exhibited increased weight loss and mortality. At 100 ppm, AKR mice manifested severe lymphocytopenia and mild anemia while the rats gave only indications of depressed lymphocyte and erythrocyte levels. None of the treated animals at either exposure level displayed granulocytopenia but mild to moderate granulocytosis appeared in all of the treated mice. Two rats which are still being exposed to 100 ppm of benzene have displayed elevated white counts combined with a shift to the left suggestive of possible chronic granulocytic leukemia. Two CD-1 mice exposed to 300 ppm of benzene developed pathologically confirmed myelogenous leukemia, one acute and one chronic. A third CD-1 mouse died with a blood profile suggestive of chronic myelogenous leukemia; this diagnosis awaits pathological confirmation. (Supported by Contract U-150-14 (PS-7) from the American Petroleum Institute, NIEHS Grant ES00260, and NCI Grant CA 13343.)

107. Effects of Diethylnitrosamine on Murine Hepatic Mixed-Function Oxidase Activities. A. N. Tucker, T. Tang, and M. A. Friedman, Departments of Pharmacology and Microbiology, Medical College of Virginia, Richmond, Virginia.

Chronic administration of diethylnitrosamine (DEN) to mice has been shown to alter liver morphology and function. The present study was undertaken to examine more closely the effects of DEN on hepatic mixed-function oxidase activities. DEN was administered to male BALB/c mice in the drinking water at 50 ppm. Aminopyrine demethylase and amline hydroxylase were
assayed in vitro using livers from mice treated for up to 24 weeks with DEN. These two enzymes declined within 4 weeks, finally reaching values which were 30% of control at 24 weeks. The yield of microsomal protein was affected to a lesser extent, being 70% of control at 24 weeks. Cytochrome P-450 was decreased also by 24-week DEN treatment to 50% of control. These effects were similar to those produced 24 hr after a single ip injection of DEN at 100 mg/kg. Liver homogenates from the mice treated chronically were also used as a source of activating enzymes in the Ames test, using several compounds representative of classes of mutagens. In spite of marked decreases in cytochrome P-450 and associated activities, these liver homogenates were generally able to activate the test compound as well as control samples. The relationship between the ability of DEN to inhibit mixed-function oxidase activity and its carcinogenic and toxic effects is unclear. However, DEN is clearly a potent inhibitor of murine hepatic mixed-function oxidase activity. (Supported by NIH Fellowship CA05745 and NIH Grant ES00701.)


Dimethylnitrosamine (DMN) is a potent carcinogen and hepatotoxin that requires biotransformation to reactive intermediates, probably by microsomal DMN-N-demethylase. Oral pretreatment of rats with acetone enhances the in vitro activity of DMN-N-demethylase and potentiates DMN-induced hepatotoxicity. To investigate further the effects of acetone on DMN-N-demethylase activity, male C57BL/6J mice were pretreated with acetone (15–60 mmol/kg ip) and sacrificed after 2, 4, 16, 24, and 36 hr, and the livers were removed for isolation of microsomes. In vitro DMN-N-demethylase activity was determined at 37°C in a 3.0-ml incubation containing DMN (0.1–200 mM), NADPH, and microsomal protein. Formaldehyde formed from DMN was measured according to the Nash procedure. To establish the levels of reactive intermediates produced, [14C]DMN (0.15 mM, sp act 4.5 Ci/mol) was substituted in the reaction mixture and RNA, protein, and exogenously added calf thymus DNA were isolated and analyzed for covalently bound 14C. Pretreatment with acetone enhanced the activity of microsomal DMN-demethylase (100% increase at 16 hr) and the in vitro covalent binding of 14C to DNA, RNA, and protein. Four distinct $V_{max}$ values dependent on the DMN concentration were found for the N-demethylase present in control microsomes. Acetone pretreatment resulted in an increased $V_{max}$ and decreased $K_m$. At concentrations of 1 and 10 mM, acetone pretreatment increased N-demethylation of DMN as compared to control, but not at 100 mM, suggesting activation or induction of a high affinity enzyme. Because alkylation of DNA has been implicated as the initiating event in DMN-induced carcinogenesis, the ability of acetone to enhance DMN bioactivation and DNA binding warrants added concern due to the combined presence of these chemicals in the environment.


The effect of polymers of varying charge, concentration, and molecular weight (MW) on the leakage of hemoglobin (spectrophotometrically monitored at 545 nm) from human erythrocytes (RBCs) under osmotic stress was investigated in order to assess the ability of polymers of defined properties to alter the mechanical stability of biological membranes, thereby changing their permeability to macromolecules. The polymers studied included dextran (DT), diethylaminoethyl-dextran (DEAE-DT), dextran sulfate (DS), heparin (H), chondroitin sulfate (CS), and iota carrageenan (CG). The RBC volume was 0.6%. All neutral and anionic polymers stabilized RBCs as their concentration increased, while DEAE-DT showed complex effects by having a stabilizing effect at high ionic strength (above 0.45% NaCl) but destabilizing the RBCs
at low ionic strength (below 0.45% NaCl). DT effected increased stabilization of RBCs with increasing MW while CG and DS showed optimum stabilization of RBCs at intermediate MWs. Increasing anionic charge (degree of sulfation) on DS enhanced the stabilizing effect of DS on RBCs. At 0.75 mg/ml, neutral and anionic polymers protected RBCs against 50% osmotic hemolysis in the order CG > CS > H > DT > DS, while DEAE-DT had a destabilizing effect. The results are discussed in terms of the ability of polymers to become systemic and alter cellular and organelle permeability and function. (Supported by NIEHS Grants 2-P01-ES00226-10 and 2-T01-ES00703-10.)

110. Species and Strain Differences in Tissue Alkylation and Toxicity by 4-Ipomeanol: Predictive Value of Covalent Binding in the Study of Target Organ Toxicity. J. S. DUTCHER and M. R. BOYD. Clinical Pharmacology Branch, National Cancer Institute, Bethesda, Maryland.

We are exploring the possibility that species and strain differences in target organ alkylation and toxicity by 4-ipomeanol (IPO) can be utilized to help elucidate metabolic and dispositional factors determining the target organ specificity of metabolically activated cytotoxins. Previous studies have demonstrated highly preferential alkylation of rat lungs in vivo by a metabolite of IPO (Boyd, Environ. Health Persps. 16, 127, 1976; Nature (London), in press, 1977). The covalently bound IPO metabolite was localized in the pulmonary bronchioles; pulmonary bronchiolar necrosis was the initial lung lesion produced by IPO. We have found a similar pattern of target organ selectivity for IPO in the rabbit and the guinea pig. No renal or hepatic lesions were seen in any of these species. In contrast, in addition to pulmonary bronchiolar lesions, IPO produced striking renal cortical necrosis in mice and centrilobular hepatic necrosis in hamsters. IPO metabolite was covalently bound preferentially in the kidneys of mice and in the livers of hamsters. For a given dose of IPO the tissue levels of covalently bound IPO metabolite were much lower in the hamster than in any other species. Correspondingly, the hamster was much less susceptible to IPO toxicity than any other species. Similarly, with different strains of rats and mice, there was a positive correlation between the level of target organ alkylation by an IPO metabolite and the relative level of susceptibility to IPO-induced toxicity. These studies indicate that the easily determined index of in vivo covalent binding may have predictive utility in screening for both target organ selectivity and potency of toxins or drugs producing lesions via alkylation metabolites.

111. Pulmonary Bronchiolar Alkylation and Necrosis by 3-Methylfuran, a Potential Atmospheric Contaminant Derived from Natural Sources. C. N. STATHAM, R. B. FRANKLIN, and M. R. BOYD, Clinical Pharmacology Branch, National Cancer Institute, Bethesda, Maryland.

Saunders and co-workers (Biomed. Mass Spectrom. 1, 192, 1974) reported that 3-methylfuran (3MF) was a major atmospheric contaminant during a smog alert in Washington, D.C., and they speculated that the 3MF was derived from photodegradation of terpenes produced primarily by deciduous trees. Previous studies (Boyd, Environ. Health Persps. 16, 127, 1976; Nature (London), in press, 1977) have shown that the furan derivative, 4-ipomeanol, is highly toxic to pulmonary bronchioles due to its metabolism in situ to a potent alkylating agent. With this precedent, we have examined the metabolism and toxicity of 3MF in mice. In vitro studies demonstrated that 3MF is converted to a highly reactive electrophilic metabolite via cytochrome P-450-dependent mixed-function oxidase activity present in mouse lung microsomes. Autoradiographic studies of lungs of mice given radiolabeled 3MF either ip or by inhalation showed highly preferential accumulation of a covalently bound 3MF metabolite in the pulmonary bronchioles. Striking bronchiolar necrosis was present in lungs of animals 24 hr after exposure to 3MF. Administration (ip) of piperonyl butoxide, an inhibitor of the metabolic activation of 3MF, prevented both the bronchiolar alkylation and necrosis by inhaled 3MF. Prior depletion of tissues of reduced glutathione by diethylmaleate increased both the bronchiolar alkylation and toxicity of 3MF. These results are consistent with the view that a
metabolite of 3MF is formed in situ which alkylates lung bronchioles, leading to their necrosis. These studies further suggest that during atmospheric conditions in which the compound is formed and sufficiently concentrated in the atmosphere, 3MF could play a significant role in the pathogenesis of lung disease in man.

112. Acute and Delayed Neuronal Toxicity in Rat and Chick Brains after Embryonic X Irradiation. BERNARD F. SCHNEIDER and SISTA NORTON, Department of Pharmacology, University of Kansas Medical Center, Kansas City, Kansas.

Three types of neuronal damage are found following prenatal X irradiation: (1) cell loss, (2) abnormal migration, and (3) delayed alterations of cell morphology. Whole body X irradiation of 125 R to pregnant rats on gestational Day 15 reproducibly damages telencephalic structures through loss of ependymal cells (presumably mitotic cells) and interference with normal neuroblast migration. Surviving neurons, examined with the Golgi stain, show no morphological alteration up to the time of general CNS maturation. Rats at 3 to 4 months (postnatal age) begin to show severe alterations in neuronal morphology (shortened, twisted dendrites with varicosities) in the caudate nucleus and frontal cortex. Chick embryos exposed to 100 to 200 R on incubation Days 11 and 13 and examined at time of hatching appear to have reduced numbers of cells in comparable telencephalic areas. Further studies are in progress to confirm these findings in the chick brain and to examine the development of the surviving neurons. The reasons for the delay in appearance of the abnormal neuronal morphology, found so far in the rat, are not known. The possibility is being investigated that neuronal deafferentation is involved in the delayed toxicity.


Primary axonal degeneration resulting from the section or crush of peripheral nerve is accompanied by a number of dramatic biochemical changes. We have examined the possibility that some of these changes could serve as biochemical markers for detecting peripheral neuropathies induced by chemicals. A biochemical method for detecting nerve damage would be rapid and economical and would yield quantitative data which are statistically analyzable. From studies on acrylamide and methylmercury-induced neuropathies in rats evidence has been obtained that the measurement of the lysosomal enzymes, β-glucuronidase and β-galactosidase, offers such a method. In Wallerian degeneration, increases of as much as 2000 to 3000% in the activities of these enzymes coincide with the proliferation and alteration in the functional state of Schwann cells and endoneurial cells. In chemically induced neuropathy, changes in excess of 300% have been observed in affected areas of the peripheral nervous system, e.g., the distal parts of the posterior tibial nerve in acrylamide neuropathy and throughout the length of the sciatic nerve and in sensory ganglia in methylmercury neuropathy. The increases in enzyme activities were related to the dose of neurotoxicant administered and no such increases were detectable after administration of nonneurotoxic compounds even in near lethal doses. This technique has been applied successfully to the screening of pesticides for neurotoxic effects in rats.

114. Kepone Inhibition of Mouse Brain Synaptosomal ATPase Activities. D. DESAIAH, H. M. MEHENDALE, and I. K. HO, Department of Pharmacology and Toxicology, The University of Mississippi Medical Center, Jackson, Mississippi.

Our previous studies have demonstrated that Mg-dependent ATPase activity in rat liver mitochondria was inhibited by kepone both in vitro and in vivo. In the present study the effect of kepone on mouse brain synaptosomal ATPase activities was investigated. The Na+-, K+- and
Mg\(^{2+}\)-ATPases were inhibited by kepone in vitro with ID50 of 4 and 5 \(\mu M\), respectively. K\(^{+}\)-stimulated PNPPase (\(p\)-nitrophenyl phosphatase), which represents the dephosphorylation step of the Na\(^{+}\)-, K\(^{+}\)-ATPase reaction, was also inhibited in vitro. The ID50 was 4 \(\mu M\). The in vivo effect of kepone on mouse brain synaptosomal ATPase activities was also investigated. Mice were fed with kepone at 0, 25, and 50 mg/kg for 2 to 3 days until tremors, characteristic of kepone toxicity, appeared. Then the mice were sacrificed and the brain synaptosomes were prepared. The Na\(^{+}\)-, K\(^{+}\)- and Mg\(^{2+}\)-ATPase activities in the synaptosomes of kepone-fed mice showed 30 and 60% reductions, respectively, as compared to controls. Oligomycin-sensitive (mitochondrial) Mg\(^{2+}\)-ATPase was more sensitive to kepone treatment than Na\(^{+}\)-, K\(^{+}\)-ATPase activity. The oligomycin-insensitive Mg\(^{2+}\)-ATPase activity was not altered by kepone both in vitro and in vivo. The results suggest that the observed toxic symptoms (tremors, etc.) in kepone-treated mice may be related to the inhibition of ATPase activities in brain synaptosomes. (Supported by NIDA, DA 01310 and BRSG 5 S07 RR05386.)


2,5-Hexanediol, a metabolite common to both methyl n-butyl ketone and n-hexane, was tested for evidence of neurotoxicity by mixing it in the drinking water at concentrations of 0.25, 0.5, and 1.0% or by intraperitoneal injection at 200 mg/kg/day to male Sprague–Dawley rats. Clinical signs of peripheral neuropathy and morphologic changes both in the peripheral and central nervous systems were found in all groups receiving 2,5-hexanediol in water. The rats receiving 2,5-hexanediol ip did not show clinical signs of peripheral neuropathy but did develop morphologic changes. The morphologic changes consisted of focal fusiform swelling of the axoplasm and secondary myelin degeneration. Electron microscopy showed that the swellings contained large numbers of neurofilaments and clumping of axonal organelles, including neurotubules, mitochondria, and glycogen granules. The thickness of myelin surrounding the swollen axons was reduced. In comparison, the clinical signs of the neuropathy induced by 2,5-hexanediol are indistinguishable from those caused by MnBK except that they occur earlier and are more severe at comparable doses. The neuropathologic changes produced by both compounds are similar, if not identical, both in morphology and distribution. In addition to neurotoxicity, all animals in the 1.0 and 0.5% groups and one in the 0.25% group developed testicular atrophy. This atrophy occurred before clinical signs of neuropathy and light microscopic evidence of axonal swelling were seen.


Hen brain and spinal cord contain a number of esterases that hydrolyze phenyl valerate. Most of this activity is inhibited by low levels of paraoxon. Included among the paraoxon-resistant esterases is neurotoxic esterase (NTE), which is inhibited in vivo and in vitro by organophosphorus esters, such as mipafox, which cause delayed neurotoxicity. Since published information on NTE content of nonneural tissues was lacking, a comprehensive study of the occurrence of this enzyme in tissues of adult white Leghorn hen was undertaken. For each tissue, a complete differential titration curve was obtained by incubating tissue homogenate with a range of paraoxon concentrations, typically 0.01 to 1000 \(\mu M\), for 20 min and then assaying for esterase activity with phenyl valerate. Paraoxon-resistant esterase activity manifested itself as a tailing off of the titration curve at the higher inhibitor concentrations. NTE activity was operationally confirmed by further titration of any residual activity with mipafox over the concentration range 0.001 to 1000 \(\mu M\), while at a paraoxon concentration sufficient to inhibit all
of the paraxoxon-sensitive esterases. Under these assay conditions, brain NTE activity was 2426 ± 104 nmol/min/g wet wt (mean ± SE). Titration of other tissues resulted in the following NTE activities, expressed as a percentage of brain activity: spinal cord, 23%; liver, 0%; kidney, 0%; white muscle, 0%; red muscle, 0%; peripheral nerve, 0%; and spleen, 70%. These results indicate that NTE, as defined here, has limited distribution among the tissues of adult hen and is present in nonneural as well as neural tissue. (Supported in part by a Faculty Research Grant from the Horace H. Rackham School of Graduate Studies.)

117. Hallucinogen Effects on Cyclic GMP Levels in Newborn Rat Colliculus. M. S. RAPPAORT and H. CORNISH, Interdepartmental Toxicology Program and Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan.

It has been proposed that phenethylamine and indoleamine hallucinogens may exert their effects by action on a common receptor. Electrophysiologic evidence indicates that in mammalian brain, the presynaptic serotonin receptor is the site of action for indoleamine hallucinogens. In pursuing a report that neonatal rat colliculus contains a serotonin-sensitive adenylate cyclase, we found that the reported serotonin agonist and indoleamine hallucinogen, 5-methoxy-N,N-dimethyltryptamine (5-methoxy-DMT) caused an increase in levels of guanosine 3':5'-cyclic monophosphate (cyclic GMP) in colliculi of rats less than 3 days old. The increase in cyclic GMP levels was maximal (190%) at 10 min following an ip administration of 0.05 mmol of 5-methoxy-DMT/kg and was blocked in animals treated ip with the serotonin antagonist methysergide. 0.05 mmol/kg, given 20 min before the 5-methoxy-DMT. Levels of cyclic GMP remained unchanged at 10 min following ip injection of a representative phenethylamine hallucinogen, 3,4-methylenedioxymethylamphetamine (MDA; 0.1 mmol/kg). All animals were killed by exposure to 1.2 kW of focused microwave irradiation. Cyclic GMP was determined by radioimmunooassay. The results indicate that the two hallucinogens may be acting by different mechanisms. (Supported by USPHS Grant ES00138.)

118. Early Lead Exposure and Ontogeny of Seizure Responses in the Rat. D. A. FOX, S. R. OVERMANN, and D. E. WOOLLEY, Departments of Animal Physiology and Environmental Toxicology, University of California, Davis, California.

Clinical evidence indicates that lead encephalopathy in children produces recurrent seizures. To determine experimentally the conditions under which seizure responses are altered by Pb exposure, Long-Evans rat pups were Pb-exposed (Days 0–21) via the milk of dams drinking either tapwater (C) or Pb(AC)₃ solutions (0.02 or 0.2% Pb) and maximal electroshock seizure (MES) responses were determined at 10, 12, 14, 16, 18, and 20 days of age. MES seizure severity in rats is characterized by the sequential appearance of graded seizures of increasing severity: grade 1, hyperkinesis or clonus; 2, forelimb flexion (FF); 3, FF followed by forelimb extension (FE); 4, FF, FE, and hindlimb flexion (HF); and 4, FF, FE, HF, and hindlimb extension (HE). Lead treatment had no effect on pup body growth, age of incisor eruption, eye opening, or appearance of auditory startle response. Repeated MES testing decreased body weight at weaning. On Days 10 and 12, the 0.2% Pb exhibited more severe seizures than either C or 0.02% Pb. The latter predominantly exhibited grade 1 seizures, whereas the 0.2% Pb typically exhibited tonic–clonic seizures and required longer to recover the righting reflex. On Days 14 through 20 all groups showed comparable development of the seizure pattern, although at 18 and 20 days of age the 0.2% group showed both increased duration of HF and increased duration of HE, indicative of increased seizure severity. Thus, levels of Pb exposure which do not affect body growth increase MES seizure severity. These observations suggest that early lead exposure acts to increase the excitatory to inhibitory ratio on the cerebrospinal axis (see also Fox et al., Pharmacologist 19, 243, 1977). (Supported by NIEHS Postdoctoral Fellowships ES05094 (DAF) and ES05057 (S.R.O.).)
119. *Persistent Neurotoxic Effects of Mild Prenatal Carbon Monoxide Exposure* L. Fechter,
Department of Environmental Health Sciences, Johns Hopkins University School of Hygiene
and Public Health, Baltimore, Maryland. (Robert J. Rubin)

Carbon monoxide has long been recognized to be an environmental hazard and both
occupational and ambient exposure standards have been established. There is now reason
to believe that the fetus may be particularly sensitive to CO and that many adults may be
surpassing exposure levels which would compromise the normal development of the fetus. The
major source of CO in blood is tobacco smoking. Since CO readily crosses the placenta,
producing equivalent levels in maternal and fetal blood, it is possible that we already are seeing
signs of CO toxicity among the offspring of cigarette-smoking women in the form of increased
perinatal mortality and reduced birth weights. Neurotoxic consequences of maternal cigarette
smoking have not clearly been established in humans. Long–Evans rats were bred in the
laboratory and following a sperm-positive vaginal smear the females were randomly assigned to
chambers where they were exposed either to room air or to room air diluted to contain 150 ppm
of CO for the duration of pregnancy. At birth, the litters were removed from the exposure
chambers and the offspring were weighed and counted. Litters were equalized to contain eight
pups. Subjects were either tested on a series of behavioral tasks including the righting reflex,
negative geotaxis, and homing or were taken for neurochemical and biochemical assay. The
results show that the offspring of CO-exposed rats weigh less at birth and grow at a slower rate
than normal subjects. Further, the CO-exposed neonates show evidence of slower behavioral
development during the neonatal period.

120. *Studies on Delayed Neurotoxicity Produced by Oral Administration of O-Ethyl O-4-
Nitrophenyl Phenylphosphorothioate in the Hen*. M. B. Abou-Dania and D. G. Graham,
Departments of Pharmacology and Pathology, Duke University Medical Center, Durham,
North Carolina.

Delayed neurotoxicity in hens was produced following daily oral administration of sub-
neurotoxic doses (0.1, 0.5, 1.0, 2.5, 5.0 and 10.0 mg/kg) of technical (85%) EPN (O-ethyl O-4-
nitrophenyl phenylphosphorothioate) in gelatin capsules for 90 days. A group of hens that was
given a daily dose of 0.01 mg/kg of EPN showed no abnormality in gait or behavior. Three
groups of hens were given daily empty gelatin capsules, 10 mg/kg of TOCP (tri-o-cresyl
phosphate) and 1 mg/kg of parathion (O,O-diethyl O-4-nitrophenylphosphorothioate) and
deposited as gelatin capsule, positive, and negative controls, respectively. While positive control
hens developed neurotoxicity, negative controls showed leg weakness with subsequent recovery
when the administration of parathion had stopped. The clinical condition of most ataxic hens
deteriorated during the 30-day observation period following the end of the oral administration of
EPN. Severity of the clinical condition depended on the size of the daily ingested dose, i.e., while
hens given small doses showed only ataxia, those treated with large doses progressed to paralysis
and died. Days of administration and “total administered dose” before onset of ataxia depended
on the daily dose. The effect was not only cumulative but the total dose required to produce
ataxia decreased with decrease of the daily dose. Degeneration of myelin and axons in the spinal
cord were the most consistent histologic changes, and were identical to those found in TOCP
control hens. Only one hen showed sciatic nerve degeneration. Livers from two hens given the
highest dose of EPN showed a moderate degree of hemosiderosis. (Supported in part by NIEHS
Grant No. ES01186 and EPA Contract. No. 68-02-2452.)

121. *Mechanisms of Organophosphate Axonopathy in Hens*. E. Olajos and I. Rosenblum,
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University, Albany, New York.

Many organophosphorous esters inhibit esterases, and the role of various esterases in distal
axonopathy has been considered. Previous studies have demonstrated the possible relationship
between distal axonopathy with the inhibition of a particular esterase (“neurotoxic esterase”).
Adult white leghorn hens were given a single oral dose (1 mg/kg) of diisopropyl phosphorofluoridate (DFP) and killed 24 hr after administration of organophosphate. The neurotoxic esterase activity of whole brain and sciatic nerve homogenates and that of subcellular fractions from brain and sciatic nerve of treated and control hens was studied. Multiple criteria (enzyme activities, electron microscopy, and polyacrylamide gel electrophoresis) have been utilized to characterize the fractions. The microsomal fractions were found to have the highest neurotoxic esterase activity. Substrate hydrolysis by hen sciatic nerve preparations was considerably less when compared to hen brain homogenates using equivalent weights of tissue. It was also shown that the percentage neurotoxic esterase of total paraxon-resistant activity was lower in sciatic nerve preparations compared to hen brain preparations. The effects of DFP on the neurotoxic esterase activities of the various subcellular fractions from brain and sciatic nerve preparations was studied and found to be definitely inhibitory. One may conclude that quantitative differences exist between neurotoxic esterase from brain and sciatic nerve.

122. Comparative Metabolism and Toxicokinetics of Aflatoxin B₁: An Overview. DENNIS P. H. HSIEH, Department of Environmental Toxicology, University of California, Davis, California. (G. L. Henderson)

Available experimental evidence has indicated that marked differences in susceptibility to the carcinogenic effect of aflatoxin B₁ (AFB₁) among animal species is correlated with differences in the activity of aflatoxin metabolism and other processes that govern the bioavailability of AFB₁ to the target hepatocytes. Under in vitro conditions, the liver preparations of relatively susceptible species such as rat and duck possess higher activities of converting AFB₁ to aflatoxicol (AFL), a reduced metabolite, and to a metabolite mutagenic to the bacterium Salmonella typhimurium, presumably an epoxide, than the liver preparations of relatively resistant species such as mouse. The latter, however, possess higher activity of converting AFB₁ to AFQ₁ and/or water-soluble metabolites. While the significance of AFQ₁ remains obscure, AFL has recently been identified as the major metabolite of AFB₁ in the plasma of the rats dosed with AFB₁ intravenously or orally. When similar experiments were performed on resistant species such as mouse and rhesus monkey, no AFL in the plasma was detectable. The presence of AFL in the plasma of the only susceptible species lends support to the proposed correlation between susceptibility and the AFL-forming activity. The correlation between resistance and the activity to conjugate AFB₁ to form water-soluble metabolites is also supported by experimental results obtained from comparative metabolism of AFB₁ by rat and mouse primary hepatocyte cultures. Over 90% of AFB₁ was transformed to water-soluble metabolites by the mouse hepatocyte culture, but only 55% was transformed to water-soluble metabolites by the rat hepatocyte culture. The results of toxicokinetic studies using animals of different susceptibilities were not consistent with the above correlations. Upon phenobarbital pretreatment rhesus monkeys produced lesser amounts of plasma water-soluble AFB₁ metabolite than the control animals, even though the drug has a protective effect. Fischer rats produced more water-soluble AFB₁ metabolites than Sprague-Dawley rats despite the greater susceptibility of the former. The water-soluble AFB₁ metabolites appeared to be detoxification products of AFB₁. Upon hydrolysis by glucuronidase and sulfatase, the freed AF metabolites became significantly mutagenic to S. typhimurium, indicating that the fate of these conjugates such as hydrolysis by the gastrointestinal microflora may be another determinant of AFB₁ toxicity in an animal. (Supported by PHS Grant ES00612 and Western Regional Research Project W-122.)

123. The Effects of Phenobarbital Pretreatment on the Metabolism and Toxicokinetics of Aflatoxin B₁ in the Rhesus Monkey. Z. A. WONG and D. P. H. HSIEH, Department of Environmental Toxicology, University of California, Davis, California. (J. J. Menn)

Our previous study on the toxicokinetics of aflatoxin B₁ (AFB₁) in the Rhesus monkey established kinetic profiles on the metabolism, distribution, and elimination of AFB₁ in vivo. We noted that toxicokinetic studies of AFB₁ can provide theoretical information toward explaining
species susceptibility to aflatoxicosis. In the present study, the toxicokinetics of AFB$_1$ was further determined in Rhesus monkeys pretreated with phenobarbital, a treatment known to protect animals from the carcinogenic effects of AFB$_1$, to examine the kinetic changes corresponding to reduced susceptibility and metabolic induction. Male rhesus monkeys (Macaca mulatta) were given [14C]AFB$_1$ intravenously at a dose range of 0.1 to 1.0 mg/kg. Blood samples were then collected at selected intervals for 5 hr. Blood, urine, and feces were also collected over a 10-day period for AF analysis. After establishing control values, the monkeys were administered phenobarbital (15 mg/kg, i.m) twice daily for 6 days, and the kinetic study was repeated. Liver biopsies enabled measurement of in vitro metabolism activity which confirmed metabolic induction by phenobarbital. Phenobarbital pretreatment at this dose level did not induce significant changes in the volume or rate of AFB$_1$ distribution after intravenous administration: $t_{1/2} = 35.5$ min, $K_{1} = 5.87$ hr$^{-1}$, $K_{12} = 13.7$ hr$^{-1}$, $K_{21} = 4.6$ hr$^{-1}$, and $V_{p} = 66\%$ of total body weight. However, the total AF plasma radioactivity was reduced by as much as 40\% of control values at peak periods. The observed decrease in plasma levels was primarily due to a decrease in water-soluble metabolites of AFB$_1$. Plasma protein-bound radioactivity, however, increased slightly over control levels. Phenobarbital administration also caused a 25\% reduction in total urinary excretion and a 64\% reduction in fecal elimination of AF. Although higher urinary levels of polar AFB$_1$ metabolites were observed, the decrease in urinary excretion appeared to be largely attributed to a 60\% decrease of free AFM$_1$ in the urine. The maintained high transfer rate constant ($K_{12}/K_{1}$) of AFB$_1$ to tissues and the reduction of total AF radioactivity in plasma, urine, and feces suggest that phenobarbital pretreatment caused the animals to retain greater intracellular proportions of AF as a result of the induction of microsomal oxidase activity in target hepatocytes. Data supporting reduced susceptibility to AFB$_1$-induced carcinogenesis was not evident; however, the apparent increase in tissue levels of AF may actually indicate an increased susceptibility to the acute effects of AFB$_1$. (Supported by NIEHS Training Grant ES00125-09.)

124. Strain-Dependent Toxicokinetics of Aflatoxin B$_1$ in the Rat. J. J. Wong and D. P. H. Hsien, Department of Environmental Toxicology, University of California, Davis, California.

The rat has been extensively used as an animal model for the prediction of human susceptibility to aflatoxin toxicity. Our investigations have involved studies of the relationship between the in vivo metabolic fate of aflatoxin B$_1$ (AFB$_1$) and the apparent strain-dependent susceptibility to aflatoxicosis in the rat. Using an acute surgical procedure, [14C]AFB$_1$ (0.5 \mu Ci/250 \mu g/animal) was infused directly into the mesenteric vein of ether-anesthetized male Fischer 344 (most susceptible), Sprague-Dawley, and Wistar rats. Over a 90-min time course, blood samples were taken from the inferior vena cava of individual preparations and then analyzed. Ninety percent or greater of the total radioactivity in the blood was associated with the plasma fraction. The greatest proportion of the plasma radioactivity was not chloroform-extractable. The plasma radioactivity was greatest in the Fischer rats, attributable to the greater production of water-soluble metabolites or conjugates. In all three strains, levels of radioactivity associated with this aqueous fraction increased with time, while that covalently bound to plasma protein bore no such relationship. Levels of covalently bound radioactivity were comparable in all three strains. Free AFB$_1$ represented up to 25\% of the total plasma radioactivity in the Wistar and Fischer rat. The Sprague-Dawley rat maintained the highest plasma levels of AFB$_1$, representing up to 37\%. The apparent plasma half-lives of AFB$_1$ in the Fischer, Sprague-Dawley, and Wistar rats were 34, 38, and 43 min, respectively. In agreement with the recent findings of this laboratory, aflatoxicol (AFL) was the major plasma metabolite. In all strains the plasma concentrations of AFL paralleled those of AFB$_1$, with AFL representing up to 80, 65, and 49\% of the radioactivity attributable to AFB$_1$ in the Fischer, Wistar, and Sprague-Dawley rats, respectively. Trace amounts of aflatoxin M$_1$ was also observed in the plasma of all three strains. Previous in vitro metabolism studies have demonstrated the production of AFL and water-soluble metabolites to be the greatest in susceptible and resistant animal species, respectively. In the present study, under in vivo conditions, the Fischer rat was found to produce
the greatest amount of water-soluble metabolites. While the absolute plasma levels of AFL were not the highest in the Fischer rat, the greater AFL to residual AFB, ratio may be related to this strain's increased susceptibility. The higher circulating levels of AFB, in the Sprague-Dawley rat may account for its increased susceptibility to AFB, induced renal carcinomas. Analysis of different organs for the amount of covalent binding of AFB, to various cellular macromolecules of these three rat strains is in progress. (Supported by USPHS Grant ES00612 and Western Regional Research Project W-122.)

125. Comparative Metabolism of Aflatoxin B, in Mouse and Rat Primary Hepatocyte Cultures.
GARRY M. DECAD, K. K. DOUGHERTY, D. P. H. HSIEH, and JAMES L. BYARD, Department of Environmental Toxicology, University of California, Davis, California.

The metabolism of aflatoxin B, was compared in primary hepatocyte cultures from mice and rats to determine whether differences in metabolism could be correlated with differences in carcinogenic susceptibility in vivo. Hepatocytes were prepared from adult male Charles River CD-1 mice and Sprague-Dawley rats by a collagenase perfusion technique. The hepatocytes were cultured on collagen-coated plastic petri dishes in a chemically defined supplemented Waymouth 752/1 medium. After 20 hr in culture, the hepatocytes had formed a confluent monolayer of 15 x 10^6 cells/150-mm plate. The medium was aspirated and 5 μg of [14C]aflatoxin B, (0.25 μCi) was added in fresh medium to each plate. Aflatoxin B, was completely metabolized in a 10-hr incubation. In mouse hepatocyte cultures, 90% of the input radioactivity was found in the aqueous phase of the culture medium. Most of this radioactivity was characterized as water-soluble aflatoxin conjugates. In addition, only 0.6% of the input radioactivity was covalently bound to cellular macromolecules. In contrast, rat hepatocytes converted only 57% of the input radioactivity into water-soluble metabolites, and more than 12% of input radioactivity was covalently bound to cellular macromolecules. The only nonpolar metabolite detected in hepatocyte culture media was aflatoxin M, which at the end of a 10-hr incubation accounted for 0.4% of the input radioactivity in mouse hepatocyte cultures and 5.0% in rat hepatocyte cultures. Available evidence suggests that the covalently bound aflatoxin is the basis for its toxicity and carcinogenicity. The 20-fold greater amount of covalent binding of aflatoxin to hepatocytes cultured from the rat compared with the mouse, correlates with the greater in vivo susceptibility of the rat to the carcinogenicity of aflatoxin B,. Thus, mouse and rat primary hepatocyte cultures provide a useful model for studying the comparative metabolism and mechanisms of action of environmental chemicals and support the feasibility of doing similar comparative studies between rodents and humans. (Supported by NIEHS Training Grant ES00125-09).

126. Characterization and Mutagenicity of Water-Soluble Conjugates of Aflatoxin B,.
CHENG-I WEN, G. M. DECAD, Z. A. WONG, J. L. BYARD, and D. P. H. HSIEH, Department of Environmental Toxicology, University of California, Davis, California. (G. L. Henderson)

The mutagenic activities of water-soluble aflatoxin B, (AFB,) conjugates were compared before and after β-glucuronidase and sulfatase hydrolysis. The conjugates were obtained from urine of rhesus monkeys and primary hepatocyte cultures prepared from adult mice (Charles River CD-1) and rats (Sprague-Dawley). The monkeys and the hepatocyte cultures were dosed and incubated, respectively, with [14C]AFB,. The conjugates were concentrated on XAD-4 resin columns, dissolved in sodium acetate buffer (0.2 M, pH 5.0), and subjected to enzymatic hydrolysis for 48 hr at 37°C. The percentages of hydrolyzable conjugates were: rat, 37%; mice, 56%; monkey (ip), 71%; monkey (iv), 37%. The distributions of radioactivity in the chloroform extract of hydrolyzed conjugates were: AFB, (3.7%), AFB, (25.3–28.4%), AFM, (15.4%), and a more polar blue fluorescent metabolite (PM) (5.4–6.7%) in rat; AFB, (12.6–17%), AFB, (14.6–15.3%), AFM, (19.21.4%), and PM (5.2–7.7%) in mice; AFB, (0.9–1.4%), AFB, (0.5%), AFB, (4.7–8.1%), and AFM, (75.5–79.5%) in monkeys (ip); and AFB, (7.2–8.2%), AFB, (44–53.1%), and AFM, (5.9–8.4%) in monkeys (iv). The water-soluble conjugates were found to
have no or very low mutagenic activity as measured by the Ames mutagen assay. After enzymatic hydrolysis, the activity was increased severalfold. The results indicate that the conjugates of the various species differ not only in the total amount but also in the metabolite make-up of the conjugates. The results also suggest that the intestinal microflora, which can cleave these conjugates, may play an important role in the determination of the toxic effect of aflatoxins. (Supported by USPHS Grant ES00612 and Western Regional Research Project W-122.)

127. Comparative Toxicokinetics of AFB\textsubscript{1} in the Rhesus Monkey, Rat, and Mouse. Z. A. Wong, D. W. Rice, and D. P. H. HsiEH, Department of Environmental Toxicology, University of California, Davis, California. (J. J. Menn)

Comparative metabolism studies of AFB\textsubscript{1} have shown that species differences in hepatic metabolism of AFB\textsubscript{1} may account for the marked differences in species susceptibility. Hepatic metabolism alone, however, may not account entirely for susceptibility since other biological processes such as absorption, distribution, and elimination may also contribute in the etiology of aflatoxicosis. Hence, a comparative toxicokinetic study of AFB\textsubscript{1} was conducted in the Rhesus monkey, rat, and mouse to determine if kinetic profiles can reflect species susceptibility to AFB\textsubscript{1}. The mouse represents a "totally" resistant animal model, while the monkey and rat are sensitive to acute and carcinogenic aflatoxicosis, respectively. Rhesus monkeys (Macaca mulatta), Sprague-Dawley rats, and Swiss-Webster mice were administered [\textsuperscript{14}C]AFB\textsubscript{1} intravenously. At selected time intervals, venous blood samples were collected, and the plasma was analyzed for AF and radioactivity. AFB\textsubscript{1} demonstrated a rapid tissue distribution in the Rhesus monkey, manifesting a blood to tissue transfer rate constant (K\textsubscript{12}) of 13.7 hr\textsuperscript{-1}, which is about three times that for the transfer from tissue to blood (K\textsubscript{13}), 4.6 hr\textsuperscript{-1}. In contrast to the monkey, the rat showed a lower K\textsubscript{13}, 9.2 hr\textsuperscript{-1}, but a much higher K\textsubscript{12}, 11.0 hr\textsuperscript{-1}. The kinetic profile for the mouse and rat were similar, namely K\textsubscript{13} > K\textsubscript{12}. The apparent volume of distribution for AFB\textsubscript{1} was the largest in Rhesus monkey, 66% of body weight, while in the rat, the distribution volume was about 45%. The plasma half-lives of AFB\textsubscript{1} in the monkey and rat were 13.6 and 35.5 min, respectively. The typical profile for the monkey was a high level of water-soluble metabolites of AFB\textsubscript{1}, producing a rise in plasma radioactivity. The rat characteristically metabolized AFB\textsubscript{1} to AFL, which represented the major plasma metabolite. The mouse metabolized AFB\textsubscript{1} to AFM\textsubscript{1} and several unidentified plasma chloroform-extractable products. The data suggest that susceptibility to acute aflatoxicosis may be attributed to a high transfer rate (K\textsubscript{13}/K\textsubscript{12}) of AFB\textsubscript{1} to target tissues in which species resistance to AFB\textsubscript{1} may be reflected in their plasma half-life. The presence of AFL in the only susceptible species to carcinogenic aflatoxicosis favours the proposed concept that AFL formation may be correlated with species susceptibility. (Supported by NIEHS Training Grant ES00125-09.)

128. Acute Toxicity of Patulin. A. Wallace Hayes, Timothy D. Phillips, and W. Lane Williams, Departments of Pharmacology and Toxicology and Anatomy, University of Mississippi Medical Center, Jackson, Mississippi.

Patulin is a toxic, carcinogenic, heterocyclic lactone whose importance in the etiology of mycotoxicoses has not been fully evaluated. It is produced by fungi known to occur in foods and feeds and has been reported in apples and in apple cider products. Patulin had the following LD\textsubscript{50} values after a single ip dose, dissolved in physiological saline, pH 7.2: mice, 7.6 mg/kg; neonate rats, 6.8 mg/kg; and weanling rats, 5.9 mg/kg. Administration by stomach tube increased the LD\textsubscript{50} values to 17 and 108–118 mg/kg in mice and weanling rats, respectively. The effects of SKF-525A and pentobarbital on the LD\textsubscript{50} value of patulin suggest that the toxicity of this mycotoxin does not arise from toxic metabolite. Patulin also potentiated pentobarbital-induced narcosis and depressed body weight gains in adult mice. A dose response reflected in decreased weight gain in neonatal rats given patulin 24 hr after birth compared with that in control animals was observed over 21 days. Death generally occurred within 24 to 48 hr.
regardless of the route of patulin administration. Postmortem examination of animals that died revealed congestion in lungs, stomach, and liver. Hemorrhagic and degenerative changes were observed in sections of liver and kidneys from animals that died; however, animals that survived presented normal histopathology. (Supported by ES 01351 and ES 01352.)

129. Distribution and Excretion of $[^{14}C] $Citrinin in Rats. R. D. PHILLIPS, W. O. BENOT, and A. W. HAYES, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi.

The distribution and excretion of $[^{14}C] $citrinin was determined in male rats given 3.0 mg/kg iv. Between 10 min and 6 hr after administration of $[^{14}C] $citrinin, 7.5 to 14.7% of the total $^{14}C$ activity was detected in the liver, 2.6 to 5.6% in the kidneys, and 4.7 to 9.2% in serum. Three elimination rates from plasma were observed, having half-lives of 3.3, 9.1, and 70.4 hr. Seventy-seven percent of the $^{14}C$ activity was excreted by 24 hr after administration. A second group of rats was pretreated with 50 mg/kg of citrinin, ip. 4 days prior to administration of 3 mg/kg of $[^{14}C] $citrinin, iv. Thirty percent of the pretreated animals died and the remaining animals could be divided into two groups, animals with damaged kidneys and animals without damage to kidneys. The damaged kidneys showed gross morphological disruption and increased weights. Urine output from animals with damaged kidneys was approximately twice that of controls. Twenty-four hours after treatment with $[^{14}C] $citrinin, 18.1% of $^{14}C$ activity was detected in the urine and 13.7% in the feces. The plasma decay curve appeared to be from a single compartment compared to three compartments for controls. Animals with damaged kidneys showed levels of radioactivity in the liver of 7.5% of the administered dose compared to 1.3% in the normal subjects at 24 hr after treatment. These results indicate that if the kidneys are damaged, the liver will begin to eliminate the radioactive material from the animal. (Supported by ES 01352 and Training Grant ES 07045.)

130. In Vitro Binding of Rubratoxin B and Its Hydrogenated Analog to Mouse Hepatic Microsomes. SHARON A. WATSON and A. WALLACE HAYES, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi.

Rubratoxin B, a mycotoxin produced by Pencillium rubrum, is a potent hepatotoxin. It also is teratogenic and mutagenic in the mouse, and preliminary results indicate it binds to mouse liver DNA. The unsaturated lactone ring has been shown to be a structural requirement for these effects. Rubratoxin B also inhibits mouse hepatic microsomal ATPase activity and the activity of several drug-metabolizing microsomal enzymes. Binding of the toxin and its hydrogenated analog to microsomes was studied by uv difference spectra. Binding of radiolabeled rubratoxin B and its hydrogenated analog to mouse liver microsomal fraction was investigated using differential centrifugation. Rubratoxin B binds in vitro to hepatic microsomes forming an adduct with a difference spectra peak at 237 nm. When uniformly labeled $[^{14}C] $rubratoxin B was incubated for 40 min at 37°C with microsomes, 60 to 65% of the labeled toxin was associated with the 105,000 g fraction. The labeled rubratoxin B–microsome complex was not dissociated by Sephadex G-25 gel filtration. The labeled hydrogenated analog of rubratoxin B was associated with the supernatant fraction rather than the 105,000 g fraction. The binding of rubratoxin B and its hydrogenated analog to microsomes also was investigated as a function of incubation time. These data indicate that rubratoxin B binds strongly to mouse liver hepatic microsomes and that the unsaturated lactone ring is a structural requirement of such binding. (Supported by Research Grants ES 01351 and Training Grant ES 07045.)


$[^{14}C] $Tetrachloroethylene (Perc) was administered to adult male Sprague–Dawley rats by oral gavage (1 or 500 mg/kg) or by inhalation (10 or 600 ppm, 6 hr in duration). Within 72 hr
following oral administration of 1 mg/kg or inhalation of 10 ppm of $[^{14}C]$Perc approximately 70% of the body burden of radioactivity was excreted as Perc in expired air, 26% was trapped from expired air as $^{14}$CO$_2$ and collected as nonvolatile metabolites in urine and feces, and 3 to 4% remained in the carcass. After oral administration of 500 mg/kg or inhalation of 600 ppm of $[^{14}C]$Perc, 89% of the radioactivity was recovered in expired air as Perc and 9% as $^{14}$CO$_2$ and urinary and fecal metabolites, and 1 to 2% remained in the carcass. The half-life for elimination of Perc in expired air was approximately 7 hr and there was no significant difference with dose or route of administration. Radioactivity remaining in the carcass 72 hr after exposure by either route was primarily distributed within liver, kidney, and fat tissue. In liver, volatile (Perc) and extractable radioactivity was cleared within 24 hr of inhalation exposure to 10 or 600 ppm. The clearance of radioactivity bound to hepatic macromolecules was comparatively slower. Thus, the data indicate that the proportion of $[^{14}C]$Perc metabolized and eliminated by rats is dependent upon dose following either route of administration. Although the slow turnover of radioactivity associated with hepatic macromolecules could indicate a potential for accumulation of bound species with repeated exposure, exposure of Sprague–Dawley rats to 600 ppm of Perc vapor 6 hr/day, 5 days/week for 12 months followed by lifetime observation did not result in organ toxicity.


The pharmacokinetics of hexachlorophene (HCP) was studied in sexually mature virgin Wistar rats. $[^{14}C]HCP$ was injected (i.v., 0.87 or 3.87 mg/kg in saline) or into the vaginal orifice (i.v.g., 0.87 mg/kg in corn oil). Radioactivity was determined by liquid scintillation spectrophotometry. The disappearance of $^{14}$C from the blood, after iv administration, followed the kinetics of a two-compartment open-system model. The blood $^{14}$C profiles were superimposable, suggesting that the distribution and elimination rate constants for both doses were similar. A rapid distribution phase (half-life, 20 min) was followed by a first-order elimination phase (half-life, 8 hr). The apparent volume of distribution was 20% of the body weight, corresponding to the total extracellular water. After ivg application of $[^{14}C]HCP$, $^{14}$C was detected in tail blood at 0.5 hr, peaked between 2 and 4 hr, and disappeared slowly to 12 hr and more rapidly thereafter. Less than 10% of the ivg dose of $[^{14}C]HCP$ remained in the vagina after 4 hr. The cumulative recoveries of $^{14}$C in the feces and urine 5 days after iv administration were 85 and 4.6% of the dose, respectively. Comparable recoveries following ivg administration were 72 and 3.7%. The results suggest that HCP readily penetrates through the vaginal mucosa of the rat.


Sulfapyrazone (Anturan) is an antithrombotic drug which acts by inhibiting platelet aggregation. The pharmacokinetics of sulfapyrazone were tested in rats for interaction with salicylate. Adult male Wistar rats were fasted overnight and salicylate (aspirin, 100 mg/kg) was administered by gavage 30 min before an ip injection of $[^{14}C]$sulfapyrazone (50 mg/kg). The disappearance of $[^{14}C]$sulfapyrazone-derived radioactivity from the blood and its appearance in the urine, feces, and bile were followed. Salicylate decreased the blood radioactivity level during the initial 48 hr after drug administration, reduced the area under the blood radioactivity vs time curve, and shortened the $\alpha$ phase (0–5 hr) of elimination of radioactivity from the blood but had no effect on the $\beta$ (5–48 hr) elimination phase. Total urinary and fecal excretion was little affected. Biliary excretion of radioactivity was increased from 44.4 to 59.5% of the dose during the first 3 hr by salicylate, but the proportion of unchanged sulfapyrazone to its metabolites in the bile was unaffected. An in vitro protein binding study showed that about 0.35% of sulfapyrazone was unbound to plasma protein and this was increased to 1.98% by salicylate.
These results suggest that, in rats, salicylate enhances the initial rate of sulfinpyrazone elimination by displacing some of the bound drug from plasma protein and that this accounts for the decreased concentration in blood.

134. Characterization of Normal and Induced Cytochrome(s) P-450 of Rainbow Trout. C. R. Elcombe and J. J. Lech, Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin.

Although there have been extensive studies of the hepatic cytochrome P-450 system in mammals, little is known concerning these cytochromes in fish. In addition to the possible implications of the state of the P-450 system in the toxicity of chemicals to fish, investigations of this system in fish may contribute to a better understanding of the induction process in higher animals. Rainbow trout (Salmo gairdneri), approximately 75 g, were injected ip with phenobarbital (PB) (65 mg/kg), Arochlor 1242 (PCB) (100 mg/kg), or β-naphthoflavone (βNF) (100 mg/kg) and control fish received vehicle alone. Model monoxygenase reactions examined were ethylmorphine-N-demethylation (EM), ethoxycoumarin-O-deethylation (EC), benzaldehyde hydroxylation (AHH), and ethoxyresorufin O-deethylation (ER). The specific activity of EM was not altered by pretreatment of fish with PB, PCB, or βNF, while AHH was dramatically increased by βNF (4081% of control) and PCB (1059%) and unchanged by PB (104%). V̇max values for EC and ER deethylations were unaffected by PB but were increased by βNF (1178 and 4455%, respectively) and PCB (283 and 1367%, respectively). a-Naphthoflavone (αNF) has been utilized in vitro as a selective inhibitor of P-448-mediated monoxygenation reactions. In this study αNF almost totally inhibited PCB-induced and βNF-induced AHH, but had little effect upon the hydroxylation in microsomes from control or PB-pretreated rainbow trout. P-450 hemoprotein concentrations in microsomes from control and PB-, βNF-, and PCB-pretreated fish were 0.22, 0.25, 0.48 and 0.30 nmol/mg of microsomal protein, respectively. The Soret absorption maxima of carboxyferrocytochrome(s) P-450 were at 449 nm (control, PB, PCB) and 448 nm (βNF). In summary, it appears that rainbow trout possess an anomalous cytochrome P-450 system, which may be induced by pretreatment with βNF or PCB but not by PB. (Supported by Research Career Development Award ES 00002; NIEHS Grant ES-01080, and EPA Grant R803971010.)

135. The Metabolism/Pharmacokinetics of Pentachlorophenol in Man, and a Comparison with the Rat and Monkey Model. W. H. Braun, G. E. Blau, and M. B. Chenoweth, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan (B. A. Schwetz)

Using gas chromatography–mass spectrometry, the pharmacokinetics and metabolism of pentachlorophenol (PCP) were determined in four healthy male volunteers after ingestion of 0.1 mg of PCP/kg body wt. A pharmacokinetic model was constructed to describe the fate of PCP in humans and compared to models constructed previously for PCP in rats and monkeys. The dynamics of PCP absorption and elimination in humans could be described by a one-compartment open-system model with first-order absorption, enterohepatic circulation, and first-order elimination. The PCP pharmacokinetic model in man was more like the rat model than the monkey model. The half-lives for absorption and elimination of PCP from plasma were 1.3 ± 0.4 and 30.2 ± 4.0 hr, respectively. The half-lives for elimination of PCP and PCP glucuronide in urine were 33.1 ± 5.4 and 12.7 ± 5.4 hr, respectively. Approximately 74 and 12% of the doses were eliminated as PCP and PCP glucuronide in the urine, respectively, within 168 hr after ingestion. Additionally, 4% of the dose was eliminated as PCP and PCP glucuronide in the feces. The peak of urinary excretion occurred at 42 hr postingestion as compared to a peak plasma concentration at 4 hr which is possibly caused by enterohepatic circulation. The plasma concentration maximum was 0.248 μg/ml. A simulation of repeated daily ingestion of 0.1 mg/kg of PCP indicated that PCP would reach 99% of steady state in 8.4 days with a plasma concentration maximum of 0.491 μg/ml. The results of the study conducted in human volunteers support the conclusion that PCP would not cause cumulative toxicity even with repeated daily low-level exposures.
136. Comparative in Vivo and in Vitro Metabolism of p-Nitroanisole in Mice. T. D. Trautman and R. I. Krieger, Department of Environmental Toxicology, University of California, Davis, California.

Though in vivo studies of p-nitroanisole (PNA) disposition in mice are lacking in spite of its widespread in vitro use as an indicator of liver monooxygenase activity, ring-labeled PNA (specific activity 0.17 mCi/mmol) was administered in an Emulphor: saline carrier (170 mg/kg, ip) to male Swiss–Webster mice (22–30 g). Absorption was rapid: CNS effects were observed within 5 min and within 15 min urine contained significant (4.7% of dose) amounts of radioactivity. Excreta were collected at 6, 12, and 24 hr. The mouse was then killed and tissues were taken for analysis of residual radioactivity. Recovery of radioactivity from urine, feces, body, expired air, and cage wash was 80 to 85%. Urine contained 75 to 80% of the dose. Hydrolysis of urine with β-glucuronidase, aryl sulfatase, β-glucosidase, and/or concentrated hydrochloric acid released p-nitrophenol (PNP). Hydrolysis was monitored using corresponding standard conjugates of PNP. Following PNA (170 mg/kg) treatment, PNP present in urine was conjugated with glucuronic acid, sulfate, and glucose (8:3:1) with 1 to 5% of total PNP present in unconjugated form. β-Glucosidase had no detectable activity on PNP glucuronide or PNP sulfate standards. Studies were performed to determine quantitative and qualitative effects of dosage and pretreatment with phenobarbital or SKF-525A. Rapid metabolism, low toxicity, and straightforward analysis make the in vivo metabolism of PNA a useful tool for studying primary and secondary metabolism and drug interactions. Comparisons between these studies and previous in vitro work (Trautman et al., Toxicol. Appl. Pharmacol. 41, 216, 1977) indicate the usefulness of PNA in vivo metabolism as an indicator of in vivo oxidative metabolic capability. (Supported in part by NIH Grants ES00125 and ES00054.)

137. Studies on Toxic, Alkylating Metabolites of 2-Methylfuran. R. B. Franklin and M. R. Boyd, Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin, and Clinical Pharmacology Branch, National Cancer Institute, Bethesda, Maryland.

We have undertaken studies on the chemical nature of toxic, alkylating metabolite(s) produced by cytochrome P-450-mediated metabolism of 2-methylfuran (2MF). 2MF causes pulmonary bronchiolar lesions in mice like those produced by other lung-toxic furans such as 3-methylfuran and 4-ipomeanol. However, it is relatively less selective, also frequently producing marked hepatic and renal necrosis. NADPH-dependent microsomal metabolism of 2MF results in the formation of a highly electrophilic metabolite which alkylates the microsomal protein; addition of cysteine to the incubations results in the formation of a water-soluble cysteine conjugate and a decrease in the microsomal alkylation. Since an initially formed furan-epoxide of 2MF could possibly rearrange to α- or β-angelicalactones, we have synthesized both the lactones, as well as their cysteine conjugates. Using a combination of techniques including high-pressure liquid chromatography, gas chromatography, and mass spectrometry, we have developed separation and analytical techniques for these standards, as well as for 2MF itself, and the conjugate formed in microsomal incubations with 2MF and cysteine. Comparison of the properties of the potential metabolites we have synthesized with those produced from 2MF in vitro and in vivo will facilitate the further characterization of the proximate toxic metabolite of 2MF.

138. NADH-Supported Oxidations of Foreign Compounds by Rhesus Monkey (Macaca mulatta) Liver. David R. Dohn, Shirley J. Gee, and Robert I. Krieger, Department of Environmental Toxicology, University of California, Davis, California.

NADH- and NADPH-supported mixed-function oxidation of xenobiotics by liver microsomal preparations from the rhesus monkey (Macaca mulatta) has been reported (Drug Metab. Disp. 4, 28, 1976). N-Demethylation of p-chloro-N-methylaniline (PCMA) is measured by a colorimetric procedure and the epoxidation of the chlorinated cyclohexene aldrin is determined by electron capture gas chromatography. The in vitro assay system contains an NADPH-
generating system consisting of 2.3 mM glucose 6-phosphate and 3 IU of glucose 6-phosphate dehydrogenase. The NADPH-generating system when added to incubations containing NADH, but no added NADP or NADPH, greatly stimulates product formation when compared to assays containing only NADH. NADH-supported aldrin epoxidation is 18 to 26% of the level of NADPH-supported oxidation. However, when NADH together with G6P and G6PDH are present, the level of substrate oxidation is 51 to 64% of that observed for NADPH. The NADH-supported N-demethylation of PCMA is 23 to 42% of the level observed for NADPH. When NADH and NADPH-generating system are added together, the rate of substrate oxidation increases to 50 to 90% of the level seen with NADPH alone. No contaminating NADP or NADPH could be found in the NADH, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, or microsomal suspensions, as judged by enzymatic assay and high-performance liquid chromatography. Redissolution of microsomes through high ionic strength buffer (0.1 M Na-pyrophosphate) had no effect on observed activities. Aldrin epoxidation supported by NADH and the NADPH-generating system is sensitive to the inhibitors SKF-525A, piperonyl butoxide, paraquat, diquat, and carbon monoxide, but is insensitive to 0.5 mM cyanide. (Supported in part by NIH Grant ES00054.)

139. Pharmacokinetics and Metabolism of an Oral Dose of Leptophos in the Cat. M. B. Abou-Donia and M. A. Ashry, Department of Pharmacology, Duke University Medical Center, Durham, North Carolina.

The pharmacokinetics and metabolism of $[^{14}C]phenyl lepto$hos $[O-(4-bromo-2,5-dichlorophenyl)$-$O$-methyl phenyl$-$[^{14}C]$]phosphonothioate$ were studied in the male cat following a single oral dose of 20 mg/kg (1.0 $\mu$Ci/kg). An oral dose of aqueous solution of atropine sulfate (15 mg/kg) was given prior to the administration of Leptophos. Radioactivity was mainly excreted from the animal body via feces. After 8 days, the total radioactivity recovered in the feces was 67.42% of the administered dose. A total of 27.33% of the radioactivity was recovered in the urine. Radioactivity was least excreted via expired air; only 1.78% of the dose was recovered in the expired CO$_2$ collected continually for 8 days. One day after the administration, the tissues had 20.38% of the administered dose, which decreased to 2.57% at 8 days. Twelve hours after administration the highest $^{14}$C content was present in the muscles, mesentery, and small intestine, followed by skin and liver. Identification of metabolites was carried out by mass spectrometry in connection with sequential thin-layer chromatography. While most of the radioactivity in the urine was identified as polar metabolites of Leptophos, the radioactivity in the feces and liver was characterized as unchanged Leptophos and desbromoleptophos. The results of this investigation indicate that the susceptibility of the cat to organophosphorus compound-induced delayed neurotoxicity may be related to the stability and metabolism of Leptophos to the more neurotoxic Desbromoleptophos and to the slow disappearance of the compound from the animal tissues. (Supported in part by NIEN Grant No. ES00056 and EPA Contract No. 68-02-2452.)

140. Pharmacokinetics and Metabolism of $[methyl^{14}C]$Paraquat Chloride in Syrian Golden Hamster following Intratracheal Instillation. M. B. Abou-Donia and A. A. Komeh, Department of Pharmacology, Duke University Medical Center, Durham, North Carolina.

Male Syrian golden hamsters (100–130 g) were given single intratracheal instillations of 10 mg/kg (7.7 $\mu$Ci/kg) of $[methyl^{14}C]$paraquat chloride (bis-$N$-$[methyl^{14}C]4,4'-bipyridylium chloride) in saline. The time-course change in lung contents of $^{14}$C radioactivity following intratracheal instillation of $[^{14}C]$paraquat was biphasic. The physiological disposition of paraquat in the lung may therefore be defined in terms of a two-compartment open-system model. The half-life for the absorption of radioactivity from the lung was 21 hr, which corresponds to a $\beta$ value of 0.033 hr$^{-1}$. A major part of the absorbed radioactivity was excreted in the urine (45.18%) during the 48 hr experiment. A significant portion (32.84%) was recovered in the expired air. Only
3.54% of the dose was excreted in the feces. One hour after administration the highest \(^{14}C\) level was present in the lung, followed by the kidney. Following intratracheal instillation of \([^{14}C]\)paraquat, a biexponential body burden curve was observed. This herbicide was eliminated at slow rate, \(\beta\) value of 0.014 hr\(^{-1}\), corresponding to a half-life of 50 hr. Most of the radioactivity in the urine was identified using mass spectrometry in connection with sequential thin-layer chromatography, as polar metabolites of paraquat. (Supported in part by NIEH Grant No. ES01186 and EPA Contract No. 68-02-2452.)

141. Selective Changes in the Hepatic Microsomal Electron Transport System following Repeated Oral L-\(\alpha\)-Acetylmethadon Administration in the Mouse. T. B. Barnes and L. W. Masten, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi. (W. M. Davis)

In light of recent work from our laboratory demonstrating the ability of L-\(\alpha\)-acetylmethadon (LAAM) to induce its own metabolism as well as significantly increase the microsomal metabolism of other substrates following oral administration, we decided to examine portions of the hepatic microsomal electron transport system (HMETS) in order to characterize this phenomenon further. Male ICR mice (27–30 g) were dosed daily with 7, 14, or 28 mg/kg of LAAM HCl and were sacrificed 24 hr after 1, 2, 3, and 6 days of administration. Upon sacrifice, animals were anesthetized and the livers were perfused with an ice-cold solution of 0.05 M Tris-0.15 M KCl buffer (pH 7.4). Cytochromes P-450 and \(b_5\) as well as two NADPH-dependent enzymes, cytochrome \(c\) reductase, and NADPH oxidase were monitored in a microsomal suspension from these mice according to standard procedures. By Day 3 of LAAM administration, the microsomal concentrations of cytochrome P-450, cytochrome \(c\) reductase, and NADPH oxidase were significantly elevated to approximately twofold at the highest dose compared to the water control values. The observed elevation appeared to be dose-related at both Days 3 and 6. In contrast, only minor changes were noted in the microsomal concentration of cytochrome \(b_5\). These results indicate a selective induction of the HMETS responsible for the majority of microsomal oxidation. Furthermore, the magnitude of this elevation correlated well with previous elevations in LAAM \(N\)-demethylase, aminopyrine \(N\)-demethylase, and aniline hydroxylase activities observed under identical dosing conditions in the mouse. (Supported in part by NIDA Grant DA 01331-02 and by the University of Mississippi Research Institute of Pharmaceutical Sciences.)

142. Toxicokinetics of Intravenously Administered Bumetanide in Beagles. J. E. Manno, R. D. Brown, B. R. Manno, J. A. Sterling, and J. W. Landreneau, Department of Pharmacology and Therapeutics, Louisiana State University Medical School, Shreveport, Louisiana.

Bumetanide was administered in doses of 0.5, 1, 2, 5, 10, 20, and 100 mg/kg by a bolus iv injection to White Eagle pure-bred beagle dogs (8–18 months old, of both sexes). The dogs were anesthetized with iv pentobarbital, and bumetanide was administered into the inferior vena cava by catheter through the femoral vein and the catheter was flushed with saline. Blood samples were withdrawn at intervals of 0.5, 1, 2, 3, 5, 10, 15, 30, 40, and 60 min and at 1.5, 2, 2.5, 3, 3.5, and 4 hr after drug administration. Serum was analyzed for bumetanide by high-pressure liquid chromatography (Manno et al., Fed. Proc. 36, 377, 1977). Data were analyzed by the use of a BASIC exponential stripping program. To determine the polyexponential equation that best fit the data, stripping was performed for one through five exponents. Results indicate that a triexponential equation adequately describes the data through 20 mg/kg. However, the 100 mg/kg data are described by a biexponential equation. At this dose, blood concentrations remained essentially constant from 5 through 60 min, indicating a plateau after the initial distribution phase. No plateau was detected at the lower doses. The above results suggest that the model that describes the 100 mg/kg dose differs from that describing the lower doses.

Weanling female BALB/c mice were continuously fed diets containing combinations of 12 or 24% protein, 4 or 24% corn oil, and 0 or 500 mg/kg 2-AAF for approximately 77 weeks to determine the effects of protein and fat on 2-AAF-induced carcinogenic lesions. After 77 weeks on test, mice were reassigned to one of the test diets that they had not previously been fed and, at periodic intervals over an 11-day period, mice from each treatment group were anesthetized with diethyl ether and sacrificed by cervical dislocation. Livers were removed aseptically and homogenized and the supernatant (S-9) from a 9000g centrifugation of each sample was tested in the Ames mutagenicity assay system using Salmonella typhimurium, TA-1538, as the indicator strain and 4 µg of 2-AAF per plate as the test chemical. The data indicate that liver S-9 fraction mixed-function oxidase activity is directly and inversely proportional to dietary 2-AAF and fat levels, respectively. The effects of dietary protein levels on the activities of these enzymes was minimal when compared to 2-AAF and fat. These data underscore the importance of dietary pretreatment in mice used in the Ames assay by illustrating the dramatic effects of changes in diet on liver S-9 fraction mixed-function oxidase activity.


Effects of butylated hydroxytoluene (BHT) and a new polymeric antioxidant, Poly AO-79, on hepatic mixed-function oxidase (MFO) were compared after dietary administration for 60 and 90 days, respectively. Male Sprague-Dawley rats received BHT at a daily dosage of 250 mg kg⁻¹ and Poly AO-79 at 5% by weight in the diet (2.8–5.0 g kg⁻¹ day⁻¹). On Days 15, 30, 60, and 90 animals were sacrificed and hepatic S-9 homogenates were prepared. Definite inductive effects were observed in all animals fed BHT. Relative liver weights were significantly higher, as were cytochrome P-450 levels. Cytochrome c reductase was unaffected. Hepatic MFO activities for p-nitroanisole demethylase and aminopyrine demethylase were significantly elevated; whereas hexobarbital oxidase and benzo[a]pyrene hydroxylase activities were lower than control. The lower values were attributed to an enzymatic competitive inhibition by residual BHT in the S-9 homogenate. There was no indication of induction or any significant differences between control and Poly AO-79-fed animals in liver weight, cytochrome P-450, cytochrome c reductase, or any of the MFO enzymes evaluated. Lack of inductive effects can be attributed to the lack of intestinal absorption of the polymeric antioxidant demonstrated in rats and several other species.


A comparison of the kinetics, body distribution, and metabolism of 2,2'-dichlorobiphenyl (2,2'-DCB), 2,4',5-trichlorobiphenyl (2,4',5-TCB), 2,2',4,4',6-pentachlorobiphenyl (2,2',4,4',6-PCB), and chloralkylene (a mixture of mono-, di-, tri-, and tetra-isopropylated derivatives of 2,4'-dichlorobiphenyl) has been performed in the rat and rhesus monkey. Rats and rhesus monkeys were dosed with the ¹⁴C-labeled compounds at levels of from 1 to 20 ppm by body weight for single oral dose studies. In long-term feeding studies with rhesus monkeys the dose was 5 ppm of the daily diet. Biological half-lives range from 24 hr or less for the chloralkylene
mixture and 2,2'-DCB in both rats and rhesus monkeys, 4.5 to 4.8 days for 2,4',5-TCB in rhesus monkeys, and 28 days for 2,2',4,4',6-PCB in the rhesus monkey. Body accumulations are low for the low chlorine content compounds but increase with increasing chlorine content of the molecule. Compounds with higher chlorine content did accumulate substantially in the lymph nodes, bone marrow, and thymus. Hydroxylation, with monohydroxylation predominating, is the major metabolic fate for the investigated compounds.


After thorough investigation of hexachlorobenzene (HCB) in rhesus monkeys, pentachlorobenzene (PCB), one of the major metabolites, was investigated and the results were compared to those obtained with HCB. Two male and two female rhesus monkeys were administered a single dose of 1 mg/kg body wt of 14C-labeled PCB orally. Urinary and fecal excretion were measured and the biological half-life of PCB was determined. The excreta were extracted and purified and PCB and its metabolites were identified. After one biological half-life of PCB, one male and one female monkey were sacrificed and the body distribution of the radioactivity was determined. PCB is metabolized and excreted more rapidly than HCB. Body distribution shows similar patterns to HCB.


The metabolic fate, kinetic behavior, autoradiographic profile, and histopathology of pentachloronitrobenzene (PCNB) were studied in adult rhesus monkeys. Studies of the initial absorption, metabolism, body distribution, and elimination were made on two rhesus monkeys. The first received 2 mg/kg body wt of 14C-labeled PCNB by gavage 24 hr prior to sacrifice. The second received the same amount 48 hr prior to sacrifice. In a second chronic study, two rhesus monkeys (one male and one female) were given 14C-labeled PCNB at a 2-ppm level of the daily diet until equilibrium in storage was reached, which was around the 72nd day. At that time they were sacrificed. Throughout the study hormone levels, blood chemistry and hematology were monitored. It appeared that PCNB was very rapidly absorbed from the gastrointestinal tract and transported to the liver, mainly via the portal venous system, where it was rapidly metabolized and excreted through the bile. High concentrations were found in the gall bladder, cecal wall, mesenteric fat, and thymus. The major metabolite was pentachloroaniline, although in addition a variety of more polar metabolites was also found. From all the monitored clinical parameters, no variation due to PCNB could be established. The only histopathologic change was present in the kidneys, where degenerative changes were observed in the small renal vessels, and in the glomerular endothelial cells, where traces of radioactivity were found as in the wall of the gall bladder.


The effects of dieldrin, pentachloronitrobenzene (PCNB), and hexachlorobenzene (HCB) were compared in rhesus monkeys. 14C-labeled dieldrin was given in the amount of 2 ppm of the
daily diet for 260 days to one male and one female rhesus monkey. The administered amount of $^{14}$C-labeled PCNB was 2 ppm of the daily diet and was given for 70 days to one male and one female monkey. One male rhesus received $^{14}$C-labeled HCB at 10 ppm of the daily food intake for 540 days. Dieldrin was rapidly absorbed from the gastrointestinal tract and transported to the liver mainly via the portal venous system. There it was metabolized and excreted with redistribution and subsequent storage in the adipose tissue, adrenal cortex, bone marrow, and thymus. The main product was a monohydroxy metabolite. PCNB was similarly absorbed and transported to the liver, but was metabolized much faster, with only small amounts of radioactivity in the gall bladder, cecal wall, mesenteric fat, and thymus. Pentachloroaniline was the major detectable metabolite. HCB was more slowly absorbed with minor involvement of the portal venous system and major absorption by the lymphatic system. The subsequent deposition took place in the fat, thymus, and bone marrow. The only affected organs in the dieldrin monkeys were the liver and kidney, which showed the advanced parenchymal degeneration associated with nodular hyperplasia in the liver. Radioactivity was evident within the cytoplasm of the hepatocytes and the cells of the proximal tubules. In the PCNB animals the kidney was the only organ involved. Degenerative changes were observed in the small renal vessels and in the glomerular endothelial cells, where traces of radioactivity were also found. In the HCB monkey, thymic cortical atrophy and degenerative changes in the cerebellum and kidney were evident without any traces of radioactivity present anywhere.

149. A Comparison of the Acute Inhalation Toxicity of Hydrogen Chloride Versus the Thermal Decomposition Products of Polyvinyl Chloride. C. S. BARROW, H. LUCIA, and Y. ALARIE, Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

The evolution of hydrogen chloride during the thermal decomposition of polyvinyl chloride has been implicated as the principal contributor to the overall toxicity from the thermal decomposition products of polyvinyl chloride. The objective of this study was to compare the acute inhalation toxicity from exposure to hydrogen chloride versus the thermal decomposition products of a plasticized polyvinyl chloride formulation which was 60% homopolymer (PVC-A). Male Swiss–Webster mice were exposed in groups of four each to hydrogen chloride ranging from approximately 20 to 20,000 ppm (0.025 to 29.0 mg/liter) or to the thermal decomposition products of PVC-A ranging from 0.01 to 39.0 mg/liter. The exposure duration was limited to 10 min. An animal model was developed to quantitate the physiological stress imposed due to the sensory irritation characteristics of hydrogen chloride or the thermal decomposition products of PVC-A and was termed the sensory irritation stress index (SISI). In addition, acute lethality and histopathological data were obtained. The results indicated that the SISI and acute lethality due to exposure to the thermal decomposition products of PVC-A was due to more than only the hydrogen chloride evolved. The pathological changes noted from exposure to the thermal decomposition products of PVC-A were very similar to those for hydrogen chloride. These data suggest that although hydrogen chloride appears to be the principal contributor to the toxicity of the thermal decomposition products of PVC-A, other gases and/or aerosols evolved are also toxicologically significant. (Supported under NBS Grant 5-9005.)


Groups of 30 albino rats, equally divided by sex, were exposed to either 0, 30, 100, 300, or 1000 ppm of toluene for approximately 6 hr/day, 5 days/week for 13 weeks (64 exposures). Mortality, clinical reactions, food consumption, and body weight gains were noted daily. Clinical laboratory studies, including hematology and clinical chemistry as well as urinalyses, were conducted on control animals and the highest exposure group on Days 5, 45, and 89. All organs
were inspected at necropsy and 42 tissue specimens were microscopically examined. No exposure-related mortality occurred. Weekly mean body weights and total weight gains, and mean weekly and total food consumption were not significantly altered when untreated control and test animal data were compared. The hematology, clinical chemistry and urinalyses results also failed to reveal any differences between control and test animals. No gross or histopathologic alterations in any of the treated rats could be attributed to the test material. Significantly lower mean liver weights were found in all exposed groups except the highest. A lack of corroborating evidence, such as the absence of morphological lesions and an inverse relationship to dose, suggested that this was a spontaneous aberration. Toluene vapor is well-tolerated by rats at exposure levels up to 100 ppm for 90 days.


Groups of 30 albino rats, equally divided by sex, were exposed to either 0, 300, 1000, 3000, or 10,000 ppm of ethylene for approximately 6 hr/day, 5 days/week for 13 weeks. Mortality, clinical reactions, food consumption, and body weight gains were noted daily. Clinical laboratory studies, including hematology and clinical chemistry as well as urinalyses, were conducted on control animals and the highest exposure group on Days 6, 45, and 83. All organs were inspected at necropsy and 42 tissue specimens were microscopically examined. No exposure-related mortality occurred. Weekly mean body weights and total weight gains, and mean weekly and total food consumption were not significantly altered when untreated control and test animal data were compared. The hematology, clinical chemistry, and urinalyses results also failed to reveal any differences between control and test animals. No gross or histopathological alterations in any of the treated rats could be attributed to the test material. Significantly lower mean liver weights were found in all exposed groups except the highest. A lack of corroborating evidence, such as the absence of morphological lesions and an inverse relationship to dose, suggested that this was a spontaneous aberration. Ethylene gas is well tolerated by rats at exposure levels up to 10,000 ppm for 90 days.

152. The Role of Saturable Enzymatic Activation in the Expression of the Inhalation Toxicity of 1,1-Dichloroethylene. MELVIN E. ANDERSEN, MICHAEL L. GARGAS, and LAWRENCE J. JENKINS, JR., Naval Medical Research Institute, Toxicology Detachment, Wright-Patterson AFB, Ohio.

Studies of the oral toxicity of 1,1-dichloroethylene (1,1-DCE; vinylidene chloride) and of its fate after oral dosing suggested that it was activated to a more toxic metabolite by a saturable, microsomal reaction. If the toxicity is due to saturable, enzymatic activation at high concentrations the inhalation toxicity of 1,1-DCE should be dependent on the time of exposure but relatively independent of the ambient concentration. Immature male rats, previously shown to be most susceptible to 1,1-DCE, were exposed to various concentrations for up to 2 hr. Pentobarbital sleep times were significantly increased after only 0.5 hr of exposure. Young rats then were not a suitable model since the competence of the microsomal oxidases appeared to decrease rapidly with exposure to 1,1-DCE. Pentobarbital sleep times were not changed during the exposure when larger (~200 g) rats were used and mature male rats were therefore judged to provide a better model system. With large rats, the LT50 (the time of exposure required to kill 50% of an exposed group at a given concentration) varied only between 2.2 and 4.0 hr as dose was varied between 1000 and 200 ppm. Failure of 1,1-DCE to adhere to a concentration-time relationship is further evidence of a role of saturable activation in the expression of its toxicity. This departure from traditional dose-response relationships has several implications for the interpretation of inhalation toxicity data in general.

The measurement of changes in pulmonary resistance and compliance in unanesthetized guinea pigs exposed to environmental pollutants has been employed for toxicologic evaluation of deep lung irritants (Amdur and Mead, Proc. Third National Air Pollution Symposium, Pasadena, p. 150, 1955). The data obtained for guinea pigs have not been consistent with toxicologic data obtained using other animal species. Perhaps this divergence in irritant response is greatest for sulfuric acid mist where the LC50 for guinea pigs is 25 to 30 mg/m³ while for mice, rats, and rabbits, the LC50 is greater than 718 mg/m³ (Treon et al., Arch. Ind. Hyg. Occup. Med. 2, 719, 1950). In 6-month exposures of rats and guinea pigs to 10 mg/m³ of sulfuric acid mist, 6 hr/day, 5 days/week, no significant histopathologic lesions were found in the respiratory tracts of either species. In acute exposures of guinea pigs to sulfuric acid mist, exposure-related microscopic alterations in the lungs were characterized by a diffuse regional, nonsuppurative alveolitis. These acute pulmonary changes following 1 or 2 days of exposure contrasted with the finding of no severe lesions after 6 months of exposure led to studies on the pathogenesis of acid mist-induced lesions. Three studies were initiated. Acute exposures of guinea pigs to 25 mg/m³ of sulfuric acid mist with interim sacrifices following 1, 2, 4, and 6 hr of exposure revealed zones of demarcation between injured and more normal lung. In an identical exposure, animals were injected prior to sacrifice with horseradish peroxidase (HRP) in order to assess pulmonary vascular injury. This experiment resulted in focal extravasation of blood in control as well as exposed lungs, suggesting a direct effect of HRP. The third study involved the same exposure and sacrifice regimen as above except that guinea pigs were exposed to histamine in order to compare the early pulmonary effects of sulfuric acid mist with a vasoconstrictor amine. The developing lesion in histamine exposures appears similar to that seen in acid mist exposures. (Supported by Contract No. NIH-ES-77-21.)


There is ample opportunity for occupational exposure to 2-nitropropane, a solvent used in the production of plastics, rubber, inks and adhesives. The purpose of this study was to determine the subchronic inhalation toxicity of 2-nitropropane in order to establish acceptable exposure levels in the workplace. Fifty male rats and 15 male rabbits were exposed to either 27 or 207 ppm of 2-nitropropane 7 hr/day, 5 days/week for periods up to 24 weeks. Fifty rats and 15 rabbits were exposed to filtered air for similar lengths of time and served as controls. Ten rats from each exposure and control group were sacrificed following 2 days, 10 days, 1 month, 3 months, and 6 months of exposure. Five rabbits from each of the exposure or control group were sacrificed following 1, 3, and 6 months of exposure. The animals were exposed in 6-m³ chambers. The intake air to the chamber was conditioned to 21.5°C and 50% relative humidity. The air flow rate through the chambers was 1000 liters/min. 2-Nitropropane was generated by metering commercial grade 2-nitropropane into a vaporization chamber heated between 75 and 100°C. The concentration of 2-nitropropane in each chamber was measured using infrared analysis. Body weights for each animal were recorded biweekly throughout the exposure period. Blood samples were drawn for hematological and clinical biochemical determinations at each sacrifice interval. Organ weights for the liver, kidney, lungs, brain, and thyroid were recorded at each sacrifice. No exposure-related effects were seen in the body weight or hematological determinations. The liver weights were significantly elevated in the rats exposed to 207 ppm of 2-nitropropane for 1, 3, and 6 months. No exposure-related gross or microscopic alterations were seen in any of the tissues examined from the rats or rabbits exposed to 27 ppm of 2-nitropropane. No gross or microscopic alterations were seen in the tissues from the rabbits exposed to 207 ppm of 2-nitropropane. Liver neoplasms were seen in all 10 rats killed following 6 months of
exposure to 207 ppm of 2-nitropropane. Hepatocellular hypertrophy, hyperplasia, and necrosis were seen in the rats exposed to 207 ppm of 2-nitropropane for 3 months. The development of hyperplastic and hypertrophic lesions of the liver in rats following 3 months of exposure to 2-nitropropane coupled with the development of frank neoplasms following 6 months of exposure indicate that 2-nitropropane is a potent carcinogen in the rat.


The acute respiratory irritancy of SO₂ can be reduced when simultaneously administered with a submicron aerosol of motor oil. Although this antagonism correlated qualitatively with the ability of the motor oil to chemically react with SO₂, the reaction kinetics did not support an “SO₂-scrubbing” hypothesis. Preexposure to 100 mg/m³ of motor oil alone did not alter the response to 50 ppm of SO₂. However, preexposure to the SO₂-motor oil combination negated the irritancy of subsequent SO₂ exposure. This evidence and the results of sequential exposures of motor oil, SO₂, and/or their combination suggest that the SO₂ has reacted with an additive component in the motor oil to produce the observed protection. When characteristic dilutions of the detergent or dispersant fractions of the complete additive package were made in mineral oil only partial protection resulted. Similar results were obtained with acid-treated motor oil. The naphthene mineral oil used as a control in this study did not protect when given simultaneously with SO₂. However, some protection was observed with long-chain hydrocarbon-based paraffin oil. The differences in the protective abilities of the pure mineral oils appears to be dependent on the physical structure of the component hydrocarbons. This hydrocarbon-dependent antagonism may explain part of the antagonism observed with the motor oil. No oil provided protection against the irritancy of formaldehyde.


Male Syrian hamsters of the BIO 15.16 inbred strain were exposed to cigarette smoke from tobacco, from Cytral tobacco supplement, or from blends of these two materials for up to 100 weeks. The animals inhaled smoke for 12 min twice daily for 7 days a week using a modified Walton reverse smoking machine. Dosages of 22 and 11% smoke were supplied for a period of 27 sec each min followed by fresh air for 33 sec. The higher dosage of tobacco smoke produced maximum tolerated carboxyhemoglobin levels. The effects of these smoke exposures on mortality, body weights, and carboxyhemoglobin levels are outlined. The chemical composition of smoke from the modified Walton machine is essentially the same as that obtained with conventional analytical smoking machines. The smoke from the various types of test cigarettes used is compared and contrasted as to chemical composition. The modified Walton machine provides the experimental animals with fresh, as opposed to aged, smoke. Using a decachlorobiphenyl tracer, deposition studies of smoke in the lungs and larynx of the test animals have been conducted and the results are described. This deposition follows the smoke delivery of the test cigarettes and correlates for individual animals with carboxyhemoglobin.


Male inbred Syrian hamsters (BIO 15.16) exposed for 59 to 100 weeks to smoke from filtered, flue-cured tobacco cigarettes showed various degrees of histological changes in the larynx. These ranged from hyperplasia in almost all animals, metaplasia, dysplasia, and preneoplasia, to
invasive carcinoma in 37% of the animals at the high-dose level. Less pronounced and generally insignificant effects were observed at other sites of the respiratory tract. The dosage regimen described in the previous abstract was used, and was more severe than that employed in our previous publication (Bernfeld et al., JNCI 53, 1141, 1974), where the same strain of animals exposed to smoke from the 1R1 reference cigarette yielded 19% laryngeal neoplasms. At a lower, half-dose level, less extensive effects were observed and only 7% carcinomas were observed. Cigarettes were also tested containing 20, 50, and 100% Cytril tobacco supplement. The 100% supplement cigarettes gave no carcinomas and only minimal histopathological changes in the larynges and other respiratory organs. The changes caused by smoke from blends were clearly less than those observed for the all-tobacco cigarette. A dose–response effect was again found for these cigarettes. A method is thus available for qualifying the carcinogenic properties of cigarette smoke by inhalation. By these tests a tobacco supplement has been shown to be inactive and to moderate the activity of tobacco when used in blends.

158. Pulmonary Pathology in Rats Exposed to Marijuana Smoke for One Year. HARRIS ROSENKRANTZ, ROBERT W. FLEISCHMAN, and JOHN R. BAKER, Mason Research Institute, Worcester, Massachusetts.

In a previous 87-day study of the effect of marijuana smoke in rodents, focal pneumonitis characterized by aggregates of alveolar macrophages was discerned (Toxicol. Appl. Pharmacol. 34, 467, 1975). In this 1-year inhalation study in Fischer rats, emphasis was placed on monitoring exacerbation or reversal of the lung irritation, defining cellular elements involved in the pneumonitis and relating the toxicity to plasma THC levels. Groups of 10 rats were given a daily exposure to 5, 8, or 15 puffs of marijuana or 12 puffs of placebo smoke or were sham-treated. The smoking apparatus automatically provided a 50-ml puff from each of three cigarettes in a 2-sec period which was retained for 30-sec followed by a 30-sec purge with fresh air each minute. Estimated Δ4-THC doses were 0.4, 0.8, and 1.5 mg/kg which were related to carboxyhemoglobin levels of 15, 30, and 51% and plasma THC levels of 58, 156, and 234 ng/ml. THC doses were similar to those of man based in body surface area (0.40–0.21 mg/kg). The biphasic response of CNS inhibition and stimulation followed by tolerance development was seen in the first and fourth months. The numbers of pneumonitis foci were sex- and dose-related and were absent in controls. The pulmonary toxic response to marijuana smoke was not reversed during a 30-day recovery.


Male Sprague–Dawley rats were intraperitoneally injected (ip) with paraquat (PQ) at a dose of 27 mg/kg and the lungs were removed 24, 48, and 72 hr later. Lung slices were then prepared, and oxygen consumption (QO₂) and the oxidation of [1-14C]glucose were determined. In slices taken from different lungs the QO₂ and glucose oxidation were increased, but varied by as much as 45 and 30%, respectively, whereas the variation between slices taken from control animals was less than 6%. The increase in the QO₂ and the oxidation of [1-14C]glucose was shown to be dependent on the PQ concentration in the medium. Therefore, one possible explanation of these results could be that different concentrations of PQ were present in the individual lung slices studied. The QO₂ in slices taken from the same lung of ip PQ animals varied by as much as 15%; analysis of the distribution of ip [14C]PQ indicated as much as a twofold variation in [14C]PQ in different parts of the same lung. This suggests that the regional distribution of PQ throughout the same lung was not uniform. Kinetic studies on the uptake of [14C]PQ into the isolated perfused rat lung (IPL) also revealed considerable variation in the amount of PQ accumulated by
comparable lungs. These studies with the IPL also confirmed the presence of a transport system for the pulmonary uptake of PQ from the circulation. Since accumulation of PQ into the lung is thought to be related to the development of pulmonary toxicity, considerable variation in the lung concentration of PQ may be of significance in explaining reported differences in the toxic response to PQ. The relative efficiency of the pulmonary removal system for PQ may be an important factor governing an individual's susceptibility to PQ toxicity and the duration of the onset of toxicity.

160. *Lipid Requirement of the Rabbit Lung Mixed-Function Oxidase System.* P. J. Harkinnen and M. Voře, Department of Pharmacology and Toxicology, University of California Medical Center, San Francisco, California.

Aromatic hydrocarbons and other environmental pollutants may be inhaled and result in lung tumorigenesis. These compounds are thought to undergo metabolism via the lung microsomal mixed-function oxidase (MFO) system to chemically reactive intermediates. In order to determine if lipid is an essential component of the lung MFO system, lyophilized rabbit lung microsomes (20–40 mg) were extracted twice with 1-butanol and twice with acetone at −70°C using a modification of a procedure described previously (Mol. Pharmacol. 10, 963–974, 1974). Extraction removed all of the neutral lipids and approximately 90% of the phospholipids; recovery of cytochrome P-450 averaged 35%. Activities were calculated per nanomole of cytochrome P-450. Extraction decreased benzphetamine N-demethylase (BPhet) activity to 65% of activity in unextracted microsomes and addition of di[14]lauroylphosphatidylcholine (diLPC) to extracted microsomes restored activity to control levels. In contrast, extraction increased benzo[a]pyrene hydroxylase (BP-OH) activity to approximately 150% and addition of diLPC increased activity up to 200% of that in unextracted microsomes. Addition of diLPC to unextracted microsomes inhibited both BPhet and BP-OH activities. These data indicate that lipid is required for BPhet but do not establish lipid requirement for BP-OH activity. The increased BP-OH activity observed following extraction suggests that the microsomal membrane contains inhibitors of BP-OH which are removed by extraction. (Supported by Grants USPHS-HL 19605 and USPHS-GM 00475.)


Viable cells have been released from rabbit lung by digestion with 0.1% Pronase in Hepes buffered balanced salt solution, pH 7.4, instilled through the trachea. These cells have been separated into five fractions on the basis of size using the Beckman JE-6 Elutriator centrifuge. Cytochrome P-450 content, N,N-dimethylaniline N-oxidase, coumarin hydroxylase, and 7-ethoxycoumarin deethylase activities have been compared in the cell digest, elutriator fractions, and isolated macrophages. Fraction 1 from the elutriator consists mostly of cell debris and contains some drug metabolism activity when NADPH is added. Fraction 2 contains small cells (RBCs, lymphocytes, 5–10% alveolar type II cells) and very low mixed-function oxidase (MFO) activity. Of fraction 3, 30 to 50% is composed of alveolar type II, although many other cell types are present (ciliated cells, goblet cells, macrophages, mast cells). This fraction also contains low xenobiotic metabolism activity. Fractions 4 and 5, which contain the largest cells of the lung, also exhibit the greatest amount of drug metabolism activity in viable cells. These fractions consist of Clara cells, macrophages, some alveolar type II cells (0–25%), and other large cells—possibly some alveolar type I cells. When comparing the presence of alveolar type II cells and foreign compound metabolism, the amounts are not proportional in the elutriator fractions. Alveolar type II cells may exhibit some drug-metabolizing activity. However, they do not comprise the major cellular pool of MFO activity in the lung.

The purpose of this investigation was to determine the inhalation toxicity of chlorine. A subacute study was completed in which groups of 10 male and 10 female Fischer 344 rats were exposed to air (controls), 1, 3, or 9 ppm of chlorine for 6 hr/day, 5 days/week, for 6 weeks. The results demonstrated significant decreases in body weight at all exposure concentrations in females and 3 and 9 ppm in males. Additionally, three females at 9 ppm died prior to the end of the 30-day exposure. Urinalysis, hematology, and clinical chemistry was completed for the surviving animals. The urinary specific gravity was elevated at all exposure concentrations in the females and at 3 and 9 ppm in the males. The hematocrit and white blood cell count was increased in the females exposed to 9 ppm. Clinical chemistry results included elevations in alkaline phosphatase, BUN, γ-glutamyl transpeptidase, and SGPT at 9 ppm and alkaline phosphatase at 3 ppm. Rats exposed to 9 ppm showed gross evidence of inflammatory reactions of the upper and/or lower respiratory tract which included hyperemia and accumulation of inflammatory material within the nasal passageways. There were also various degrees of atelectasis and/or consolidation of the lungs. These observations were also made, but to a much lesser degree, at 3 ppm. The kidneys of rats exposed to 9 ppm were found to be darkened in appearance. These data indicated that repeated exposures of chlorine in rats at 3 and 9 ppm resulted in gross pathological changes of the respiratory tract, significantly decreased body weight, altered kidney function, and a greater sensitivity of females than males to chlorine's overall effects.

163. *Inhalation Toxicology of Air-Borne Particulate Manganese in Rats and Rhesus Monkeys.* T. B. Griffin and F. Coulston, International Center of Environmental Safety, Albany Medical College, and Holloman Air Force Base, New Mexico.

Rats and rhesus monkeys were exposed to air-borne particulate manganese at 100 μg/m³ of air daily for 23 hr/day. The particulate manganese was generated by the combustion of methylcyclopentadienyl manganese tricarbonyl. Among rats exposed for periods of 8 weeks, there was a small increase of manganese in urinary and fecal excretions as well as an increased concentration of the metal in lung and brain tissue. Manganese levels in tissue returned to normal within 1 week when the exposed animals were removed from the chamber. Similar small increases of excretion of manganese in the urine and feces were observed in monkeys exposed for 1 year. Among the exposed monkeys the concentration of manganese was elevated in the lungs, liver, pancreas, kidney, myocardium, and tissues from the central nervous system. The greatest increase of manganese was in the lung tissue. While the greatest exposure to manganese was through diet, and the inhalation procedure did not contribute greatly to total intake, the significant shifts in tissue levels suggested an important role for pulmonary absorption of the metal. Throughout the study no other signs of toxicity or changes in various clinical parameters were observed in rats or monkeys. There was no change in morphology, either gross or microscopic, which could be attributed to exposure to manganese.


Ethylenethiourea (ETU), a degradation product of the widely used ethylenebisdithiocarbamate group of fungicides, was administered orally in single daily doses of 0, 5, 10, 30, 60, or 120 mg/kg to time-mated cats. Daily dosing was started on Day 16 of gestation in all test groups. It was discontinued on appearance of toxicity signs (between Days 29 and 34 of gestation in the 120 mg/kg group and Day 35 of gestation in all other groups). Signs of toxicity were observed in cats from all treatment groups, except those from the 5 mg/kg and control
groups. Signs of toxicity were delayed in onset and were characterized by progressive loss in body weight, loss of body balance, tremors, and hindlimb paralysis. The number of cats that died or were killed in moribund condition versus the total number on test were: 5 of 10 from the 120 mg/kg dose; 2 of 7 from the 60 mg/kg dose; and 4 of 8 from the 30 mg/kg dose group. (In rats, 40 mg of ETU/kg/day given from 42 days preconception until Day 15 of gestation manifested no obvious signs of toxicity). An incidence of abortion, apparently unrelated to the treatment, occurred in all groups, including the control. Eleven of the 35 fetuses obtained from six cats (four from the 30 mg/kg and one each from the 60 and 120 mg/kg groups) that died or were killed in a moribund state were malformed and presented coloboma in four, cleft palate in two, spina bifida in one, and umbilical hernia in four fetuses. All surviving cats were necropsied on Days 44 to 46 of gestation and fetuses were collected. These were evaluated following routine teratologic methods. No clear evidence of teratogenicity of ETU was found in live fetuses from the surviving cats.

165. A Biochemical Basis for Insecticide-Induced Changes in the Male Reproductive System. J. A. Thomas, M. P. Donovan, and L. G. Schein, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

Previous studies have revealed that the toxic effects of certain pesticides on the mammalian reproductive system are due, in part, to their ability to interfere with the metabolism and/or uptake of steroid hormones. The present studies sought to elucidate the action(s) of certain insecticides (e.g., parathion, dieldrin, carbaryl, heptachlor, and methoxychlor) on hormone-receptor interactions. Using mouse prostate glands as a hormone target organ and isolating a cytosolic fraction containing receptor protein(s) for dihydrotestosterone (DHT), varying in vitro concentrations (10^{-8} to 10^{-3} M) of pesticides were studied with respect to their inhibitory activities on androgen-receptor interactions. Two cytosolic proteins can be separated by gel electrophoresis and both can effectively bind DHT. The binding of DHT to either of these two cytoplasmic components was effectively inhibited by 10^{-6} M concentration of dieldrin. Likewise, these cytoplasmic receptors were inhibited by parathion (10^{-3} M). Carbaryl was ineffective. Studies involving [14C]parathion or [14C]dieldrin failed to reveal an association of either pesticide with the cytoplasmic proteins on the gels. This failure to associate does not necessarily exclude binding to other proteins of the cytoplasm. These findings provide further insight into the biochemical or molecular basis for pesticide-induced changes in hormonal actions. (Supported in part by EPA Grant R803-578-030.)

166. Monofluoroacetate and Trifluoroethanol as Testicular Poisons in the Rat. J. L. Sullivan, F. A. Smith, R. M. Wilkenfeld, R. H. Garman, and P. J. Kostyniak, Department of Radiation Biology and Biophysics, and Environmental Health Sciences Center, University of Rochester School of Medicine and Dentistry, Rochester, New York.

During the course of investigations into the toxicology of aliphatic fluorocarbons, it was noted that trifluoroethanol (TFE) produced testicular damage in the rat. Fluoroacetate (FAc) is already recognized as a testicular poison, and it seemed worthwhile to compare effects of this nature produced by each compound. Previous work in our laboratory showed that 20 ppm of FAc in the drinking water of rats decreased the testis weight, elevated citrate concentration in the testis, and produced early signs of seminiferous epithelial damage and involved both spermatocytes and spermatids. Early signs of regeneration were evident by 7 days post-treatment, but this was not complete at 21 days. New information from a breeding experiment extend these morphological observations through a 10-week post-treatment period. Also, reproductive performance was impaired after 1 week post-treatment, completely inhibited during Weeks 3 to 6 post-treatment and improved thereafter, being near-normal when the experiment was terminated after 12 weeks. No unusual incidence of gross abnormalities was seen in the fetus taken 11 days post-conception. The 2-hr LC50 for male rats inhaling TFE was 4850 ppm (deaths were recorded at 24 hr postexposure). Damage to spermatocytes, spermatogonia and
Sertoli cells was observed at 400 ppm for 2 hr, the lowest concentration used in the LC50 determination and persisted for at least 86 hr postexposure. Single doses of 50 mg of TFE/kg of injected ip induced similar testicular damage through 7 days postinjection. TFE was not defluorinated in vitro by a rat liver fraction capable of defluorinating FAc. TFE appears to differ somewhat from FAc in that the former compound affects spermatogonia whereas FAc does not. (Supported in part by (NIEHS) Grant ES 00779, Grant GM-01781, and by U.S. Energy Research and Development Administration Report No. UR-3490-1240.)


Captan and Folpet are widely used fungicides generally considered of low toxicity with oral LD50's in the 6000 to 10,000 mg/kg range. However, with repeated exposures or altered routes of exposure the toxicity increased. Thus, three routes of exposure (oral, subcutaneous, and inhalation) were used for the teratology studies. Timed pregnant CD-1 mice were exposed to Captan or Folpet daily during organogenesis. The inhalation route was by whole body exposure for 4 hr/day from Day 6 through 13 of gestation. Sham control animals were housed in an identical chamber and subjected to the same air flow for 4 hr/day. The inhalation doses were one-eighth the LC50's, which approximate 488 mg/hr/m³ for Captan and 830 mg/hr/m³ for Folpet. The particle sizes were measured during exposure using photometry. The average particle size was in the respirable range, e.g., less than 5 µm. The dose level for the subcutaneous or oral routes was 100 mg/kg/day for both Captan and Folpet. These mice were treated from Day 6 through 15 of gestation. For teratogenic evaluation, all fetuses were recovered by cesarean section on Day 17 of gestation and examined by necropsy and alizarin staining. Maternal body weight reduction and a slight increase in fetal mortality demonstrated some toxicity of the fungicides by these routes of exposure. Normal formations were produced by Captan or Folpet using oral, subcutaneous, or inhalation routes of exposure.


Cefaclor (3-chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid) is a new cephalosporin antibiotic. Animals were treated orally with cefaclor during all phases of the reproductive cycle. Cefaclor treatment (0.5 or 1.0%, w/w, in the diet) did not affect either fertility, or the survival and development of the offspring when adult male rats were fed cefaclor diets for ca. 10 weeks prior to mating and females were fed the respective diets 2 weeks prior to mating through the lactation period. There was no evidence of a teratogenic effect when cefaclor doses of 250, 500, or 1000 mg/kg were administered daily by gavage to female rats and mice on gestation Days 6 through 15. The rabbit was an unsuitable species for teratology trials; daily cefaclor doses of 200 mg/kg (gestation Days 6 through 18) resulted in maternal mortality. In the ferret doses of cefaclor (40, 80, or 120 mg/kg b.i.d.), administered from gestation Day 14 through 26, produced delayed emesis; this treatment was without evidence of a teratogenic response. The mean peak plasma concentration of radiocarbon (36 µg-equiv/ml) occurred 90 min after ferrets were given single oral doses of [14C]cefylor (120 mg/kg). The t½ for the disappearance of plasma radiocarbon was ca. 2 hr. When rats were given cefaclor at doses of 500 or 1000 mg/kg/day during the perinatal–postnatal period (gestation Day 14 through postpartum Day 20), initial progeny weights were slightly depressed but litter size and neonatal survival were normal. Lactating rats given single oral doses [14C]cefylor (1000 mg/kg) excreted 56% of the radiocarbon dose in the urine by 24 hr. The peak serum concentration of radioactivity (278 µg-equiv/ml) occurring at 30 min was over fivefold greater than the peak level found in milk.

This study was conducted to determine if chronic ingestion of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has a detrimental effect on reproduction and development of the resultant offspring of rats. Male and female Sprague-Dawley rats 3 weeks of age were fed lab chow containing 0, 3, 10, or 30 mg/kg/day of 2,4,5-T for 90 days and then bred. At Day 21 of lactation, pups for the following generation were randomly selected and the rest of the pups were necropsied. Fertility was decreased in the matings for the f₁ₚ litters at the 10 mg/kg/day dose level. Neonatal survival was significantly decreased in the f₁ (10 and 30 mg/kg on days 14 and 21) and f₂ (3 mg/kg on Days 14 and 10 and 30 mg/kg on Day 21) litters. At the 3 mg/kg/day dose level, a decrease in neonatal survival was seen only on Day 14 in only one of four matings in three generations and is considered to be due to chance and not an effect of 2,4,5-T. The relative liver weight of weanlings was significantly increased at the 30 mg/kg/day dose level in the f₁, f₁ₚ, and f₁ₚ generations. A significant decrease in relative thymus weight was seen only in the f₁ₚ generation fed the 30 mg/kg/day level. Hydronephrosis was seen in a diminishing incidence through the generations. Thus, dose levels of 2,4,5-T which were sufficiently high to cause signs of toxicity in adults and neonates had no effect on the reproductive capacity of rats except for a tendency toward a reduction in neonatal survival at dose levels of 10 and 30 mg/kg/day. No significant effects were seen at the lowest dose level, 3 mg/kg/day.


As part of a program to evaluate hydrogen-containing fluorocarbons for industrial use, groups of 25 pregnant Sprague-Dawley rats were exposed for 6 hr/day to chlorodifluoroethane (FC-142b) at 0.1 or 1.0% (v/v) on Days 4 through 15 of gestation. Similarly, dams were exposed to 1.0% of 2,2-dichloro-1,1,1-trifluoroethane (FC-123) or dichlorofluoromethane (FC-21) on Days 6 through 15. At sacrifice on Day 21, dams and fetuses were examined for gross changes and fetuses were fixed in Bouin's solution for free-hand sectioning or preserved in alcohol for skeletal examination. Exposure to FC-21 adversely affected maternal weight gain and caused a total preimplantation loss of fertilized ova in 15 of 25 dams, but was not teratogenic. FC-142b and FC-123 caused no embryotoxic or teratogenic effects at these levels.


Beagle dogs were given styrene in a peanut oil suspension by gavage 7 days/week for 560 days. Dose levels were 200, 400, or 600 mg/kg body wt/day. The controls received peanut oil only. The red blood cells, especially of the dogs of the high-dose level, contained increased numbers of Heinz bodies, with a concurrent decrease in packed cell volume and sporadic decreases in hemoglobin and red blood cell counts. Marked individual variations were noted for these values in dogs receiving the same level of test material. Within 34 to 50 days after temporary cessation of the high-dose level of treatment, Heinz bodies were not detected. Within 28 days of resumption of administering the test material, Heinz bodies were again present, equal to the previous levels. No treatment-related alterations were detected in body weight, organ weights, urinalyses, clinical chemistry determinations of serum urea nitrogen, glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, or alkaline phosphatase. Minimal histo-
pathologic changes were found in the liver. These were characterized by increased iron deposits within the reticuloendothelial cells of dogs with elevated numbers of Heinz bodies. Dogs receiving the highest dose level of styrene also had increased numbers of intranuclear acidophilic crystalline inclusions in the hepatocytes when compared to controls. All of the dogs receiving the lowest dose level of styrene showed no alterations in any parameter evaluated, with the possible exception of a single female in this group which had equivocal observations in the form of sporadic low-level occurrence of Heinz bodies and had a very slight increase in iron deposits within the liver. In the teratogenic study, pregnant Sprague-Dawley rats and New Zealand rabbits were exposed to 300 or 600 ppm of styrene for 7 hr/day on Days 6–15 (rats) and 6–18 (rabbits) of gestation. Additional groups of pregnant rats were given 90 or 150 mg of styrene/kg body wt by gavage twice daily on Days 6–15 of gestation. No evidence of a teratogenic effect was discerned in either species under the conditions of the test. (Supported by the Manufacturing Chemists Association.)

172. 2-Benzoylthethyltrimethylammonium, a New Potent Inhibitor of Choline Acetyltransferase and Sperm Motility. B. V. Rama Sastry, A. K. Chaturvedi, V. Janson, M. L. Anderson, and P. Soupart, Departments of Pharmacology and Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Previous investigations have indicated that spermatozoa from several species (rat, rabbit, bull, and man) contain choline acetyltransferase (Cha) and acetylcholinesterase (AChE). Further, acetylcholine (ACh) has been shown to occur in mammalian spermatozoa. The lack of membrane stores for ACh in spermatozoa indicates that ACh synthesis, the stimulation of the receptor by ACh, and the hydrolysis of ACh by AChE are closely linked and may be localized within the same compartment. In view of this unique organization of the Cha–ACh–AChE system in spermatozoa, we have investigated the effects of 2-benzoylthethyltrimethylammonium (BETA), a newly synthesized Cha inhibitor in our laboratory. BETA is a potent inhibitor of Cha from monkey brain (IC50, 4.8 × 10^-6 M), human placenta (IC50, 3 × 10^-6 M), and rat spermatozoa (IC50, 6.4 × 10^-5 M). It inhibited the motility index of human spermatozoa (IC50, 8.5 × 10^-8 M) at concentrations higher than 10^-8 M after a contact time of 5 to 60 min. It depressed the motility index by about 80% after 5 min and by 95% after 1 hr at a concentration of 10^-6 M. Therefore, BETA and related compounds are suitable agents for studying the role of Cha–ACh–AChE system on motility. (Supported by USPHS-NIH Grant HD-10607.)


Mice (CD-1 strain) were given Durham drinking water that had either been distilled and passed through cartridges designed to reduce organics and remove inorganics, or water that came directly from the tap. After a 2-week acclimation period, animals were bred and pregnancy (Day 0) confirmed by the presence of a sperm plug. During the course of the study, approximately 500 pregnant mice were sacrificed on Day 18 and litters were subsequently examined for visceral and skeletal anomalies. Data on fetal weight and mortality were also obtained. The data indicate that the average number of live fetuses/litter was identical (10.4) in both groups. In the tapwater group, there were increases in the average number of dead (2.2 vs 1.7), total number of implants (12.6 vs 12.1), and percentage of fetuses with supernumerary ribs (27.5 vs 21.1). No differences were noted in either the type or number of anomalies found in the two groups. Analyses of the data by month (August–March 1976–1977) showed that some statistically significant differences occurred between groups during specific months, but these differences were often subsequently reversed and appeared to be random occurrences. Anomalies which were found were distributed randomly throughout the year.
174. Acute and Sub-acute Toxicity of Chlorinated Guaiacols in the Rat. D. C. Villeneuve, L. Ritter, I. A. Marino, and I. Chu, Biochemical Toxicology Section and Pathology Section, Environmental Health Directorate, Health Protection Branch, Ottawa, Ontario, Canada.

The following studies were carried out as part of a general program to assess the toxicity of chemicals found in pulp mill effluents. Acute oral toxicity studies in adult male Sprague–Dawley rats showed that trichloroguaiacol had an LD50 of 2980 mg/kg body wt, whereas tetrachloroguaiacol had an LD50 of 1690 mg/kg body wt. In a subacute study, male weanling rats (six per group) were fed diets containing 0, 50, 500, or 5000 ppm of each chlorinated guaiacol for 28 days. No consistent dose-related changes were observed in the following parameters: body weight gain, wet tissue weights (liver, spleen, kidney, heart, brain), food consumption, and hematology. Gross pathology and histological examination of thyroid, heart, lungs, liver, spleen, kidney, and brain showed no abnormalities. Both tri- and tetrachloroguaiacol induced hepatic microsomal enzyme activity. No residues of trichloroguaiacol were detected (sensitivity, 0.1 ppm) in the liver or kidney at any dose level. Tetrachloroguaiacol residues were detected in both tissues at the two highest dose levels. The data support the conclusion that both tri- and tetrachloroguaiacol are only moderately toxic to the rat.

175. Aldrin Epoxidase Activity in the Developing Rabbit Lung. H. M. Meendale, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi.

Studies of the mechanism of uptake and formation of epoxides of various xenobiotics are becoming of increased importance because of the highly reactive nature of epoxides with macromolecules of the lung. The objective of these studies was to determine epoxidase activity in the developing rabbit lung using the conversion of aldrin to dieldrin as a model. Artificially ventilated isolated perfused rabbit lung preparations as well as subcellular in vitro preparations (9000g supernatant) were utilized to measure aldrin epoxidase activity. Male New Zealand white rabbits ranging from ages 2 to 16 weeks were used in these investigations. Since the wet weight of lungs varied with age of the animals, aldrin used in these perfusion experiments was weighted with respect to the lung weight. Thus, aldrin used in these perfusion experiments was 2.5 μmol/10 g of lung or an apportionment thereof, depending upon the wet lung weight. Aldrin was introduced to the recirculating perfusate and timed samples of the perfusate and the lung homogenate at the end of the experiment were analyzed for aldrin and dieldrin. The rate of conversion of aldrin to dieldrin reached a maximum in 30 min of perfusion. Aldrin epoxidase activity increased from 2 to 8 weeks of age and reached a plateau thereafter. Although direct comparisons between perfused lung and in vitro studies are difficult, studies in which aldrin was incubated with subcellular preparations also indicate an age-related increase in aldrin epoxidase activity in the lung tissue. (Supported by a grant from the Mississippi Lung Association.)


F344 rats and B6C3F1 mice received a polybrominated biphenyl mixture (Firemaster FF-1) in corn oil by daily gavage, 5 days/week for a total of 22 doses. The dose administered was either 0, 0.03, 0.3, 3, or 30 mg/kg/day. Six animals from each dose were killed and necropsied 15, 31, 46, and 64 days into the study; the latter two time periods occurred approximately 2 and 4 weeks subsequent to the 22nd (final) dose. Only male mice receiving 30 mg/kg had decreased body weights. In rats of both sexes, significant body weight decreases were evident after eight doses of FF-1 at 30 mg/kg, which persisted for the remainder of the study. Time-, and dose-dependent increases in liver weight occurred in rats and mice receiving 3.0 and 30.0 mg/kg of FF-1. Histopathologic effects, primarily confined to the liver, were observed in both rats and
mice that received 3.0 and 30.0 mg/kg of FF-1. The liver changes were characterized by hepatocellular swelling, increased lipid deposition, and microabscessation (the latter occurring only at the 30 mg/kg dose). Decreased responsiveness of splenic lymphocytes to the T-cell mitogens, PHA, and Con A were found in the 3.0 and particularly 30 mg/kg dosage groups for both mice and rats. Splenic mitogen response to LPS, a B-cell mitogen, was depressed in the 30 mg/kg dosage group in mice. In mice, serum IgC levels were slightly decreased in the 30 mg/kg group only. Serum IgM and IgA levels in exposed mice were similar to controls. Differences in the IgM antibody plaque assay, were not found in mice or rats for any dosage group.

177. Comparison of a Commercial Polybrominated Mixture (Firemaster BP-6) with 2,4,5,2',4',5'-Hexachlorobiphenyl and a Tetrabromonaphthalene as Inducers of Hepatic Mixed-Function Oxidases. J. A. GOLDSTEIN and P. HICKMAN, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

This study compares FF-1 (Firemaster BP-6 containing 1.3% silica) with its major component, 2,4,5,2',4',5'-hexabromobiphenyl (HBB), and 2,3,6,7-tetrabromonaphthalene (TBN) as inducers of drug-metabolizing enzymes. Female Fischer rats (5–6 weeks old) were injected ip with single doses of 1.6, 8, 40, 200, or 1000 nmol/kg of FF-1 or HBB or 0.04, 0.32, 1.6, 8, or 40 nmol/kg of TBN and sacrificed 4 days later. Aryl hydrocarbon hydroxylase (AHH) was increased 52-fold by FF-1 but only 7-fold by HBB. However, HBB was a better inducer of aminopyrine N-demethylase (ND) than FF-1. FF-1 and HBB produced comparable increases in cytochrome P-450 (5-fold). However, FF-1 shifted the cytochrome P-450-CO peak to 449 nm and reversed the ratio of the 455:430 peaks of the ethyl isocyanide difference spectra, while HBB did not. In contrast, TBN increased AHH but not ND. The lowest dose of TBN which induced AHH appreciably was very high (40 nmol/kg) with respect to the 200-ppm concentration of bromonaphthalenes (penta- and hexa-) found in FF-1. In another experiment, rats were dosed Po 5 days/week with 0.03, 0.3, 3, and 30 mg/kg FF-1 or with the amount of HBB contained in these doses of FF-1. Rats were killed on Days 14 and 31 and after 15 and 33 days of recovery. The differences between FF-1 and HBB were comparable to those reported above. During recovery, the amount of FF-1 in the liver decreased 75%, but the amount in fat remained stable. Liver enzymes recovered only 30% at the high dose. Hepatic porphyria developed after 60 days recovery from 30 mg/kg of FF-1. These results show that the effects of FF-1 cannot be reproduced by its major component regardless of dose. FF-1 is a mixed inducer of drug-metabolizing enzymes, while HBB is a pure phenobarbital-type inducer. TBN resembles 3,4-benzpyrene.

178. Effect of Hexachlorobutadiene on Renal Function in the Rat. W. O. BERNDT and H. M. MEHENDALE, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi.

Although assertions have been made concerning the potential nephrotoxicity of HCBD, few controlled laboratory studies have been undertaken to document these. The present study was designed to examine the effects of HCBD on a variety of renal functions in the Sprague–Dawley rat. Doses of 25, 50, or 100 mg/kg were employed and these were administered ip on each of 4 days. Control and experimental animals were housed individually in metabolism cages and 24 hr urine samples were collected on 1 control and 4 treatment days. On the fifth day the animals were sacrificed and renal cortical slices were prepared for in vitro studies on organic and inorganic ion transport. The transport of p-aminohippurate (PAH), tetraethylammonium (TEA), and α-aminoisobutyrate (AIB) were monitored using radioisotopes. At the lowest dose of HCBD studied, PAH uptake was significantly reduced. At the highest dose studied, the uptake of all three organic compounds was reduced. Only minimal effects were found on total tissue water, inulin space, or intracellular Na or K concentrations. The highest dose of HCBD also
affected in vivo renal function. Marked increases in glucose and urinary protein excretion and a marked decrease in urine osmolality were observed 24 hr after the first injection. At lower doses delayed responses were seen. For example, at 25 mg/kg no significant alterations in urine osmolality were observed until the 3rd treatment day. These data indicate that HCBD is capable of producing effects on renal function that are entirely consistent with a nephrotoxic response. (Supported by NIH Grant AM 18124.)

179. 2,3,7,8-Tetrachlorodibenzo-p-dioxin Induction of Aryl Hydrocarbon Hydroxylase (AHH) in Hepatic Microsomes from Female Rats. KIRK T. KITCHIN and JAMES S. WOODS, Laboratory of Environmental Toxicology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina.

These studies investigated the time and dose dependence of TCDD, a potent hepatotoxin, in the induction of AHH in female rat liver. Hepatic microsomal AHH activity was elevated from 5 to 10, 32, 49, 68, and 122 amol of polar products/mg of protein/hr at 6, 9, 12, 18, and 24 hr after TCDD (2 μg/kg, ip) treatment, respectively. Concurrent administration of the protein synthesis inhibitors actinomycin D (2 mg/kg, ip) or cycloheximide (5 mg/kg, ip) completely prevented TCDD induction of AHH. To substantiate the extreme potency of TCDD in increasing hepatic AHH activity, the dose–response relationship was determined using 10 different single oral doses ranging from 0.0006 to 20 μg/kg. The ED50 was 0.62 μg/kg, and the lowest dose which significantly increased AHH activity was 0.002 μg/kg. It was estimated from radiotracer experiments in which [1,6-3H]TCDD (0.002 μg/kg, 0.05 μCi) was orally administered to animals 3 days prior to sacrifice that only 65 molecules of TCDD per hepatocyte are necessary to produce a significantly increased hepatic microsomal AHH activity. At this dose 1.1% of the total [3H]TCDD was incorporated per gram liver. These studies indicate that TCDD acts via de novo protein synthesis to induce AHH and that this effect occurs in doses as small as 1/100th of those previously reported to produce biological responses in mammals. (Supported by a CIIT postdoctoral fellowship.)

180. Studies on the Mechanism of the Metabolism of Haloforms to Carbon Monoxide. J. L. STEVENS and M. W. ANDERS, Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota.

Haloforms are widely used in commerce and industry and have recently been shown to be widespread contaminants of municipal drinking water supplies. Furthermore, chloroform is a suspected carcinogen. Previous studies in this laboratory have shown that haloforms are metabolized to carbon monoxide by hepatic microsomal cytochrome P-450-dependent enzymes. This reaction was markedly stimulated by various sulphydryl compounds. The objective of the current studies was to investigate the role of sulphydryl compounds in the biotransformation of haloforms to carbon monoxide. Hepatic microsomal fractions isolated from male Sprague/Dawley rats were used. Incubation mixtures, contained buffer. NADPH-generating system and various concentrations of substrate (bromoform) and glutathione. Carbon monoxide was measured gas chromatographically. It was observed that 0.5 mM glutathione stimulated the reaction maximally. Glutathione added to incubation mixtures was found to decrease with the concomitant appearance of carbon monoxide in a ratio of 2:1 (GSH:CO). In addition, it was found that oxidized glutathione was formed in a ratio of 1.04:1.0 (GSSG:CO). These studies suggest that glutathione plays a role in the in vivo metabolism of haloforms to carbon monoxide since normal hepatic glutathione levels are about 10-fold greater than that required for maximal stimulation of the reaction. Furthermore, the formation of oxidized glutathione indicates an important mechanistic role for glutathione in the biotransformation of haloforms to carbon monoxide.

Rats were maintained for 2 years on diets supplying 0.1, 0.01, and 0.001 μg of TCDD/kg/day. Analysis of these diets indicated 2200, 210, and 22 ppt of TCDD. Ingestion of 0.1 μg/kg/day caused an increased incidence of hepatocellular carcinomas and squamous cell carcinomas of the lung, hard palate/nasal turbinates, or tongue. There was also a decreased incidence of tumors of the pituitary, uterus, mammary glands, pancreas, and adrenal gland. Other indications of toxicity at this dose level included increased mortality, decreased weight gain, slight depression of erythroid parameters, increased urinary excretion of porphyrins and δ-amino-levulinic acid plus increased serum levels of alkaline phosphatase, γ-glutamyl transferase, and glutamic pyruvic transaminase. Morphologic changes were noted in the hepatic, lymphoid, respiratory, and vascular tissues. Terminal liver and fat samples from rats at this high dose level contained 24,000 and 8100 ppt of TCDD, respectively. Rats given 0.01 μg of TCDD/kg/day for 2 years had a lesser degree of toxicity than those at the highest dose level. This included increased urinary excretion of porphyrins in females, liver lesions (including hepatocellular nodules), and lung lesions (including focal alveolar hyperplasia). Terminal liver and fat samples contained 5100 and 1700 ppt of TCDD, respectively. Ingestion of 0.001 μg of TCDD/kg/day caused no discernible effects in male rats and an increased incidence of swollen hepatocytes in livers of female rats that was judged to be reversible in nature and likely associated with the hepatic detoxification process for TCDD. Terminal liver and fat samples each contained 540 ppt of TCDD. These data indicate that continuous doses of TCDD sufficient to induce severe toxicity increased the incidence of some types of tumors, while reducing other types. No increase in tumors occurred in rats receiving sufficient TCDD to induce slight or no manifestations of exposure during the 2-year study.

182. Exquisite Toxicity in the Guinea Pig to Structurally Similar Halogenated Dioxins, Furans, Biphenyls, and Naphthalenes. ERNEST E. MCCONNELL and JAMES D. MCKINNEY, Environmental Biology and Chemistry Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (Joyce A. Goldstein)

Previous studies of the subject compounds have shown that the guinea pig is extremely sensitive to their toxic effects. In addition, the most toxic isomers of each of the compounds have certain common chemical criteria, i.e., the lateral positions of the aromatic nucleus must be fully halogenated while the peri-positions are not. With this in mind, we gave the most toxic isomer of each compound class to the same species (Harley strain guinea pig), using the same route of exposure (single oral gavage), to compare the toxicity and lesions produced by each compound and relate them to chemical structure. Each compound was dissolved in reagent-grade acetone or benzene, and subsequently diluted in corn oil after which the original solvent was removed in vacuo. Dilutions were prepared so that each guinea pig received 0.2 ml of the solution/100 g body wt. The animals were held for 30 days postexposure, after which they were killed and subjected to a complete gross and histopathologic examination. Based on the LD50–30 the relative toxicity was: dibenzo-p-dioxin > dibenzofuran > biphenyl > naphthalene. At the LD50–30 the signs of toxicity, median time to death, and lesions were essentially the same for all of these compounds. This suggests that there is also a commonality for the molecular basis of disease which may involve a specific biological receptor.

183. Biochemical Changes Seen in Guinea Pigs after Inhalation of Formaldehyde and Nitrogen Dioxide. FRANCIS J. MECLER, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts. (Mary O. Amdur)

Guinea pigs were exposed to either 10 ppm of formaldehyde, 10 ppm of nitrogen dioxide, or control air for 4 hr/day, 5 days/week for 13 weeks. Three days after the last exposure animals
were sacrificed. Liver, kidney, and lung were analyzed for glutathione content and alkaline phosphatase and glutathione reductase activity. In the formaldehyde-exposed animals lung glutathione was 38.0% higher than the control. This difference was significant by the unpaired t test. The liver and kidney glutathione levels were 26.3 and 10.3% lower than control values, but these differences were not significant. The glutathione reductase activities of lung, liver, and kidney from formaldehyde-exposed guinea pigs were 32.8, 57.3, and 64.1%, respectively, lower than control values. The liver and kidney decreases were significant. Alkaline phosphatase levels were similar to the controls. The nitrogen dioxide-exposed animals exhibited slightly lowered glutathione reductase activities than control and slightly higher glutathione values than control but the differences were not significant. Alkaline phosphatase activity was not significantly different. These data suggest that nitrogen dioxide does not affect the same level of biochemical change in the guinea pig as it is reported to effect in other experimental animals. These data also suggest that formaldehyde is a compound worthy of more than acute toxicologic study.

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184. Acute Effects of Paraquat and Ozone on Cytochrome-Dependent Enzyme Systems in the Lung. MARK R. MONTGOMERY, PATRICK J. CASEY, and DENNIS E. NIEWOEHNER, VA Hospital and University of Minnesota, Minneapolis, Minnesota.

The pulmonary, microsomal generation of two toxic oxygen species (superoxide and peroxide) is known to be markedly stimulated in vivo by low concentrations of paraquat (Pq). Sublethal doses of Pq (20 mg/kg, ip) or O₃ (1 ppm for 24 hr) were evaluated at 1, 4, or 7 days after administration for their effectiveness in altering pulmonary microsomal mixed-function oxidations (MFO) which are dependent upon oxidizable cytochromes. For cytochrome b₅-mediated stearoyl-CoA desaturation, the anorexic effect of acute Pq or O₃ administration resulted in a 90% depression of desaturation activity at 1 day with no change in cytochrome b₅ concentration. At 4 and 7 days both the desaturation activity and the cytochrome b₅ levels were unchanged from controls. The pattern of Pq- and O₃-mediated alterations in benzphetamine N-demethylation and concomitant concentration of the obligatory cytochrome P-450 were markedly different. Pq caused a progressive decrease in both parameters over the 7-day period. Ozone significantly decreased both the enzymatic activity (50%) and cytochrome P-450 concentration (25%) at 1 day; by 4 days both indices had returned to control values. Histologically, these low doses produced only mild alterations in lung architecture, characterized by focal inflammation and mild alveolar hypercellularity. When followed longer than the 7-day acute phase, there was no evidence of the fibrotic response which is characteristic of higher doses of either agent. These results suggest that pulmonary MFOs exhibit differential responses to acute, low level oxidant-induced damage. The cytochrome P-450 system appears to be more sensitive than the cytochrome b₅ system.

185. Effects of Chlorphentermine on Properties of the Alveolar Macrophage. MARK J. REASOR and ELIZABETH R. WALKER, West Virginia University Medical Center, Morgantown, West Virginia. (John U. Bell)

Chlorphentermine (CPM) is one of a number of amphiphilic drugs which when administered chronically to experimental animals results in a generalized lipidosis in many cell types, particularly the alveolar macrophage (AM). Therefore, we have investigated the effects of CPM on rabbit AM properties and function. AMs were obtained by pulmonary lavage from adult, male New Zealand white rabbits. The administration of CPM (30 mg/kg, ip) for 7 weeks (5 days/week) failed to produce a noticeable appearance of the characteristic lipid-rich, lysosomal "myeloid bodies" within the macrophage. However, when compared to same-day controls, there was an increased stabilization (decreased latency) of macrophage lysosomes when incubated for 15 min in hypo-osmotic sucrose solution. This was reflected in a 37.5% reduction in release of acid phosphatase (AP) and a 32.5% reduction in release of β-N-acetyl glucosaminidase (βNAG). Studies were also undertaken to determine the effects of incubation of
control AMs with CPM in vitro. A similar stabilization was observed for AP (10^{-3} to 5 	imes 10^{-5} M) and βNAG (10^{-3} and 5 	imes 10^{-4} M) when a lysosomal-rich fraction from AMs was incubated with CPM. In addition, CPM (10^{-3}, 10^{-4}, 10^{-5} M) inhibited βNAG by 32, 15, and 11%, respectively, suggesting the drug may have multiple effects at the level of the lysosome. The phagocytosis of particles of bisdecyl phthalate containing oil red O was significantly diminished when control AMs were incubated in vitro with CPM (10^{-3} to 10^{-5} M). Although the administration of chlorpheniramine to rabbits in vivo did not lead to characteristic morphological change in AMs, this drug caused potentially deleterious effects on lysosomal and cellular function following both in vivo and in vitro challenge. (Supported by a PMA Foundation Research Starter Grant and the WVU Medical Corp.)

186. Induction of Aryl Hydrocarbon Hydroxylase in Rodent Tissues following Intratracheal Instillation or Interperitoneal Administration of Benzo(a)pyrene. C. E. Mitchell, Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, New Mexico. (Frederick W. Oehme)

Aryl hydrocarbon hydroxylase (AHH) activity is increased in many tissues of rodents following administration of polycyclic aromatic hydrocarbon (PAH). Although the lung is one of the primary sites of contact with PAH, relatively few studies have examined the metabolism and fate of PAH in the lung. In this study AHH levels have been measured in the lung, kidney, and liver following intratracheal instillation of benzo(a)pyrene (BP). AHH was induced in lungs of Chinese hamsters, Syrian hamsters, Fischer 344 rats, and CD-1 mice following intratracheal instillation of BP (10 mg/kg). The level of AHH induced in the lung was lowest in Chinese hamsters and highest in mice. After intraperitoneal administration of BP (50 mg/kg to Chinese hamsters), AHH level in the liver increased to a maximum value at 24 hr followed by a return to 50% of maximum at 72 hr postadministration and AHH levels in lung and kidney were not significantly higher than in controls. In contrast, when BP (10 mg/kg) was intratracheally instilled into Chinese hamsters, AHH levels in lung increased to maximum levels at 12 hr following administration. The level remained high for 1 week before returning to control values. In addition, AHH levels in the liver increased to twofold at 24 hr followed by a rapid return to control values after intratracheal administration. The level in kidney was not significantly increased. AHH induction in mice showed a similar pattern following intratracheal administration. Although higher inducibility was observed. These studies show that following intraperitoneal administration of BP the liver is inducible within 24 hr, followed by a rapid return to baseline levels, whereas lung and kidney show no change. In contrast, following intratracheal administration of BP, maximum levels in the lung are maintained for days before returning to baseline levels, with liver showing a modest increase in AHH levels. Further studies in this area may help to identify the role of AHH in lung cancer induction and, in addition, the fate and translocation of PAH to other organs. (Research performed under USERDA Contract No. EY-76-C-04-1013.)

187. Pulmonary Toxicity of 3-Substituted Furans from the Mint Plant Perilla frutescens Britton, B. J. Wilson, J. E. Gakst, and R. D. Linnabary, Center in Toxicology, School of Medicine, Vanderbilt University, Nashville, Tennessee.

Perilla frutescens, also called purple mint plant and various other names, is an import from Asia that now grows wild over much of Eastern United States and various European countries. Several varieties contain perilla ketone, egomaketen, and isoeogomaketen which are closely related chemically to the toxic ipomeanols from the moldy sweet potato. Since no biological data existed for these compounds, they were synthesized and tested for their toxicity in laboratory animals and certain ruminants. The intraperitoneal LD50's for all three perilla 3-substituted furans in both male and female white mice was less than 10 mg/kg and the oral doses were proportionately low. Death in about 24 hr resulted from extensive pulmonary edema and pleural effusion, which was indistinguishable from that caused by ipomeanols. Female goats were killed by intravenous doses of perilla ketone at 10 mg/kg but were not affected by 40 mg/kg injected
intraruminally. An Angus heifer was killed by 30 mg/kg intravenously (the lowest dosage tested) but was not affected by 40 mg/kg injected into the rumen. A sheep was made ill by 19 mg/kg intravenously but recovered somewhat prior to postmortem examination. The ruminants made ill or killed by intravenous doses of perilla ketone showed pulmonary pathology similar to that seen in natural outbreaks of acute bovine pulmonary emphysema (ABPE). Both the mold-damaged sweet potato and perilla mint have been implicated as causative agents of ABPE. The authors propose that perilla, as well as the mold-damaged sweet potato, is probably a cause of ABPE due to the β-substituted furans found in the plant. The widespread use of perilla varieties in oriental countries for food flavoring and for medical purposes suggests possible hazards also to human health.

188. Assessment of Ozone Toxicity Using a Biomedical Model. F. J. Miller,
Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle
Park, North Carolina. (D. B. Menzel)

Environmental toxicologists must make judgments concerning the validity of extrapolating to man the results obtained from animal experiments. With a highly reactive gas, such as ozone, the problem of estimating the effective dose delivered to the target organ is compounded. Species differences in the ratio of effective dose to exposure concentration must also be considered in establishing ambient air quality standards adequate for the protection of the public health. An approach for evaluating the pulmonary toxicity of ozone will be presented which involves experimentally determining nasopharyngeal removal of ozone and mathematically modeling pulmonary uptake. Experimental estimates of nasopharyngeal removal serve to determine boundary conditions when using gas transport equations to model lower airway deposition. The lower airway model characterizes the transport and removal of O3 within model segments that are partitions of individual airway generations and predicts the amount of ozone absorbed in each generation of the lung. The effects of convection, axial diffusion, and radial diffusion, as well as the chemical reactions of O3 with various components of mucus are included. Results of model analyses indicate that a general similarity exists among guinea pigs, rabbits, and man in the shape of the predicted pulmonary dose curves. In man, for any given tracheal concentration, the model predicts that the first generation of respiratory bronchioles receives the maximum tissue dose of ozone, a result which is in agreement with the finding from primate studies. The effects of respiratory frequency, tidal volume, and mucus production on pulmonary deposition of ozone are illustrative of the physiological and toxicological comparisons that can be made using this modeling approach.

189. Factors Effecting the Efflux of Paraquat from the Lung. L. L. Smith, I. Wyatt, and M. S.
Rose. Imperial Chemical Industries Ltd., C.T.L., Alderley Park, Near Macclesfield, Cheshire,
England.

Rats given paraquat by various routes have been shown to die primarily as a result of lung damage. Paraquat is accumulated in the rat lung by an energy-dependent uptake process which appears to be specific to the lung. This process is inhibited by CN⁻, iodoacetate, rotenone, and a variety of endogenous and exogenous compounds. The net amount of paraquat accumulated by the lung is determined by the rate of uptake into the lung and the rate of efflux from the lung. Therefore, compounds which inhibit the accumulation may produce their effect by (1) reducing the rate of uptake of paraquat into the lung, (2) increasing the rate of efflux of paraquat from the lung, or (3) a combination of 1 and 2. The studies reported here were undertaken to determine the effect of various compounds on the efflux of paraquat from the lung. Rats were dosed intravenously with paraquat, the lungs were removed 2 hr later, and the efflux of paraquat from slices of lung was determined over a 4-hr period. The t½ of the efflux of paraquat in medium alone was found to be ≈17 hr, which is similar to that found in vivo. Promethazine (10⁻⁴ M), histamine (10⁻⁴ M), and rotenone (10⁻⁴ M) in the incubation medium did not effect the efflux of paraquat from lung slices whereas iodoacetate (10⁻³ M), CN⁻ (10⁻³ M), putrescine (10⁻⁴ M), bromthymol blue (3 x 10⁻⁴ M), and valinomycin (10⁻⁶ M) increased the rate
of efflux, as did incubation of the lung slices under nitrogen instead of air. The effect of these various metabolic inhibitors on oxygen consumption, $^{14}$CO$_2$ evolution from [U-$^{14}$C]glucose and protein efflux from lung slices were also determined. Iodoacetate ($10^{-3}$ M), CN$^-$ ($10^{-3}$ M), and rotenone ($10^{-4}$ M) almost totally abolished oxygen consumption and $^{14}$CO$_2$ evolution whereas bromthymol blue ($3 \times 10^{-4}$ M) and valinomycin ($10^{-6}$ M) had little or no effect. Iodoacetate ($10^{-3}$ M), CN$^-$ ($10^{-3}$ M), and incubation under nitrogen greatly increased the efflux of protein from lung slices suggesting the cells were damaged. From these data we have concluded that some compounds which inhibit the accumulation of paraquat into lung slices do so by increasing the rate of efflux of paraquat from the lung directly or by damaging lung cells.

190. Experimental Respiratory Hypersensitivity to a Toluene Isocyanate Hapten. Meryl Karol, E. Joan Riley, Holly Ioset, and Yves Alarie, Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

Toluene diisocyanate (TDI) has frequently been associated with occupational respiratory hypersensitivity reactions. Sensitivity to TDI can be demonstrated by bronchial provocation testing. However, the immunologic basis of this hypersensitivity remains uncertain since TDI-specific antibodies have not been detected in clinically "sensitized" individuals. To assess the sensitizing ability of the toluene isocyanate determinant, p-tolylisocyanate (T) was reacted with ovalbumin (OA) and the resulting haptenconjugate (T-OA) was used to sensitize guinea pigs. By repeatedly exposing guinea pigs to an aerosol of the T-OA conjugate, respiratory hypersensitivity to this antigen was produced. Sensitivity toward the "toly" portion of the antigen was demonstrated by: (a) respiratory response to challenge with T-OA but not with OA. (b) respiratory reactivity toward an antigen composed of tolyl linked to an unrelated carrier protein (bovine serum albumin). (c) inhibition of respiratory hypersensitivity reactions using a tolyl-amino caproate hapten. Serological analyses of sensitized guinea pigs revealed tolyl-specific antibodies. The method for inducing hapten-specific hypersensitivity can be applied to screen various industrial chemicals for their sensitizing abilities toward the respiratory tract. (Supported by NIEHS Grant R01-ES01532, and a grant from the PPG Foundation.)


The rate of removal of low levels of $^{63}$NiCl$_2$ from the airways of the rat lung was measured in vivo and in vitro. Isolated, ventilated, and perfused lungs were prepared as previously described using negative pressure ventilation and perfusion with Tyrode's solution modified by the addition of 35 g/liter of polyvinylpyrrolidone (Charles and Menzel, Res. Commun. Chem. Pathol. Pharmacol. 12, 389–396, 1975). Removal in vivo was studied by intratracheal instillation of $^{63}$NiCl$_2$ solutions using the technique described by Schanker. In both in vivo and in vitro studies, 100 µl of isotonic sucrose (ph 7.40) was instilled into the trachea containing 1, 10, or 100 nmol of $^{63}$NiCl$_2$. Aliquots of the perfusion fluid were measured for $^{63}$Ni radioactivity and lungs from animals exposed in vivo were digested in perchloric acid, decolorized with hydrogen peroxide, and counted for $^{63}$Ni in Triton X-100/Toluene-based liquid scintillation fluid. $^{63}$Ni$^{2+}$ appears to be removed by a very slow simple diffusional process. Using a computer-based data reduction program, the rate of removal was calculated to have a $t_{1/2}$ of 187 ± 27 min in vivo. The $t_{1/2}$ observed in vivo was approximately 197 min. These data are the first reported for the removal of soluble Ni salts at nanomole doses per lung. The levels of Ni$^{2+}$ ion used in our studies are well within the estimated environmental exposure levels. The slow removal of NiCl$_2$ may indicate the existence of high-affinity binding sites in the lung. These data suggest the need for studies of long-term exposure to NiCl$_2$ to investigate potential pulmonary accumulation and toxicity. (Supported by EPA Contract 68-02-2436 and by NIH Grants 5 T32 ES07002 and 5 T32 GM07105.)
192. The Effect of Cadmium on Calcium Absorption from the Rat Intestine. E. M. Yuhas, T. S. Miya, and R. C. Schnell, Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana.

The effect of cadmium on gastrointestinal calcium absorption in male rats was examined using an in situ single-pass intestinal perfusion technique. The addition of cadmium (10^-3, 10^-4, 10^-5, or 10^-2 M) to the perfusion medium caused a decrease in net calcium absorption. At concentrations of Cd greater than 10^-4 M, a net negative calcium balance occurred. The decrease in net calcium absorption resulted from both a decrease in the lumen-to-plasma flux of calcium (10^-2 to 10^-4 M Cd) and an increase in the plasma-to-lumen flux of calcium (10^-4 to 10^-2 M Cd). Intestinal calcium absorption was unaltered 3 days following a single acute injection of cadmium acetate (2 mg/kg, ip). In another experiment, rats were given cadmium (0, 1, 10, or 100 ppm) in the drinking water for 13 weeks and then in situ intestinal calcium absorption was determined. At 1 or 10 ppm of Cd, there was no effect on net calcium absorption or lumen-to-plasma or plasma-to-lumen fluxes of calcium. At 100 ppm of Cd, there was an increase in net calcium absorption which resulted from an increase in the lumen-to-plasma flux of calcium. There was no effect on the plasma-to-lumen flux of calcium. The study indicated that while cadmium when added to the perfusate may exert a direct inhibitory effect on intestinal calcium absorption, the in vivo treatment of rats with cadmium, either acutely or chronically, does not decrease intestinal calcium absorption. (Supported by NIEHS Grant ES-00921 and NIEHS Training Grant T01-00071.)

193. Role of Hepatic Metallothionein in the Tolerance to Cadmium-Induced Inhibition of Drug Metabolism in the Rat. S. A. Roberts, G. S. Probst, and R. C. Schnell, Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana.

Previous studies in this laboratory have shown that rats exhibit a tolerance to cadmium (0.84 mg/kg, ip)-induced inhibition of hepatic hexobarbital metabolism when pretreated with lower doses of Cd (0.21-0.42 mg/kg, ip). Since this tolerance was correlated with increased hepatic metallothionein (MT) concentrations, studies were undertaken to examine further the role of MT in the tolerance phenomenon. Initial studies were directed at defining the time course of tolerance development. Male Sprague-Dawley rats received a tolerance-producing dose of Cd (0.21 mg/kg) at intervals of 4 to 336 hr prior to a challenge dose of Cd (0.84 mg/kg). Subsequent measures of hepatic drug metabolism (hexobarbital, ethylmorphine, or aniline) were made 72 hr following the Cd challenge. The minimum time required for tolerance development was 14 to 16 hr, with maximal tolerance being demonstrated from 16 to 144 hr. The tolerance began to decline at 240 and 336 hr. Kinetic studies were conducted to determine the time course of MT synthesis. The rate of hepatic synthesis of Cd-induced MT, determined by a 15-min pulse label with [35S]cystine (500 μCi/kg), was maximal 2 hr after Cd treatment. Total hepatic metallothionein levels increased rapidly and were maximal at 8 to 67 hr post-Cd but remained significantly increased over control values at 336 hr. Subcellular distribution of a challenge Cd dose (0.84 mg of Cd/kg; 50 μCi of 106Cd/kg) revealed an increase in total cytosol and MT bound 106Cd in Cd-tolerant animals. These data indicate that the development of the tolerance phenomenon is temporally correlated with the kinetics of MT synthesis and indicate that performed hepatic MT alters the subcellular distribution of Cd. These data support a proposed protective role for MT in cadmium toxicity. (Supported by NIEHS ES-00921 and NIH Toxicology Training Grant T32-GM07095.)

194. Cadmium-Induced Alteration in Hepatic Microsomal Drug Metabolism: Sex-Related Differences in the Rat. D. H. Pence and R. C. Schnell, Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana.

In the rat a sex-related difference exists in the ability of cadmium to alter drug action. Following either in vivo Cd treatment (2 mg of Cd/kg, ip, 72 hr) or in vitro addition of Cd (10^-6
to $10^{-3}$ M) to untreated hepatic microsomes, a significant inhibition of metabolism of hexobarbital and ethylmorphine occurred at all Cd concentrations in male, but not in female-derived microsomes. However, similar treatments produced a significant decrease in metabolism aniline by both male and female rat liver microsomes. Partial protection from the inhibitory effects of cadmium was observed in castrated male rats treated with cadmium (2 mg of Cd/kg, ip) immediately following surgery. The potentiation of hexobarbital-induced hypnosis was only 54% in these animals compared to 235% in sham-operated male rats receiving Cd. Similarly, hepatic microsomal metabolism of hexobarbital was inhibited 39% in Cd-treated castrated male rats, compared to 75% in Cd-treated sham-operated males. The increase in metabolism of hexobarbital, ethylmorphine, and aniline by hepatic microsomes isolated from female rats which had received testosterone propionate (20 mg/kg, sc) for 4 weeks and then challenged with Cd (2 mg Cd/kg, ip) was significantly decreased for all substrates. These data support the possibility that sex-related differences in the ability of Cd to alter drug response in the rat is mediated through the testes by decreasing testosterone levels, and thus, decreasing the metabolic destruction of the sex-dependent substrates, hexobarbital and ethylmorphine. (Supported by NIEHS Grant ES-00921 and NIEHS Training Grant TO1-00071.)

195. Role of Metallothionein in Accumulation, Transport, and Excretion of Cadmium in Rats. K. Hirayama and Z. A. Shaikh, Department of Pharmacology and Toxicology, University of Rochester Medical School, Rochester, New York. (T. W. Clarkson)

It has been suggested that metallothionein might play a central role in the metabolism and toxicity of cadmium. This study was conducted to elucidate the metabolic function of metallothionein in rats chronically exposed to cadmium. Male rats of Wistar strain were given daily subcutaneous injections of 5 μmol $^{109}$CdCl$_2$/kg (sp act 0.4 μCi/μmol), 5 days/week for up to 14 weeks. The animals were sacrificed at periodic intervals of 4, 6, 8, 10, 12, and 14 weeks. Total tissue $^{109}$Cd and $^{160}$Cd bound to tissue metallothionein were determined. During the 12-week period there was a continuous increase in $^{109}$Cd levels of all tissues. In liver, kidney, and pancreas, 80, 70, and 65% of the respective tissue $^{109}$Cd was bound to metallothionein. After 12 weeks the $^{109}$Cd levels in all tissues declined markedly. The decrease in tissue levels was also reflected in the amount of $^{109}$Cd bound to metallothionein. In plasma as well as red blood cells, as early as 4 weeks, a significant portion of the $^{109}$Cd was bound to a protein having the same molecular weight as metallothionein. Excretion of injected $^{109}$Cd in urine during the first 10 weeks, although small, was proportional to the body burden. After 10 weeks the animals developed proteinuria and glucosuria. The urinary excretion of $^{109}$Cd increased markedly and reached a peak at 13 weeks. This was followed by a sharp decline. The high excretion of $^{109}$Cd in urine was accompanied by appearance of metallothionein in the urine. A considerable portion of the urinary $^{109}$Cd was bound to metallothionein after 10 weeks. The above results suggest that metallothionein is involved in sequestration of Cd in various tissues. The presence of metallothionein in plasma indicates that it may play a role in the transport of Cd. At later stages of Cd intoxication, when renal damage has occurred, metallothionein also seems to function as an excretion vehicle for the element. (Supported by NIH Grants ES 01448 and ES 01247.)

196. Increase in Metal-Binding by Hepatic Metallothionein after Treatment with Alkylating Agents. F.N. Kotsonis and C. D. Klaassen, Department of Pharmacology, University of Kansas Medical Center, College of Health Sciences and Hospital, Kansas City, Kansas.

Several physiological functions for metallothionein have been suggested; however, only those related to zinc metabolism and heavy metal detoxification have been supported by experimetal evidence. In order to determine the effect of nonmetals on the concentration and function of hepatic metallothionein, rats were treated with several alkylating agents and the metal-binding of hepatic metallothionein was determined. Male Sprague-Dawley rats were injected (ip, 2 ml/kg) with Sodium iodocacetate (30 mg/kg), bromobenzene (1200 mg/kg), diethylmaleate (560 mg/kg), iodomethane (46 mg/kg), saline, or corn oil 40 and 16 hr prior to being sacrificed. The metal binding of hepatic metallothionein was measured by adding $^{203}$HgCl$_2$ to the liver homogenate, deproteinizing the homogenate with 10% TCA, and determining the amount of $^{203}$Hg bound to
metallothionein by chromatographing (Sephadex G-75, 0.01 M citrate, pH 2.5) the TCA supernatant. The metal-binding of hepatic metallothionein in the rats treated with alkylating agents was significantly greater (2- to 5-fold) than in ad libitum-fed controls of pair-fed control rats. The hepatic protein which bound the metal after treatment with sodium iodoacetate was isolated and characterized as a metallothionein because of its chromatographic behavior, high affinity for Zn, Cd, and Hg, and absence of absorbance at 280 nm. These results indicate that alkylating agents, which may alter the sulfhydryl status and/or glutathione levels of the liver, can also increase the metal-binding of hepatic metallothionein. The increased metal-binding may be due to either an increase in the concentration of metallothionein or an increase in the ratio of metal to protein. (Supported by USPHS Grant ES-01142.)

197. Residues of Cadmium in Edible Tissues or Products of Lactating Cows, Swine, and Layer Hens after Low-Level Dietary Exposures. J. C. Street, R. P. Sharma, J. L. Shupe, and D. J. Wagstaff, Utah State University, Logan, Utah, and Bureau of Veterinary Medicine, Food and Drug Administration, Rockville, Maryland.

Possible indirect contamination of human foods through increased levels of heavy metals in animal feeds is of concern in relation to food safety. To evaluate the potential hazard from cadmium residues, dairy cows and growing pigs were treated with cadmium chloride (added to the diet at levels approximating 2 and 10 ppm of cadmium above the normal dietary intake) for 3 months. After this continuous treatment, some of the animals were maintained on control feed to allow cadmium depletion from the body. Laying hens were similarly treated, but with a 6-week period of continuous treatment followed by a 6-week depletion period. Cadmium was determined in skeletal muscle, liver, kidney, and bone of each species, in milk from cows, and in eggs using atomic absorption spectrophotometry. No increase in the cadmium concentration in milk or eggs (yolks and whites) was observed during the treatment and depletion periods; control values averaged 0.018 ppm (milk) and 0.11 ppm (egg yolk, wet basis). Muscle samples from each species also showed no consistent effect of cadmium treatment. Cadmium in bone was slightly elevated but only at the high treatment level. Consistent dose-time-related elevations of cadmium in liver and kidney were observed in all three species. The depletion periods resulted in no decrease in the cadmium content of those organs. The results suggest that only the cadmium-accumulating organs represent potentially unsafe food products from animals environmentally exposed to cadmium residues. (Supported in part by Contract FDA-223-74-7195.)

198. Castration Fails to Prevent Cadmium Carcinogenesis. Arthur Furst, Institute of Chemical Biology, University of San Francisco, San Francisco, California.

An early toxic manifestation of cadmium administration to male rats is the appearance of interstitial cell hyperplasia in the testes. Later the testes may atrophy. Interstitial cell tumors have also been reported after a single subcutaneous injection of cadmium chloride (Lucis et al., Oncology 26, 53-67, 1972). In our laboratories an intramuscular injection of cadmium powder always results in fibrosarcomas at the site. To ascertain if the testes play a role in cadmium carcinogenesis, castrated male rats were evaluated. Two groups, 40 intact and 40 recovered castrated male Fischer-344 rats weighing about 100 g were placed on experiment. Each group was divided in half. The first served as the vehicle controls receiving four monthly injections of 0.2 ml of pure triocanoin deep in the right thigh muscle. The treated group was injected similarly with an equal volume of the vehicle containing 3 mg of a suspension of cadmium powder, 300+ mesh, 99.9+ pure. Tumors appeared between 5 and 13 months in both treated groups, with 70% appearing in the intact and 75% in the castrates. In the main the tumors were described as poorly differentiated fibrosarcomas. All tumors were transplanted six consecutive times in unconditioned Fischer rats; no tumor regressed. The castrated animals which came to necropsy were found with partially degenerated seminal vessels and complete atrophy of the prostate gland. It is concluded that castration has no effect on the induction of tumors at the site of injection of pure cadmium powder. (Supported by NCI Contract No. N01-CP-3329.)
199. Effect of Cadmium on the Biochemical Components of the Hepatic Microsomal Monoxygenase System of the Male Rat. J. R. MEANS, G. P. CARLSON, and R. C. SCHNELL, Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Purdue University, West Lafayette, Indiana.

Cadmium is a potent inhibitor of hepatic drug metabolism but little is known about the biochemical mechanisms involved. Male Sprague-Dawley rats receiving a single dose of cadmium acetate (2 mg of Cd\(^{2+}\)/kg, ip) exhibited significant decreases in hepatic microsomal metabolism of hexobarbital (79%), ethylmorphine (71%), and aniline (47%), as well as decreased levels of cytochrome P-450 (39%) 72 hr after administration of the metal, but NADPH-cytochrome c reductase activity was not changed. The magnitude of microsomal spectral binding of hexobarbital, ethylmorphine, and aniline was significantly reduced. In vitro addition of Cd\(^{2+}\) (10\(^{-6}\) to 10\(^{-3}\) M) to microsomes produced a concentration-dependent inhibition of the metabolism of these substrates and a reduction in the level of cytochrome P-450, which was converted to its inactive form, P-420. In kinetic studies, apparent \( V_{\text{max}} \) values were significantly lowered for aniline hydroxylase (57%) and ethylmorphine N-demethylase (75%) 72 hr following administration of cadmium. The apparent \( K_{m} \) value for aniline hydroxylation was not altered, but that for the N-demethylation of ethylmorphine was significantly decreased. After in vitro cadmium addition, concentration-dependent decreases were observed in the apparent \( V_{\text{max}} \) and \( K_{m} \) values for both microsomal reactions. These data indicate that inhibition of the microsomal metabolism of aniline following cadmium treatment parallels the decreased cytochrome P-450 content, but the greater inhibition of ethylmorphine metabolism by cadmium in vivo suggests that factors in addition to cytochrome P-450 are involved. (Supported by NIEHS Grant ES-00921 and NIEHS Training Grant TO1-ES-00071.)

200. Absorption and Retention of Orally Administered \(^{109}\)Cd in Humans. Z. A. SHAIKH and J. C. SMITH, Department of Pharmacology and Toxicology, University of Rochester, School of Medicine, Rochester, New York. (T. W. Clarkson)

The general population is exposed to cadmium through the diet. To obtain the information about the gastrointestinal absorption of cadmium and the fate of the absorbed dose, the present study was carried out in adult male volunteers. Each volunteer was given orally 5 or 10 \( \mu \)Ci of \(^{109}\)CdCl\(_2\) mixed with a homogenate of 1 g of beef kidney. The fecal output was monitored to calculate the absorbed dose. To determine the total radioactivity in the body, the subjects were counted using a whole-body counter. In addition, the radioactivity in liver and left kidney was also monitored. The majority of the absorbed dose was excreted within the first 3 days. This was followed by a slower phase of excretion. It was estimated that 3 to 7% of the orally administered cadmium was absorbed. The retention half-time of the absorbed dose appeared to be rather long. The present estimates indicate a minimum half-time of approximately 1 year and a maximum half-time of several years. The amount of \(^{109}\)Cd taken up by the liver and kidney did not appear to change markedly during the 6 months following the dose. There was, however, indication of a slow increase in the renal levels. The retention half-time of the element in the liver and kidney is definitely much longer than that of the whole body. These observations in humans are in general agreement with those reported in animals. (Supported by NIH Grants ES 01247 and ES 01248.)

201. The Distribution of Vanadium in Rat Tissues following Continuous Ingestion of Vanadyl Sulfate and Sodium Ortho-vanadate. ROBERT D. R. PARKER and RAGHUBIR P. SHARMA, Department of Biology and Toxicology Program, Utah State University, Logan, Utah.

Vanadium is considered essential for the rat and other animals in trace amounts but at higher concentrations it can lead to a number of biological effects involving respiratory and gastrointestinal systems. Experiments were conducted to determine the distribution and effects of selected salts in male rats following continuous ingestion. Wistar rats were continuously fed drinking water containing 5 and 50 ppm of vanadyl sulfate and sodium metavanadate for a 3-month period. At the end of the 3rd, 6th, 9th, and 12th weeks, blood, kidney, liver, bone, muscle, and digestive tract were collected and analyzed for V utilizing flame atomic absorption spectro-
photometry. At the 5- and 50-ppm levels, V had no consistent effect on body weights. In the tissues of animals given 5 ppm of V salt, the V contents were essentially the same as those in controls. The tissues of animals given 50 ppm of V salt showed increased concentrations of vanadium. Kidney had the highest vanadium concentration followed by bone, liver, and muscle. In general, the tissue concentrations of V in animals given 50 ppm as sodium ortho-vanadate were higher than those exposed to similar levels as vanadyl sulfate. The V concentration of the kidney in the animals given 50 ppm of sodium ortho-vanadate showed a sharp increase from 9.8 μg/g in the 3rd week to 19.8 μg/g in the 9th week. With this exception, the V content of the tissues for the treated animals plateaued from the 3rd week.

202. Toxicodynamics of Intratracheally Administered Vanadium in Rats. STEVEN G. OBERG, ROBERT D. R. PARKER, and RAGHUHIN P. SHARMA, Department of Biology and Toxicology Program, Utah State University, Logan, Utah.

Investigations have been performed to determine baseline parameters for further study of the essentiality and/or toxicity of various vanadium compounds in mammals. Intratracheal injection techniques, chosen to imitate the inhalation mode of administration, and a fairly soluble form of the element, in order to maximize distribution kinetics, were selected as principal variables in the present case. 4VOCl₂ was administered intratracheally to juvenile male Wistar rats. Each 1-ml dose contained approximately 12.66 μCi of the radioactive vanadium. Members randomly selected from each injection group were sacrificed at postexposure survival times of 15 min, 4 hr, 1, 7, 14, 28, and 63 days. Samples of all major organ tissues were analyzed for 48V content by NaI(Tl) γ-ray spectrometry. More than half of the deposited 48V was removed from the lungs within the first day. Lung clearance rates slowed in later phases of the study with about 3% of the burden remaining after 63 days. Within 15 min of exposure, the vanadium isotope translocated to all measured organs, except the brain, with the blood, heart, spleen, liver, and kidneys receiving the largest fractions of the activity. Peak uptake activities for most organs occurred between 4 and 24 hr after injection, with the kidneys maintaining the largest fraction of 48V in the early phases of the study. Bones accumulated activity through the first day and maintained relatively large burdens throughout the 9-week period. The testes also received small fractions of the isotope which persisted during all sacrifice intervals. The clearance of the isotope from the organs examined displayed multiple-order rate kinetics. Excretion of the radiotracer occurred by both urinary and fecal means although the urinary route predominated.

203. Evidence for Specific Lead–δ-Aminolevulinate Complex Formation by 11C Nuclear Magnetic Resonance Spectroscopy. C. STUART BAXTER, HOWARD E. WEY, and ALAN B. CARDIN, Departments of Environmental Health and Biological Chemistry, University of Cincinnati College of Medicine, Cincinnati, Ohio. (P. B. Hammon)

Lead is known to have toxic effects on haem synthesis, being an especially potent, and, relative to other metals, specific inhibitor of the enzyme δ-aminolevulinate dehydratase (ALAD). The mechanism of inhibition of ALAD by lead is uncertain, but binding of lead to ALAD sulfhydryl groups has been assumed. Mechanisms involving lead interaction with the enzyme substrate (ALA), rather than the enzyme are also reasonable, but have not as yet been considered; therefore, the interaction of lead acetate with ALA was investigated by carbon-13 nmr spectroscopy, a technique shown to be very powerful in examining ligand–metal interactions. Addition of lead acetate solution to a 0.25 M solution of ALA at pH 6.5 resulted in a pronounced shift of the carbon resonances of the latter, a maximal effect occurring at a ALA : Pb concentration ratio of 2. No similar effects were observed with acetates of zinc, cadmium, silver, or mercury. Studies on the variation in shift of resonances from individual carbon atoms with lead acetate concentration suggested that lead interacted most strongly at the carboxyl and α-carbon atoms of ALA. The variation of shift with pH showed that at an ALA : Pb ratio of 1 the pKₐ of the ALA carboxyl group was decreased by 1.2 units. These studies demonstrate that lead is specific among the metals tested in forming a complex with δ-aminolevulinate acid. Such complex formation may be important in the mechanism of lead toxicity on the haem biosynthetic pathway. (Supported by NIH Grants ES 00159 and 00127.)
204. Mercury Exhalation in Mice Given HgCl₂, JAN DOTZLER DUNN, THOMAS W. CLARKSON, and LASZLO MAGOS, Environmental Health Sciences Center, Department of Radiation Biology and Biophysics, University of Rochester School of Medicine, Rochester, New York, and Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey, England.

The current model by which ethanol modifies the metabolism of mercury is that ethanol acts specifically to depress the rapid unidirectional conversion of mercury vapor to its ionic form (Hg²⁺). We report that (1) volatile mercury is exhaled by mice injected several days beforehand with HgCl₂ and (2) the amount exhaled can be dramatically increased by treatment with ethanol. These findings suggest that mammalian reduction of ionized mercury occurs and that the process is also subject to modification by ethanol. As a corollary, it is conceivable that the administration of a standard dose of ethanol and quantitation of the mercury exhaled could be used as an index of the body-burden of inorganic mercury.


Methylmercury is excreted into the bile at a relatively slow rate. This slow excretion and the fact that methylmercury undergoes extensive enterohepatic recirculation accounts for the long biological half-life of methylmercury. The purpose of this study was to synthesize a chelating agent which would enhance the biliary excretion of methylmercury. Male Sprague-Dawley descendent rats were treated with test compounds 1 hr prior to administration of methylmercury. Test compounds were administered intraperitoneally and methylmercury was administered intravenously. Animals were anesthetized with urethane. A laparotomy was performed on anesthetized rats and the bile duct was cannulated to make serial collections of bile during the following 5 hr. The dosage of CH₃²⁰⁹Hg was 1 mg/kg. Two new compounds were found to significantly increase the biliary excretion of methylmercury. Cystecholic acid, a bile acid derivative, more than doubled the biliary excretion of methylmercury from 30 to 300 min following treatment. This increase in biliary excretion of methylmercury was similar to that seen for pregnenolone-16α-carbonitrile. Adamantane-imidacetic acid, synthesized by a basic condensation of adamantan amine and chloroacetic acid, also increased the biliary excretion of methylmercury. Structure activity relationship studies indicate that a large molecular weight polycyclic structure is required for stimulation of biliary excretion of methylmercury. Several aliphatic compounds possessing sulfur donor atoms and amine groups did not enhance the biliary excretion of methylmercury. (Supported by USPHS Grant ES01018 and ES00782.)

206. Effects of Arsenic on Mitochondrial and Microsomal Oxidative Interactions. BRUCE A. FOWLER, JAMES S. WOODS, and CAROL M. SCHILLER, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina.

Previous ultrastructural–biochemical studies from our laboratories have shown that prolonged oral exposure to arsenate produces in situ mitochondrial swelling and decreased mitochondrial oxidation of NAD-linked substrates in mammalian liver. The present studies were initiated to examine the effects of these changes on interactions between hepatic mitochondrial and microsomal oxidative functions following exposure of rats to 0 or 40 ppm of sodium arsenate (As) in drinking water for 6 weeks. Ultrastructural morphometric studies disclosed a two-fold increase in the ratio between the surface density of rough endoplasmic reticulum and mitochondrial volume density in livers of As-treated animals relative to those of controls, indicating an alteration of the normal physical relationship which exists between these two organelle systems in vivo. This effect was accompanied by a twofold increase in the mitochondrial NAD/NADH ratio, secondary to decreased mitochondrial reduction of NAD. The
specific activity of aminopyrine demethylase (AD), a microsomal mixed-function oxidase which
generates aldehydes metabolized by an NAD-dependent mitochondrial dehydrogenase, was
unchanged from that of controls when measured in microsomes isolated from As-treated rats. In
contrast, when microsomes from As-treated rats were incubated in the presence of mito-
chondria from livers of the same animals, AD activity was decreased 30% as compared with
that seen in microsomes combined with mitochondria from control liver. Microsomes isolated
from As-treated rats, incubated with mitochondria from control livers, contained 20% less AD
activity than mitochondria plus microsomes from controls alone. The results of this study
suggest that a functional interaction exists in vivo between mitochondria and microsomes with
respect to maintaining oxidative functions in normal liver. Perturbation of this interaction during
arsenic exposure appears to be characterized in part by decreased mitochondrial NAD-linked
oxidative capability and a secondary reduction of microsomal mixed-function oxidative activity.

207. Perturbation of Hepatic Heme Biosynthesis and Urinary Porphyrin Levels as an Early
Response to Prolonged Arsenic Exposure. JAMES S. WOODS and BRUCE A. FOWLER,
National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North
Carolina.

These studies investigated the effects of prolonged exposure to sodium arsenate (As\(^{5+}\)) on
hepatic heme biosynthesis and urinary porphyrin levels in rats and mice. Continuous exposure to
As\(^{5+}\) at 20, 40, or 85 ppm in drinking water for 6 weeks resulted in depression of δ-ami-
nolevulinic acid (ALA) synthetase and heme synthetase (HS), the first and last enzymes in heme
biosynthesis, respectively. ALA synthetase was maximally depressed to 79% of control values at
40 ppm in both species, whereas HS activity was decreased to 63 and 75% of control at 85 ppm
in rats and mice, respectively. Concomitantly, urinary uroporphyrin levels were increased 12-
fold, and coproporphyrin levels 9-fold, with respect to control values in both species. In contrast,
no changes were observed in the activity of hepatic ALA dehydratase or in the levels of cyto-
chrome oxidase or cytochrome P-450, indicators of mitochondrial and microsomal hemoprotein
function, respectively. Moreover, no changes in body weight, general appearance or behavior, or
in liver morphology aside from mitochondrial swelling occurred in As\(^{5+}\)-treated animals. These
findings demonstrate that prolonged exposure to As\(^{5+}\) results in perturbation of selective hepatic
heme biosynthetic pathway enzymes with concomitant increases in urinary porphyrin levels.
These changes occur independently of alterations in hepatic hemoprotein function or other toxic
effects and may serve in the early indication of pretoxic arsenic exposure.

208. Heavy Metals in Dairy Products. D. J. WAGSTAFF, K. MAHAFFYE, and J. C. STREET,
Food and Drug Administration, Washington, DC, and Cincinnati, Ohio and Utah State
University, Logan, Utah.

This is a review of heavy metal (Pb, Cd, Hg) concentrations in American dairy food products,
factors controlling these concentrations, potential human toxicity, and options for minimizing
risks to the consumer. Young children are at higher risk than adults due to their higher intake of
milk products (89 g/kg body wt/day in children under 1 year of age compared to 3 to 4 g/kg
body wt/day in adults of working age) and the greater susceptibility of growing children to some
of the adverse effects of heavy metals. Milk at the time of secretion by the cow is generally lower
in heavy metal content than other types of food but some handling and processing steps can
increase residue levels in dairy products. Therefore, the major steps necessary to control heavy
metals in dairy products should be directed at milk handling and processing. This differs from
some other contaminants such as fat-soluble pesticides whose residues in milk are best
minimized by controlling the ration and environment of the cow. Also the steps needed for
control of heavy metals in dairy products are different from those for other types of animal
products and other types of foods. Dairy food safety decisions should be based on human
consumption patterns for dairy products, residue levels in those products, and the susceptibility
of each class of consumer.
ABSTRACTS: SEVENTEENTH ANNUAL MEETING

209. Effects of Zinc and Other Metal Ions on the in vitro Binding of Androgens to Cytoplasmic Proteins of Mouse Prostate Gland. M. P. Donovan, L. G. Schein, and J. A. Thomas, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

A possible mechanism of metal ion toxicity in hormone-dependent tissues is inhibition of binding of hormones to cytoplasmic receptors. In this study a series of divalent and trivalent ions were studied as inhibitors of in vitro binding of ([3H]dihydrotestosterone ([3H]DHT) to cytoplasmic receptors in mouse anterior prostate glands. Endogenous metals were removed from cytosol fractions by treatment with Dowex chelating resin, and then the cytosols were incubated with [3H]DHT and exogenous metal ions. Zinc (10^{-5} M) was a very effective inhibitor of specific hormone binding, but exerted little influence on non-specific binding. Toxic metals such as cadmium, mercury, and copper were also inhibitory. Calcium and magnesium did not inhibit binding even at concentrations up to 1 mM. Mercaptoethanol (1 mM) tended to reverse the inhibition of binding by metal ions, suggesting that sulphydryl groups are important in the binding reaction. These data indicate that inhibition of hormonereceptor interactions can be an important aspect of metal ion toxicity. (Supported by EPA Grant R803-578-030.)


Isopropylphenyl phosphate esters are used as flame retardants in plastics and fire-resistant hydraulic fluids. Isopropylphenyl phosphate esters have previously shown low toxicity in a battery of standard acute toxicity tests. (oral LD50 > 20,000 mg/kg (rat); dermal LD50 > 10,000 mg/kg (rabbit); inhalation LC50 > 200 mg/liter (rat)). Subacute dermal toxicity was assessed using the hen since this species is more sensitive to delayed neurotoxic effects than rodents. The purpose of this study was to determine whether subacute dermal exposure to an isopropylphenyl phosphate mixture causes toxic effects in hens. The sample was applied to the combs of hens at a dose level of 50 mg/kg/day, 5 days/week for 4 months. A positive control group received pure tri-o-cresyl phosphate at the same dose level. Treatment with isopropylphenyl phosphate caused no neurotoxic effects compared to untreated hens as determined by histopathological, visual, and biochemical examination. This treatment also did not show any other toxic effects compared to untreated hens as shown by hematology, clinical chemistry, and gross and histopathological examination of tissues and organs. The positive control using tri-o-cresyl phosphate at the same level showed visual, clinical neurotoxic response in 15 to 19 days. This approach might be useful for evaluating subacute dermal toxicity of other similar materials in the hen.

211. Results of a Two-Year Inhalation Toxicity Study of Hydroxyethyl Acrylate in Rats. L. W. Rampy, R. J. Kociba, M. F. Balmer, D. G. Keyes, J. D. Schuetz, and H. O. Yakei, Toxicology Research Laboratory, Health and Environmental Research, 1803 Building, Dow Chemical Company, Midland, Michigan.

Groups of 100 male and 100 female rats were exposed to atmospheres containing 0, 0.5, or 5.0 ppm of hydroxyethyl acrylate (HEA) vapor. Exposures occurred on weekdays for 18 months followed by a 5-month (males) or 6-month (females) observation period. Evaluation of urinalysis, clinical chemistry tests, body weight gain, terminal organ weights, and cumulative mortality revealed no alterations related to exposure to HEA vapor. Examination of chromosomes from bone marrow cells revealed no cytogenetic alterations. Minor hematological changes noted after 12 months of exposure to 5.0 but not 0.5 ppm of HEA were not observed in the latter phases of the study. Gross and histopathologic examination revealed a yellow staining of the haircoat and an increased incidence, increased severity, and earlier onset of lesions associated with chronic murine pneumonia in rats exposed to 5.0 but not 0.5 ppm of HEA. Rats exposed to HEA did not have increases in the parameters used to assess carcinogenic potential. The results of this toxicity study indicate that chronic inhalation by rats of atmospheres containing 5.0 ppm of HEA caused a minimal degree of toxicity; no toxicity was observed in rats exposed to 0.5 ppm of HEA. There was no indication of a carcinogenic effect in the groups exposed to either 5.0 or 0.5 ppm of HEA.

Carnauba wax, an exudate from the pores of the leaves of the Brazilian wax palm, Copernicia prunifera, is used in cosmetic materials, in foods, in pharmacy as a tablet coating, and to raise the melting point of other waxes when a hard high-polish wax is desired. However, despite its widespread use, subchronic toxicity data on the effects of carnauba wax consumption are not available. In these experiments, FDRL Wistar rats and purebred beagle dogs consumed carnauba wax as 0, 0.1, 0.3, and 1.0% (w/w) of their diets. Rats were exposed to the test material through one full generation (F₁A) starting with its ingestion by pregnant dams and continuing through maturation. F₂B litters were used to examine the teratogenic potential of carnauba wax. The dogs consumed the test diets for 6 months. Hematological and biochemical tests, body weights, food consumption, reproductive indexes (rat), and gross and microscopic examination of tissues were used to evaluate the toxicity of carnauba wax. Results of hematological and biochemical testing of the F₁A rats and young Beagle dogs revealed no toxicologically significant dose effects. Carnauba wax did not affect body weights or food consumption in rats or dogs. Reproductive performance in rats was not affected during either the first or second generation breedings. Organ weight data indicated no treatment effects, and histopathological examinations revealed no difference between any test level and the control. Moreover, dietary exposure to carnauba wax produced no evidence of any treatment-related effects in rats or dogs at a level of 1% of the diet.


The purpose of the bioassay was to evaluate the effects of chronic exposure to an organosilicon chemical, TX-1319 (30.4% active), on Daphnia magna. Daphnia are freshwater crustaceans that are recognized as food for fish and a sensitive laboratory test species. Following preliminary screening, test concentrations of 10, 18, 32, 56, and 100 ppm of TX-1319 were chosen for the bioassay. Daphnia in the first instar stage were introduced into each of four vessels of each test concentration and the untreated control. The animals were observed for 21 days and survival and fecundity of the animals were recorded as well as the number of offspring produced through asexual reproduction. Each week, the adult animals were transferred into fresh test water. Water samples were collected and analyzed by atomic absorption spectrophotometry to determine the concentrations of organosilicon in the test water. Three tests were conducted and the data from each test and the composite data of the three tests were statistically analyzed. Significant differences were noted in tests for analysis of variance in survival data of adult animals, total number of offspring produced, and number of surviving offspring produced. Tests for multiple comparison revealed the survival of adult Daphnia in 100 ppm of TX-1319 was significantly lower than that of the lower test concentrations and untreated control and the total number of offspring produced in 32, 56, and 100 ppm of TX-1319 were also significantly lower. A 17 and 25% reduction in total offspring production was also noted at 10 and 18 ppm of TX-1319, respectively. Tests for multiple comparison revealed the number of surviving offspring produced at 32, 56, and 100 ppm of TX-1319 were significantly lower; however, when the percentage survival of offspring in each test concentration was examined, no appreciable difference was noted.


A flowthrough bioassay was conducted to determine the chronic effects of an organosilicon chemical, TX-1319 (30.4% active) on fathead minnows (Pimephales promelas). Following pre-
liminary screening, test concentrations of 0.63, 1.25, 2.50, 5.00, and 10.00 ppm of TX-1319 were chosen for the bioassay. Water samples were collected each week and analyzed by atomic absorption spectrophotometry to determine the concentration of organosilicon in the test water. Fathead minnow eggs were obtained from the Newtown Fish Toxicology Station of the U.S. Environmental Protection Agency and these eggs were hatched in the vessels of each test concentration and the untreated control. These fish became the parent generation (F₀). Fish production based on survival, growth, and reproduction of the F₀ generation and survival, growth, and maturation of the eggs and fry of the F₁ generation were investigated. No adverse effects were noted in the parameters investigated in both the F₀ and F₁ generations of fathead minnows continuously exposed to the chosen concentrations of TX-1319.


The toxicity of methoxyacetone administered in drinking water to female Wistar rats was compared to that of methyl n-butyl ketone and a series of four other aliphatic ketones (diethyl ketone, ethyl n-butyl ketone, methyl isobutyl ketone, methyl tert-butyl ketone) with particular reference to neurotoxicity. The administration of approximately 1 g/kg/day of methyl n-butyl ketone produced the most severe toxicity, reflected in reduced food and water consumption, reduced body weight gain, increased kidney weight/body weight ratio, peripheral neuropathy, and neurologic impairment reflected in hindlimb weakness and muscle atrophy. Methoxyacetone, at final dosage levels of 2 and 4 g/kg/day, decreased food and water consumption and increased liver weight. Minimal muscle atrophy of the hindlimbs without associated peripheral neuropathy was present in one of five rats given the lower dosage of methoxyacetone. Effects produced by the other ketones included reduced body weight gain decreased relative kidney weight. None of these other ketones produced significant neurologic alterations. It is concluded that 1 g/kg/day of methyl n-butyl ketone administered to rats in their drinking water for 120 days produced muscle weakness and atrophy and peripheral neuropathy. Methoxyacetone administration at a dosage approximately four times that of methyl n-butyl ketone produced slight muscle atrophy without attendant neuropathology. None of the ketones studied produced discernible neurotoxic effects.

216. The Effect of Carboxylesterase Inhibition on the Toxicity of Methyl Acrylate, Ethyl Acrylate, and Acrylic Acid. E. H. Silver and S. D. Murphy, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts, and Division of Toxicology, University of Texas Medical School, Houston, Texas.

The effect of carboxylesterase inhibition on lethality and on tissue sulfhydryl depletion resulting from inhalation of acrylate esters and acrylic acid was investigated in male rats. Hydrolysis of methyl acrylate and ethyl acrylate was demonstrated in plasma and in homogenates of rat liver, lung, and kidney. Treatment of rats with 5 mg/kg of trioctylphosphoramide (TOPO) significantly inhibited this hydrolysis. TOTP, 125 mg/kg, maximally inhibited the hydrolysis of methyl acrylate and ethyl acrylate in vitro and potentiated the acute toxicity of both esters. During a 4-hr exposure to 750 or 1000 ppm of methyl acrylate or 1000 ppm of ethyl acrylate mortality was 20, 80, and 80%, respectively, in TOTP-pretreated rats, whereas no deaths occurred in corn oil-pretreated rats exposed to these concentrations of esters. Mortality in rats exposed to acrylic acid was not altered by pretreatment with TOTP. Dose-response studies with methyl acrylate and ethyl acrylate indicated that lung nonprotein sulfhydryl (NPSH) was decreased to a greater extent than that of blood or liver. Acrylate ester-induced depletion of tissue NPSH was more pronounced following TOTP pretreatment. A 4-hr exposure to 135, 370, 490, or 720 ppm of methyl acrylate decreased lung NPSH 0, 26, 27, and 52%, respectively, in corn oil-pretreated rats, versus 34, 55, 69, and 83%, respectively in TOTP-pretreated rats. Both methyl acrylate and ethyl acrylate significantly decreased kidney NPSH only in rats pretreated
with TOTP. Exposure to 1000 ppm of acrylic acid was necessary in order to significantly decrease tissue NPSH. The results of these studies indicate that tissue carboxylesterases are involved in the detoxification of methyl acrylate and ethyl acrylate and that potentiation of acrylate ester toxicity may occur when carboxylesterase activity is inhibited. (Supported by NIEHS Grants ES-00002 and ES-00084 and a grant from Procter & Gamble Company.)

217. Species-Dependent Toxicity of a Thiourea Derivative in Rats and Monkeys. S. D. WARNER and J. E. LEBEAU, Toxicology Laboratory, Health and Consumer Products Department, Dow Chemical U.S.A., Indianapolis, Indiana. (D. J. Thompson)

In 90-day preclinical toxicity studies of a thiourea derivative, 1-(2-hydroxyethyl)-2-thio-3-(3,4-xylyl) urea (HXTU), the compound was administered in the diet of Sprague-Dawley rats (15/sex/group) at levels sufficient to provide 0, 5, 15, 45, 90, 180, and 360 mg/kg/day. In rhesus monkeys (3/sex/group), the compound was suspended in 0.5% METHOCEL and administered po at dose levels of 0, 25, 50, 100, and 400 mg/kg/day. Doses of 15 mg/kg or greater in male and female rats produced significant decreases in body weight and food consumption, changes in blood chemistry (increased BUN and cholesterol levels), involution of the thymus, and atrophy of the male accessory sex glands. Doses of 45 mg/kg and greater resulted in 40 mortalities; 33 occurred during the first 2 weeks. Deaths were frequently preceded by dyspnea and associated major pathology including pulmonary edema and congestion, pleural effusions and congestion of the liver and kidneys. Major histopathologic observations included pyknosis and loss of cortical thymocytes with associated increase of reticuloepithelial and stromal components in the thymus, decreased secretory content and increased height of secretory epithelium of male accessory sex glands, and diminished colloid and increased height of thyroid follicular cells. Doses of 5 mg/kg/day of HXTU were without effect in the rat. Toxic effects were not observed in monkeys at any dose level of HXTU. Pronounced differences in metabolism of HXTU have been observed between rats and monkeys. Toxic effects in the rat are attributed to species-dependent biotransformation of the compound to a toxic metabolite associated with the hydroxyethyl portion of HXTU.


The rodenticide RH-787 (N-3-pyridylmethyl-N'-p-nitrophenyl urea) appears to be a nicotinamide/NAD antagonist (Decker et al., Fed. Proc. 36, 990, 1977), whose species selectivity could be due to differences in its ADME. These studies compared the disposition of pyridyl- and nitrophenyl[14C]RH-787 in tolerant and susceptible species. The compound was rapidly absorbed by rats, mice, and dogs after oral administration. Blood levels peaked in 1 to 6 hr, depending on species and label, and were higher after NP- than Py(14C) administration in each species. Doses of 30 mg/kg produced higher 14C blood levels in dogs than in rats—yet such dose levels are lethal to rats, but not dogs. G.I. transit of 14C was more rapid in dogs than in rats. Urinary and fecal excretion were of similar importance in all three species; NP label was more rapidly eliminated. Tissue distribution of the two 14C labels varied, especially in dogs—suggesting more extensive metabolism. Liver contained more of the dose than any other single organ. Most hepatic 14C was located in the cytosol of each species, but rats and dogs differed in nuclear and microsomal fractions. Label differences in subcellular distribution were small when expressed as percentages of total liver 14C. Most 14C was reversibly bound to various organelle pellets. Rats tolerated, metabolized, and eliminated single or multiple sublethal RH-787 doses (5 mg/kg), but their metabolic systems were less efficient than those of dogs for detoxifying larger doses (>20 mg/kg). Pretreatment of rats with 3-MC enhanced their hepatic extraction of RH-787, increased biliary secretion of its metabolites, and protected rats against RH-787 toxicity. Thus, RH-787 tolerance in dogs seems to be related to efficient hepatic extraction, followed by rapid metabolic degradation and excretion.

Based on the known metabolic fate of vinyl chloride, wherein carcinogenic/mutagenic activity has been associated with intermediate epoxide formation, a model has been developed which assumes that a series of related substituted alkenes will be capable of forming epoxides in vivo and therefore have the potential to elicit similar biological effects. By using the reference compounds, vinylidene fluoride and tetrachloroethylene, as probable generators of biologically inactive epoxides and vinylidene chloride (VDC) as producing a biologically active epoxide, the calculated molecular strain energy of each epoxide was plotted against an arbitrary scale of biological activity, with VDC placed at the maximum of a smooth curve. The theoretical ring strain energy in epoxides derived from 16 other substituted alkenes was then used to predict their relative biological activity. The predicted activity pattern was then compared with the reported genotoxic properties of the parent alkene, and a good correlation was observed. Activities were predicted for a further 14 substituted alkenes for which no genotoxicity data have been reported.

220. Response of Rats to Repeated Administration of Δ⁹-Tetrahydrocannabinol: Serum Protein, Lipoprotein, and LDH-Iszyme Electrophoretic Patterns. B. R. Man no, J. E. Man no, C. S. Kaga, and J. L. Schaller, Department of Pharmacology and Therapeutics, Louisiana State University Medical School, Shreveport, Louisiana.

Subtle changes in serum protein constituents, lipoproteins, and LDH-isozymes were evaluated after repeated low, oral doses of Δ⁹-tetrahydrocannabinol (THC) to male Sprague-Dawley rats. Treatment conditions included: sodium glycocholate (vehicle) controls, and 0.05, 0.25, and 1.25 mg/kg/day of Δ⁹-THC. The rats for each treatment condition were administered vehicle or Δ⁹-THC by daily gastric intubation. Blood and tissue specimens were collected from ether anesthetized animals from each treatment group 24 hr after the last delivery of vehicle or Δ⁹-THC. These collections were made at 7-, 14-, 21-, and 28-day intervals. Serum protein, lipoprotein, and LDH-isozyme thin-gel agarose electrophoresis was performed using the Pol-E-Film System (Pfizer Diagnostics Division, Pfizer, Inc.). One-way analysis of variance with Tukey's A test was performed on data for significance in relation to duration of treatment and treatment conditions. Significant differences within the individual treatment conditions were observed with lipoprotein A, lipoprotein pre-β, α₁ protein, α₂ protein, β protein, LDH-1 (heart), LDH-3, LDH-4, and LDH-5 (liver) (p < 0.05). Other lipoprotein, protein, and LDH-isozyme fractions were not significantly altered. Fewer changes occurred between Δ⁹-THC groups at 7-, 14-, 21-, and 28-days of treatment. Serum protein, lipoprotein, and LDH-isozymes fractions were not significantly changed between treatment conditions after given dosing periods except for an α₂ protein, LDH-1 (heart), and LDH-2 (heart) (p < 0.05).

221. Interaction of Diethanolamine with Phospholipid Metabolism in Vitro and in Vivo. S. J. Barbee and R. Hartung, Department of Environmental and Industrial Health, School of Public Health, University of Michigan, Ann Arbor, Michigan.

Previous studies have shown that diethanolamine (DEA) is incorporated into hepatic phospholipids in vivo. However, little information is available on the effect of DEA on phospholipid metabolism in vitro, and no information exists evaluating its effect on phospholipid metabolism in vitro. We investigated the effect of DEA on in vitro and in vivo phospholipid metabolism in hepatic tissue from the male Sprague-Dawley rat. DEA inhibited the in vitro synthesis of phosphatidyl choline and phosphatidyl ethanolamine. In each case, the $K_m$ was approximately 3 mm. The apparent $K_m$ and $V_{max}$ for the incorporation of DEA into a phospholipid derivative were 11.6 mm and 21.0 nmol/mg of protein/60 min, respectively. Administration of a single oral dose of 250 mg of DEA/kg failed to produce inhibition of in vivo synthesis of phosphoglycerides of ethanolamine and choline, but 330 mg of DEA/kg did cause inhibition of the synthesis of these phosphoglycerides upon repeated oral administration. The synthesis of ethanolamine phosphoglycerides declined to 27% of the control value after 1 week of exposure.
of animals to DEA; no further reduction was noted for the remainder of the 3-week dosing regimen. The synthesis of phospholipid derivatives of choline fell to 82, 47, and 41% of the control value following 1, 2, and 3 weeks of DEA administration, respectively. A study of the in vivo kinetics showed that the phosphoglycerides of choline and ethanolamine were catabolized at a rate faster than those of DEA. This investigation indicates that DEA inhibits phospholipid synthesis in vitro and in vivo. These data suggest that two mechanisms are involved in this inhibitory response.


Carbon tetrachloride (CCl₄) administration to rats leads to an early dilation, vesiculation, and disorganization of the liver endoplasmic reticulum (ER). This hepatotoxin also causes detachment of ribosomes from rough ER membranes, dilation of the Golge cisternae and occasionally dilation of the perinuclear membrane. Prior treatment of the rats with pyrazole, which decreases the intensity of the irreversible binding of CCl₄-reactive metabolites to cellular constituents without modifying the intensity of the CCl₄-induced lipid peroxidation process, is able to completely prevent CCl₄-induced ultrastructural observable alterations at 3 hr and to slightly ameliorate those effects at 6 hr. Results suggest that interaction of reactive metabolites rather than lipid peroxidation mediates deleterious effects of CCl₄ on the liver ER.


¹⁴CCl₄ irreversibly binds in vivo to liver DNA from strain A/J mice and Sprague-Dawley rats. Binding of ¹⁴CCl₄ to DNA was also observed in vitro in incubation mixtures containing microsomes and an NADPH-generating system as well as in tissues slice experiments. Chemically induced ·CCl₃(CCl₄ + benzoyl peroxide) intensively bind to DNA. Liver nuclear proteins also irreversibly bind CCl₄ metabolites. Nuclear protein fractionation studies revealed that deoxyribonucleoproteins, acidic proteins, histones, and residual proteins are the favorite targets of metabolic interaction. Nuclear sap proteins are less intensively labeled by CCl₄-reactive metabolites. Most of the label is in the phospholipid fraction and diphosphatidylglycerol is the phospholipid more intensively labeled. Phosphatidylethanolamine, phosphatidylinoline, lysophosphatidylcholine, and sphingomyelin are also labeled by metabolites. Results suggest that CCl₄ is activated to ·CCl₃ at the nuclear envelope and that interaction of the ·CCl₃ with DNA and nuclear proteins could be relevant to CCl₄ liver tumor induction and hepatotoxicity.


SQ 14,225 is L-(3-mercapto-2-methyl-1-oxopropyl)-L-proline (SS). The intravenous LD₅₀ in mice was about 1000 mg/kg, and the oral LD₅₀ values in mice and rats were approximately 6000 mg/kg. The maximal single doses (in mg/kg) that produced no overt effects were: mice, 700 (iv) and 2800 (po); rats, 4000 (po); dogs, 200 (po); and monkeys, 375 (po). SQ 14,225 was administered orally for 1 month to rats, dogs, and monkeys. Groups of rats were given total daily doses of 0, 50, 150, and 450 mg/kg; one additional group received daily doses that were gradually increased from 600 to 3000 mg/kg. Changes in the highest-dose group included growth retardation, slight decreases in erythrocytic parameters, slight leukocytosis, and a moderate increase in serum urea nitrogen. Rats given 150 or 450 mg/kg daily showed only slight retardation of growth and a slight increase in serum urea nitrogen. There were no changes in rats given 50 mg/kg daily. Groups of dogs and monkeys were given oral doses of 1, 25, 75, and 225
mg/kg daily for 1 month. Slight decreases in erythrocytic parameters were observed in all high-dose and some intermediate-dose dogs. There were no adverse effects in dogs at 50 mg/kg daily or in monkeys at any dose. In both species, plasma renin activity was elevated by the third day of dosing, but was returning toward normal by the fourth week. SQ 14,225 was also administered orally to monkeys at total daily doses of 0, 50, 150, and 450 mg/kg for 3 months. Monkeys given the highest dose had decreases in erythrocytic parameters and increases in serum urea nitrogen. Histopathological examination of the kidneys revealed hyperplasia of juxtaglomerular cells in all monkeys given 450 mg/kg daily and half of those given 150 mg/kg. Monkeys that received SQ 14,225 at daily doses of 50 mg/kg for 3 months showed no changes.

225. Modulation of Pulmonary Drug Uptake by Preexposure to Xenobiotics. H. M. Mehendale, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi.

A number of drugs and other xenobiotics have been reported to be accumulated in lung tissue. Studies directed at understanding the mechanism(s) by which these chemicals are sequestered in the lung would be aided by the development of suitable models for modulation of pulmonary drug uptake and sequestration. Male New Zealand white rabbits (2.5 to 3 kg) were exposed to aerosolized piperonyl butoxide (8 ppm for 30 min) using an inhalation system which allowed exposure only through the nose. Twenty-four hours after exposure, steady-state uptake of [14C]imipramine (IMP) was measured using artificially ventilated isolated perfused lung preparations. In another series of experiments, a group of animals were treated with bisolvon (bromhexine; 200 mg/kg/day) orally in gelatin capsules for 5 days. Isolated perfused lung preparations obtained from these and control animals receiving gelatin capsules were used for steady-state drug uptake determinations. Steady-state IMP uptake was doubled in lung preparations obtained from animals exposed to piperonyl butoxide. Preexposure to bisolvon also enhanced the steady-state uptake of IMP by the lung preparations. The increase in pulmonary drug uptake induced by bisolvon was also accompanied by enlarged and more numerous concentric lamellar bodies in the type II pneumocytes. While other investigators have noted increases in the size and number of lamellar bodies in the type II pneumocytes, it is not clear whether this bears a cause-effect relationship to enhanced drug uptake. Such pretreatment models might be useful in studies directed at the mechanism(s) pulmonary drug sequestration. (Supported by PHS Grant HL-20622.)


Adriamycin (ADR), an anthracycline antibiotic, is a potent anticancer drug which is used clinically against leukemias and solid tumors. At total doses exceeding 600 mg/m², ADR produces cardiomyopathies that frequently result in congestive heart failure. ADR has been shown to produce free radicals in an NADPH-microsomal system. These free radicals might produce the cardiotoxicity of ADR, if sufficient protective reducing substances are not present in the heart. ADR, 15 mg/kg, ip, reduced myocardial, red blood cell, and liver glutathione levels. Recently, we have shown that the acute toxicity of ADR in mice can be decreased by concurrent administration of two sulphydryl reagents, cysteamine (CYS) or N-acetylcysteine (NAC). The purpose of this study was to assess the ability of CYS and NAC to protect against ADR-induced cardiomyopathy and lethality without altering its antitumor effect in Swiss-origin mice bearing a nonresistant transplantable Ehrlich ascites tumor. Four daily intravenous doses of ADR up to a total dose of 14 mg/kg did not significantly alter the life span of the mice when compared to untreated tumor bearing mice. However, when ADR 3.0 mg/kg daily for 4 days was administered intraperitoneally (ip) to tumor bearing mice, a significant increase in survival time was noted. This increased survival was not affected by concurrent ip administration of CYS (50 mg/kg twice daily). In vitro studies on the uptake and DNA binding of ADR by Ehrlich ascites cells indicate that neither CYS nor NAC affect either parameter. These data demonstrate that sulphydryl reagents can significantly alter the toxicity of ADR and not affect the antitumor
properties of the drug. (Supported in part by USPHS Grants GM00058, GM15431, and ES00782.)

227. Effect of Nonsteroidal Anti-inflammatory Agents on Toxicity of Retinoic Acid in Mice. E. Jane Hixson and E. Paul Denine, Southern Research Institute, Birmingham, Alabama.

The toxicity of vitamin A (retinol) and its structural analogs (retinoids) seems to be mediated by prostaglandins (Harrison et al., Nature (London) in press), since the incidence of mortality in mice treated with the subacute LD50 (qd x 21) of all-trans-retinoic acid (RA), 30 mg/kg, ip, was significantly lowered by concurrent administration of aspirin. In the present study, a sublethal dose of RA was used to determine whether this model, using a clinically relevant dose of RA, is sufficiently sensitive to compare the protective effects of other inhibitors of prostaglandin synthesis. In each of two experiments, 40 adult male and female Swiss mice were distributed into two groups of 20 each and were treated daily for 21 days. Mice were treated with the subacute LD10 (qd x 21) of RA, 14 mg/kg, ip. One group received RA alone; the other group received RA plus a daily dose of aspirin, 150 mg/kg, po. Bone fractures, a consistent index of sublethal retinoid intoxication, were counted on X-ray films obtained weekly. Because χ² analysis indicated that the incidences of fractures in the two experiments were comparable, the two sets of data were pooled. On treatment Day 15 and the day after last treatment, the number of mice with fractures was significantly lower in the group treated with RA plus aspirin (p < 0.01); the number of mice having two or more fractures on the day after last treatment was significantly lower (p < 0.05) in recipients of the combination. Comparison of these results with those of previous studies using the LD50 of RA suggests that protection from retinoid toxicity can be studied effectively in mice delivered clinically relevant doses of this drug. Similar experiments suggest that ibuprofen is about twice as potent as aspirin in protecting against retinoid toxicity. Ibuprofen is also a more potent inhibitor of prostaglandin synthesis, and this observation strengthens the suggestion that prostaglandins mediate retinoid toxicity. (Supported by Contract No. N01-CP-22064, DCCP, NCI, NIH, DHEW.)

228. The Effect of Organophosphate Insecticides on the Action and Metabolism of Local Anesthetic Esters. Richard E. Ouellette, Brian T. Laplanche, and Steven D. Cohen, Section of Pharmacology and Toxicology, School of Pharmacy, University of Connecticut, Storrs, Connecticut.

Previously we demonstrated that in mice, inhibition of liver esterases by noncholinesterase-inhibiting doses of triorthotolyl phosphate (TOTP) and Dasanit was closely correlated with potentiation of procaine-induced loss of righting ability (LRA) and lethality. The present studies provide additional evidence for carboxylesterase inhibition-dependent interactions between organophosphate insecticides and local anesthetics. Eighteen hours after 0.5 to 25 mg of EPN/kg (ip) brain cholinesterase (CHE) was not inhibited, yet 50 mg/kg killed four of six male CD-1 mice. Liver CHE was not inhibited by less than 5 mg of EPN/kg. Liver hydrolysis of the carboxylesterase substrates, diethylsuccinate, o-napthyl acetate, and procaine was inhibited in a dose-dependent manner by 0.5 to 25 mg of EPN/kg. Mean durations of LRA after procaine (175 mg/kg, ip) were 8.1, 14.6, 9.9, 15.6, 32.6, and 31.9 min in mice pretreated with 0.5, 1, 2.5, 5, 10, and 25 mg of EPN/kg, respectively. Mean (±SE) LRA in control (corn oil-pretreated) mice was 3.8 ± 0.3 min. Other groups of mice were pretreated with 7.5 mg of Dasanit/kg and 1 or 24 hr later they were either sacrificed without challenge or challenged with procaine (150 mg/kg, ip) and sacrificed 3 min later. Mouse liver hydrolysis of procaine was 83 and 39% inhibited 1 and 24 hr after Dasanit, respectively. Plasma procaine concentrations (μg/ml) were 24.2 in controls and 40.6 and 28.0 in mice challenged 1 or 24 hr after Dasanit, respectively. In male Sprague–Dawley rats 2.5 mg of Dasanit/kg, ip, produced only 10% inhibition of brain CHE within 1 hr; yet it produced 50 to 75% inhibition of liver carboxylesterases. Tricaine methanesulphonate (500 mg/kg, ip) had no effect in control rats yet it induced LRA and cyanosis in Dasanit-pretreated rats. These findings with insecticidal organophosphates strongly suggest the potential for dangerous interactions with local anesthetic esters. (Supported by grants from the University of Connecticut Research Foundation and NIEHS Grant No. ES01093-01A.)

Four structural analogs of spiro (isobenzofuran-1(3H), 4'-piperidine) were given to groups of 10 male Sprague-Dawley (CD) rats for 12 weeks (0.075% of diet). An identical control group had cellulose only mixed in the diet. Measurements of body weight gain, food consumption, and clinical condition were made weekly. Selected serum chemistry and hematological tests were done at 0, 1, 4, and 12 weeks. Postmortem analyses included gross necropsy, liver weights, selected enzyme histochemistry, and light microscopy. Varying degrees of hepatotoxicity were seen in all four treated groups. Significant elevations in GOT, GPT, and alkaline phosphatase were only seen in the most severely affected group. Findings characteristic of this group were increased liver weights, moderate hypertrophy of midzonal/cenrilobular hepatocytes, and moderate midzonal vacuolization and lipid accumulation. Decreased levels of triglycerides were present in all four groups and in general, paralleled the degree of hepatotoxicity. Two of the groups (but not the most severely affected group) showed increased levels of glucose 6-phosphatase activity in midzonal and centrilobular hepatocytes. No significant increases in cytochrome oxidase or acid phosphatase activity occurred in any group.


The objective of this investigation was to determine in the normoglycemic dog if sodium dichloroacetate (DCA) can prevent or correct the hyperlactatemia and subsequent acidosis induced by high intraduodenal doses of phenformin. Fasted purebred beagles were surgically prepared for dosing and administered phenformin or DCA alone or in combinations as follows: (1) phenformin intravenously at 10 mg/kg; (2) phenformin intraduodenally (id) at doses of 30, 45, or 60 mg/kg; (3) phenformin intraduodenally at 45 mg/kg followed immediately by an intravenous injection of 100 mg of DCA/kg; (4) phenformin intraduodenally at a dose of 45 mg/kg followed in 1 hr by infusions of 25 or 50 mg of DCA/kg/hr for 4 hr; (5) DCA as an infusion of 50 mg/kg/hr followed in 0.5 hr by 45 mg of phenformin/kg intraduodenally with the infusion lasting for 5 hr; and (6) DCA alone as an infusion of 50 mg/kg/hr for 4 hr. Doses of phenformin at 10 mg/kg, iv or 30 mg/kg, id caused no changes, while 45 and 60 mg of phenformin/kg, id produced hyperlactatemia, hyperglycemia, and decreased arterial pH and bicarbonate. Concomitant administration of 45 mg of phenformin/kg, id and 100 mg of DCA/kg, iv or 25 mg of DCA/kg/hr infusion caused only a transient reversal of these effects. Infusion of 50 mg of DCA/kg/hr administered 1 hr after the phenformin bolus maintained these parameters within predose levels in most dogs. Infusion of 50 mg of DCA/kg/hr that was given 0.5 hr prior to the 45 mg of phenformin/kg bolus maintained parameters within predose levels in all dogs. Infusion of 50 mg of DCA/kg/hr alone caused only progressively low lactic acid levels. In conclusion, the results indicate that high intraduodenal doses of phenformin cause hyperlactatemia, hyperglycemia, and acidosis in the normoglycemic dog and these changes can be reversed with selected doses and carefully timed infusions of DCA.

231. Studies on Cefazolin-Induced Depression of Aminotransferase Activity in Laboratory Animals. S. A. Turnipseed, J. S. Wold, and J. S. Wells, Toxicology Division, Lilly Research Laboratories, Greenfield, Indiana.

Subacute toxicity studies have been conducted with the parenteral cephalosporin antibiotic cefazolin. The intravenous administration of cefazolin to dogs at 500 mg/kg/day for 14 days resulted in a significant decrease in serum alanine aminotransferase (SGPT) activity compared to saline controls (1.5 ± 1.5 vs 12.8 ± 1.5). Serum aspartate aminotransferase (SGOT) activity in dogs, however, was unaffected (40.8 ± 3.0 vs 40.5 ± 3.7). The administration of a single intravenous dose of cefazolin, 500 mg/kg, to dogs resulted in a significantly lower SGPT value at 3 hr
and the effect was maximal at 24 hr. Single subcutaneous doses of cefazolin, 800 mg/kg, to Dutch-belted rabbits also significantly decreased SGPT values measured at 48 hr after administration. The direct addition of cefazolin to dog serum did not affect SGPT values. The addition of pyridoxal phosphate, a cofactor for SGPT, to serum from cefazolin-treated dogs slightly increased the lowered SGPT activity. Exhaustive dialysis of serum from cefazolin-treated dogs, followed by replenishment of pyridoxal phosphate, resulted in a marked increase in SGPT activity. Examination of the effects of a series of experimental cephalosporins on SGPT in rabbits indicated that only cephalosporins with the thiazolothiazide substituent at the 3-methyl position produced lowered SGPT values.

232. The Mechanism of Enhanced Cephaloridine Nephrotoxicity after Inhibition of Cation Transport. J. S. Wold, S. A. Turnipseed, and B. L. Miller, Toxicology Division, Lilly Research Laboratories, Greenfield, Indiana. (J. L. Emmerson)

Pretreatment with the cyanine dye, 1-ethyl-2(1,4-dimethyl-2-phenyl-6-pyrimidinylidine)methylquinolinium chloride (cyanine), enhances the nephrotoxicity of the zwitterionic cephalosporin antibiotic cephaloridine in rabbits (Fed. Proc. 36, 953, 1977). Cyanine pretreatment did not significantly increase peak cephaloridine concentrations in the rabbit renal cortex in vivo. Cyanine pretreatment did, however, result in increased cortical cephaloridine concentrations at time points after the 3-hr peak, suggesting that the efflux of cephaloridine from renal cortical tissue was inhibited. The effect of cyanine (1.28 x 10^-8 M) on the efflux of [14C]p-aminobenzophenone tetraethylammonium (TEA), and [14C]cephaloridine was studied by determining the rate of disappearance of the substrate from preloaded rabbit renal cortical slices transferred through beakers of substrate-free buffer. The rate constant (min^-1) for TEA efflux decreased from 0.021 ± 0.003 to 0.016 ± 0.001 in the presence of cyanine. PAH efflux was unchanged (0.030 ± 0.001 vs 0.031 ± 0.004). The efflux of cephaloridine was considerably slower than that of either PAH or TEA and was further decreased by cyanine (0.012 ± 0.0005 vs 0.010 ± 0.0005). Thus, although the antibiotic enters the cells of the renal cortex via the anionic transport system, cephaloridine relies, at least in part, upon cation transport for exit from the cells. This transport step, which can be inhibited by cyanine, leads to prolonged cortical concentrations, resulting in enhanced nephrotoxicity.

233. Toxicology of Glyphosate in Rats and Mice. E. A. Babu, O. O. Olorunsogo, and O. Bassir, University of Ibadan, Department of Biochemistry, Ibadan, Nigeria.

Toxicological studies on glyphosate, a broad spectrum and nonselective herbicide, have been carried out using the rat and the mouse. In both species the acute oral LD50 values (4873 mg/kg, rat; 1568 mg/kg, mouse) were significantly higher than the acute intraperitoneal LD50 values (238 mg/kg, rat; 134 mg/kg, mouse). Severe stress, enhanced breathing, elevated rectal temperature (hyperthermia), occasional asphyxiating convulsive movements, and rigor include the symptoms which preceded the death of the animals which received lethal doses of the herbicide. Hyperemia in the lungs was the major lesion observed in glyphosate poisoning. Although, daily intraperitoneal administration of 15, 30, 45, and 60 mg/kg to rats for 28 days resulted in reductions in daily body weight gain, blood hemoglobin, red blood cell count, and hematocrit values during the experimental period, the levels of serum glutamate pyruvate transaminase (SGPT), and leucine aminopeptidase were elevated. In view of the conditions of elevated rectal temperature and asphyxiating convolution induced by glyphosate, the effect of the herbicide on some mitochondrial functions were examined. Mitochondria isolated from the livers of rats 5 hr after a single intraperitoneal dose of 15, 30, 60, or 120 mg of glyphosate/kg exhibited diminished respiratory control ratios, enhanced adenosine triphosphatase activity and a stimulation of ADP-less respiration. The activities of succinate, NAD-specific isocitrate, glutamate, and β-hydroxybutyrate dehydrogenases were slightly elevated at all the dosage levels. The activities of cytochrome reductase and cytochrome oxidase systems remained, however, unchanged. These findings suggest that although the acute toxicity of glyphosate is significantly dependent on the route of administration of the herbicide and species of the animal, it is not sex dependent. These
findings also suggest that uncoupling of oxidative phosphorylation in mitochondria may be a primary lesion in acute intoxication by glyphosate.


Acute toxicity studies were conducted with technical captan (68%) and folpet (87%) to assess their relative inhalation hazard. The 2-hr inhalation LC50 values (expressed in terms of active ingredient) for Sherman rats and Swiss–Webster mice of both sexes after exposure to dust atmospheres of captan in a continuous flow system were greater than 5.7 mg/liter of air (gravimetric) for rats and 4.5 (3.9–5.2) and 5.0 (4.2–6.0) mg/liter of air for male and female mice, respectively. Folpet dust atmospheres produced LC50 values of greater than 5.0 mg/liter of air for rats and greater than 6.0 mg/liter of air for mice. Mechanical milling produced 2-hr LC50 values for captan of 1.7 (1.3–2.2) mg/liter of air for male mice and 3.7 (2.8–4.8) mg/liter of air for female mice. This sex difference was not seen with unmilled captan, nor was it apparent after intraperitoneal and oral administration of an aqueous suspension of captan. Intraperitoneal dosing resulted in LD50 values of 518 (405–633) and 462 (402–531) mg/kg for male and female mice, respectively; oral LD50 values were 7840 (6482–9485) for males and 7000 (6940–8050) mg/kg for female mice. Estimated inhalation LC50 values (assuming total absorption of inhaled dust atmospheres and resting minute volume of 23 ml/min) would be approximately 142 mg/kg for males exposed to milled captan and 310 mg/kg for female mice. These data indicate that captan is slightly more toxic to the rat and mouse by the inhalation route than folpet. Milling captan markedly increases the toxicity of captan to male mice, but not the female. This sex difference is not seen after oral or intraperitoneal routes. Extrapolation of inhalation data would suggest that the toxicity resulting from inhalation exposure to captan is at least as great as after intraperitoneal dosing.

235. Comparative Penetration (in Vivo) of Insecticides through the Skin and Gastrointestinal Tract of Mice. S. M. Ahmed, P. V. Shah, and F. E. Guthrie, Toxicology Program, North Carolina State University, Raleigh, North Carolina.

The in vivo penetration of 10 insecticides representing five chemical classes has been compared via the dermal and gastrointestinal routes, the first such comprehensive comparison of penetration with insecticides. Female mice were treated dermally (2 cm shaved back) or orally (stomach tube) with single doses (0.1 ml dermally in acetone and 0.1 ml orally in corn oil) at 1 mg/kg to 8-week-old female mice (ICR strain). Mice were sacrificed at 5, 15, 30, and 60 min and 8 and 48 hr and the radioactivity was determined in blood, brain, feces, heart, liver, urine, and at the site of application. Penetration was approximately twice as rapid through the gastrointestinal as through the dermal route for the insecticides tested. There was a general correlation for individual compounds through the two routes. Parathion and carbaryl penetrated most rapidly while diephrin was the slowest among the compounds tested. As measured by appearance of radioactivity in blood, penetration did not appear to follow first-order kinetics. The distribution of these compounds was concentrated in liver, urine, and feces. Following oral application, approximately 50% of absorption occurred in the stomach. Although the intestine is the favored site of penetration, absorption in that area is delayed until the stomach is emptied.


A need exists for a standard procedure for evaluating the inductive effects of pesticides on hepatic xenobiotic metabolizing enzymes. As part of a program to develop such a protocol, 16 pesticides were either injected ip or give po to adult male mice daily for 3 days. In addition, one known carcinogen (3-methylcholanthrene), one cotton defoliant (DEF), two drugs (phenobarbital
and chloral hydrate), and two insecticide synergists (piperonyl butoxide and sesamex) were also tested. On the 4th day, livers from control and treated mice were compared with respect to weight, microsomal N- and O-demethylation activity, and various spectral characteristics of microsomal cytochrome P-450. While several of the more recently developed insecticides tested showed induction, TH-6040 (a substituted pyrazolone) showed the highest level (> threefold) with respect to the parameters tested. These included significant increases in liver weight/body weight ratio, O-demethylation of p-nitroanisole, N-demethylation of aminopyrene, and CO spectrum. This procedure appears to be useful for the measurement of inductive effects of drugs and industrial compounds as well as pesticides.


Increased concern for the deleterious effects of pesticides has, in recent years, led to considerations of their ability to induce xenobiotic metabolizing enzymes. Several studies on rats have shown only modest, if any, induction of GSH S-transferase activity while similar data on other mammals is lacking. We have investigated the effect of pretreatment (>100 mg/kg for 3 days, ip or po) of several compounds on mouse hepatic GSH S-aryl-, aralkyl-, or -epoxide transferase activity. Small depressions of GSH S-epoxide transferase activity were noticed following treatment with either chlorpyrifos, etrimfos, CGA-19255 (an azidotriazine insecticide), trreflan, endosulfan, or low doses of permethrin (<50 mg/kg). No change was observed in any of the three enzyme activities following treatment with 2,4-D or higher levels of permethrin (100 mg/kg). Compounds causing modest induction (10–50%) of one or more activities included chlorpyrifos, SD-35651 (a substituted nitromethane insecticide), CGA-19255, etrimfos, 2,4,5-T, dimilan, TH-6038 (a dichloro analog of dimilan, gardona, DEF, and trifluran. Greater induction (50–100%) in one or more activities was noted with CGA-19255, 2,4,5-T, dimilan, gardona, and endosulfan. TH-6042 (a substituted pyrazolone insecticide) caused more than a 2-fold increase in all three enzyme activities tested. Phenobarbital administered ad libitum in the drinking water (1 mg/ml) caused a more than 4.0-fold increase in GSH S-aryl transferase activity while GSH S-epoxide and GSH S-aralkyl transferase activities were increased only 30 and 50%, respectively. The results suggest that, not only are these enzyme activities inducible, but since mice are more responsive than rats, that species differences may be important.

238. The Metabolism and Disposition of Ethalfluralin, a Dinitroaniline Herbicide, in Male Rats. G. K. Hanasono, J. L. Occolowitz, and R. L. Wolen, Toxicology Division, Lilly Research Laboratories, Greenfield, Indiana. (J. L. Emmerson)

The excretion, biotransformation, and enterohepatic circulation of ring-labeled [14C]ethalfluralin (N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4(trifluoromethyl) benzeneamine) was investigated in male rats given single oral doses (100 mg/kg). The 7-day urinary and fecal excretion of radiolabeled materials by intact animals accounted for 24 and 71%, respectively, of the administered radioactivity. Three urinary metabolites of ethalfluralin were identified by GC–MS and by high-resolution mass spectrometry. A major urinary metabolite (ca. 25% of urinary 14C) was identified as 2-(hydroxypropyl)-3-[2,6-dinitro-4-(trifluoromethyl)phenylamino] propionic acid. Two minor urinary metabolites (i.e., each less than 7% of urinary 14C) were found to be 2,6-dinitro-4-(trifluoromethyl) benzeneamine and 7-nitro-5-(trifluoromethyl)-1H-benzimidazole. Animals with biliary fistulas excreted 32% of the radiocarbon dose into the bile by 24 hr and 41% by 48 hr. When bile containing 14C-labeled metabolites of ethalfluralin was infused into the duodenum of other bile duct-cannulated rats, 45% of the administered radioactivity reappeared in the 24-hr bile collection. This indicated that an appreciable portion of the labeled materials excreted into the bile undergoes enterohepatic circulation.
239. Disposition of Tricyclazole, a Rice Blasticide, in the Rat. C. L. Pierson and L. C. Howard, Toxicology Division, Lilly Research Laboratories, Greenfield, Indiana. (L. Golber)

In order to support the toxicologic evaluation of tricyclazole (5-methyl-1,2,4-triazolo-[3,4-b]benzothiazole) studies were conducted to investigate the metabolic fate of this systemic fungicide used for the control of rice blast disease. Experiments were designed using [14C]tricyclazole (uniformly labeled in the benzene ring) administered by gavage to male rats. After a 60 mg/kg dose, the disappearance of radioactivity from plasma was biphasic—initial phase $t_{1/2} = 2.5$ hr, followed by a more prolonged decay. Peak plasma concentrations of tricyclazole of 5.8 µg/ml were found 1 hr after oral administration. Radioactivity was rapidly distributed to all major organs with the highest levels found in the liver and kidneys. Upon repeated administration of tricyclazole (seven daily doses of 60 mg/kg), no accumulation of radioactivity was observed in tissues. Radioactivity was completely eliminated (98.2%) within 4 days, urinary excretion being the major route of elimination (65.6%). In the bile duct cannulated rat, biliary excretion accounted for 75.6% of an orally administered dose of tricyclazole over a 24-hr interval. Two urinary metabolites were identified by thin-layer chromatography and GC-MS as the alcohol (1,2,4-triazolo[3,4-b]benzothiazole-5-methanol) and acid (1,2,4-triazolo[3,4-b]benzothiazole-5-carboxylic acid) derivatives of tricyclazole.


Tricyclazole is a fungicide effective in the control of rice blast disease. As part of the toxicologic evaluation of this compound a chronic toxicity—oncogenic study was initiated in Wistar-derived albino rats. Rats were maintained continuously for 2 years on diets of 0, 50, 100, 275, or 620 ppm of tricyclazole, providing doses of 2.2 to 43.9 mg/kg/day (calculated during 52nd week). An additional group of animals was maintained on a diet containing 1600 ppm of tricyclazole for 3 months, and the control ration for 21 months. A significant ($p < 0.05$) decrease in body weight was observed for both male and female rats given 620 or 1600 ppm of tricyclazole. Survival, terminal hematology, and serum chemistry measurements and urinalyses determinations were not altered by tricyclazole treatment. Analysis of terminal organ weight data indicated a dose-related increase in mean relative (g/100 g body wt) liver, kidney, and heart weights which paralleled decreases in body weight. Mean absolute organ weights for rats given tricyclazole were not significantly ($p > 0.05$) different from mean absolute organ weights for control animals. The results of gross and histopathologic examination of tissues and organs indicated that incorporation of tricyclazole in the diet of rats at levels up to and including 620 ppm for 2 years of 1600 ppm for 3 months had no adverse effects.


Transplacental crosse of pesticides and fetal toxicity have been documented in normotensive rats but no reports appear in the literature regarding the susceptibility of spontaneously hypertensive perinates to pesticide challenge. It is the intent of this study to examine the effects of long-term subacute parathion exposure on perinatal rats born to hypertensive mothers. Ninety-day-old spontaneously hypertensive male and female rats (WKY strain) were utilized. The animals were bred and vaginal smears were examined for the presence of sperm. On Day 1 of gestation, doses of parathion of 0.01, 0.1, and 1.0 mg/kg body wt were administered daily to the dam via oral intubation in a peanut oil vehicle throughout gestation and lactation. Peanut oil was administered as a dose control solution. On Day 24 postpartum, the perinates were examined for possible toxicological effects. Hypertensive mothers exhibited no significant reduction in the
number of pups born per litter. Cholinesterase levels were not inhibited in the hypertensive mothers nor in female perinates. However, male spontaneously hypertensive perinates exhibited a significant reduction in red blood cell and plasma cholinesterase at the 1.0 mg/kg dose level. Relative organ weights were altered, indicating involvement of the organs of detoxification. Hematological parameters and serum enzymes were altered in accord with toxicological exposure. Heart rate was significantly reduced at the 0.01 and 0.1 mg/kg dose level. The results of this study indicate that exposure of hypertensive mothers to parathion during gestation and lactation did indeed result in toxicological effects in the perinate basically similar to those seen in normotensive perinates.

242. Carcinogenesis Study in Mice with Hexachlorobenzene. J. R. P. Cabral, T. Mollner, F. Raitano, and P. Shubik, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska.

Hexachlorobenzene (HCB) is used as a fungicide in agriculture in various parts of the world. HCB is also an important contaminant of several widely used pesticides, including Dacthal, pentachloronitrobenzene and others. A severe episode of HCB poisoning in humans occurred in Turkey between 1955 and 1959. The ingestion of HCB-treated wheat seeds caused an epidemic of toxic porphyria, which involved about 5000 people, predominantly young children. The annual mortality ranged from 3 to 11%. In the only previous experiment (Cabral et al., Tox. Appl. Pharmacol. 41, 155, 1977), Syrian golden hamsters were fed HCB for life. A significant increase was found in hepatomas and liver hemangioendotheliomas. The purpose of the present experiment was to verify whether HCB induces tumors in mice. Swiss mice were fed for life a diet containing, respectively, 50 ppm (30 females and 30 males), 100 ppm (30 females and 30 males), and 200 ppm (50 females and 50 males). A control group of 50 females and 50 males was also provided. Hepatomas developed in treated animals. The hepatoma incidence was 10% in both females and males treated with the median dose of HCB, and 29.7 and 14.0%, respectively, in females and males treated with the highest dose of HCB. None of the hepatomas metastasized or occurred in the control group. The present results indicate the carcinogenicity of HCB.

243. Long-Term Study of Potential Carcinogenicity of Inorganic Arsenic Aerosols to Mice. P. E. Berbeau, J. O. Flom, R. L. Dimnick, and A. R. Boyd, University of California, School of Public Health, Naval Biosciences Laboratory, Naval Supply Center, Oakland, California. (W. F. Durham)

Limited epidemiological data have indicated a relationship between exposure of humans to arsenic and increased incidence of cancer. Exposure of laboratory animals to arsenical compounds has not led to a clear indication of tumor formation. Whereas animal exposure studies have been by the oral or dermal routes, human exposure is often by the inhalation route. We have found no reports of long-term inhalation studies of arsenic compounds with laboratory animals. For this reason, we have exposed a tumor-susceptible strain of female mice, 20 to 40 min daily, 5 days/week, to a respirable aerosol of 1% (w/w) aqueous solution of sodium metarsenate for a total of 55 weeks. Initially the experiment consisted of 60 exposed mice, 30 cage-mates, and 30 controls. Exposed mice were housed with cage-mates in a ratio of 7 to 3. Daily doses were about 1.5% of the measured inhalation LD50 value of sodium arsenite. Aerosol concentration of arsenic was determined by chemical analysis of the air sampled by an impinger, using a colorimetric method. After 270 days, the exposed mice weighed significantly less than the controls \( p < 0.001 \) and slightly less than the cage-mates \( p = 0.02-0.05 \). Organ to body weight ratios of mice sacrificed after 207 to 208 days of exposure did not vary significantly between groups. No gross evidence of neoplasia has been encountered; limited histological studies have supported this observation. Apart from weight gain, no other differences have been encountered between the two groups. Thus, based upon periodic inhalation for over half their expected lifespans there is no indication that sodium arsenite is a carcinogen to mice. (Supported by EPA Contract No. IAG-D6-009 and by the Office of Naval Research.)

The degranulation test for chemical carcinogenesis has been studied in a number of laboratories. In general, these studies have been based on measurements of a loss of RNA from rough endoplasmic reticulum (RER) preparations incubated with test compounds and then subjected to prolonged (ca. 20 hr) isopycnic centrifugation. This approach, however, has severe limitations, particularly in that it gives no quantitative information on the event(s) occurring in the system. As there are at least five different mechanisms that could account for the loss of RNA, we have developed a system that gives not only precise quantitative data but also simultaneously yields detailed qualitative data. Essentially small aliquots of test and control incubates are analyzed by rate-sedimentation in sucrose gradients (2–4 hr, 40,000 rpm). The centrifuged gradients are then fractionated automatically while the uv absorbance pattern is monitored. When RNA is prelabeled in vivo with $^3$H- or $^{14}$C-chorotate, quantitative and qualitative distributions may be assessed by liquid scintillation counting of the gradient fractions. A set of BASIC programs has been developed to analyze the results. While the number of compounds examined is yet small, the system has already shown great potential. For example, benzol[al]pyrene, which in our laboratory has proved somewhat refractory in a number of tests (e.g., the Ames test) based on rat liver, has been found to produce a small quantitative change which, however, is qualitatively marked.

245. Chemical Modification of DNA in Rats Treated with Hydrazine. L. R. Barrows and R. C. Shank, Department of Medical Pharmacology and Therapeutics and Department of Community and Environmental Medicine, University of California at Irvine, Irvine, California.

Hydrazine is an important rocket fuel and a powerful reducing agent used for oxygen scavenging in boilers; annual production is over 10,000 tons. Other hydrazine derivatives are used in the production of plastics, soaps, herbicides, and drugs; hydrazines can be an environmental contaminant. Hydrazine is neurotoxic at high doses (120 mg/kg) and hepatotoxic and nephrotoxic at lower doses. In examining mechanisms of hydrazine hepatotoxicity in the rat, liver DNA methylation was observed. Male Fischer 344 rats (200–250 g) were intubated with 0, 30, or 60 mg of hydrazine/kg. At time 0 and hourly thereafter for 4 hr 20 μCl of $[^{14}$C]-methylmethionine was injected ip. The rats were killed 5 hr after intubation, and the liver DNA was isolated, subjected to mild hydrolysis, and fractionated by high-pressure liquid chromatography. Ultraviolet-absorbing peaks were collected and counted for radioactivity by liquid scintillation spectrometry. Results from liver DNA hydrolysates from high-dose animals indicate an increase in radioactivity cochromatographing with authentic 7-methylguanine carrier, compared to hydrolysates from control or low-dose animals. Radioactivity was observed in the pyrimidine oligonucleotide fractions derived from all animals, but detectable radioactivity cochromatographed with O-methylguanine carrier in hydrolysates only from high-dose animals. (Research supported by U.S. Air Force Contract F33615-76-C-5005.)


Male and female CD-1 mice were exposed to 50, 250, or 1000 ppm of vinyl chloride (VC) 6 hr/day, 5 days/week for 49 weeks. To determine the effect of disulfiram (DS) on VC-induced carcinogenesis, one-half of the mice received 0.1% DS in the daily diet for the duration of the study. Bronchiolo-alveolar adenomas were found in all mice exposed to 1000 ppm of VC both with and without DS treatment. However, the tumors tended to occur at a later time in the DS-treated mice. The incidence of hepatic hemangiosarcoma in the mice exposed to 1000 ppm of VC
was less in the mice treated with DS than mice without DS treatment. The incidence of mammary gland tumors in females exposed to 1000 ppm of VC was also reduced with DS treatment. In mice exposed to 250 ppm of VC, the incidence of bronchiolo-alveolar adenoma was not affected by DS treatment. The incidence of hepatic hemangiosarcoma in 250-ppm VC exposed mice was reduced by DS treatment. Studies of the activity of hepatic mixed function oxidase enzymes showed DS treatment to reduce the activity. DS plus VC resulted in a partial decrease in activity. These data suggest that chronic DS treatment may produce at least partial protection against VC-induced carcinogenesis and that the effect of DS on mixed-function oxidase activity may be involved in this protective effect. (Supported by Contract No. NIEHS-N01-ES-2-2084.)


To study the effects of prolonged estrogen administration, virgin female C3H/HeJ mice are being fed diets containing 0, 10, 100, or 500 ppb of diethylstilbestrol (DES) or 0, 100, 1000, or 5000 ppb of 17β-estradiol (E₂) from 6 to 110 weeks of age, while C3H/HeJ mice are being fed DES from 6 to 136 weeks. This updates the findings reported to this Society in 1976. Changes observed in over 1500 mice, some given estrogens for more than 2 years, increased in incidence with dose and duration of estrogen treatment. They include cervical adenosis, adenomyosis with glandular hyperplasia, and hyalin changes in the uterine horns, ovarian atrophy, and proliferation of bony trabeculae and focal fibrosis in the sternal marrow. Tumors observed to date only in DES- or E₂-treated mice included 4 endometrial and 17 cervical adenocarcinomas, a cornual adenocanthoma, a vaginal and 2 perianal epidermoid carcinomas, 5 mesotheliomas, 5 osteosarcomas, and 4 granular cell myoblastomas. Most malignancies, including mammary tumors, occurred in C3H/HeJ mice. These findings indicate that the mammary tumor virus factor facilitates DES-induced mammary tumorigenesis in C3H mice and may contribute to other DES-induced malignant lesions.


Nitrosodiethanolamine has been reported to occur in metal-cutting fluids, in some medicated hair products, and in tobacco. It arises as a result of the reaction of diethanolamine (or triethanolamine) and nitrite, the latter usually a bacterial reduction product of nitrate. Because of the recent interest in this nitrosamine, we synthesized sufficient quantities for bioassay in male and female Syrian golden hamsters. The bioassay protocol consisted of subcutaneous injections (in saline) twice weekly for 3 to 54 weeks. The total dose of nitrosodiethanolamine per hamster was 1.54 g. applied in either 7 (high) or 28 (low) subdoses. Doses applied per injection were based on acute toxicity, that is, 1/5th or 1/20th of the LD50. Within 80 weeks after the first application, 40 of 60 hamsters treated with nitrosodiethanolamine had developed tumors. The tumors were primarily carcinomas of the nasal cavity. Tracheal tumors were also observed in addition to several hepatomas and sarcomas at the site of injection. Control animals were free of these tumors. Previous studies on the rat (Druckrey et al., Z. Krebsforsch. 69, 103, 1967) showed nitrosodiethanolamine to be a weak hepatic carcinogen. The analogous hydrazine, 1,1-diethanolhydrazine, obtained by chemical reduction of the nitrosamine, exhibited no carcinogenic activity in the hamster, although it is much more toxic than the nitrosamine. Experiments are also underway to elucidate the mechanisms of action of the nitrosamine. The results of the bioassay, taken together with those of the earlier study, raise some concern over the use of consumer and industrial products that have the potential to give rise to nitrosodiethanolamine.
249. Chemical Carcinogenesis in Non-Human Primates. R. H. Adamson, S. M. Sieber, P. Correa, and D. W. Dalgaard, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Maryland; Louisiana State University Medical Center, New Orleans, Louisiana; and Hazleton Laboratories, Vienna, Virginia.

For several years we have been treating non-human primates with known rodent carcinogens and materials suspected of being carcinogenic to humans. Among the objectives of the program were to obtain comparative data on the response of non-human primates to these materials, to evaluate the long-term carcinogenic effects of clinically useful antineoplastic agents, to assess the value of chemoprotection in chemical carcinogenesis, to obtain model tumor systems in non-human primates for evaluation of antitumor agents, and to develop biological markers and diagnostic tests for detecting preneoplastic changes as well as overt neoplasia. Thus far, seven compounds have shown carcinogenic activity in non-human primates. Three nitrosamines, N-nitosodiethylamine, N-nitrosodipropylamine, and 1-nitrosopiperidine, have induced primary liver carcinoma in over 100 rhesus and cynomolgus monkeys. Aflatoxin B1 has induced primary liver tumors and osteosarcomas, and methylazoxymethanol acetate has induced primary liver and kidney tumors. A high incidence of squamous cell carcinomas of the larynx and esophagus were diagnosed in monkeys receiving methylazoxymethanol, and 20% of monkeys treated with procabazine have developed acute myelogenous leukemia, hemangiosarcoma, or osteogenic sarcoma. a-Fetoprotein has been found to be a useful marker for diagnosis of primary liver carcinoma as well as for following response to therapy.

250. DNA Repair in Primary Rat Hepatocytes Induced by 2-Acetylaminofluorene and N-Hydroxy-2-Acetylaminofluorene. J. W. Oldham, D. A. Casciano, and M. D. Cave, The National Center for Toxicological Research, Jefferson, Arkansas, and the Interdisciplinary Toxicology Graduate Training Program, University of Arkansas for Medical Sciences, Little Rock, Arkansas. (Thomas J. Haley)

Viable hepatocytes, isolated by in situ perfusion of the liver of Sprague-Dawley rats were placed in primary culture and used to study the dose–response induction of DNA repair (excision repair) after treatment with 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene. These nonproliferating parenchymal cells were simultaneously exposed to media containing 10 μCi/ml of [3H]thymidine and one of several doses of the chemicals for either 4 or 24 hr. Cells irradiated with various fluences of 254 nm of ultraviolet (uv) light served as a positive control for repair induction. Simultaneous exposure to uv light and the chemical carcinogen was used to detect chemically induced cytotoxicity. Induced DNA repair was assessed by measuring incorporation of [3H]thymidine using direct scintillation counting of acid precipitable material, cesium chloride gradient analysis, and quantitative autoradiography. Scintillation counting of acid-precipitable material indicates a dose-dependent induction of DNA repair with 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene from 2.5 to 20.0 and 0.25 to 2.0 μg/ml, respectively. Cesium chloride gradient analysis supports these findings, showing a dose-dependent increase in specific activity of the DNA with increasing doses of 2-acetylaminofluorene or N-hydroxy-2-acetylaminofluorene. Nuclear grain counts made on autoradiographs of treated cells also show a dose-dependent increase in repair induction with both chemicals. 2-Acetylaminofluorene and N-hydroxy-2-acetylaminofluorene require enzymatic activation to the reactive (ultimate) carcinogen. These intact hepatocytes retain enzymatic capabilities when isolated from the whole animal and placed in primary culture. These findings support the idea that induction of DNA repair by primary rat liver parenchymal cells may be used as a screening system for promutagens and procarcinogens as well as direct-acting mutagens and carcinogens.


Previous studies have demonstrated a profound impairment in humoral immunity in mice fed 167 ppm of PCB 1242 or HCB. A significant reduction in splenic direct antibody plaque-forming cells was observed in the absence of any histopathological alterations of lymphoid
tissues. A high concentration of PCB and HCB in primary and secondary lymphoid organs suggested that the environmental chemicals were inducing a functional alteration without any concomitant morphological changes. These results suggested that these two chemicals may be immunosuppressive and that the suppression may include an impairment in cellular as well as humoral immunity. To evaluate this possibility, the influence of PCB 1016 and HCB on the donor cells of the graft-versus-host reaction was studied. Male C57 B1/6 mice (18–20 g) received 167 ppm of PCB 1016 or HCB in their diets for 3 weeks. Spleen cells were suspended in Hank's balanced salt solution (HBSS) and were injected ip into neonatal (<24 hr) BDF1 mice (C57 B1/6 × D2B6A) in a volume of 0.05 ml containing 1 × 10^6, 6 × 10^6, or 10 × 10^6 spleen cells. Nine days following cell injection the spleens of the recipient BDF1 mice were removed and weighed, and spleen index = (relative spleen weight of experimental)/(relative spleen weight of noninjected littermates) was calculated. A dose-related response was observed in neonatal BDF1 mice which received the three different concentrations of spleen cells from donor mice on control diet. However, the dose-related response was not as pronounced in neonates which received spleen cells from PCB-treated donor mice and even less pronounced in neonates which received spleen cells from HCB-treated donor mice. The BDF1 mice which received 1 × 10^6 spleen cells from C57 B1/6 mice treated with PCB 1016 had a 44% increased spleen index while BDF1 mice which received cells from HCB-treated C57 B1/6 mice had a 35% increased spleen index over control values. The data indicate an enhanced cell-mediated immunity and the disparate dose response may be interpreted to mean that perhaps PCB and HCB may activate in situ donor lymphocytes. (Supported, in part, by NIEHS 2-P01-ES00226-10 and by a joint program between the Gesellschaft für Strahlen- und Umweltforschung Munich, Germany and the Institute of Comparative and Human Toxicology.)


Male rats and rabbits were exposed at 0.75, or 200 ppm of chlorobenzene vapors for 7 hr/day, 5 days/week, for up to 120 exposure days (24 weeks). There was a suggestion of decreased food utilization in rats. Groups of animals from both species were sacrificed after 5, 11, and 24 weeks of treatment and examined for hematology, clinical chemistry, and gross and histopathological changes resulting from the chlorobenzene treatment. A statistical analysis of the data suggested an increased liver weight in rats and lung weights in rabbits. Changes in rat hematology included an increase in platelets after 11 weeks of treatment and a decrease in microcell volume with increased microhematocrit, suggesting a microcytic anemia. An interesting clinical chemistry change was a decrease in SGOT activity in rats at all sacrifice periods and in rabbits at the 11-week sacrifice. Histopathological changes were confined to rats, where occasional focal lesions in the adrenal cortex, tubular lesions in the kidneys, and congestion in the liver and kidneys suggested a treatment relationship. (Supported by the National Institute of Occupational Health and Safety, under Contract No. 210-76-0126.)


The microsomal dechlorination of a series of haloalkanes has been measured by Van Dyke. Subsequently Loew et al. correlated these values with a molecular parameter of each compound as calculated by the extended Hückel method. In order to examine further this apparent correlation we have performed a kinetic analysis of the dechlorination reactions for a series of haloethanes. Liver microsomes from rats pretreated with 500 mg/kg of Aroclor 1254 were used. The maximum velocity data for each compound were analyzed by Loew's original method and by more refined molecular orbital methods. The relationship between microsomal dechlorination rate and molecular structure parameters shows a maximum for 1,1-dichloroethane. We have
attempted to compare such theoretical and experimental observations with any known toxicological properties of the compounds. It would appear that compounds having a high reactivity, such as 1,1-dichloroethane \((V_{\text{max}} = 41.6 \text{ nmol of chloride ion/mg of protein/min})\) show notable toxic properties whereas 1,1,1-trichloroethane which has a low order of reactivity \((V_{\text{max}} = 2.94 \text{ nmol of chloride ion/mg of protein/min})\) is inactive \textit{in vivo}. The possible predictive value of this correlation is explored. In particular, the fact that 1,1,1-trichloroethane has a low yet finite ability to undergo microsomal dechlorination but fails to produce tumors \textit{in vivo} may indicate that a threshold effect may be operating.

254. \textit{Effect of Acute and Subchronic Chloroform Exposure on Cholinergic Parameters in Mouse Brain.} F. J. VOCCI, B. R. MARTIN, S. PETTY, and W. L. DEWEY, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia. (J. Borzelleca)

Several halogenated hydrocarbons have been identified as contaminants in drinking water available for human consumption. We have recently examined the acute, subchronic, and chronic effects of chloroform (CHCl\(_3\)) on the following cholinergic parameters in mouse brain: (1) \textit{in vitro} forebrain synaptosomal uptake of \(^{3}H\)choline; and (2) whole brain \(^{3}H\)acetylcholine biosynthesis following a pulse injection of \(^{3}H\)choline. In the \(^{3}H\)choline uptake studies, male Swiss Webster ICR mice were acutely gavaged with vehicle (emulphor--saline) or chloroform (30 or 300 mg/kg; 3000 and 30,000 times average daily human consumption) and sacrificed 15 min after administration. In subchronic and chronic studies, animals were gavaged with 14 or 90 daily doses of chloroform (3 or 30 mg/kg) and sacrificed 18 hr after the last administration. Neither acute nor chronic administration of chloroform had any effect on \textit{in vitro} \(^{3}H\)choline uptake into synaptosomes. In the \(^{3}H\)acetylcholine synthesis experiments, mice were injected with \(^{3}H\)choline (250 \(\mu\)Ci/mouse) via a tail vein 15 min after acute gavage with vehicle or chloroform (30 mg/kg) and sacrificed by microwave irradiation 1 min after tracer injection. Chloroform decreased \(^{3}H\)acetylcholine synthesis (57% of control) while vehicle had no effect. Subchronic (14 day) treatment of chloroform (3 mg/kg), 18 hr after the last administration, produced a reduction in \(^{3}H\)acetylcholine synthesis (57% of control). The results indicate that acute and subchronic chloroform administration may have potentially adverse effects on cholinergic neuronal systems. (Supported by EPA Grant 804701010.)

255. \textit{The Effects of Organic Water Contaminants on Catecholaminergic Neurons in Mouse Brain.} WILLIAM L. DEWEY, BILLY R. MARTIN, BARBARA BAGSHAW, ANN MARSTON, and JENNY MONTGOMERY, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia. (J. Borzelleca)

Chloroform (CHCl\(_3\)), dichlorobromomethane (CHCl\(_2\)Br), chlorodibromomethane (CHClBr\(_2\)), and bromoform (CHBr\(_3\)) at concentrations of \(8 \times 10^{-4} \text{M}\) did not alter the uptake of norepinephrine (NE) or dopamine (DA) into mouse brain synaptosomes \textit{in vitro}. The acute administration of 3000 or 30,000 times the average daily human consumption of these haloalkanes did not significantly affect the uptake of these transmitters. Similarly, daily oral administration of these haloalkanes at 300 or 3000 times the average daily human consumption for 14 days did not cause a change in overt behavior, body weight, brain weight, or the uptake of NE or DA. In another series of experiments CHCl\(_3\), CHCl\(_2\)Br, CHClBr\(_2\), and CHBr\(_3\) were found not to alter the uptake of tyrosine into the brain, the level of endogenous tyrosine, NE or DA in the brain, the rate of synthesis of \(^{3}H\)NE and \(^{3}H\)DA from \(^{3}H\)tyrosine in brain or the incorporation of \(^{3}H\)tyrosine into brain protein. Chloroform was administered orally to mice at 300 and 3000 times the average daily human consumption for 90 days. The animals were sacrificed 24 hr after the last gavage and 10 min following the intravenous administration of 50 \(\mu\)Ci of \(^{3}H\)tyrosine. There were no significant effects on any of the parameters mentioned above as well as no change in brain to body weight ratio. The data from these experiments show that long-term oral dosing of mice with the high concentrations of haloalkanes which have been found as contaminants in drinking water do not have significant effects on noradrenergic or dopaminergic systems of the brain. (Supported by EPA Grant 804701010.)
256. Synthesis and Metabolism of Brain Serotonin in Mice following Acute Exposure to Several Haloalkanes. B. R. Martin, W. L. Dewey, J. S. Beckner, and J. F. Borzelleca, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia.

Several haloalkanes that have been identified as contaminants in drinking water are being investigated in order to determine whether or not they produce neurochemical changes. Chloroform (CHCl₃), bromodichloromethane (CHBr₂Cl₂), dibromochloromethane (CHBr₃Cl), and bromoform (CHBr₃) are being evaluated for their effects on transport of [³H]tryptophan into brain, synthesis of serotonin (5-HT), and turnover of 5-HT. Four groups of six male Swiss Webster ICR mice received either no drug, vehicle (emulphor and saline), a low dose (30 mg/kg), or a high dose (300 mg/kg) of the haloalkane by gavage. Five minutes later mice received [³H]tryptophan (50 µCi/mouse) via the tail vein and were sacrificed after a 10 min pulse time. None of the haloalkanes altered the transport of [³H]tryptophan into brain. CHBr₂Cl₂, CHBr₃Cl, and CHBr₃ did not alter the synthesis and metabolism of 5-HT. Only the lower dose of CHCl₃ increased the accumulation of 5-HT. The higher dose of CHCl₃ increased the metabolism of 5-HT while the lower dose was ineffective. These data show that of the haloalkanes tested, only CHCl₃ has any effect on the synthesis and turnover of 5-HT. It would appear that acute exposure to high doses of either CHBr₂Cl₂, CHBr₃Cl, or CHBr₃ does not alter the neurochemistry of 5-HT in mice. (Supported by EPA Grant 80-4701010.)

257. Effect of Four Haloalkanes on Humoral and Cell-Mediated Immunity in Mice. G. B. Schuller, B. M. Kaufman, J. F. Borzelleca, V. M. Sanders, and A. E. Munson, Departments of Pharmacology and Microbiology, Medical College of Virginia, Richmond, Virginia.

ICR male and female mice were exposed by gastric gavage for 90 days, beginning 7 days after birth, with chloroform (CHCl₃), dibromochloromethane (CHBr₂Cl₂), bromoform (CHBr₃), and bromodichloromethane (CHBr₃Cl₂). The doses of trichloromethane included 0.5, 50, and 150 mg/kg, while the other haloalkanes were given at 0.2, 12.5, and 125 mg/kg. Delayed hypersensitivity (DH) was assayed by ¹²⁵I-labeled albumin extravasation into the footpad at 18 hr after a second challenge with sheep erythrocytes. CHBr₂Cl and CHBr₃ did not appear to alter DH in male or female mice. In contrast, in males at 150 mg/kg, trichloroethylene demonstrated 54% inhibition of the vehicle control stimulation index (4.623 ± 0.75). There was no significant difference in the females in this group at any dose. Females exposed to 125 mg/kg of CHBr₂Cl demonstrated 69% inhibition of control response. Males in this group showed a similar depression of DH. Serum hemolytic antibody levels to sheep erythrocytes were used to quantify the humoral immune response. Twofold serum dilutions were made and incubated with ⁵¹Cr sheep erythrocytes and guinea pig complement. The release of ⁵¹Cr was plotted semilog versus serum dilutions and the reciprocal of the dilution intercept was taken as the antibody titer. CHBr₂Cl suppressed humoral immunity in females by 43% as compared to the vehicle control titer of 3568 ± 124. CHCl₃ treated females produced 41, 48, and 76% suppression at 0.5, 50, and 150 mg/kg, respectively. In contrast, males showed no difference in antibody titers at any dose. Other toxicological parameters, including liver function, kidney function, and hematology, showed no alterations at the three doses examined. These studies suggest that the immune response may be a sensitive indication of the toxicity of the haloalkanes in mice. (Supported by EPA Grant R804701010.)

258. Reticuloendothelial System Function in Mice Exposed to Four Haloalkanes: Drinking Water Contaminants. A. E. Munson, V. M. Sanders, J. F. Borzelleca, R. G. Tardiff, B. A. Barrett, Department of Pharmacology, Medical College of Virginia, Richmond, Virginia, and Health Effects Laboratory, Environmental Protection Agency, Cincinnati, Ohio.

Chloroform (CHCl₃), bromodichloromethane (CHBr₂Cl₂), dibromochloromethane (CHBr₃Cl), and bromoform (CHBr₃) were administered by gastric gavage to ICR male and female mice for 90 days, beginning 7 days after birth. The functional activity of the reticuloendothelial system (RES) was assessed by measuring the vascular clearance rate indicated as
phagocytic index (P.I.), and organ distribution of $^{131}$I-labeled *Listeria monocytogenes*. CHCl$_3$, at doses of 0.5, 5.0, and 150 mg/kg caused no changes in P.I. In female mice, uptake of *Listeria* (cpm/mg of tissue) by the liver showed 9 and 24% decreases at 50 and 150 mg/kg, respectively. Splenic phagocytosis was increased by 29 and 12% at 0.5 and 50 mg/kg, respectively, and suppressed by 43% at 150 mg/kg. In males, CHCl$_3$, caused a dose-dependent decrease in hepatic phagocytosis, but no changes in splenic or pulmonary localization. CHBrCl$_2$, at doses of 0.3, 12.5, and 125 mg/kg, caused no changes in P.I. in males or females. Hepatic phagocytosis showed a dose-dependent suppression in males with the highest dose causing a 45% decrease, while female mice were suppressed 18% at the highest dose. CHBr$_2$Cl produced no changes in the P.I., but caused a dose-dependent decrease in hepatic phagocytosis in males and females. Splenic phagocytosis was depressed 27 and 40% in the 12.5 and 125 mg/kg treated males. CHBr$_3$, at doses of 0.3, 12.5, and 125 mg/kg, caused dose-dependent changes in hepatic phagocytosis with a maximum suppression of 53% in females and 28% in males. All other RES parameters assessed were within normal ranges. These studies indicate that the major effect of the haloalkanes on RES function is related to a suppression of hepatic phagocytosis. (Supported by EPA Grant R-804701010.)

259. *Immunotoxicological Evaluation on Mice Exposed to Polychlorinated Biphenyls*. S. H. Smith, V. M. Sanders, B. A. Barrett, J. F. Borzelleca, A. E. Munson, Department of Pharmacology and MCV/VCU Cancer Center, Medical College of Virginia, Richmond, Virginia.

ICR adult and neonatal mice of both sexes were gavaged acutely or for 14 days (SC) with polychlorinated biphenyls (Aroclor 1254) at doses ranging from 0.3 to 75 mg/kg in adults and 0.3 to 12.5 mg/kg in neonates. Evaluations were done on reticuloendothelial system function using $^{131}$I-labeled *Listeria monocytogenes* and on cell-mediated immunity using sheep red blood cells and $^{131}$I-labeled serum albumin to measure footpad swelling. In adult mice, at both dosing schedules, liver uptake of *Listeria* and specific activity (SA) were depressed dose-dependently. Liver weights of SC-dosed adult mice showed a twofold increase at 75 mg/kg. SC-dosed neonates exhibited no increase in liver size and only the males showed a depression in liver uptake and SA of 24% at 12.5 mg/kg. In adult females dosed SC at 75 mg/kg there was a depression in splenic uptake and SA. This splenic depression was seen only in neonatal males dosed SC at 0.3 and 12.5 mg/kg. Kidney localization of *Listeria* was increased in all mice exposed to the contaminant. Erythrocyte counts were depressed in mice exposed SC. Cell-mediated immunity in SC-dosed adults of both sexes exhibited an inhibition of $^{131}$I-labeled albumin incorporation at 75 mg/kg. These investigations indicate that the polychlorinated biphenyls are deleterious to the monocyte macrophage system. (Supported by EPA Grant R804701010.)

260. *Failure of Kepone and Hexachlorobenzene to Induce Dominant Lethal Mutations in the Rat*. Glenn Stuart Simon, Barbara R. Kipps, Robert G. Tardiff, and Joseph F. Borzelleca, Division of Toxicology, Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, and Health Effects Laboratory, Environmental Protection Agency, Cincinnati, Ohio.

Previous investigations have shown that both Kepone (1,3,4-metheno-2H-cyclobuta (CD) pentalene-2-one) and hexachlorobenzene (HCB) may adversely affect reproductive function and performance. Exposure of germ cells to these agents could elicit mutagenic effects. These effects were evaluated using the dominant lethal assay in the rat. Five groups of 10 male rats each were administered (po) 0, 3.6, or 11.4 mg of Kepone or 70 or 221 mg of HCB per kilogram body weight daily for 5 consecutive days. The higher doses of Kepone and HCB were the maximally tolerated doses for 5 days administration. Ten other male rats received a single oral dose of 0.5 mg of triethylene melamine (TEM)/kg body wt. All test compounds were dissolved in corn oil. Each male rat was allowed to mate with two naive, nulliparous females per week for 14 consecutive weeks. Females were sacrificed on the 14th day of gestation, and their uteri and
ovaries were exposed and examined for numbers of corpora lutea, and total viable and non-viable implantations. Appropriate statistical analyses were conducted. TEM elicited dominant lethal mutations. Kepone and HCB failed to induce any apparent compound-related effects. At the doses tested, Kepone and HCB do not appear to be potent mutagens. (Supported in part by EPA Grant R-804290010.)

261. Metabolic Study of 2,4,5,2',4',5'-Hexachlorobiphenyl in Rhesus Monkeys. D. H. Norback, E. Mack, K. A. Blomquist, and J. R. Allen, Department of Pathology, Department of Surgery, and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin.

Gastrointestinal absorption, tissue distribution, and biliary and urinary excretion of 2,4,5,2',4',5'-hexachlorobiphenyl (HCB), a component of commercial polychlorinated biphenyl (PCB) mixtures, in two rhesus monkeys was studied. Bile duct and duodenal cannulation performed at laparotomy allowed externalization of the bile flow for quantification, collection of a fraction for metabolic studies, and return of the remainder to the duodenum of the animals. Following a postsurgical period of stabilization, [3H]HCB (1 g/kg dissolved in less than 6 ml of corn oil) was administered by nasogastric intubation. In a young mature female monkey, the major portion of the administered [3H]HCB (87%) passed unabsorbed through the intestine and was recovered from the feces during the first week. Over a 3-week period, 1% was excreted in the bile with the peak excretory rate occurring between 24 and 48 hr. In a juvenile male monkey, 59.3% was recovered from the feces within the first week. During the first 4 weeks, 12% was excreted in the bile; during the total 7-week period 17% was excreted in the bile. The period of highest excretory activity occurred between 48 and 96 hr. There was no detectable radioactivity in the urine of either animal. In both animals the organs with the highest concentration of the material included the adipose tissue, bone marrow, skin, peripheral nerve, and adrenal tissues. The skin, followed by muscle due to its large mass, was the major reservoir of the compound. (Supported by U.S. Public Health Service Grants ES00472, CA22140, and RR00167 from the National Institutes of Health.)

262. Acute, Subchronic, and Chronic Toxicological Studies with Kepone. Paul S. Larson, Gordon R. Hennigar, Richard W. Lane, and Joseph F. Borzelleca, Division of Toxicology, Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

The toxicological effects of Kepone (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta (CD) pentalene-2-one), a potent insecticide, were evaluated in several animal species. The acute oral LD50 was determined in male and female rats, male rabbits, and mongrel dogs. A 5% solution in corn oil was used. The acute percutaneous LD50 in male rabbits was obtained using a 20% solution of Kepone. Oral LD50 values (mg/kg) were 132 ± 8 for male rats and 126 ± 12 for females, 71 ± 6 for male rabbits, and 250 for dogs. The percutaneous LD50 for male rabbits was 410 ± 65 mg/kg. Severe tremors preceded death. In two separate studies, rats received Kepone at levels of 0 and 1 ppm in the diet and 0, 3, 10, 25, 50, and 80 ppm for up to 2 years. All rats receiving 50 and 80 ppm of Kepone died during the first 6 months. Depressed growth occurred at dietary concentrations estimated to be as low as 6 ppm for females and 25 ppm for males. Food consumption (expressed as grams of feed per kilogram body weight) increased with increasing concentration of Kepone. Excessive bleeding when sampling occurred was noted in the rats receiving 50 and 80 ppm, but no clotting defect was evident. There was no dose-related effect noted in reducing substances in the urine. However, proteinuria was observed in all rats receiving Kepone at levels of 5 ppm and greater. The concentration of Kepone in body fat ranged from 10 ppm in rats receiving the 5-ppm diet to 400 ppm at the 80-ppm diet. Degenerative changes in liver cells and testicular atrophy were noted at 3 months at levels of 25, 50, and 80 ppm. In rats surviving between 1 and 2 years, evidence of liver and kidney damage was noted. The presence of hepatocarcinomas could not be definitively established. In addition, 16 purebred beagle dogs were divided into four groups of two males and two females and
received kepone in the diet at levels of 0, 1, 5, and 25 ppm for up to 127 weeks. The dogs showed no compound-related effects (body weight, blood, and urine data, histopathologic findings). (Supported in part by a grant from the Allied Chemical Corp.)

263. Short-term Toxicity of Photomirex in the Rat. L. Ritter, R. Norstrum, G. Felsky, I. Chu, I. A. Marino, V. E. Valli, and D. C. Villeneuve, Biochemical Toxicology Section and Pathology Section, Environmental Health Directorate, Ottawa, Ontario; Toxic Chemicals Division, Canadian Wildlife Service, Ottawa, Ontario; and Bow Valley Holdings Ltd., Guelph, Ontario, Canada.

Photomirex (8-monohydro Mirex), a photo degradation product of Mirex, has recently been identified as the fourth highest organochlorine contaminant in Lake Ontario (Hallet et al., 1976). The toxic potential of this compound has not been fully defined and the following study was designed to determine the subacute toxicity and tissue distribution of Photomirex in the rat. Male Sprague-Dawley weanling rats were divided into groups of 10 animals and fed diets containing 0, 0.5, 5.0, 50.9, or 500.0 ppm of Photomirex for 28 days. The results showed that Photomirex accumulated in a dose-dependent manner, with fat containing the highest levels, followed by liver, kidney, heart, brain, and spleen. The highest dose level was toxic and caused death in 9 of 10 animals. Animals fed 50 ppm of Photomirex showed an increased food intake, body weight gain, liver, heart, spleen, and kidney weight gain. Animals fed 0.5 and 5.0 ppm of Photomirex showed significant increases in liver weight while only those receiving 50 ppm of Photomirex showed any significant increase in spleen weight. All hematological parameters remained unchanged except for white blood cells which increased significantly in animals receiving 50 ppm Photomirex. Biochemical analysis has shown that serum sorbitol dehydrogenase activity increased in all groups while the groups receiving 5.0 and 50 ppm of Photomirex showed increased hepatic microsomal enzyme activity. Microscopic examination of tissues showed a progressive reduction in thyroid colloid, possible cessation of spermatogenesis, and mid-zonal ballooning of hepatocytes accompanied by regressive changes in hepatocellular nuclei. Experiments designed to study the significance of these changes are now underway.


Photomirex (8-monohydro Mirex) is an environmental contaminant the toxicity of which remains to be determined. Therefore, the present study was designed to provide information on the teratogenic potential of this compound. Adult New Zealand white does were intubated orally with single daily doses of 0, 5, or 10 mg of Photomirex/kg body wt on Days 6–18 of gestation. Pregnancies were terminated at term by cesarian section and fetuses were evaluated by following routine teratologic methods. Both maternal and fetal tissues were analyzed for residues of Photomirex. None of the treated does showed any sign of toxicity. Except for a significant reduction in mean fetal weight in the 10 mg/kg group all other parameters evaluating fetal survival and fetal development were within the control range. Photomirex was found in all tissues examined. The highest maternal levels were found in fat followed by liver, kidney, spleen, heart, brain, and blood. Highest fetal levels were found in the heart, followed by liver, brain, and blood. The results from the present study show that Photomirex is transferred across the placenta and accumulates in the fetus. However, there were no teratogenic effects associated with the doses used in this study.

265. Subchronic Toxicity Studies of 1,2,4-Trichlorobenzene in Experimental Animals. P. G. Watanabe, R. J. Kociba, R. E. Heffner, Jr., H. O. Yakel, and B. K. J. Leong, Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan.

The subchronic toxicity of inhaled 1,2,4-trichlorobenzene was assessed in male rats, rabbits, and dogs exposed 7 hr/day, 5 days/week for 30 exposures in 44 days. No significant effects on body weight, hematology, or gross or microscopic pathologic examination were observed in any species. However, the liver weights of rats and dogs were increased at the 100-ppm exposure
level. Furthermore, rats exposed to 30 and 100 ppm of TCB showed elevated urinary excretion of uroporphyrin and coproporphyrin. A subsequent study was conducted to determine the level which would not increase the excretion of urinary porphyrin in rats. Male and female Sprague–Dawley rats were exposed to 10 or 3 ppm of TCB, 6 hr/day, 5 days/week for 3 months. Exposure to 10 ppm of TCB resulted in a slightly increased urinary uroporphyrin excretion over the 90-day period. This effect was reversible and no increase was observed 2 or 4 months following termination of exposure. Exposure of rats to 3 ppm of TCB 6 hr/day, 5 days/week for 90 days did not cause any increase in urinary porphyrin excretion, and thus, under the conditions of this study can be considered a no-adverse-effect level for rats.

266. Immunotoxicologic Effects of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin in Laboratory Animals. R. P. Sharma, R. J. Kociba, and P. J. Gehring, The Dow Chemical Company, Midland, Michigan.

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) has been reported to be capable of inducing immunologic alterations in animals given high dosages. In the study reported herein, male CD-1 mice and White New Zealand rabbits were given 0, 0.01, 0.1, 1, and 10 μg of TCDD/kg/wk for a period up to 8 weeks. The mice were sacrificed after 2, 4, and 8 weeks of exposure, with lesions primarily in the liver and to some extent in the thymus. The thymic changes in mice were pronounced after 4 weeks but not after 8 weeks of exposure. Splenic lymphocytes of both species were cultured in vitro with or without the presence of selective blast-forming agents (phytohemagglutinin and pokeweed mitogen) and the incorporation of [3H]thymidine was measured. Exposure of both species to TCDD, even at the lowest level (0.01 μg of TCDD/kg/wk), caused an increase in the thymidine uptake by nonstimulated splenic lymphocytes. The blastogenic response to phytomitogens was decreased at the higher levels (>0.1 μg of TCDD/kg/week) of treatment. Additional groups of mice and the rabbits were inoculated with an antigenic mixture (tatanus toxoid and Freund’s adjuvant) simultaneously with TCDD treatment to evaluate the induced immune reactivity. Dose levels of 1 or 10 μg of TCDD/kg/wk reduced the serum anti-tatanus concentrations in both species and skin reactivity to tuberculin and the antibody producing cells in the popliteal lymph nodes of rabbits. The concentration of serum immunoglobulins were markedly reduced with the higher dose levels of TCDD, whereas they were elevated with the lower dose levels. The results indicated that TCDD at low levels of exposure caused an enhancement of immune responsiveness similar to that produced by antigenic substances. Immunosuppression occurred at toxic levels of TCDD exposure, and there was suggestion of an adaption to this effect.

267. Influence of Alterations in Drug Metabolism on Trichloroethylene-Induced Cardiac Arrhythmias. J. F. White and G. P. Carlson, Department of Pharmacology and Toxicology, Purdue University, West Lafayette, Indiana.

Many clinical reports have noted cardiac arrhythmias in patients intoxicated with trichloroethylene (TCE). No recent studies using laboratory species substantiate or refute that claim, and there have been no investigations on the relationship between blood concentrations of TCI and its metabolites, trichloroethanol (TCE), and trichloroacetic acid (TCA), and cardiac arrhythmias. Studies examined the effect of TCI on the cardiac rhythm of the rat and rabbit and the influence of alterations in drug metabolism on the occurrence of spontaneous and epinephrine-induced arrhythmias. Rabbits and rats were pretreated with saline or the inducing agents phenobarbital or Aracol or 1254, or the inhibiting agents SKF-525A or Lilly 18947 and were exposed to TCI (3000 and 25,000 ppm, respectively) in a dynamic inhalation chamber for 1 hr. Serial blood samples were collected from rabbits via an indwelling jugular cannula and from TR, TCE, and TCA by gc. Epinephrine was administered to the rat via a jugular cannula and to the rabbit via a catheter in the marginal ear vein. Lead I ECG’s were monitored using a Grass polygraph. After 7.5, 15, 30, 45, and 60 min of exposure animals were given increasing doses of epinephrine, up to 4 μg/kg, until arrhythmias occurred. In rats, only those pretreated with SKF-525A developed either spontaneous or epinephrine-induced arrhythmias. Rabbit controls exhibited premature ventricular beats and extrasystoles when challenged with epinephrine after a 30-min exposure to 3000 ppm of TCI. Rabbits pretreated with SKF-525A or Lilly 18947
developed more arrhythmias after shorter times and at lower doses of epinephrine and had higher blood levels of TRI. Phenobarbital-pretreated rabbits developed fewer arrhythmias and had lower blood levels of TRI. Aroclor 1254 did not alter either TRI metabolism or the development of arrhythmias in the rabbit. No correlations were found between the blood concentrations of TCE and TCA and cardiac arrhythmias. The data indicate that TRI can cause spontaneous and epinephrine-induced arrhythmias in the rat and rabbit, and the rate of metabolism of TRI can alter the susceptibility to the development of these arrhythmias.

268. Behavioral Toxicologic Effects of Polybrominated Biphenyl Compounds in Rodents. H. A. Tilson and P. A. Cabe. Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. A. Moore)

Male and female rats (Fisher-344/N) and mice (B6C3F1) were administered Firemaster (FF-1), a mixture of polybrominated biphenyls (PBBs), and 2,4,5,2',4',5'-hexabromobiphenyl (HBB), the major component of FF-1. Dosing (0.03–30 mg/kg/day of FF-1 and 0.168–16.8 mg/kg/day of HBB) was by gavage 5 days/week for a total of 22 doses. At the end of the 30-day dosing regimen, the animals were given a battery of tests (30-day test) designed to evaluate behavioral and neurological dysfunction. Thirty days after cessation of dosing, the animals were reevaluated on the same battery of tests (60-day test). Exposure to PBBs resulted in a profile of effects consisting of decreased body weight, depressed neuromuscular reflexes, decreased motor activity in the open field, and decreased forelimb grip strength. Rats were generally more affected on these measures than mice. PBBs also decreased the number of visual placement responses made by rats, decreased the rectal temperature of mice, and had no effect on the number of defecations and urinations made by rats or mice in the open field maze. In general, rats tended to remain the same or get worse following cessation of dosing, while mice tended to improve. The results of this study indicate that oral dosing with relatively low amounts of PBBs results in behavioral toxicologic effects. Additional studies are underway to characterize the mechanisms by which these effects are produced.


PCBs, have been reported to be carcinogenic in laboratory animals. The carcinogenicity of most chemical carcinogens is due to their metabolic activation to highly reactive electrophilic intermediates which bind covalently to cellular macromolecules. In order to investigate the covalent binding of PCBs, two hexachlorobiphenyls, 2,3,6,2',3',6'-trichlorobiphenyl (PCB I), a rapidly metabolized PCB, and 2,4,5,2',4',5'-pentachlorobiphenyl (PCB II), a more slowly metabolized PCB, were administered daily for 5 days, to male mice. The animals were sacrificed on Day 6 and the liver macromolecules were isolated and extracted thoroughly with organic solvents. The concentration of PCB I in liver was 45.24 pm/mg, the inextractable radioactivity in isolated and purified macromolecules was 149.3 pm/mg of RNA, 57.2 pm/mg of protein, and 7.09 pm/mg of DNA. The concentration of PCB II in liver was higher, 54.45 pm/mg; however, the inextractable radioactivity in isolated and purified macromolecules was considerably lower—4.08 pm/mg of RNA, 2.2 pm/mg of protein, and 0.17 pm/mg of DNA. Liver macromolecules isolated from controls spiked in vitro with either PCB I or II, showed no radioactivity associated with the nucleic acids and only traces were detected in protein. The results indicate that PCBs bind covalently not only to RNA and proteins, the macromolecules more likely to be in close contact with the reactive metabolic intermediates, but also to DNA.


The relative importance of different sites on the biphenyl molecule to the metabolism of polychlorinated biphenyls (PCBs) can be most accurately estimated when the number of sites
available for enzymatic attack are limited and are the same in each phenyl ring, i.e., symmetrical. The sites of enzymatic attack are effectively limited by increasing the degree of PCB chlorination. However, symmetrical PCBs with as many as six chlorines, three on each phenyl ring, may be metabolized at a rate which severely limits the amount of metabolite which can be isolated for chemical characterization. In an effort to circumvent this problem and to study the metabolism of a series of symmetrical hexachlorobiphenyls by the male rat, the anticipated major metabolites of each PCB in the series have been synthesized and used to identify the major metabolites excreted by rats. The structures of the synthetic metabolites were confirmed by mass spectrometry and proton and $^{13}$C nuclear magnetic resonance spectroscopy. The authentic metabolites were isolated from the feces and bile of animals which had received an iv dose of the respective $^{13}$C-labeled hexachlorobiphenyl. Identification of the authentic metabolites was by cochromatography on thin-layer and high-pressure liquid chromatography and by isotope dilution with the synthetic metabolites. The half-lives of these hexachlorobiphenyls varied from approximately 1 day to near infinity and could be attributed almost solely to the effect of chlorine position on the metabolism of these PCBs. The hexachlorobiphenyl metabolites, which were isolated and identified, illustrate the importance of two adjacent unsubstituted carbon atoms, preferably in the meta-para positions, to the metabolism of PCBs. The absence of two adjacent unsubstituted carbon atoms inhibits, but does not prevent, the metabolism of PCBs. The nature of the major metabolites of these PCBs strongly implies that their metabolism involves an aren oxide intermediate.

271. Effect of Polychlorinated Biphenyl and Other Inducing Agents on the Microsomal Cytochrome P-448-Mediated Reaction, Ethoxyresorufin O-Deethylation. T. C. ORTON and J. E. HIGGINS, Drug Metabolism Section, ICI Pharmaceuticals Division, Alderley Park, Near Macclesfield, Cheshire, England (M. S. Rose)

Burke et al. (Drug Metab. Disposition 5, 1, 1977) have shown that the O-deethylation of ethoxyresorufin is catalyzed by microsomal cytochrome P-448, which is involved in the metabolism of many polycyclic hydrocarbons via carcinogenic intermediates. This work was undertaken to study the effect of polychlorinated biphenyl (PCB), an environmental pollutant, and other inducers of the hepatic microsomal drug metabolising enzymes on ethoxyresorufin O-deethylation (EROD) by liver microsomes of male rats (R) and hamsters (H). Biphenyl 2- and 4-hydroxylation (Bδ20H and Bδ40H), aminopyrine N-demethylation (APND), and cytochrome P-448/450 content were also studied. The following rates of EROD (nmol/mg of microsomal protein/min at 250 nm substrate concentration) were obtained: Control (arachis oil or saline), 0.014 (R) and 0.10 (H); Aroclor 1254 (PCB), 5.68 (R) and 0.60 (H); phenobarbitone (PB), 0.053 (R); 3-methylcholanthrene (3MC), 10.87 (R); B-naphthoflavone (BNF), 5.76 (R); and PB + BNF, 3.55 (R). EROD was low in control and PB rat and was markedly induced after 3MC and BNF treatment which raised the level of cytochrome P-448. EROD, Bδ20H, Bδ40H, APND, and cytochrome level (peak at 449 nm) were induced in PCB rat. EROD had a high basal rate in hamster which was induced to a smaller extent than in the rat after PCB treatment. In the rat, the combination of PB + BNF treatment gave a broad spectrum of induction of metabolism similar to PCB and may prove a safer alternative. It is concluded that EROD is a useful substrate for monitoring the activity of microsomal cytochrome P-448.


Mice (male weanlings) were administered 30 ppm of Mirex in Wayne mouse laboratory diet for 12 weeks in order to determine the effect of this compound on the state of hepatic microsomal proteins. Control mice were treated identically as experimentals except that Mirex was not added to the diet. At sacrifice, the livers were removed and tared, and hepatic microsomes prepared by the citrate-pyrophosphate method of Bailey et al. (Canad. J. Biochem. 52, 1003, 1974), a procedure that removes ribosomes from the microsomal preparation. Protein content of the
hepatic microsomes was determined by the method of Lowry et al. (J. Biol. Chem. 251, 4659, 1951). The SDS–PAGE gels of hepatic microsomes were stained with Coomassie blue, photographed, and spectrophotometrically scanned at 600 nm. The results of these experiments show that (a) the livers of Mirex-treated mice were grossly enlarged (4.1 g av) compared to controls (2.0 g av); (b) Mirex-treated mice had 1.6 times greater protein content in electrophoretic bands corresponding to cytochrome P-450 positions than did controls; (c) Mirex induced a microsomal protein band not seen in SDS–PAGE patterns of microsomes of controls or in SDS–PAGE patterns of species treated with phenobarbital, 3-methylcholanthrene and benzo(a)pyrene but noted in species treated with 2-acetylaminofluorene and diethylnitrosamine. These results are discussed in terms of the effects of Mirex on the metabolism of the other environmental chemicals and drugs. (Supported by NIEHS Grant No. 2-F01-ES00226-10 and Grant No. 2-T01-ES00703-10.)

273. Chlorohydrocarbon Excretion and Liver Monoxygenases in Rhesus Monkeys following Chronic DDT Exposure and SKF 525-A Treatment. CHARLES R. CLARK, PAUL W. FERGUSON, SHIRLEY J. GEE, and ROBERT I. KRIEGER, Department of Environmental Toxicology, University of California, Davis, California.

Male rhesus monkeys, Macaca mulatta, were exposed to 5, 10, 25, 50, and 100 ppm of DDT in the diet over a period of 18 months. Blood levels and urinary excretion of chlorohydrocarbon (DDT, DDE, DDD, and DDA) were measured. Antipyrine plasma half-life (APH) and monooxygenase activity in liver biopsy homogenates were measured before and after cessation of DDT exposure. Blood levels of DDT dropped (750 to 300, 450 to 150, and 500 to 200 ppb) in the three animals over a 35-day period after discontinuing DDT. Urinary excretion of DDT and DDA decreased (4000 to 500, 5000 to 300, and 2000 to 300 ng/day and 900 to 150, 800 to 50, and 500 to 100 µg/day, respectively) over this same period. Excretion of DDA, which accounted for ca. 99% of total chlorohydrocarbon measured in urine, was slightly reduced 1 day following treatment with SKF 525-A (20 mg/kg, im) and increased 180, 170, and 144 above pretreatment rates over the next 7 to 8 days. Serial administration of SKF 525-A (20 mg/kg, im, 1/day × 3 days) decreased urinary excretion rates of DDA to approximately the same level as following a single dose. A smaller increase in DDA excretion was observed. Rates of oxidation of model substrates by liver biopsy homogenates decreased (aldrin, 10–15%; dihydrodioxin, 5–33%; p-nitroanisole, 8–25%; benzo(a)pyrene, 64–75%) and APH increased (25–31%) over values measured during DDT feeding (100 ppm) within 32 and 29 days, respectively. When these rates were measured 6 days after serial SKF 525-A treatments, three oxidations were increased (DHI, 20–31%; PNA, 49–100%; BP, 88–500%). Increased chlorohydrocarbon excretion may be related to SKF 525-A-enhanced cyclohexene uptake in mice (Toxicol. Appl. Pharmacol. 38, 315–323, 1976). (Supported by NIH ES 00054 and 00125.)

274. The Effects of Bromochlorodifluoromethane on Contractility and Cardiac Tissue Levels of High-Energy Metabolites. R. N. TERPOLILLI, R. A. DAVIS, G. R. PETERSON, and K. C. BACK, Toxic Hazards Division, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, and Departments of Biological Chemistry and Pharmacology, Wright State University, Dayton, Ohio.

The purpose of this study was to determine whether a depletion of ATP and creatine phosphate (CP) is related to the negative inotropic effects of bromochlorodifluromethane (BCF). Guinea pigs were urethane-anesthetized, respired on 100% O₂, and instrumented to record left ventricular blood pressure (LVBP), dLVBP/dt, arterial blood pressure (artBP), heart rate (HR), lead I EKG, arterial pH, pO₂, and pCO₂, body temperature, and ventricular contractility (peak dLVBP/dt + LVBP). A 20% vapor concentration of BCF was selected and produced approximately a 50% decrease in contractility. Following a control period and 30-min exposure, the apex of the heart was freeze-clamped and extracted, and tissue levels of ATP and
CP were determined using an enzymatic assay. Similar experiments conducted substituting N₂ for BCE served as controls. Pharmacokinetic studies demonstrated that BCF blood levels stabilized within 30 sec of exposure and remained constant. Significant physiological effects included: (1) a reversible decrease in contractility to 45.5% of control within 5 min which remained constant throughout the exposure, (2) a fall in artBP, HR, and arterial pH, and (3) an increase in the PR and QT intervals. Arterial pO₂ was maintained above 100 mm Hg at all times. Control levels of ATP and CP were 3.62 and 5.35 mmol/kg compared with exposure values of 3.67 and 5.88 mmol/kg, respectively. Consequently, these results suggest that the negative inotropic effect of BCF is probably not related to a decreased availability of ATP and CP. (Supported in part by a research grant, Miami Valley Heart Chapter, American Heart Association.)


The fate of 2,4,5,2',4',5'-hexabromobiphenyl (HBB), the major component of Firemaster BP-6, was determined in rats in an effort to elucidate the pharmacokinetic factors likely to affect the tissue distribution and excretion of this compound during chronic exposure. Male Sprague-Dawley rats were given either a single iv or four oral doses of 1 mg/kg of [14C]HBB. Biliary excretion was determined by bile duct cannulation of iv dosed animals. Parent HBB concentration as well as total radioactive material was determined in tissue and excreta samples collected at various time points. Adipose tissue was the major long-term storage site. Excretion was negligible in urine and very slow in feces. Less than 7% of an iv dose was excreted after 42 days. Animals that received one oral dose excreted 7.9 to 13.9% during the first 24 hr, compared to 0.35 to 1.5% for animals that received the same amount iv. A physiological compartmental model was constructed from the iv data and used to simulate the pharmacokinetics of repeated oral HBB ingestion. The model showed that following each oral dose, net intestinal absorption prevailed for the first 18 hr due to concentration differences between material in the gut lumen and its surrounding tissue. Thereafter, oral dose disposition kinetics was similar to that for an iv dose. The slow decline in tissue concentrations over a period of 6 weeks was primarily the result of dilution due to growing tissue volumes and only minimally due to excretion.

276. Reductive Biotransformation of Chlordecone in Man and Rat. M. W. Fariss, R. V. Blanke, J. J. Boylan, S. T. King, and P. S. Guzelian, Department of Pathology and Medicine, Medical College of Virginia, Richmond, Virginia.

Chlordecone (Kepone, CD), an organochlorine pesticide, was recently implicated in the industrial poisoning of 35 male workers at a Hopewell, Virginia manufacturing plant. The eaged structure of CD is very similar to that of Mirex except for the presence of a bridge head carbonyl group. The existence of this polar group suggested the possibility of a more extensive biotransformation for CD than has been reported for Mirex. In this study, we collected and examined samples from CD-poisoned humans and rats. Samples of serum, urine, bile, and feces were analyzed by GLC-ECF for Cd and possible metabolites. In human faecal samples, a reduced form of CD, chlordecone alcohol (chlordecol, CDOL) was isolated and identified by GLC-MS. This metabolite appears to be present in human stool in quantities at least equivalent to CD. Small amounts of CDOL were demonstrated in human bile, whereas negligible amounts were found in human urine or plasma. Analytical evidence also indicated the existence of small quantities of CDOL in rat stool. However, negligible quantities of this metabolite were detected in rat urine, plasma, or bile. Recent experiments also suggest the existence of conjugated forms of CDOL but unequivocal confirmation of these forms has not yet been established. (Supported by NIEHS Contract 1-N01-ES-6-2117, NIEHS Grant 1-R01-ES-01519 and a grant from Allied Chemical Corp.)
ABSTRACTS: SEVENTEENTH ANNUAL MEETING


A histopathologic and autoradiographic comparison of a low chlorinated biphenyl (2,5,4'-trichlorobiphenyl (TCB) and 2,4,6,2',4'-pentachlorobiphenyl (PCB) was conducted. Two rhesus monkeys (one male and one female) received 14C-labeled TCB at 5 ppm of the daily diet for 84 days and one (male) received 14C-labeled PCB at 5 ppm for 300 days. In the TCB monkeys primary induced injury of the arterioles, capillaries, and venules of the organs of all three body cavities was responsible for degenerative changes in the kidney, liver, cerebellum, and hippocampal area of the cerebrum. The cerebellar changes consisted of swelling of Purkinje cells and the thinning of the granular cell layers. In the liver focal periportal infiltrates an increase in the number of Kupffer cells were observed. In the PCB monkey, along the minor curvature there was an extensive area of glandular metaplasia in which the gastric pit epithelium has replaced the deeper situated gastric glands. In addition, there was cytoplasmic shrinkage and nuclear damage in the Purkinje cells with demyelination of the submarginal and ventral corticospinal tracts of the cervical spinal cord. The liver changes consisted mainly of hepatocytic degeneration and proliferation of cholangiocytes. It appears that TCB damage involves mainly the small vessels, whereas PCB interferes with the parenchymal cells of the stomach, cerebellum, and liver.

278. Effects of Cysteine, Diethylmaleate, and Trichloropropane Oxide on Acute Vinyl Chloride Hepatotoxicity. R. B. CONOLLY and R. J. JAEGGER, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts.

Vinyl chloride (VC) is acutely hepatotoxic to male rats pretreated with polychlorinated biphenyl (PCB). In vivo, VC may be activated to chloroethylen oxide (CEO). If CEO is a substrate, epoxide hydrase (EH) would hydrate it to the glycol. CEO and its derivatives may also be conjugated with glutathione (GSH). We have studied several compounds which could interact with these detoxification pathways. Cysteine, a precursor of GSH, should protect against VC toxicity. Diethylmaleate (DEM) depletes hepatic GSH and should increase VC toxicity. Trichloropropane oxide (TCPO) inhibits EH and may thereby increase VC toxicity. PCB (300 µmol/kg) was given once a day for 3 days prior to a 4-hr inhalation exposure to VC. Cysteine (500 mg/kg), DEM (0.5 ml/kg), and TCPO (0.1 ml/kg) were given immediately before VC exposure. All treatments were by gavage. Hepatic injury was estimated by measurement of serum sorbitol dehydrogenase (SDH) and GSH levels by measurement of total nonprotein sulfhydryl (NPSH). Cysteine blocked depletion of hepatic NPSH during exposure of fasted rats to 8000 ppm of VC and significantly decreased SDH elevations due to this exposure. Fasted rats have about 25% less hepatic GSH and are about five times more sensitive than fed rats to VC toxicity. Treatment of fed rats with DEM such that their hepatic NPSH levels during VC exposure were identical to those of fasted rats did not increase the sensitivity of the fed rats to VC toxicity. TCPO significantly increased the toxicity of VC in fasted rats over a range of concentrations from 4000 to 8000 ppm. Hepatic NPSH levels did not differ between vehicle and TCPO-treated groups during these exposures. TCPO did not alter the sensitivity of fed rats to VC toxicity. These data suggest the importance of GSH in the detoxification of VC metabolites in PCB-pretreated, fasted rats. It appears that the sensitization of fasted rats to VC toxicity by TCPO is not mediated by GSH depletion. Thus, EH seems more important in the fasted rat where GSH is less available. The absence of enhanced toxicity after DEM may be explained by alteration of VC uptake. (Supported by NIEHS Grant ES 00002.)

279. Effects of 2,2'-Dibromobiphenyl and 2,2',3,4,4',5,5'-Heptabromobiphenyl on Microsomal on Drug Metabolizing Enzymes. ROBERT W. MOORE and STEVEN D. AUST, Department of Biochemistry, Michigan State University, East Lansing, Michigan.

Polybrominated biphenyls (PBBs) cause a mixed-type induction of liver microsomal drug-metabolizing enzymes. We have shown that 2,2',4,4',5,5'-hexabromobiphenyl (HBB), the
major component (56%) of PBBs (Firemaster), is strictly a phenobarbital-type inducer, and are examining the effects of other purified PBB components on these hepatic enzymes. Male rats were given one 90 mg/kg ip injection of either DBB, a trace component of PBBs, or of HBB, a major (27%) congener, and were sacrificed 1 to 22 days later. DBB had little, if any, effect on any parameter examined. In contrast, HBB, increased liver weights, and strongly induced microsomal protein, NADPH-cytochrome P-450 reductase, cytochrome P-450, aminopyrine demethylase, and epoxide hydratase. These effects were apparent within several days after treatment, and were still pronounced at Day 22. HBB, caused only small increases in benzopyrene hydroxylation and UDP-glucuronyltransferase, and failed to shift the cytochrome P-450 spectral maximum from 450 nm. These results, and the results of SDS-polyacrylamide gel electrophoresis, indicate that HBB, like HBBb, is strictly a phenobarbital-type inducer of hepatic microsomal drug metabolizing enzymes, and that all brominated biphenyls are not inducers. Seventeen percent by weight of Firemaster remains uncharacterized; one or more of these components is responsible for the 3-methylcholanthrene-like aspects of the mixed-type induction caused by the PBB mixture.

280. Neurotoxic Effects of Dieldrin. Karen Swanson and Dorothy Woolley, Departments of Animal Physiology and Environmental Toxicology, University of California, Davis, California.

In an effort to understand better the mechanism of dieldrin neurotoxicity, we examined dieldrin's effects on electroshock seizures and brain electrical activity in adult rats. The effects of a single dose on maximal electroshock seizure (MES) pattern and electroshock seizure threshold (EST) in male and female rats and on evoked potentials (EPs), spontaneous electrical activity (SEA), and behavior in female rats with chronically implanted brain electrodes in limbic and cortical areas were determined. Dieldrin in corn oil was administered by intubation. In the MES test a dose of 45, 50, or 55 mg/kg significantly increased duration of tonic flexion 6 hr later, increased duration of total tonic 6, 12, 24, and 48 hr later, and hastened recovery of the righting reflex after the seizure. The EST was markedly lowered 4 hr after 40 mg/kg and 3 days were required for recovery, whereas 12.5 and 25 mg/kg only slightly lowered EST after 4 hr. Amplitudes of hippocampal SEA and of hippocampal EPs elicited by stimulation of the olfactory cortex were greatly increased from 3 to 9 hr after 25 mg/kg. Amplitudes of hippocampal EPs elicited by septal stimulation were also increased but less so than were olfactory-evoked hippocampal potentials. Spontaneous repetitive forepaw clonus was associated with high amplitude slow waves (5 to 7 c/s), which were especially prominent in the cortex and septum. We propose that the convulsant effect of dieldrin may be mediated via effects on the hippocampus and other limbic structures.


There is a paucity of information on effects of halogenated benzenes on the pancreas. We have found that bromobenzene (BB, 5 mmol/kg, ip, 24 hr) increases pancreatic juice flow 800% in the rat. The concentration of protein, bicarbonate, sodium, and potassium in pancreatic juice of BB-treated rats was lower than that of control animals (p < 0.05) while chloride concentration was similar. Cyclic AMP content of the pancreas was also similar in control and BB-treated rats when measured 24 hr after treatment, a time when pancreatic juice flow was different. Pretreatment with 3-methylcholanthrene (20 mg/kg, ip, 88, 72 and 64 hr before BB) protected against the BB-induced increase in pancreatic juice flow and the decrease in its protein concentration. Pretreatment with cystein (1.9 g/kg, po, 30 min before BB) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 10 μg/kg, po, 20 days before BB) decreased juice flow from that seen with BB alone (p < 0.05) but flow was still higher than control rats not receiving BB. In control rats and rats treated with a small dose of BB (1.4 mmol/kg, ip, 24 hr) pancreatic flow was similar. Phenobarbital treatment (80 mg/kg/day, 3 days) followed 1 day later by a small dose of BB did not increase pancreatic flow even though liver damage was more severe. Finally treatment with the small dose of BB (1.4 mmol/kg) 1 day before a large dose of BB (5 mmol/kg)
did not prevent the increase in pancreatic flow. Effects of halogenated benzenes on the pancreas were not limited to BB. Pretreatment for 24 hr with 5 mmol/kg, ip, of chlorobenzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene, and to a lesser extent 1,3,5-trichlorobenzene also increased pancreatic juice flow and decreased its protein content. The only chlorinated benzene tested which had no effect on pancreatic excretory function was 1,4-dichlorobenzene. (Supported by NIH Grant ES01332.)

282. Effects of Pregnenolone-16α-Carbonitrile and Spironolactone on Biliary Excretion of Ouabain in 2,3,7,8-Tetrachlorodibenzo-p-dioxin Treated Rats. R. E. Peterson and N. Hamada, University of Wisconsin, Madison, Wisconsin.

Pretreatment of rats with a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, 25 μg/kg, po) for 10 days depressed bile flow, plasma disappearance, and biliary excretion of ouabain in vivo and in the isolated perfused rat liver preparation. Administration to TCDD-treated rats of pregnenolone-16α-carbonitrile (PCN, 75 mg/kg/day) or spironolactone (S, 75 mg/kg/day) on days 6 to 9 after TCDD caused plasma disappearance and biliary excretion of ouabain on Day 10 to be similar to control rats not given TCDD. This effect of PCN and S was seen both in vivo and isolated perfused liver experiments. Bile flow in TCDD + PCN and TCDD + S groups was intermediate between TCDD and control. To determine if PCN or S would protect against the depressant effect of TCDD on ouabain excretion, rats were treated with PCN or S (75 mg/kg/day) for 4 days before TCDD (25 μg/kg) and excretory function was assessed 10 days after TCDD. Biliary excretion of ouabain in the PCN + TCDD group was similar to control while S + TCDD was similar to rats given TCDD only. PCN or S (75 mg/kg/day) was also given on Days 6 to 9 after TCDD (25 μg/kg) and biliary function assessed 20 days after TCDD. Again, excretion of ouabain in the TCDD + PCN group was similar to rats not given TCDD whereas TCDD + S was similar to rats given TCDD only. Finally, administration of PCN or S for 4 days before [3H]TCDD (25 μg/kg, po) or on Days 6 to 9 after [3H]TCDD did not alter the amount of TCDD derived 3H in the liver measured on Day 10. Thus, PCN and S improve hepatic excretory function for ouabain in TCDD-treated rats by a mechanism that does not appear to involve a reduction in hepatic content of TCDD. Also PCN treatment protected against and reversed for a longer time than S, the depressant effect of TCDD on ouabain biliary excretion. (Supported by NIH Grant ES01332.)

283. Toxicity of 1,2,4-Trichlorobenzene in Rhesus Monkeys: Comparison of Two in Vivo Methods for Estimating P-450 Activity. S. T. Cragg, G. F. Wolfe, and C. C. Smith, Department of Environmental Health, University of Cincinnati, College of Medicine, Cincinnati, Ohio.

1,2,4-Trichlorobenzene (TCB) was administered orally in daily doses of 1 to 25 mg/kg to groups of four rhesus monkeys for 120 days. To investigate the effects of TCB on enzyme induction two methods were employed. The first involved determination at monthly intervals of three urinary metabolites of labeled chloroguanide ([14C]CG). These data indicated that oral doses of TCB from 1 to 25 mg/kg caused no significant induction of cytochrome P-450 or P-448. This conclusion was corroborated by the second method consisting of comparing the \( t_{1/2} \) of TCB in animals treated with 25 mg/kg and in control animals. Data on weight changes, hematology, and clinical chemistry, as well as a clinical examination, indicated no evidence of toxicity for doses up to 25 mg/kg. Administration of 125 mg/kg caused temporary weight loss and was lethal to one of four monkeys. The surviving animals of this group showed a significant change in the urinary profile of CG metabolites indicating induction of P-450. A second experiment to better define the toxic and inductive effects of TCB is under way using doses of 90, 125, and 173.6 mg/kg/day. (Supported in part by NIEHS Training Grant ES-00127 and USEPA Grant R-803963.)

284. Responses of Rhesus Monkeys to Polybrominated Biphenyls. J. R. Allen and L. Lambrecht, Department of Pathology and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin.

Rhesus monkeys have been fed diets containing 0.3, 1.5, and 25 ppm of a commercial mixture of polybrominated biphenyls (PBB) (Firemaster, FF-1). Seven adult females have been on the
0.3 ppm of PBB for over 1 year and during this period have consumed over 25 mg of the PBB mixture. In addition to a loss of weight, the monkeys experienced changes in the levels of serum estradiol and progesterone during the initial 6 months of exposure which could be correlated with altered menstrual cycles. After 6 months of exposure the animals were bred and all conceived after one to four breedings. Two of the seven animals aborted, and the remaining five had term infants. The monkeys consuming 1.5 ppm of PBB have been on the diet for over 5 months, during which they have consumed approximately 75 mg of PBB. These animals experienced a moderate weight loss and periorbital edema. The third group, which received 25 ppm of PBB in the diet for 10 weeks consumed approximately 350 mg of PBB. They experienced a weight loss, abdominal distension, and diarrhea. In addition, the latter two groups of animals show alterations in B and T cell function. These data suggest that relatively low levels of PBB exposure may affect reproduction, weight gain, and immunologic competence of rhesus monkeys.


Teratology and reproduction studies of vinylidene chloride (VDC) were conducted in Sprague-Dawley rats and New Zealand rabbits. The teratogenic potential of inhaled VDC was evaluated in both species. Bred rats were exposed to VDC at concentrations of 0, 20, 80, or 160 ppm for 7 hr/day on Days 6 through 15 of gestation. Bred rabbits were exposed to 0, 80, or 160 ppm of VDC for 7 hr/day on Days 6–18 of gestation. The teratogenic potential of ingested VDC was evaluated in rats maintained on drinking water containing 0 or 200 ppm of VDC during Days 6 through 15 of gestation. Vinylidene chloride was not teratogenic in rats or rabbits inhaling up to 160 ppm or in rats given water containing 200 ppm of the compound. Some evidence of embryotoxicity and fetotoxicity was observed in both rats and rabbits inhaling VDC at maternally toxic levels. A three-generation reproduction study was conducted in Sprague-Dawley rats. Male and female rats were continuously maintained on drinking water containing 0, 50, 100, or 200 ppm of VDC. Some reproductive parameters were affected on a sporadic basis. These latter changes were not related to dose level and were not consistent from one generation to the next, and thus, did not appear to be an effect of VDC on reproduction.


In an earlier 4-week study (Villeneuve et al., *Sci. Total Environ.*, in press) food deprivation was observed to increase the biological activity of hexachlorobenzene (HCB) in rats. The present study was carried out in order to determine the effects of a longer period of food deprivation on the toxicity, distribution, and excretion of HCB. Female rats (30–90 g) were divided into 10 groups of 10 animals each. Groups 1, 3, 5, 7, and 9 were fed diets ad libitum containing 0, 4, 20, 100, and 500 ppm of HCB. Groups 2, 4, 6, 8, and 10 were fed 50% of the amount consumed by groups 1, 3, 5, 7, and 9, respectively, and their diets contained 0, 8, 40, 200, and 1000 ppm of HCB. The above feeding regimen continued for 15 weeks, during which time food intake and body weight changes were measured routinely. The animals were then killed; their tissues were excised, weighed, and frozen pending residue analysis. Other parameters measured included SGPT, alkaline phosphatase, and urea levels in serum, liver microsomal enzyme activity, triglyceride and porphyrin content of the liver, and porphyrin levels in the urine. Routine hematology tests and histological examinations of lung, liver, spleen, kidney, thymus, and adrenals were also carried out. Tissue profiles were established for serum, liver, and brain. Food deprivation was observed to increase the ability of HCB to cause liver hypertrophy and induce microsomal enzyme activity. Food deprivation also increased HCB-induced porphyrin
accumulation in the liver and excretion in the urine. The accumulation of HCB in brain and liver and fecal excretion of HCB was increased in food deprived animals. These results confirm earlier findings and indicate that food deprivation can alter the biological activity of HCB at levels in the diet of 20 ppm or higher.


Cd, a pollutant which is widely distributed in the environment, has been shown to inhibit hepatic drug-metabolizing enzymes in mammals. Some studies indicate that the Cd-induced alterations are time dependent and tolerance to Cd inhibition has been reported. This study reports the effects of acute and chronic Cd pretreatment on the metabolism of lindane by rats both in vivo and in vitro, at 3 days and 1, 2, 3, and 5 weeks after exposure. Eighty adult male Wistar rats were divided into four exposure groups. One group was injected sc, three times a week throughout the experiment, with 0.75 mg/kg of Cd. The other three groups received one sc injection of either 0, 1.04, or 3.20 mg/kg of Cd. Twenty-four hours prior to sacrifice four rats from each group received a po dose of 1.83 mg of lindane (containing 2.5 μCi of 14C-lindane). Three days after exposure, the control rats excreted significantly more radioactivity than the Cd-treated groups. While no significant differences were noted at 1 or 2 weeks after exposure, at 3 weeks both the control rats and those receiving repeated Cd injections excreted significantly more radioactivity than the other two groups. At 5 weeks, moreover, the controls excreted significantly more radioactivity than any of the rats exposed to Cd. The distribution of neutral and polar lindane metabolites was significantly altered by Cd exposure. Dehydrogenation of lindane to hexachlorocyclohexene was significantly inhibited at 3 days and 2, 3, and 5 weeks after Cd exposure. The dechlorination of lindane to 3,4,5,6-tetrachlorocyclohexene was inhibited at all time intervals and the hydroxylation of hexachlorocyclohexene to 2,3,4,5,6-pentachloro-2-cyclohexene-1-ol was inhibited at 1, 2, 3, and 5 weeks. Results of this study indicate that Cd pretreatment causes inhibition of lindane metabolism which is of long duration and which is not relieved by chronic exposure to the metal.


There is evidence that Cd affects microsomal enzymes which metabolize pesticides such as lindane. This study was designed to determine if acute or chronic exposure to Cd affected the liver microsomal enzyme activities and lindane metabolism. This paper reports on the microsomal enzymes and tissue Cd and Zn concentrations. Three groups of 20 male Wistar rats were given 0, 1.04, or 3.20 mg/kg of Cd as a single sc dose. A fourth group was given repeated doses of 0.75 mg/kg of Cd, 3 days/week throughout the experiment. Animals from each group were killed at 3 days and 1, 2, 3, and 5 weeks. At 24 hr prior to sacrifice, animals were given a po dose of 1.83 mg of lindane (containing 2.5 μCi of 14C-lindane). Liver, kidney, and lung tissue were assayed for Cd and Zn by atomic absorption spectroscopy. Microsomal β-glucuronidase, cytochrome P-450, cytochrome c reductase, and aminopyrine demethylase were measured. The liver Cd concentrations in the 1.04 and 3.20 mg/kg dose treatments were 40 and 60 ppm, respectively, throughout the experiment. The liver Cd concentration in the repeatedly dosed animals increased from 33 to 116 ppm from 3 days to 5 weeks of treatment. The liver Zn concentration in all Cd treated animals were between two and three times the control values throughout the experiment. The microsomal enzyme activities for the singly injected groups were 50 to 60% that of control on Day 3 and rose to within 80 to 90% of control values by the fifth week. The repeatedly dosed animals showed no effect until the fifth week, when there was a small but significant decrease in all enzyme activities. The recovery of the initial inhibition of microsomal enzyme activity and lack of inhibition in the divided group is consistent with known induction of the protective Cd binding protein, Cd metallothionein.
289. **Long-Term Turnover and Stability of Cd-Metallothionein and Translocation of Cd following an Initial Low Cd Dose in Rats.** J. W. RIDDLINGTON, D. R. WINGE, and B. A. FOWLER, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina, and Biochemistry Department, Duke University, Durham, North Carolina.

Male rats were injected subcutaneously on two consecutive days with either saline or 2 mg of Cd/kg to induce Cd-metallothionein. At the end of 1, 2, 3, 4, and 6 months, control and Cd-treated animals were pulse-labeled with \[^{35}\text{S} \]cysteine (20 \( \mu \)Ci/animal) 2 hr prior to sacrifice. The kidneys and livers were immediately removed and the Cd concentrations in heat-treated supernatant material from liver and kidney determined by atomic absorption spectrophotometry to ascertain the rate of Cd translocation from liver to kidney. The kidney to liver ratio for total organ Cd content was found to be 0.137, 0.201, 0.244, 0.287, and 0.315 for Months 1, 2, 3, 4, and 6, respectively, suggesting a slow but definite translocation of Cd. Total animal Cd content remained constant during the study indicating basically no Cd excretion during this time period. The metallothionein-containing heat-treated supernatant fraction was further examined by Sephadex G-75 column chromatography with 10 mM phosphate buffer, pH 7.8. Following chromatography, the ratio of [Cd]:cystine incorporated in the metallothionein peak was calculated for both liver and kidney at each monthly time period. For each month there was a significant incorporation of \[^{35}\text{S} \]cysteine into metallothionein while the [Cd]:cystine ratio remained constant demonstrating that one initial exposure to Cd does not lead to a permanent Cd-metallothionein but rather that the protein continues to turnover at a constant rate. Further purification of metallothionein by DEAE-column chromatography of material from the 4- and 6-month animals showed that both A and B forms were present in relatively constant amounts indicating similar turnover rates and stabilities for either form with time.


Thirty male dogs, 11 to 13 months of age, were divided into five groups and given 0, 0.0625, 0.25, 1.0, and 4.0 mg/kg of Firemaster BP-6 orally for 61 days. Weight loss was significant after 30 days in animals at the highest dose level. The supplemental feeding of meat to all dogs in the study reversed the decline but body weight did not return to pretreatment levels. One dog in the 4 mg/kg group had convulsions on the 50th and 52nd days and appeared to be blind thereafter. Another dog in the 4 mg/kg group died on the 58th day; gross autopsy revealed gastrointestinal hemorrhage. Blood smears taken at the 30th and 61st days of the study revealed juvenile lymphocytes along with cells that appeared to be degenerating lymphocytes in some dogs at all levels; these changes were particularly evident at the 0.25 and 1 mg/kg dose levels. Hematopoiesis, especially erythropoiesis, was markedly reduced in bone marrow of the 4 mg/kg group and a marked increase in large reticuloendothelial cells with foamy cytoplasm was seen. Three of six dogs at the 1 mg/kg level showed similar changes but to a much lesser extent. Lymph nodes at the 4 mg/kg level showed variable degrees of depletion of lymphocytes, particularly in the T-cell zone; these changes were minimal at the 1 mg/kg level. In dogs at the 4 mg/kg level, marked extramedullary hematopoiesis, predominantly of an erythropoietic and megakaryopoietic nature, was seen in the spleen and lymphocytes were moderately reduced in the white pulp. At the 1 mg/kg level, extramedullary hematopoiesis was mild to minimal. A variable degree of involution of thymic tissue was observed at all dose levels. Anti-dog IgG fluorescein-conjugated rabbit antibody was used to determine the distribution of plasma cells in the popliteal lymph node. Plasma cell numbers of dogs at the 4 mg/kg level were markedly reduced compared with those of the control group.

291. **Determination of the Teratogenic and Mutagenic Potential of Griseofulvin.** R. L. STEELMAN and J. J. KOCSIS, Pathology/Toxicology Department, McNeil Laboratories, and Pharmacology Department, Thomas Jefferson University, Philadelphia, Pennsylvania.

The teratogenic potential of the antifungal agent griseofulvin (GF) was investigated in rats by utilizing the increased absorption made possible by solubilizing the drug in polyethylene glycol.
300. The mutagenic potential of GF was evaluated in dominant lethal, cytogenetic, and bacterial tests with and without microsomal enzymes. The teratogenic effects of micronized GF dissolved in PEG 300 at doses of 50, 250 and 500 mg/kg on Days 6 to 15 of pregnancy were studied. A dose response of increasing depression of pup birth weight, decreasing number of live fetuses, and increasing number of resorptions occurred. Internal hydrocephalus and subcutaneous hemorrhage may have been related to treatment with GF. Skeletal variations and malformations, which were minimal at the low dose and more severe at the higher doses, included cervical and thoracic asymmetric axis, misalignment, misshapen, missing or fused vertebrae, incompletely ossified or split centra, wavy, fused, or incompletely ossified ribs, misshapen clavicle, scapula, radius, and ulna. Also, parts of ossification centers in the sternum, caudal vertebrae, metacarpals, and metatarsals showed a decreased number in treated pups compared to controls. Griseofulvin was consistently without mutagenic activity in dominant lethal, rat bone marrow cytogenetic, and Ames direct and liver microsomal bacterial tests.


Linuron, malathion, and methoxychlor are among the most commonly used pesticides and their residues have been detected in various foods. Teratogenic potential of these pesticides remains to be determined. Technical grade of linuron, malathion, and methoxychlor, and formulations of linuron and methoxychlor, were intravenously in single daily doses on Days 6 to 15 of gestation in rats. Pregnancies were terminated at term by cesarean section. Fetuses were evaluated for survival, growth, and visceral and skeletal development by routine teratologic methods. No adverse effect was observed upon administration of technical grades at doses of up to 100 mg of linuron/kg body wt and 300 mg of malathion/kg and for linuron (Hoechst) formulation up to 200 mg/kg. Formulation of linuron (Dupont) produced high incidence of anomalous fetuses at 200 mg/kg but not at 100 mg/kg. Treatment with methoxychlor, technical or in the formulation, was associated with reduced maternal body weight gain during gestation at all test doses which ranged from 50 to 400 mg/kg. Both methoxychlor samples were teratogenic at 200 and 400 mg/kg and produced a dose-related increase in wavy rib anomaly at 100, 200, and 400 mg/kg/day.


Handling bred mice during early gestation lowers fertility indices. We replicated this previous finding and also determined the incidence of spontaneous anomalies in a new mouse strain [Upj : TUC(ICR)]spf for use in teratologic studies. We examined fetuses from mice in the following test groups: unhandled or untreated, untreated but weighed on gestation Days 6 to 15, treated orally with 0.25% methylcellulose vehicle on gestation Days 6 to 15, treated orally with sterile water on gestation Days 6 to 15, treated orally with cyclophosphamide at 20 mg/kg on gestation Day 10, and treated orally with 2.5 mg/kg hydrocortisone on gestation Days 11 to 14. Each test group (except for hydrocortisone) had a minimum of 25 pregnant dams. All fetuses were collected on gestation Day 18. The incidence (5.4%) and types of anomalies found in fetuses from dams that were weighed or weighed and treated with the vehicles was comparable to those found in fetuses from dams that were neither handled nor treated (6.8% incidence). The most common anomalies were cleft palate, talipes, left unibibular artery, and sternoepleural changes. The most frequent skeletal variations were cervical ribs, accessory (lumbar) ribs, and combinations thereof. With the exception of dams given cyclophosphamide, no statistically significant increase in numbers of resorption sites or decrease in numbers of live fetuses, maternal or fetal body weights occurred in any test group. The TUC(ICR) mouse was very sensitive to cyclophosphamide and hydrocortisone. Ninety-five percent of fetuses from dams given cyclophosphamide were malformed. The incidence of cleft palate in the untreated and
vehicle-treated groups was 1.2%. However, 33.3% of the fetuses from dams given hydrocortisone had cleft palates and this difference was highly significant.


It is essential to characterize the effects of prostaglandins on reproduction and development as part of the safety evaluation process. Brd Dutch-belted rabbits were given 0.0, 0.5, 0.75, 1.0, and 1.5 mg/kg, of PGE₂ by subcutaneous injection at various times between the 6th and 18th days of gestation. Doses as large as 0.75 mg/kg did not interrupt pregnancies when given daily from the 6th through the 18th day of gestation and doses as large as 1.5 mg/kg did not interrupt pregnancies when administration was limited to Days 9 to 18. Most treated does had diarrhea but there were no other signs of toxicity. The incidence of gross and visceral anomalies in fetuses produced by treated dams was low and comparable to the incidence in control fetuses. Fetuses from treated dams had a slightly higher incidence of skeletal anomalies but since that response was neither dose-related nor statistically significant, it was considered to be a nonspecific and secondary effect. These findings are consistent with results of other teratology studies involving the administration of prostaglandins to pregnant animals. The relative sensitivity of pregnant rabbits to small doses of prostaglandins (especially when given during the early phases of gestation) has been reported previously and was confirmed in this study.


The embryotoxicity and teratogenicity of nickel carbonyl, Ni(CO)₄, were investigated in Fischer rats. Rats were placed in an exposure chamber and exposed to inhalation of specified concentrations of Ni(CO)₄ for 15 min. The concentrations of Ni(CO)₄ in the chamber were monitored by gas chromatography. Based upon a pilot study of 88 nonpregnant adult Fischer rats, the LD₅₀ of Ni(CO)₄ is 0.58 (SE ± 0.09) mg of Ni(CO)₄/liter/15 min, and the LD₅ is 0.12 mg of Ni(CO)₄/liter/15 min. Pregnant rats were exposed on Day 8 of gestation to inhalation of Ni(CO)₄ in dosages of 0.06 mg of Ni(CO)₄/liter/15 min (group A, N = 6) and 0.12 mg of Ni(CO)₄/liter/15 min (group B, N = 7). Rats in a control group (N = 12) were placed in the exposure chamber for 15 min without Ni(CO)₄ exposure. The dams were killed on the 20th day of gestation. The number of live fetuses per dam (mean ± SD) was 9.2 ± 2.1 (controls), 7.2 ± 3.1 (group A), and 6.3 ± 4.7 (group B) (p < 0.02). The ratio of dead fetuses to conceptuses was 4/114 (3.5%, controls), 2/45 (4.4%, group A), and 15/59 (25.4%, group B) (p < 0.005). The body weights (mean ± SD) of live fetuses were 3.36 ± 0.19 (g controls), 3.01 ± 0.38 (g group A), and 2.79 ± 0.31 g (group B) (p < 0.001). Ophthalmic malformations were found in 0/110 (0%, controls), 12/43 (28%, group A) (p < 0.001), and 12/44 (27%, group B) (p < 0.001) of the live fetuses. The ophthalmic malformations included unilateral microphthalmia (15), bilateral microphthalmia (6), and unilateral anophthalmia (3). These findings demonstrate that Ni(CO)₄ is embryotoxic and teratogenic in Fischer rats. (Supported by ERDA Contract E(11-1)-3140.)


Ethylenethiourea (ETU), a food contaminant resulting from the degradation of ethylenebisdithiocarbamate group of fungicides, was used to study the teratogenic effects on the developing central nervous system of the rat. A single oral dose, 300 mg/kg, of 1.6% aqueous solution of ETU was administered by intubation to the 20 experimental pregnant female rats of wistar strain on Day 12 of pregnancy while 5 control rats were dosed only with distilled water. All pregnant rats were sacrificed on Day 22 of gestation. The average weight of the experimental fetuses was 3.86 ± 0.57 g as compared to the control 5.40 ± 0.36 g. The fetuses were fixed in
10% buffered neutral formalin and Bouin's fixative for histological preparations. One hundred ninety-six fetuses from the experimental group were grossly and microscopically examined for the central nervous system defects. The cranial defects were found as 38% encephaly, 30% hydrocephalus, and 20% hydronephaly associated with other abnormalities, such as agnathia micrognathia, abnormal limbs, and tail defects. None of these defects were found in the controls. In exencephalic fetuses the open neural plate was found in the midbrain region. It was suggested that ETU may reinforce the neural tube to reopen by affecting the target cells of the mesencephalon. Hydrocephalic fetuses were recognized by dome-shaped head with or without meningoencephalocele in the occipital region and those which had normal appearance of the head. These anomalies were also associated with short tail or absence of tail. The cerebral aqueduct was occluded and the choroid plexus was congested. As a result of this occlusion lateral ventricles cranial to this lesion and the duct caudal to the occlusion was dilated. Hydronephaly was identified as the round head bulging forehead and a transparent fluid filled brain cavity. Patches of brain tissue were left with the hypoplastic choroid plexus. With the scanning electron microscopic technique, the neuron was found degenerated, leaving a large intercellular space. The synaptic relation between the nerve cells appeared disrupted. Most cranial defects revealed disorganization and degeneration of nervous tissue, localized absence of ependymal cells, rosettes formation, dark staining of undifferentiated cells (H & E), and incomplete osteogenesis of the skull bones. Spinal cords of these fetuses showing cranial defects also manifested hypoplastic degeneration, distortion, and absence of the spinal canal. There was no typical inflammatory reaction in these central nervous system anomalies exhibiting degenerative changes.


Quaternary ammonium compounds are currently used as household disinfectants, adjuncts to surgery, in treatment of food contact surfaces, and in outdoor swimming pools. Aliphatic quaternary compounds representing variations in the side chains were administered to pregnant Wistar-derived rats throughout organogenesis at doses of 5, 15, and 50 mg/kg body wt. On Day 20 the dams were subjected to caesarean section and the uterine contents were examined. One-third of the pups in each litter were randomly chosen for soft tissue examination and the remaining pups were cleared and stained for skeletal examination. The results indicate the compounds did not induce terata at any dose level but a slight increase in rudimentary ribs was observed in five pups distributed among three dams of the 50 mg/kg group of one of the compounds. This is considered a result of possible maternal stress since two dams died at this dose level. However, no other signs of maternal toxicity were observed at the 50 mg/kg dosage level.


ORF 5656 (17-caproyl-17-ethinyl-19-nor-4-androstene-3-one oxime) is a long-acting antifertility agent when administered subcutaneously or intramuscularly in both the rat and the rabbit. A study was performed to assess the teratogenic potential of ORF 5656 in the rat and the rabbit. A single intramuscular injection of ORF 5656 was administered to Long-Evans derived hooded rats on Day 6 of pregnancy at dosage levels of 0.4, 1.6, and 4.8 mg/kg in an oil vehicle solution and to albino rabbits on Day 7 of pregnancy at dosage levels of 0.4, 0.8, and 1.6 mg/kg. A caesarean section was performed on the rat and the rabbit on Days 20 and 30, respectively. Fetuses were examined for gross visceral and skeletal malformations. There were dose-related increases in fetal resorptions at the 0.8 and 1.6 mg/kg dosage levels in rabbits and at the 1.6 and 4.8 mg/kg dosage levels in rats. No significant rate of fetal resorption occurred in either the rat or the rabbit at 0.4 mg/kg dosage level. There were no teratogenic effects of ORF 5656 observed in both rats and rabbits at the dosage levels tested.

Ethylene dibromide (EDB) was administered at 0, 20, 38, and 80 ppm by inhalation to pregnant Charles River CD rats and CD-1 mice for 23 hr/day. The exposures started on Day 6 of gestation and lasted for a total of 10 days. EDB produced effects on the dam, as measured either by weight change or feed consumption, in both species at all of the doses tested. In addition, a significant increase in adult mortality occurred in rats exposed to 80 ppm of EDB and in mice exposed to 38 and 80 ppm of EDB. Fetal mortality, as measured by the incidence of resorptions, was increased in rats exposed to 80 ppm of EDB and in mice exposed to 38 ppm of EDB. Body weights of fetuses from rats exposed to 38 ppm of EDB and from mice exposed to 20 and 38 ppm of EDB were reduced. These effects indicate that EDB (1) adversely affected maternal welfare in both rats and mice; (2) was more toxic in pregnant mice than pregnant rats; (3) produced morphological changes in fetuses from exposed dams only at concentrations that also affected maternal welfare; and (4) was judged to have little primary effect on development.

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Much of the available evidence suggests that the protective effect of selenium in heavy-metal toxicity is due to the formation of an insoluble metal selenide. The present study in mice shows that mercury and selenium mutually protect against each other's toxicity and raises the question as to whether this effect is due to a simple 1:1 in vivo reaction to form highly insoluble mercuric selenide (HgSe). Sodium selenite (Na₂SeO₃) (40 μmol/kg, sc) significantly lowered the mortality produced by mercuric chloride (HgCl₂) (60 μmol/kg, sc) when administered from 8 hr before to 1 hr after HgCl₂. When injected 1 hr before HgCl₂ (60 μmol/kg, sc), a dose which killed all mice, Na₂SeO₃ at 40 and 60 μmol/kg, sc, reduced mortality to 20 and 50%, respectively; at 80 μmol/kg, sc, Na₂SeO₃ did not reduce mortality. When 90 μmol/kg of Na₂SeO₃ was injected sc, 12/15 mice were killed, but a nonlethal dose of HgCl₂ (20 μmol/kg, sc) given 1 hr before Na₂SeO₃ (90 μmol/kg, sc) reduced mortality to 1/15. Higher doses of HgCl₂ did not protect. The sc L50 of mercuric selenide (HgSeO₃), a compound formed when equimolar solutions of Na₂SeO₃ and HgCl₂ are mixed, was 36.5 μmol/kg, approximately the same dose of Na₂SeO₃ (40 μmol/kg) that protected against an even larger dose of HgCl₂ (60 μmol/kg). HgSe, although extremely insoluble (KSP 1 x 10⁻³⁹ m), had an L50 of 400 μmol/kg when injected sc in suspension. These findings argue against the hypothesis that selenium counteracts the toxicity of heavy metals simply by the formation of chemically and biologically inert metal selenides.

301. Metabolism of Intravenously Injected Renal Mercurithionein in Rats. K. Hirayama and Z. A. Shaikh, Department of Pharmacology and Toxicology, University of Rochester, School of Medicine, Rochester, New York. (T. W. Clarkson)

The metabolism of rat hepatic Cd-thionein has been reported previously by us and by others. The purpose of this investigation was to study the metabolism of rat renal thionein produced in response to Hg²⁺. The protein was induced in rat kidneys by repeated injections of HgCl₂. It was labeled in vivo with either ²⁰³Hg or [³⁵S]cysteine. The protein was isolated and injected intravenously into Wistar rats (235 ± 5 g). Nearly half of the injected [²⁰³Hg]thionein was taken up by the kidneys in 3 hr at dose levels of 0.01 to 1 mg of protein. At the 5-mg dose the uptake by the kidneys was 20% lower. The excretion of [²⁰³Hg]thionein in urine was proportional to the injected dose of protein. At the highest dose level (5 mg), 45% of the injected ²⁰³Hg was recovered in urine. Further fractionation of the kidneys revealed that 60% of the tissue ²⁰³Hg was in the soluble fraction, of which two-thirds was associated with metallothionein and the remaining to the higher molecular weight proteins. The intracellular distribution of ³⁵S label in kidney at 0.5 mg of protein dose was very similar to that of the ²⁰³Hg label 1 hr after the injection. However, after 48 hr, when as much as 27% of the injected ²⁰³Hg was bound to
metallothionein, only 3% of the $^{35}$S was found in this protein. This rapid degradation of the thionein moiety was also evident from the gel filtration analysis of the urine from animals given $^{35}$S-labeled Hg-thionein. It is concluded from this study that the excretion of $^{109}$Hg-thionein in the urine is proportional to the dose of injected protein which is similar to what has previously been reported for $^{109}$Cd-thionein. It also appears that the injected Hg-thionein is degraded by the kidney at a much faster rate than that reported for the protein synthesized within the renal cells. (Supported by NIH Grants GM 15190, ES 01247, and ES 01448.)

302. Comparative Studies on the Gastrointestinal Absorption and Metabolism of Cadmium-Chloride and Cadmium-Thionein in Mice. M. George Cherian, Robert A. Goyer, and Leslie S. Valberg, Departments of Pathology and Medicine, Health Sciences Centre, University of Western Ontario, London, Ontario, Canada.

The differences in the absorption and metabolism of cadmium from its various bicomplexes in diet may have an important role in the toxicity of cadmium. Though the nutritional factors like iron deficiency may have a significant influence on the gastrointestinal absorption of cadmium there is little information on the absorption of cadmium from the different cadmium-containing compounds in the diet and its subsequent metabolism and toxicity. The largest source of cadmium in the diet is cereal or meat, especially in liver and kidney in the form of metallothionein. Since metallothionein is heat stable, this protein-metal complex is not destroyed by cooking and this may be the main dietary form of cadmium. In order to study the absorption and tissue distribution of cadmium from cadmium salts and metallothionein, two groups of mice (BL/57) were given radioactive $^{109}$Cd as cadmium chloride and cadmium-thionein in equal doses (60 µg and 2 µCi) by stomach tubes under mild ether anesthesia. Mice were counted immediately and every 24 hr for a period of 6 days in a whole body counter. At the end of the experimental period, they were sacrificed and the amount of cadmium in the organs and blood was compared in the two groups. Similar studies were carried out in iron-deficient mice. The absorption of cadmium from both forms was identical but retention of cadmium was increased in iron deficiency. However, the distribution of cadmium in the organs differed in the two forms of cadmium. A significantly greater deposition of cadmium was observed in the kidney in cadmium-thionein fed mice than in cadmium chloride groups where a major portion of cadmium was deposited in liver. These differences in distribution of cadmium were more significant in iron deficiency. A major portion of radioactive cadmium in both renal and hepatic tissues was bound to metallothionein. These results suggest that the dietary form of cadmium may have an important influence on the critical organ concentration of cadmium and this may be influenced by the iron status of the diet. (Supported by grants from the Medical Research Council of Canada.)

303. Induction of Hepatic and Renal Metallothionein in Cows, Pigs, and Chickens during Low-Level Cadmium Ingestion. Mahendra P. Verma, Raghurir P. Sharma, and Joseph C. Street, Toxicology Program, Utah State University, Logan, Utah.

Metallothionein (MT), a zinc storage protein in the animal body, has been assumed to protect the animals against cadmium (Cd) toxicity. Administration of certain metals, including Cd, induces MT synthesis in the liver and kidney of laboratory animals. In the present investigation, lactating cows, growing pigs, and laying chickens were given feed supplemented with 2 and 10 ppm of Cd as cadmium chloride for periods up to 24 weeks. Liver and kidney tissues were analyzed for MT content by a competitive mercury binding technique. Average MT contents (in milligrams per gram of wet tissues) in control cow, pig, and chicken liver were 0.16 ± 0.04, 0.84 ± 0.30, and 0.57 ± 0.54, respectively, and in kidneys were 0.06 ± 0.03, 0.20 ± 0.07, and 0.15 ± 0.10, respectively. Feeding of Cd-supplemented diet (10 ppm) for 12 weeks increased the MT content 509 and 244%, respectively, in the liver and kidney of cows. The corresponding rises in pigs were 87 and 630%, and in chickens were 215 and 3125% following a similar Cd exposure of 24 weeks. In cows, 12 weeks after the termination of 10-ppm Cd feeding, the hepatic MT values returned to control levels, whereas the renal MT showed a continued rise. In the
liver and kidney of pigs the MT values 12 weeks after cessation of Cd feeding were 188 and 675% of controls, respectively. Seven weeks after termination of 10-ppm Cd (following a 6-week exposure) feeding in chickens, the respective MT values were 204 and 1498% of controls. The increase in MT contents of the tissues were related to their Cd concentrations. (Supported in part by USPHS-FDA 223-74-7195.)

304. Inhibition by Lead and Cadmium of Human Na\(^+\)+K\(^+\)-ATPase Activity. B. R. Nechay and J. P. Saunders, Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas.

This extends our studies on inhibition characteristics of sodium- and potassium-dependent adenosine 5'-triphosphatase by metals to the enzyme of human origin. The enzyme activity in renal microsomes was measured by hydrolysis of exogenous ATP in appropriate ionic environment. PbCl\(_2\) or CdCl\(_2\) was preincubated with the enzyme and electrolytes for 30 min before starting the reaction with ATP. \(I_{50}\) was \(5 \times 10^{-5}\) M for Pb and \(10^{-4}\) M for Cd. According to Ackermann-Potter analysis the inhibition by Pb was reversible and that by Cd was irreversible. Pb, but not Cd, inhibition was antagonized by ATP and Na\(^+\). This suggests that Pb inhibits the enzyme directly at the site activated by Na\(^+\) and indirectly by chelating ATP. Cd appears to inhibit the enzyme by a different mechanism. There was certain specificity of both metals for Na\(^+\)+K\(^+\)-ATPase since this enzyme was at least 10-fold more sensitive to both metals than Mg\(^2+\)-ATPase. \(I_{50}\) remained the same when whole kidney homogenate was substituted for microsomal fractions. These results are similar to those obtained previously with the enzyme of several other species.

305. Kinetics of Cadmium and Zinc Competition in the Isolated Perfused Rat Liver. B. Stern Kingsley and J. M. Frazier, Department of Environmental Health Sciences, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland. (R. J. Rubin)

Previous investigations of cadmium transport in the isolated perfused rat liver (Frazier and Kingsley, *Toxicol. Appl. Pharmacol.* **38**, 583, 1976) suggested that cadmium uptake may involve both simple diffusion and a passive-mediated transport pathway normally operative for zinc. If this were true, cadmium and zinc should compete for carrier sites in sinusoidal membranes of hepatocytes. Livers from male Wistar rats were surgically isolated and perfused via the portal vein with Krebs-Ringer bicarbonate buffer supplemented with 4% bovine serum albumin and 0.8 g/liter of dextrose. Zinc concentrations in the perfusion medium were initially adjusted to equal the available zinc concentration in rat plasma (0.7 \(\mu\)g/ml). Following a 60-min control period, four treatments (four livers per treatment) were imposed: (1) control, (2) addition of 324 \(\mu\)g of Cd (equivalent to 1 \(\mu\)g of Cd/ml in the perfusion medium; Zn : Cd molar ratio of 1.9), (3) addition of 2350 \(\mu\)g of Zn (equivalent to 7.4 \(\mu\)g of Zn/ml), and (4) addition of 324 \(\mu\)g of Cd and 2350 \(\mu\)g of Zn (Zn : Cd molar ratio of 12.5). Samples of perfusion medium and bile were collected at fixed intervals throughout the experiment (300 min) and analyzed for cadmium and zinc. The normalized uptake rate constant (NRC, rate constant divided by liver weight) was determined. Excess zinc-suppressed cadmium uptake as indicated by the decrease in the NRC from 0.350 ± 0.019 ml/min/g (mean ± SE) in Treatment 2 to 0.142 ± 0.014 ml/min/g in Treatment 4. The uptake of cadmium enhances the net export of hepatic zinc into the perfusion medium in Treatment 2. Exposure to cadmium significantly depressed bile flow; excess zinc did not prevent this toxic response. Total cadmium excreted in the bile during the entire exposure period is significantly less in Treatment 4 (3.59 ± 1.28 \(\mu\)g) compared to Treatment 2 (9.18 ± 2.71 \(\mu\)g). Cadmium exposure increased biliary excretion of zinc from control levels of 0.42 ± 0.05 to 4.23 ± 1.01 \(\mu\)g in Treatment 2 and 4.39 ± 1.17 \(\mu\)g in Treatment 4. Gel permeation chromatography profiles of liver cytosol (106,000g 60-min supernatant) were obtained using Sephadex G-75. Cadmium was mainly bound to high molecular weight macromolecules (70–90% of total cytosol cadmium). In summary, the competitive effects seen between cadmium and zinc lend support to the hypothesis that these two metals utilize the same carrier on the hepatocyte membrane.
306. Cadmium-Induced Suppression of Cellular Immunity in Mice. D. W. Barnes and A. E. Munson, Department of Pharmacology, East Carolina University, School of Medicine, Greenville, North Carolina and Medical College of Virginia, Richmond, Virginia. (J. P. Dvanzo)

Suppression of humoral immunity has reportedly been produced in mice which have been exposed to cadmium or other heavy metals in drinking water. In the present study, the effects of cadmium on delayed-type hypersensitivity (DTH) and the functional activity of the mononuclear phagocytic system (MPS) were determined. Groups of male and female ICR adult mice were orally gavaged with single, daily doses of 0.65, 32.6, or 65.2 mg/kg of cadmium chloride for either 14 or 90 days. The functional activity of the MPS was measured by blood clearance and organ distribution of 125I-labeled Listeria monocytogenes. The effect of cadmium on DTH was determined by footpad swelling in response to sheep erythrocytes (SRBC). After 14 days, treatment with the highest dose of cadmium produced a significant suppression of footpad swelling in both male and female mice. In female mice footpad swelling was also decreased after exposure to the lower doses. After 90 days of treatment, the DTH response to SRBC was decreased only at the high dose in males. In female mice suppression of DTH was significantly greater than in males, and was dose-dependent with almost total suppression at the highest dose. MPS activity was also decreased after 90 days of treatment as evidenced by significant reduction in uptake of Listeria into the livers of both female and male mice treated with the two highest doses. This study suggests that exposure to cadmium results in suppression of macrophage activity and cellular immunity, as well as humoral immunity.

307. In Vitro Effects of Dialkyltin Compounds on Suspended Rat Thymocytes. R. R. Miller, Rolf Hartung, and H. H. Cornish, Department of Environmental and Industrial Health, School of Public Health, University of Michigan, Ann Arbor, Michigan.

Atrophy of the thymus gland characterized by a depletion of thymic lymphocytes of the cortex has been noted in rats fed dimethyltin dichloride (DMTD) and diethyltin dichloride (DETD) in their drinking water. This response is apparently mediated by a mechanism independent of glucocorticoid activity as has been reported for di-n-octyltin dichloride. DETD was more potent than DMTD in vivo, and male rats were more susceptible than female rats. The in vitro effects of DETD and DMTD on suspended rat thymocytes were evaluated using a glucose/salt medium. Both DMTD and DETD reduced the number of sulfhydryl groups titratable with 5,5'-dithiobis-(2-nitrobenzoic acid). Transport of α-aminoisobutyrate (AIB) was inhibited in resting as well as insulin-stimulated rat thymocytes. Inhibition of AIB influx occurred with 5 min of exposure to DETD at concentrations as low as 10 μM. No effect on AIB efflux was found. Addition of 17β-estradiol (10 μM) to the incubation medium substantially decreased the inhibitory effects of DETD on α-aminoisobutyrate influx, while the addition of testosterone (10 μM) had no effect. DETD was more potent than DMTD in reducing the number of titratable sulfhydryl groups, and DETD was also a more potent inhibitor of AIB influx than DMTD. The data indicate that dialkyltin compounds may bind plasma membrane sulfhydryl groups essential for the transport of amino acids by rat thymocytes. This binding of dialkyltins may also be responsible for the altered response to insulin, since insulin receptors also have essential sulfhydryl groups, and could partially explain the depletion of cortical thymic lymphocytes observed in vivo.


Since methylmercury (MeHg) is nearly 100% absorbed, elimination rate is a critical determinant of body burden resulting from ingestion of MeHg containing foods. Mouse studies
were undertaken to assess the potential significance of diet on Hg kinetics. Female mice (BALB/c, 6 months old) were fed three diets. Each group of mice received Charles River RMH 3000 pelleted diet, Pet evaporated whole milk, or GIBCO 116EC liquid diet ad lib. for 1 week prior and 2 weeks subsequent to a single po dose of MeHg (0.5 mg/kg, labeled with $^{203}$Hg). The estimated whole body Hg elimination half-time (Days 1 through 7 after MeHg administration) were 10.4 days (pelleted diet), 19.7 days (milk diet), and 5.8 days (GIBCO diet). The 95% confidence intervals for elimination half-times of each group do not overlap. Relative whole body elimination rates (as measured by whole body $\gamma$ counting) were confirmed by fecal $^{203}$Hg excretion. Urinary excretion accounted for 7% (GIBCO diet group) to 19% (milk diet group) of the whole body elimination by Day 7. Brain, blood, liver, and kidney Hg concentrations (14 days after MeHg administration) were consistent with the relative mercury body burdens. There are many differences between the three diets; this experiment alone does not define the relative importance of specific dietary factors. Non-Hg-containing dietary components can affect MeHg body burden and brain levels. Because the brain is a critical organ for MeHg toxicity, dietary factors could play a role in MeHg poisoning. (Supported by USPHS-NIH Grant 5-T01-Gm-01781, NIEHS Grants ES 01247 and ES 01248, and US/ERDA Contract UR-3490-1232.)


During a preventative isoniazid (INH) program in 1970, 19 Capitol Hill employees developed hepatotoxicity. Since 4.4 million doses of INH were sold in Canada in 1975, we became interested in INH-induced hepatotoxicity and possible toxic interactions with other chemical agents. In this study, male New Zealand White rabbits maintained on vitamin $B_6$ (25 mg/kg, po) and terramycin (0.005%, w/v, in the drinking water) were used. The effect of ethanol (500 mg/kg, po) on the toxicity of INH (50 mg/kg, po) was examined over a 5-week period. Clinical chemistry indicated that INH caused a statistically significant elevation in plasma levels of triglycerides, cholesterol, and total lipids, and ethanol treatment tended to potentiate these changes. Frozen liver sections stained with oil red O revealed that 66% of the animals in the ethanol–INH group had extensive accumulation of fat in the centrilobular region compared to 33% in the INH group and 0% in the control and ethanol-treated groups. These preliminary results suggested that ethanol increased the hepatotoxicity of INH.


Male, New Zealand White rabbits of the same acetylator phenotype were randomly divided into two groups of five. All received an oral dose of $[^{14}C]$isoniazid (50 mg/kg) and the ethanol-treated group received an oral dose of ethanol (1 g/kg) 30 min prior to the dose of isoniazid. Blood was sampled up to 12 hr and urine was collected up to 48 hr. Plasma was extracted and the extract subjected to paper chromatography that separated the $^{14}$C into three peaks corresponding to acetylsisoniazid > isonicotinic acid > isoniazid. The $t_{1/2}$ of isoniazid was 0.71 hr in the controls and 0.93 hr in the ethanol-treated rabbits, but the difference was not significant. No significant changes were observed in the elimination of acetylsisoniazid or isonicotinic acid. Paper chromatography of the urine gave more numerous peaks but the major ones corresponded to isonicotinic acid > acetylsisoniazid > isonicotinoyl glycine. Isonicotinic acid represented over 50% of the $^{14}$C found in urine. Ethanol treatment did not significantly change the excretion of any of the metabolites. It is concluded that at the dose used, ethanol does not significantly affect the pharmacokinetics of isoniazid in the rabbit.
311. Response of Anti-inflammatory and Immunosuppressive Agents in a Standard Experimental Regimen for Early Assessment of Hematologic or Gastrointestinal Toxicity. ROGER D. HEMM, Ayerst Research Laboratories, Toxicology Section, Chazy, New York. (John J. Pollock)

The proficiency of two standard protocols designed for early detection of potential hematotoxicity by prospective new anti-inflammatory or immunosuppressive agents was evaluated using the standard agents aspirin, indomethacin, hydrocortisone, Na-butanol, cyclohexan, imuran, 2-amino-6-mercaptopurine, and 6-mercaptopurine. In initial studies (Segment I) these standard agents were administered intragastrically to 16 groups of 10 CR-CD rats each at two and five times the rat polyarthritis test screen ED-50 for 3 days. Certain hematologic parameters were evaluated at Days 0, 3, and 7. Animals were sacrificed on Day 7 and grossly abnormal gastrointestinal tissue was evaluated microscopically. In a subsequent study (Segment II) each of these eight compounds was administered intragastrically for 9 days to three groups of six CR-CD rats each, at 1.5 and 6 times the estimated ED-50. Three groups of six animals each receiving saline intragastrically served as controls. Selected hematologic parameters were evaluated at 3, 5, and 9 days of treatment and on Day 14 after a 5-day period of treatment withdrawal. Anticipated effects consistent with the known effects of anti-inflammatory or immunosuppressive agents on survival, body weight gain, appearance and behavior, and on the gastrointestinal systems occurred following the regimen described in Segment I. However, the initial short duration protocol (Segment I) proved to be of limited value in early detection of drug induced hematologic changes in groups exhibiting high mortality and depression of body weight gain. The Segment II study of longer duration proved to be sufficiently sensitive for selective differentiation of the potential effects of various standard agents on components of the erythroid or leukocytic elements of blood and may provide a useful study design for early selection of potential anti-inflammatory or immunosuppressant candidates based on relative toxicity.

312. Salicylate-Induced Hypothermia of the Rat. H. W. McCAIN, R. S. TEAGUE, and R. L. MUNDY, Department of Pharmacology, University of Alabama in Birmingham, Birmingham, Alabama.

Salicylate-induced hypothermia has been well documented for rats given large ip doses of the drug and subjected to cold stress. Although salicylate apparently alters central mechanisms of both behavioral and physiological temperature control, whether it acts directly within the CNS or indirectly remains obscure. We administered sodium salicylate (SS) to 375-g rats by ip or intracerebroventricular (icv) routes. The ip injections were 0, 150, 250, and 350 mg/kg of SS dissolved in 1 ml of 0.9% saline. One group of animals received 320 mg/kg of p-chlorophenylalanine (PCPA) 48 hr before 350 mg/kg of SS. Rectal temperature measurements were made with a thermistor probe 90 min after SS injections. One group of animals received SS via icv infusion through canulas chronically implanted in their lateral ventricle. SS was dissolved in mock cerebrospinal fluid and delivered at 3 and 6 μM/hr. Temperature measurements of these animals were made each 10 min by telemetry for 2 hr after beginning of drug infusion. After ip injections or during icv infusions animals were individually housed in a 9 liter chamber with air circulation of 3 liters/min. Chamber temperature was 24 to 26°C with 100% relative humidity. Intraperitoneal SS produced a dose-dependent depression of rectal temperature which was 1.6°C at the highest dose. Pretreatment with PCPA did not alter this depression SS icv (3 μM/hr) had no effect on animal core temperature; however 6 μM/hr produced hypothermia which began 20 min after drug infusion. This hypothermia continued throughout the experiment and reached 4°C within 2 hr. These results indicate that SS may produce hypothermia via direct CNS action.

313. The Effects of Protein Deficiency on Drug Toxicity in the Neonate Rat. JOSEPH P. HANIG, PAIGE D. YODER, and STEPHEN KROP, Food and Drug Administration, Washington, D.C.

Protein deficiency during gestation and lactation has been shown to have profound effects upon growth and development in the neonate. To study the toxicity of drugs under these
conditions, pregnant female Holtzman rats were placed on either protein-deficient (8%) or normal protein diets (24%) and the resulting pups from both dietary groups were reassigned, following birth, to either protein-deficient or normal lactating dams. The observed detrimental effects of deficiency during both gestation and lactation were so severe and deficient females so poor at nursing that we soon abandoned this approach in favor of an animal model for gestational deficiency alone, e.g., all pups cross-fostered to normal lactating mothers only. Under these conditions, oral administration of sodium pentobarbital at three dose levels to pups 5 days old resulted in significantly greater survival of protein-deficient males, compared with controls at the mid-level dose (60 mg/kg). In a separate series of experiments, protein-deficient males aged 23 days, but not females, were found to be significantly more susceptible to acetaminophen (1500 mg/kg) than controls on normal diets. Our findings support an important role for nutritional influences such as protein deficiency in modifying the toxic response in the neonate to certain drugs.


Teratological studies in Sprague-Dawley rats and Dutch-belted rabbits were conducted on the antineoplastic drugs BCNU, CCNU, MeCCNU, streptozotocin (STZ), bleomycin, DTIC, adriamycin, daunomycin, hexamethylenamine (HMM), procarbazine, and L-alanosine. The drugs were administered throughout the period of organogenesis in both species as well as for various 4-day intervals during organogenesis in rats. Treatment intervals were segmented in rats to determine the period of teratogenic sensitivity. Maximum tolerated doses were predetermined in pregnant animals to aid in the selection of optimal dose levels for the teratology studies. Most of the drugs were potent teratogens in the rat and generally elicited the strongest response when given on Days 6 to 15 or 6 to 9 of gestation. Bleomycin, HMM, and STZ were comparatively weak teratogens, resulting only in increased numbers of skeletal defects and a low incidence of soft tissue anomalies. None of the drugs were clearly teratogenic in the rabbit although some resulted in a marginal response (DTIC) or abortion (BCNU, CCNU, bleomycin, DTIC, adriamycin, and STZ). Although it is conceivable that a teratogenic response might have been attained had higher doses been given for fewer days, these studies have shown the rabbit to be considerably less sensitive than the rat to a number of highly teratogenic agents when treated in the conventional manner which spans the period of organogenesis. (Sponsored by Contract N01-CM-23712 from the Division of Cancer Treatment, NCI, NIH, DHEW.)

315. Liver Heme Metabolism: Toxic Effect of Morphine in Rats. D. Gurantz and M. A. Correia, Departments of Medicine and Pharmacology and Toxicology, University of California Medical Center, San Francisco, California. (M. Vore)

Morphine administration to rats has been shown to lower cytochrome P-450 content in the liver. Such lowering can be due to decreased synthesis of the hemoprotein or to its increased turnover. Since synthesis of cytochrome P-450 requires synthesis of heme, we investigated the effect of morphine on δ-aminolevulinic acid synthetase (ALAS), the rate-limiting enzyme in the heme synthetic pathway. Male Holtzman rats were treated with a single injection of morphine sulfate (45 mg/kg, ip) and sacrificed at various intervals thereafter. Cytochrome P-450 in these rats was significantly decreased as early as 1 hr following morphine. A progressive lowering in the cytochrome content was observed reaching a maximum (31%) at 6 hr following treatment. However, morphine failed to produce a parallel decrease in ALAS activity, indicating that heme synthesis may not be impaired. In contrast, the activity of microsomal heme oxygenase (MHO), the rate-limiting enzyme in heme degradation, was significantly stimulated as early as 2 hr, reaching a fourfold maximum (0.42 ± 0.13 nmol of bilirubin formed/mg of protein/10 min) at 8 hr after morphine. Stimulation of MHO activity has been associated with accelerated turnover of cytochrome P-450 and consequent release of its heme. Since morphine enhances MHO activity, it remains to be determined whether morphine also stimulates the turnover of
cytochrome P-450 in vivo. Morphine-mediated acceleration of heme degradation represents a potential toxicity of the drug to biological systems, particularly those dependent on hemoproteins.


Tricyclic antidepressant drugs (TCAD) are used to alleviate the symptoms of depression in adults and for enuresis in children. Narrow safety margins with unpredictable dose–response effects often cause serious adverse drug reactions (ADR) on the central (neurologic and behavioral), cardiovascular (CV), autonomic-anticholinergic (ACH), and hematopoietic (BLD) systems. For developing animal models to predict age-related drug toxicity, we investigated age as a modifying factor in these ADRs from the FDA monitoring program of marketed TCAD. For the years 1969–1975, 3183 ADRs were reported to FDA for five TCAD, of which imipramine (IMP) and amitriptyline (AMT) accounted for 52.7 and 33.7%, respectively (perhaps reflecting wider use rather than increased toxicity). ADRs for these two TCAD were grouped into age blocks (<20, 21–40, 41–60, and >60), physiologic systems affected, sex, and severity. Of the total ADRs reported for IMP and AMT, 85 and 70%, were classed as significant, with 23 and 31% as serious. By increasing age blocks 22, 27, 37, and 14% of the ADRs were induced by IMP and 8, 36, 36, and 18% were caused by AMT. Sex (M/F) ratios were 44/56 for IMP and 34/66 for AMT ADRs. In decreasing occurrence by physiologic systems, ADRs were neurologic > behavioral > ACH > BLD > CV. Overdose and fatalities accounted for 2 and 0.9% for IMP; 4 and 0.7% for AMT; by severity, CV ADRs > behavioral > neurologic > BLD > ACH. In decreasing ADR frequency, CV ADRs were due to tachycardia > hypotension > arrhythmias; behavioral ADRs were due to agitation > confusion > nervousness > psychoses for IMP while confusion > psychoses > hallucinations were the major cause for AMT. Neurologic ADRs were mainly coma > convulsions > tremors > somnolence > dizziness. Major BLD ADRs were leukocytosis > anemia. ACH ADRs were due to xerostomia > constipation > sweating. With advancing age, increases were noted in CV, behavioral, and BLD ADRs and decreases were seen in ADRs for ACH and neurologic effects. These data show that age is a significant variable in modifying the toxicologic profiles of the TCAD in man.


N-(Phosphonacetyl)-L-aspartic acid (PALA, NSC224131) inhibits aspartate transcarbamylase required for pyrimidine nucleotide biosynthesis and has therapeutic activity against mammary adenocarcinoma 13/C, colon adenocarcinoma 38, B16 melanoma, and Lewis lung carcinoma in mice. The activity against Lewis lung carcinoma is marked; few other compounds can effect cures of this tumor. Preclinical toxicologic evaluations of PALA are in progress. The present study was designed to provide a preliminary indication of the qualitative and quantitative toxicity of PALA. PALA was administered ip to male BDF1 mice (22–24 g) daily for 9 days in doses of 180, 220, and 290 mg/kg. These doses approximate 0.8 LD10, LD10, and LD50, based on historical and concurrent 30-day lethality data. Five mice for each dosage group were sacrificed on Days 6, 10, 16, and 30 (day of first treatment = Day 1) for hematologic and histopathologic evaluation. Only minimal changes were detected in peripheral hematologic values. PALA produced dose-related reticulocytopenia with a nadir on Day 10. Reticulocyte counts of survivors had returned to normal by Day 16. Mild lymphopenia with a nadir on Day 10 was reversed by Day 16. Thrombocytopenia was not observed, and total marrow cell counts did not reflect hematotoxicity. Mild lymphoid depletion and diffuse enteritis were the most consistent lesions observed. The severity of the splenic and gastrointestinal lesions was greatest on Day 10. Time to recovery was dose-related for both lesions, but recovery from peripheral lymphopenia preceded gastrointestinal recovery. These results suggest that gastrointestinal
toxicity may be dose-limiting, but, unlike most other antimetabolites, PALA may be only mildly hematotoxic. (Supported by Contracts N01-CM-43756 and N01-CM-57000, DCT, NCI, NIH, DHEW.)

318. Enhancement of Hexobarbital and Barbital Hypnosis by Diphenhydramine. R. T. LOUIS-FERDINAND, F. BEUTHIN, and M. STOUT, Pharmacology Division, Wayne State University, College of Pharmacy and Allied Health Professions, Detroit, Michigan.

In order to assess the relative contribution of microsomal enzyme inhibition to the mechanism(s) responsible for the enhancement of barbiturate activity by diphenhydramine (DP), the influence of DP pretreatment on the duration of hexobarbital and barbital hypnosis was compared. Male (25–35 g) Swiss–Webster mice were pretreated with either 5, 10, or 50 mg/kg (ip) DP followed 1 hr later by hexobarbital (100 mg/kg, ip). A significant \( p < 0.05 \) increase in hexobarbital hypnosis (151–190%) was produced in 10 or 50 mg/kg DP-pretreatment animals; however, 5 mg/kg of DP did not enhance hexobarbital sleep time. In vitro incubations of mouse hepatic 9000g supernatant fractions with \( 10^{-4} \) or \( 10^{-5} \) M DP resulted in 67.6 or 46.0% inhibition of hexobarbital oxidation, respectively. The influence of DP (10 or 50 mg/kg, ip) on the activity of barbital (325 mg/kg, ip) was determined in order to evaluate the effect of DP on the action of a barbiturate which is not appreciably metabolized in vivo. Although no significant \( p > 0.05 \) enhancement of barbital hypnosis was produced by DP, 10 mg/kg, a significant \( p < 0.05 \) 368% increase in barbital hypnosis was produced in animals pretreated with 50 mg/kg of DP. These results suggest that inhibition of drug metabolism also contributes to the enhancement of barbiturate hypnosis produced at lower doses of DP.

319. The Effect of Propranolol, Diazepam, and Chlorpromazine Administration on the Response to Lethal Levels of Intravenous Cocaine in the Cynomolgus Monkey (Macaca Fascicularis). MEREDITH M. GUINN, MARVIN C. WILSON, and JOHN A. BEDFORD, Department of Pharmacology, Research Institute of Pharmacological Science, School of Pharmacy, University of Mississippi, University, Mississippi. (W. Marvin Davis).

The lethal effect of cocaine HCl delivered intravenously as an infusion of 0.5 mg/kg/ml/min was examined in three adult male cynomolgus monkeys (Macaca fascicularis). Heart rate, respiratory rate, mean arterial pressure, and rectal temperature were measured and behavioral observations made prior to and at 10-min intervals following initiation of the infusion. A mean minimal convulsant dose of 15.0 ± 0.88 mg/kg and a mean lethal dose of 25.0 ± 1.5 mg/kg were calculated from these three subjects. Convulsant activity appeared more closely correlated to cause of death than did changes in cardiorespiratory parameters and body temperature. Subsequent animals were given one of three intravenous treatments 10 min into the cocaine infusion: propranolol HCl (3 mg/kg), diazepam HCl (0.5 mg/kg), and chlorpromazine HCl (10 mg/kg). The infusion of cocaine was then continued until death or until the animal had received 39.0 mg/kg of cocaine, a value corresponding to three standard deviations above the previously calculated lethal dose. Those two subjects receiving propranol convulsed after 10.0 ± 1.0 mg/kg of cocaine and died following administration of 16.0 ± 0 mg/kg of cocaine. Both animals that received diazepam convulsed, one following 39.0 mg/kg of cocaine and the other after 22.5 mg/kg. The latter subject died following 27.5 mg/kg of cocaine. Both chlorpromazine-treated animals survived the administration of 39.0 mg/kg of cocaine; however, one animal convulsed after 28.5 mg/kg of cocaine. Results indicate that of those agents tested chlorpromazine would be the best agent to use in the clinical management of severe cocaine toxicity. Propranolol would be contraindicated in light of its apparent sensitization of the subject to the convulsant effects of intravenously administered cocaine.

320. Cocaine-Induced Hepatic Necrosis. RICHARD W. FREEMAN and RAYMOND D. HARBISON. Department of Pharmacology, Vanderbilt Medical Center, Nashville, Tennessee.

Cocaine induces hepatic necrosis when administered chronically at dosages of 20 and 30 mg/kg and when administered to phenobarbital-pretreated mice. Cocaine is an ester of benzoic acid and a nitrogen-containing base, methylecgonine. The purpose of this study was to determine
the effect of other benzoic acid esters on hepatic function as well as to determine the effect of esterase inhibition on cocaine-induced hepatic dysfunction. Male Swiss origin mice were used for study. Phenobarbital (PB) pretreatment, 60 mg/kg daily for 4 days intraperitoneally, antagonized the toxicity of procaine and lidocaine. No benzoic acid esters tested, other than cocaine, produced hepatic necrosis. Serum glutamate-pyruvate transaminase (SGPT) levels were significantly elevated in groups of animals receiving cocaine chronically or in groups of phenobarbital-pretreated animals receiving cocaine. Neither benzoic acid esters nor benzoic acid elevated SGPT levels. Administration of tropacocaine also did not induce hepatotoxicity. S,S,S-
Tributyl-phosphorotriithioate (DEF), an esterase inhibitor, administered at a dosage of 2 mg/kg intraperitoneally enhanced the toxicity of cocaine. DEF pretreatment potentiated cocaine-induced hepatic necrosis. In PB-pretreated mice, cocaine (30 mg/kg) produced marked hepatic necrosis following DEF pretreatment. However, in PB-pretreated mice, cocaine (30 mg/kg) administered as a single dosage did not produce hepatic necrosis. In PB-pretreated mice, DEF pretreatment also potentiated the effect of cocaine (30 mg/kg) to produce a greater than 10-fold elevation of SGPT levels. Cocaine induces hepatic necrosis when administered chronically and when administered to phenobarbital and DEF pretreated mice. Structure–activity relationship studies indicate that an intact benzoimidyleegonine is required to produce hepatotoxicity.
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321. **Comparison of the Effects of Quinidine and Dihydroquinidine on the Canine Heart.**

T. Balazs, E. Herman, and J. Atkinson, Food and Drug Administration, Washington, D.C.

Pharmaceutical preparations of quinidine (Q) may contain up to 20% of dihydroquinidine (DQ) (USP XIX). Questions have been raised on the activity of this contaminant. The present studies compared the potency of the tartrate salts of Q and DQ, using the heart rate, force of contraction, and coronary arterial pressure to measure the effects in isolated canine hearts perfused with autologous blood. Each dose of 5, 10, or 20 mg of Q or DQ was repeated in five separate experiments. Both alkaloids caused negative chronotropic effects, dose-related transitory negative inotropic effects, and a decrease of coronary pressure with comparable patterns and rates of recoveries. No significant differences were found in these test results except for a greater depressant effect of 20 mg of DQ on the coronary pressure. The negative dromotropic effects of the alkaloids were tested in anesthetized beagle dogs by measuring the duration of QRS complexes of the electrocardiogram. Pure Q or DQ and mixtures (25 and 50% DQ) were administered intravenously at a rate of 5 mg/kg/min for 10 min to eight dogs each. The QRS duration increased on a dose-related manner but there were no statistical differences in the effects of alkaloids alone or in mixtures. Results of these assays indicate that the pharmacological effects of Q and DQ on the canine heart are comparable.

322. **The Effects of Manganese (MnO₄⁻) Administration on the Spontaneous Behavior of Male and Female Rats.**


Chronic manganese can result from industrial exposure to high concentrations of manganese dust. Although the use of Mn compounds in fuels as a substitute for lead has been increasing, the health effects of long-term low-level exposure to manganese oxides is generally not known. The present study is a description of the effects of prolonged dietary administration of MnO₄⁻ on spontaneous behavior. Long-Evans (Blue Spruce) hooded female rats were assigned to one of eight treatments on Day 1 of gestation. The experimental design was a (4 x 2) factorial with four levels of Mn (50 ppm [control], 400, 1100, 3550 ppm) and two levels of iron (240 ppm [normal] and 200 ppm [iron-deficient]). Treatments were administered to the mothers throughout gestation and lactation. Pups weaned at 21 days of age were housed with nonsibs in unisexual groups of three and continued on their respective diets for the remainder of the experiment. At 45 and 90 days of age the exploratory activity levels of individual male and female rats (N = 244) were observed for 90 min in residential mazes. For comparative purposes, male and female rats (N = 132) were
also tested for activity in an open-field at 110 days of age (one trial, 5 min in duration) and for their reaction to handling at 120 days of age. Attempts to escape, vocalization, struggling, biting, and freezing during handling were scored. Results of these behavioral tests indicated that Mn lowered the residential maze activity in a dose-related manner. The Mn$_2$O$_4$-treated rats at the higher doses were approximately 20% less active than the controls. However, the shorter open-field activity levels, although positively correlated with the maze activity were not significantly affected and the reaction to handling was also not different. It is concluded that Mn has a subtle behavioral effect, reducing reactivity in male and female rats. Physiological data, also collected in this study, suggest that this effect may be mediated through the animals reproductive physiology. In addition to the main effects of Mn, low dietary iron interacted with Mn, enhancing Mn toxicity in weanling animals.

323. The Behavioral Effects of Subacute Exposure to Kepone or Mirex on the Weanling Rat.

Kepone (K) and Mirex (M) are two structurally related insecticides with markedly different behavioral toxicity. In the adult, the acute toxicity of K is reported to be three times higher than M. In the present study, weanling rats were exposed to either M or K for a period of 4 weeks. A total of 80 Sprague–Dawley rats (40 males and 40 females) were assigned to one of five treatment groups at 21 days of age. After 6 days on control diet (Purina chow), the pesticides were administered at dietary levels of either 0, 40, or 80 ppm. K-exposed animals showed a significantly exaggerated startle response (169 and 197% for 40 and 80 ppm of K, respectively) within 1 week of treatment which persisted throughout the 4-week exposure period. Ambulation in an open field was significantly decreased by K-exposure (25% at 80 ppm of K) when tested after 3 weeks of treatment but was unchanged when measured in a residential maze. M-exposure, on the other hand, was lethal to weanling rats and after 4 weeks of treatment produced 56% mortality in the 80-ppm M group. Animals that died showed no signs of CNS toxicity. In addition, M-exposed animals showed a reduction in both food consumption and growth rate. After 4 weeks of treatment, males showed a dose-related decrease in both testes and adrenal weights along with enlarged livers. Results of behavioral testing were negative except for a significant reduction in residential maze activity, probably reflecting a general toxicity. In summary, these results indicate that in the weanling rat during subacute exposure, M is more toxic than K. The critical organs appear to be different, M producing primarily a systemic toxicity whereas with K exposure the CNS effects predominate.

324. Comparison of Conditioned Avoidance and Unconditioned Reflex Tests in Rats Exposed by Inhalation to Carbon Monoxide, 1,1,1-Trichloroethane, Toluene, or Ethanol. N. Krivanek and L. S. Mullin, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont De Nemours and Company, Wilmington, Delaware. (C. F. Reinhardt)

The sensitivity of conditioned avoidance and unconditioned reflex tests in evaluating behavioral toxicity was studied. Male rats were exposed by inhalation up to 4 hr to 0, 200, 400, 800, or 1600 ppm of carbon monoxide; 0, 1500, 3000, 6000, or 12,000 ppm of 1, 1, 1-trichloroethane; 0, 800, 1600, 3200, or 6400 ppm of toluene; or 0, 4000, 8000, 16,000, or 32,000 ppm of ethanol. Animals were tested for behavioral changes at 0.5, 1, 2, and 4 hr during exposure and 18 hr after exposure. In unconditioned testing the presence or absence of unconditioned reflexes (corneal, grip and righting reflexes, etc.) and simple behavior patterns including locomotor activity and coordination were determined. The conditioned reflex task consisted of having trained rats avoid a shock by bar press following light and sound stimuli. Rats began to fail unconditioned reflex tests at 800 ppm of CO, 3000 ppm of trichloroethane, 800 ppm of toluene, and 8000 ppm of ethanol. Decrements in conditioned avoidance were first observed at 400 ppm of CO, 3000 ppm of trichloroethane, 3200 ppm of toluene, and 4000 ppm of ethanol. Neither the unconditioned nor the conditioned reflex test was always more sensitive than the other in detecting behavioral changes. Compared to human data for these compounds, both the unconditioned and conditioned reflex tests were less sensitive by two- to tenfold.

Maneb is a widely used fungicide that can be converted to EBIS (ethylenesbisethioocyanate sulfate) and ETU (ethylenethiocarbamate). In support of teratology studies done in our laboratory, female CD rats received oral intubations of 480 or 240 mg/kg of Maneb, 30 or 15 mg/kg of EBIS, 30 or 20 mg/kg of ETU, or vehicle alone from Day 7 of gestation to Day 15 postpartum. Litters were normalized at birth to four individuals of each sex, weighed weekly, and tested for the startle reflex and air righting. In the fifth postnatal week, the males were observed in a circular open field. Four of nine litters in the high dose ETU group died within 24 hr of birth. No morphological defects were evident in these litters. Although there was milk in the dams, they failed to nurse. Only two of these litters survived the first postnatal week, and these developed hydrocephaly. These effects were not seen in the low dose ETU litters. Maneb and EBIS did not affect development or behavior, but on the second day of open-field testing the low-dose ETU males were mildly hyperactive ($p = 0.06$) and had a greater central tendency ($p < 0.001$).


Spectrophotometric measurements of several sensory irritants in the presence of glutathione and a glutathione S-transferase preparation from bovine corneal epithelium show that none of the irritants tested acts as substrate for the transferase. However, many of them are potent inhibitors of the enzyme activity when 1-chloro-2,4-dinitrobenzene is used as substrate. The inhibitions were found to be of three types: (1) irreversible, covalent inhibition by chloracetopheonone and by N-ethylmaleimide, N-methylmaleimide, and acrolein in the absence of glutathione; (2) competitive inhibition with glutathione by the reaction products of N-ethylmaleimide, N-methylmaleimide, acrolein, and B-nitrosoyrene with glutathione; and (3) an apparently complex, mixed-type inhibition by o-chlorobenzylidenemalononitrile, benzyldiene malononitrile, d-phenylaminochloroarsine, 2-iodocinnamalmalononitrile, and 2-nitrocinnamalmalononitrile. This third type could be only partially overcome by the addition of excess glutathione; it could not be determined whether competitive inhibition with the chlorodinitrobenzene was involved. The following irritants showed no evidence of interaction with the transferase system: capsaicin, veratrine, nicotine, dicyclohexylcarbodiimide, diisopropylcarbodiimide, malononitrile, formaldehyde, dibenz(b,f)-1,4-oxazepine, N-acetyl-cis-4-cycloheximethylcyclohexylamine, and N,N-di-t-butylethylenediamine. No correlation was seen between the potency of these substances as sensory irritants and as inhibitors. Thus there is apparently no direct relationship between sensory irritation and glutathione S-transferase. (Supported by NIOSH Grant R01-OH00367.)


Male White Carneaux pigeons were trained to peck three illuminated response keys in a predetermined four-step position sequence. For one group of birds the correct sequence was the same each day (performance group) and for the other group of birds the sequence was changed each day (repeated-acquisition group). Responses on the incorrect key produced 2 sec of total darkness and reset the sequence to the original step, while responses on the correct key changed the key color and advanced the sequence to the next step. After responding stabilized so that the number of errors was stable from day to day (the weekly range of errors was from 1 to 8 per day for the performance group and from 94 to 384 per day for the repeated-acquisition group), daily oral administration of lead acetate (6.25 mg/kg), or sodium acetate was started. For two of three birds in the performance group, the administration of lead acetate increased the total number of
errors within 2 weeks and the peak effect was obtained in 6 to 8 weeks. The third bird showed little effect during the 8-week period. Peak blood lead concentration ranged between 588 and 1099 µg and these concentrations occurred between the 5th and the 10th weeks of lead administration. Over the same 8-week period of lead administration, there were no changes in the total number of errors for the repeated acquisition group, although blood-lead concentrations were even higher, ranging between 876 and 2320 µg. These data suggest that the low number of errors maintained under the performance schedule is more sensitive to the effects of lead than the higher number of errors maintained under the acquisition schedule. (Supported by NIEHS Grant 5-P01-ES01104-02.)

328. Effect of Repeated Exposure of Mice and Rats to Concentrated Toluene and Acetone Vapors. JAMES V. BRUCKNER and RICHARD G. PETERSON, Department of Pharmacology, University of Texas Medical School, Houston, Texas and Department of Anatomy, Indiana University School of Medicine, Indianapolis, Indiana.

Although self-intoxication by the intentional inhalation of hydrocarbon solvents is a widespread practice, relatively little effort has been devoted to evaluation of potential health hazards of the practice. In order to develop exposure protocols which mimic typical episodes of solvent “sniffing,” male mice (ICR Sprague–Dawley) and rats (Sprague–Dawley) were exposed by inhalation to toluene vapor levels ranging from 2600 to 12,000 ppm for as long as 3 hr. Rats were similarly subjected to acetone in concentrations varying from about 12,600 to 50,600 ppm. Intoxication, as monitored by a battery of animal performance tests, was seen within minutes at the higher toluene exposure levels. Recovery upon cessation of exposure was also rapid. In contrast, both induction of and recovery from acetone-induced intoxication were quite slow. Analysis of tissue concentrations of toluene by gas chromatography demonstrated good correlation between performance inhibition and the quantity of solvent in the brain. Based on the preliminary studies, a protocol was established in which the rats and mice were exposed to 12,000 ppm of toluene for seven 10-min periods, with 20-min solvent-free intervals between subsequent exposures. Additional groups of rats were exposed for 3 uninterrupted hours daily to 19,000 ppm of acetone. Both the toluene and the acetone regimens were repeated 5 days/week for 8 weeks. Groups of each animal species were sacrificed after 2, 4, and 8 weeks and at 2 weeks following the last exposure. The following parameters failed to reveal evidence of significant injury by acetone or toluene at any sacrifice period: lactate dehydrogenase activity; glutamic-pyruvic transaminase activity; blood urea nitrogen; liver triglyceride content; organ histopathology; lung fluid content; organ weights. Toluene was found to be a more potent, rapidly acting narcotic than acetone. No evidence was seen, however, that either solvent exerted a residual toxic effect. (Supported by NIDA Contract 271-75-3067.)


The classical antidotal combination of sodium nitrite and sodium thiosulfate is widely used in many animal species to antagonize cyanide intoxication. In sheep, a species subject to cyanide intoxication via cyanogenic plants, the recommended dosage of 10 mg/kg of sodium nitrite and 50 to 100 mg/kg of sodium thiosulfate is similar to that used in man. Other antidotes such as cobalt and oxygen also have been proposed based on their efficacy in combination with the classical antidotes. The present study was proposed to evaluate the effects of various combinations and dosage schedules of sodium nitrite, sodium thiosulfate, oxygen, and cobaltous chloride in sheep. Adult female sheep of mixed breeds received sodium cyanide orally. The various antidotes were administered at 5 min following the cyanide. Oxygen (100%) was administered by face mask for a 30-min period. Conventional doses of sodium nitrite (6.6 mg/kg) and sodium thiosulfate (67 mg/kg) were effective in increasing the LD50 of sodium cyanide from 3.7 to 21 mg/kg. Increasing the dose of sodium nitrite and sodium thiosulfate to 22 and 660 mg/kg, respectively, increased the LD50 of sodium cyanide to 50 mg/kg. The high levels of sodium thiosulfate (660 mg/kg) alone were found to be more effective than the generally
recommended dosage schedule of the nitrite-thiosulfate antidotal combination. Cobaltous chloride (10.6 mg/kg) was not effective when administered with the classic nitrite-thiosulfate therapy; however, when it was employed in combination with the higher dosage of sodium thiosulfate, a significant protective effect was observed. Oxygen was found to enhance the protective effect of the nitrite-thiosulfate antidotal combination. For maximal effectiveness in treating cyanide intoxication in sheep, it is recommended that large doses of sodium thiosulfate (660 mg/kg) be used in combination with conventional doses of sodium nitrite (6.6 mg/kg) or cobaltous chloride (10.6 mg/kg). Oxygen may also be an important addition to the therapeutic regimen.

330. The Effect of 2,4-Dichlorophenyl-p-nitrophenyl Ether on Pulmonary Surfactant Production in the Rat Fetus. L. C. Stone and J. M. Manson, Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, Ohio. (P. B. Hammond)

Recent studies have established that the herbicide, 2,4-dichlorophenyl-p-nitrophenyl ether (TOK) retards lung maturation in the rat fetus. The purpose of this study, therefore, was to determine whether the respiratory distress, exhibited by newborn pups exposed in utero to TOK, could be attributed to an inhibition of pulmonary lipid surfactant synthesis. Female rats were treated with TOK (50 mg/kg/day) on Days 8 through 18 of pregnancy. Fetuses were removed by caesarean section on Days 20 and 21 of gestation. Fetuses in TOK-exposed dams had smaller lungs both in absolute weight and relative to body weight than did controls on both days. However, the incorporation of \( ^{14}C \)cholesterol into lung slices from fetuses exposed in utero to TOK was not significantly different from controls at either Day 20 or 21 of gestation. Cholinephosphotransferase, the terminal enzyme in the choline pathway, did not show diminished activity in lungs of TOK exposed fetuses when compared to controls. Lung phospholipid content also did not differ significantly between control and experimental in either Day 20 fetuses or newborn pups. These data indicate that TOK does not inhibit surfactant synthesis but rather has a generalized inhibitory effect on lung maturation. Thus, the respiratory distress observed in newborn pups is probably due to TOK exerting an inhibitory effect upon some other step in the maturation process of the lung besides surfactant synthesis.

331. In Vitro Teratogenic Assay System with P-450 Metabolism. J. Manson and R. Simons, Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio. (P. B. Hammond)

We have examined the influence of cyclophosphamide on in vivo and in vitro mouse limb development. When pregnant mice were exposed to 20 mg/kg on Day 11 of gestation, the predominant limb malformations obtained were preaxial ectrodactyly and hemimelia. Limb buds of the same gestational age exposed to cyclophosphamide in vitro, in the absence of P-450 activity, responded identically to controls in morphology and uptake of \( ^{3}H \)thymidine and \( ^{35}SO_4 \). Exposure to a purified P-450-generated metabolite, 4-ketocyclophosphamide, resulted in the formation of limbs with malformations similar to those seen when the parent compound was administered in vivo, and with reduced uptake of \( ^{3}H \)thymidine and \( ^{35}SO_4 \). Having ascertained that P-450 metabolism of cyclophosphamide was necessary for teratogenicity, we then attempted to add a metabolizing system to limb bud culture. Three approaches were taken; in the first, the 9000g supernatant from adult mouse liver was added to tissue culture media with cofactors and cyclophosphamide, and alkylating metabolites generated; in the second, the same procedure was followed with a redissolved microsomal fraction; and in the third, a culture of hamster embryo cells capable of P-450 metabolism was used to metabolize cyclophosphamide. Of the three approaches, the last, activation with hamster embryo cells, was the most successful, both in continuous (3-day) generation of metabolites, and lack of nonspecific damage to limb buds. Hamster embryo cells, however, produced fewer metabolites than the other two systems, but the metabolites produced were capable of causing malformations in limbs developing in vitro. Addition of a P-450 metabolizing system to limb bud culture increases the applicability of this method as an in vitro screening procedure for environmental teratogens.

We have previously reported the existence of aryl hydrocarbon hydroxylase, epoxide hydrolase, and glutathione S-transferase in the testicular microsomal and soluble fraction of rat testes. Toxic effects of arene oxides upon male germ cells depend on various pharmacokinetic factors such as the steady-state level of activation and inactivation of active intermediates, the blood/testis barrier and DNA repair process. The isolated perfused testis (IPT) was utilized to develop a model of these interrelated factors. This paper presents aspects of the enzymatic inactivation of an active intermediate, benzo[a]pyrene-4,5-oxide. The IPT was perfused via the testicular artery at a constant flow rate of 20 ml min⁻¹ testis⁻¹ with 3% bovine serum albumin in Krebs-Ringer bicarbonate equilibrated with 95% O₂ and 5% CO₂ at 32°C. BP-4,5-oxide [¹H] (sp act 10 mCi/mmol) was added to the perfusate at initial concentrations of 1.3, 3.6, and 5.0 μM. Five milliliters of effluent was collected via testicular vein every 15 min for 120 min to determine the rates at which metabolites were produced at each concentration. The kinetics of the formation of both BP-4,5-diol and glutathione conjugates were 2.3, 7.7, and 14.9 pmol min⁻¹ g testis⁻¹ and 26.8, 75.3, and 103.4 pmol min⁻¹ g testis⁻¹, for the respective concentrations. These data demonstrate that the ability of testes to detoxify reactive arene oxides providing one mechanism of biological protection from reactive intermediates of benzo[a]pyrene for the germ cells.


The toxicological significance of the acylation of fetal, maternal, and adult tissues is currently being investigated. As part of this project, we have studied the teratogenicity and chemical reactivity of selected anhydrides and imides. Teratogenicity was evaluated following ip administration of the compounds to pregnant CD-1 mice on gestational Days 8 to 10 or 11 to 13. Hydrolysis of the anhydrides (2 mM) was measured in carbonate buffer (pH 7.4) using the ph-stat technique. Acylation of the model nuclease p-nitrophenol (PNP, 0.15 mM) by the anhydrides (0.1 mM) was observed spectrophotometrically. All of the anhydrides and imides caused fetal abnormalities, with both dose schedules. Based upon the minimum dose (mmol/kg) to produce a significant increase in defects for treatment Days 11 to 13, the anhydrides tested may be ranked in the following order: propionic anhydride (PA, 0.156) > acetic anhydride (AA, 0.188) > succinic anhydride (SA, 0.25) > phthalic anhydride (PHA, 0.375) > maleic anhydride (MA, 0.375). The same order occurs in the anhydrides' half-lives (sec) in buffer: PA, 345 ± 10; AA, 182 ± 6.2; SA, 118 ± 2.2; PHA, 41.1 ± 4.4; and MA, 25.4 ± 0.5. Similarly, the total amounts (μM) of PNP acylated were: PA, 27.0 ± 0.4; AA, 25.6 ± 0.4; SA, 4.42 ± 0.15; PHA, 3.36 ± 0.18; MA, not detectable. In addition, preliminary data show that the reactivities of the imides hydantoins, 2,4-oxazolidinedione, phthalimide, and succinimide are about 100-fold lower than the anhydrides and that the lowest doses to produce malformations are, concomitantly, two orders of magnitude higher (10 to 50 mmol/kg) than the anhydrides. These data suggest that there may be a correlation between acylating ability and teratogenic potential.


The flame retardant Tris(2,3-dibromopropyl) phosphate (TRIS) has been recently implicated as a mutagen and carcinogen. A teratology study of orally administered TRIS was conducted in pregnant Sprague-Dawley rats. The route of administration was by gavage on Days 6 to 15 of gestation. Dose levels in propylene glycol were 0, 5, 25, or 125 mg/kg/day. The dams were sacrificed on Day 20 of pregnancy, and the fetuses were delivered by caesarean section. Preliminary single-dose studies administrated orally by intubation in the rat gave the following
LD50 values: TRIS (10%, w/v, in propylene glycol), 1.88 (1.26–2.48) g/kg; propylene glycol (neat), 20 (20–25) g/kg. Other preliminary rat data by oral gavage with TRIS on Days 6 to 15 of gestation with 0, 250, and 1000 mg/kg/day showed mortality ratios (M/R) of 0/10, 1/10, and 10/10, respectively. The rats on the highest dose died on Days 9 to 11 of gestation. Nonpregnant female rats dosed with TRIS for 10 days with 100, 150, 500, or 1000 mg/kg/day showed M/R of 0/10, 0/10, 7/10, and 10/10, respectively. Results from earlier studies with TRIS in our laboratory indicated chronic interstitial nephritis in rats following oral administration, and chronic interstitial nephritis and testicular atrophy in rabbits with dermal application. In the present study, some renal damage was observed in the fetuses by Wilson sectionings, and these data are being further evaluated. There were no external abnormalities. Other standard teratological parameters will be reported.

335. Effects of Hormones on the Development of Enzymes Associated with Drug Metabolism.
   J. E. A. Leakey and J. R. Fouts, Laboratory of Pharmacology, National Institute of
   Environmental Health Sciences, Research Triangle Park, North Carolina.

   Hepatic cytochrome P-450-related mixed-function oxidase activity and an organism's
   capacity to metabolize toxic compounds are all at low levels during the perinatal period. In the
   rat, liver cytochrome P-450 develops neonatally from the low fetal to adult concentrations. We
   have investigated the effects of glucocorticoids and glucagon upon cytochrome P-450
   development, as these hormones control the development of other neonatally developing
   enzymes such as tyrosine aminotransferase. Dexamethasone acetate treatment (35 μg/g body wt
   in saline, ip) to 4 to 6-hr-old neonates had, after 36 hr, precociously stimulated hepatic
   cytochrome P-450 concentrations to three times those of littermate controls (10 μl of saline/g).
   Glucagon treatment (15 μg/g, ip) had no effect on cytochrome P-450 concentrations under these
   conditions. Treatment of 2.5-day-old neonates with dexamethasone as above precociously
   stimulated cytochrome P-450 to adult values, whereas such treatment of adult rats (either sex)
   caused no significant stimulation of cytochrome P-450 even at increased dosage (100 μg/g).
   Cortisol (50 μg/g every 12 hr) also precociously increased neonatal hepatic cytochrome P-450
   concentrations. When pregnant rats (18 or 20 days gestation) were treated with unlabeled or 3H-
   labeled dexamethasone (35 μg and 0.035 μCi/g), no stimulation of fetal cytochrome P-450
   occurred although label could be detected in both fetal plasma and liver. The above findings
   imply that the development of rat liver cytochrome P-450 is controlled by (1) the onset of
   competence of the fetal liver to respond to glucocorticoids occurring at birth and (2) by
   increasing plasma glucocorticoid concentrations in the neonate.
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