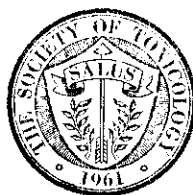


# ABSTRACTS OF PAPERS

# SOCIETY OF TOXICOLOGY

INCORPORATED



## SIXTEENTH ANNUAL MEETING

TORONTO, CANADA

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Abstracts of Papers for the Sixteenth Annual Meeting of the  
Society of Toxicology, Toronto, Canada  
March 27–30, 1977

1. *Chlorinated-s-Triazine Trione Compounds: Acute and Chronic Toxicity Studies.* J. LAVEGLIA, D. G. MACKELLAR, J. H. WEDIG, M. L. KEPLINGER, P. L. WRIGHT, and G. J. LEVINSKAS, Monsanto Co., St. Louis, Missouri; FMC Corp., Princeton, New Jersey; Olin Corp., New Haven, Connecticut; and Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

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 remain. Acute toxicity studies were conducted with the specific chlorinated-s-triazine trione compounds; chronic studies were conducted with monosodium cyanurate alone or with calcium hypochlorite. Chlorinated-s-triazine trione compounds are slightly toxic by ingestion in single oral doses (LD<sub>50</sub>, 1500 to 2100 mg/kg) and by single dermal applications (LD<sub>50</sub>, >2000 mg/kg). When applied as dry powders, they are slight eye and skin irritants, but they are corrosive when applied to the skin as moistened pastes. Feeding studies with monosodium cyanurate in rats at 15,000 and 30,000 ppm and in mice at 10,000, 20,000, and 30,000 ppm resulted in increased morbidity and mortality after 6 to 8 months. Gross autopsy revealed macroconcretions in the urinary tracts of animals from all treatment groups. These were associated with chronic renal disease characterized by the presence of calcified casts in the renal pelvis, tubule, and urinary bladder. Degenerative changes in the kidney and hyperplasia of the bladder mucosa also were noted. When dietary concentrations of 300, 1000, and 3000 ppm monosodium cyanurate were fed to mice for 18 months and to rats for 24 months, no evidence of chronic toxicity or carcinogenicity was observed. A 20:1 aqueous solution of monosodium cyanurate plus calcium hypochlorite was applied dermally (0.1 ml/day) to mice three times a week for 18 months; no evidence of chronic toxicity or carcinogenicity was observed. Monosodium cyanurate administered alone and as a 20:1 mixture with calcium hypochlorite was neither mutagenic in mice at 250 mg/kg ip nor teratogenic in rats, po, at 500 mg/kg.

2. *Comparative Toxicology of Cardiac Glycosides from the Milkweed *Asclepias eriocarpa*.* J. M. BENSON, J. N. SEIBER, R. F. KEELER, and A. E. JOHNSON, Department of Environmental Toxicology, University of California, Davis, and Poisonous Plant Research Laboratory, U.S. Department of Agriculture, Logan, Utah. (W. W. Kilgare)

Some milkweeds native to the western United States are extremely toxic to range animals. Approximately 0.2 to 0.5 lb of fresh *A. eriocarpa*, for example, can be lethal to a 100-lb sheep. The cardiac glycosides (cardenolides) in *A. eriocarpa* have been implicated as the toxic principle. Experiments were conducted to verify this, and to determine the toxicity of three individual cardenolides present in the plant. Three preparations were tested: dried ground plants, ethanolic plant extract, and plant extract from which pigments and fats had been removed. Preparations containing equivalent amounts of cardenolide were administered po at three dosages to female sheep (35–55 kg). In addition, a purified cardenolide from *A. eriocarpa*, labriformin, was administered at two dosage levels (55 and 123 mg/sheep) and digitoxin, a clinically important cardenolide, was administered at three levels (50, 80, and 300 mg/sheep). Toxic signs and gross pathology were qualitatively similar regardless of whether plants, extracts, labriformin, or digitoxin were administered. Comparable results were obtained using guinea pigs. Three *A.*

*eriocarpa* cardenolides, labriformin, cardenolide *a*, and cardenolide *c*, have been isolated and chemically characterized. They represent a new series of cardenolides similar, but not identical, to those present in *A. curassavica*, a species whose cardenolides have undergone some toxicological investigation. Acute toxicity studies on the *eriocarpa* cardenolides and uscharidin and calotropin from *A. curassavica* were performed using male Swiss Webster mice. Compounds dissolved or suspended in emulfor-water-ethanol (1:8:1) were administered ip. The cardenolides, ranked in decreasing order of toxicity, were: cardenolide *c*, calotropin  $\cong$  labriformin, uscharidin, cardenolide *a*. Each of the LD50 values is below 50 mg/kg. Cardiac glycosides present in *A. eriocarpa* represent a highly toxic group of compounds that account for the toxicity of the plant. (Supported in part by NIH ES00125, NSF BMS 75-14266, and the U.S. Department of Agriculture.)

3. *Safety Evaluation of Insect Repellent Cyclohexamethylene Carbamide*. STANTON C. LOVRE, ARTHUR H. MCCREESH, and MAURICE H. WEEKS, U.S. Army Environmental Hygiene, Aberdeen Proving Ground, Maryland.

Cyclohexamethylene carbamide has been proposed for use on U.S. Army troops as a replacement for the standard insect repellent, diethyltoluamide. It has been widely used in Russia and elsewhere, but few toxicological data were available. Potential safety evaluations were performed on the pure compound and organic solutions using laboratory animals and *in vitro* mutagen evaluations were performed prior to application to humans. Acute testing with New Zealand White rabbits indicated that the pure liquid was a primary skin and eye irritant, but was not photochemically active in alcoholic solution. Repeated skin applications (5 days/week for 3 weeks) showed that alcoholic concentrations of 5% or greater produced contact dermatitis in rabbits, while applications of 1% solutions produced no irritant effects. Testing with Dunkin-Hartley guinea pigs failed to indicate a sensitization potential. Acute oral LD50 values in male and female Sprague-Dawley rats were 4274 and 2668 mg/kg, respectively. A 14-day feeding study in rats indicated no-effect levels of 535 mg/kg/day for males and 384 mg/kg/day for females. Dermal applications of <sup>14</sup>C-labelled compound to rabbits resulted in the transfer of 52% of the applied dosage to the urine within 24 hr, and 65% by 7 days, with the remainder found at the application site. Teratologic testing in rats, using ip injections of up to lethal doses, produced no chemically related abnormal changes to indicate a fetal hazard. *In vitro* mutagenic evaluation using the Ames procedure developed no indication of mutagenic activity. Enzyme induction testing in male rats indicated that this compound was not an inducer of hepatic microsomal activity. Rapid avoidance behavioral testing resulted in significant performance decrement only after injection of doses greater than one-half of the predetermined LD50 level, doses which produced grossly obvious toxicological signs.

4. *Percutaneous Absorption of Zinc Pyridinethione in Monkeys*. W. B. GIBSON and G. CALVIN, Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio. (E. V. Buehler)

Zinc pyridinethione (ZPT) is used as an antidandruff agent in shampoo formulations. This work was undertaken to estimate the systemic exposure to ZPT that might be expected from exaggerated topical applications to primates. The penetration of ZPT through intact or abraded abdominal skin of rhesus monkeys was measured by applying lotions that contained 2% [<sup>35</sup>S]ZPT, with or without surfactant, either once or on 3 successive days. The material that remained on the skin was removed after each 3-hr exposure. The amount of <sup>35</sup>S that was absorbed was determined by measuring and summing the radioactivity of the urine, feces, and carcass. Absorption was: 0.02% of the applied dose (24  $\mu$ g), when a single application of 6-8 mg of ZPT/cm<sup>2</sup> was made on a 10-cm<sup>2</sup> area of intact skin; 0.2% of the total applied dose (315  $\mu$ g) when three successive applications (6 mg/cm<sup>2</sup>/day) were made in a medium containing 20% surfactant; (0.3% (221  $\mu$ g) when the ZPT was applied (7 mg/cm<sup>2</sup>) as a single 3-hr application to abraded skin. Levels of <sup>35</sup>S in the blood were below the threshold of detection (0.02  $\mu$ g of ZPT/g) except after repeated applications in a surfactant vehicle or after application to abraded skin. The <sup>35</sup>S that was absorbed was almost entirely excreted in the urine. Only small fractions of the absorbed radioactivity appeared in the feces or in the liver and kidney at sacrifice. The penetration of ZPT through the scalp of rhesus monkeys was found to be independent of the

duration of exposure. Absorption and subsequent excretion of  $^{14}\text{C}$ , applied to the scalp as a suspension of [ $^{14}\text{C}$ ]ZPT ( $400\ \mu\text{g}/10\ \text{cm}^2$ ), was equivalent to  $11.7 \pm 5.3\ \mu\text{g}$  of ZPT, whether the material was applied for 3 or 72 hr. Blood concentrations of  $^{14}\text{C}$  were generally below the detectable limit of  $1\ \text{ng/g}$ , calculated as ZPT. It was concluded that the amount of ZPT absorbed under the conditions used was extremely small and probably without physiological significance.

5. *Acute and Subacute Intravenous Toxicity of Hashish Oil in Monkeys.* YUGAL K. LUTHRA, HARRIS ROSENKRANTZ, ROBERT W. FLEISCHMAN, and MONIQUE C. BRAUDE, Mason Research Institute, Worcester, Massachusetts, and NIDA, Rockville, Maryland.

Toxicity findings on crude marijuana extract prepared for NIDA have been reported (Thompson *et al.*, *Toxicol. Appl. Pharmacol.* **25**, 363, 1973), but similar data on illicit hashish oil are lacking and the present study provides such data. Sex-paired rhesus monkeys (2–3 kg) received a single saphenous injection of 480, 360, 240 or 120 mg/ml in a 60-ml volume of an aqueous emulsion delivered at a rate of 2 ml/min. The drug formulation consisted of 4% hashish oil (11.6%  $\Delta^9$ -THC), 15% sesame oil, and 1% Polysorbate 80 in isotonic saline; control animals received a similar formulation but without hashish oil. In a subacute iv study, 2 monkeys/sex/dose received 260, 130, or 65 mg/kg in a volume of 6.5 ml/kg for 5 consecutive days. The acute iv LD<sub>50</sub> was 326 mg/kg and the cause of death (1–2 days) was respiratory arrest and cardiac failure. Toxic signs included muscle spasms, ataxia, asthenia, prostration, dyspnea, and hypothermia. Subacute treatment initiated delayed (Day 3 or 5) lethality in one animal of each sex at the high dose. Behavioral (CNS inhibition) and physiological (hypopnea, hypothermia, and bradycardia) changes were dose related and sex related, males being more affected than females. Tolerance to some aspects of CNS inhibition developed between Days 3 and 5. High-dosed monkeys had necrosis and ulceration at the injection site associated with edema and hemorrhage of the limb. This dose also caused hemorrhages in lungs, heart, gut, and adrenals. Moderate focal interstitial giant cell pneumonitis was common and morphological changes were associated with microembolization of drug formulation. Pathological findings in control monkeys were not vehicle related. No major aberrations in hemochemistry or urinalysis were seen for treated or control animals. An acute study with a second batch of hashish oil (31.1%  $\Delta^9$ -THC) yielded similar results in terms of behavioral and physiological changes but, surprisingly, was less toxic than the hashish oil with the lower content of  $\Delta^9$ -THC.

6. *Observations on the Toxicity of Zearalanone in the Rat.* MICHAEL A. GALLO, DAVID E. BAILEY, JOHN G. BABISH, GEORGE E. COX, and JEAN M. TAYLOR, Food and Drug Research Laboratories, Inc., East Orange, New Jersey, and Food and Drug Administration, DHEW, Washington, D.C.

Zearalanone, a metabolite of fusarium in corn mold, has been shown to be mildly estrogenic in poultry, cattle, and swine. The studies presented here are part of a major program to determine the safety of Zearalanone as a potential indirect food additive. Chronic toxicity studies are in progress with Zearalanone as a dietary additive at concentrations corresponding to 0, 0.1, 1.0, and 3.0 mg/kg/day. The animals on this phase of the study were derived from F<sub>0</sub> parents exposed to equivalent levels of Zearalanone. Exfoliative cytology suggests a prolongation of estrus cycles in females receiving 3.0 mg/kg/day but little or no change at the 0.1 mg/kg level. A decrease in body weight gain, which is dose dependent, is observed in male rats and also in high-dose female rats. The major observed pathological change has been the elongation of long bone trabeculae which apparently is related to the estrogenic action of the compound.

7. *Subacute Oral Toxicity of Two Alkylating Agents, Methyl Methanesulfonate, and Metepa, in the Mouse.* G. L. KENNEDY, JR., D. E. GORDON, M. L. KEPLINGER, and J. C. CALANDRA, Industrial Bio-Test Laboratories, Northbrook, Illinois.

Groups of 15 CD-1 mice per sex were fed either MMS (methyl methanesulfonate) or metepa [tris(2-methyl-1-aziridinyl)phosphine oxide] for 90 consecutive days. Diets, containing either 300 or 1000 ppm MMS or 100 or 300 ppm metepa were prepared weekly and refrigerated, and they were offered to the animals daily. A concurrent group of 15 mice of each sex served as

control. During the experiment, no unusual behavioral reactions were observed among the test mice. Growth and food consumption data revealed no treatment-related differences. The number of reticulocytes among males fed 300 ppm metepa, males fed 300 ppm MMS, and both sexes fed 1000 ppm MMS were increased. All other hematologic parameters and clinical blood chemistry data were unaltered. No treatment-related changes were seen in the analysis of urine samples for albumin, glucose, occult blood, and formed elements. Gross pathologic examination revealed a reduction in the size of the testes among mice fed either 1000 ppm MMS or 300 ppm metepa. This finding was confirmed by analysis of organ weight data. Animals in these groups displayed degenerative changes in the testes and epididymides. The early testicular alterations consisted of a diminution or absence of sperm within the seminiferous tubules. The more advanced lesions consisted of an absence of the germinal epithelium lining tubules with concomitant atrophy or collapse of the tubules and hyperplasia of the interstitial cells of Leydig. Testicular degeneration to a minimal degree was seen in 6 of 13 animals fed 300 ppm MMS. Testicular findings among animals fed 100 ppm metepa were similar to those seen in the controls. Microscopic evaluation of the liver, kidneys, and spleen failed to reveal any pathological changes.

8. *Information Analysis: The Toxicology Information Response Center Activities During 1976.* H. G. GERSTNER, J. E. HUFF, G. U. ULRIKSON, H. M. KISSMAN, and B. M. VASTA, Information Center Complex/Information Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, and Toxicology Information Program, National Library of Medicine, Bethesda, Maryland.

The mounting concern accompanying the increased awareness of hazards to the human environment mirrors the need for more reachable information in the broad discipline of toxicology. The steadily increasing volume of literature in all fields of science and technology has created a need for specialized information services. Particularly during the last decade, this need has culminated in the emergence of information centers that possess scientific and technical expertise to support the scientific community by updating the state of the knowledge. Recently, a symposium on *The Handling of Toxicological Information* was convened in Washington, D.C., to commemorate the tenth anniversary of the President's Science Advisory Committee report. The meeting reviewed the past, stated the present, and forecasted the future. Toxicology information is no exception to the generally accepted axiom that most published literature—even that reporting a single, well-defined subject area—appears scattered throughout the world in myriad sources. To meet these visible needs, the Toxicology Information Response Center (TIRC) was founded in the fall of 1971 at the Oak Ridge National Laboratory as a national and international center for toxicology information. Sponsored by the National Library of Medicine's Toxicology Information Program, TIRC is part of the Information Center Complex, a major scientific and technical unit within the Information Division of the Oak Ridge National Laboratory. The complex coordinates the functions and activities of several information units at the Laboratory. These associations provide a unique opportunity for scientific and technical interactions. As an information analysis center, TIRC selects, evaluates, and synthesizes comprehensive literature packages according to a user's specific request or to satisfy current needs (chlordecone, mirex, vinyl chloride, trichloroethylene). TIRC also prepares and publishes specialized bibliographies, state-of-the-knowledge overviews, and state-of-the-art reviews on topics of current concern. TIRC interacts with a large and diverse user population, both domestic and foreign: government agencies, industry, academia, private citizens groups, and other organizations. This presentation describes the immediacy of response to topical and pressing problems, the various methods for disseminating toxicology information, and the cost-effectiveness of providing information support activities.

9. *Structure-Activity Relationships of Active Uptake of Paraquat into Rat Lung as Influenced by 4,4'-Bipyridyls.* JOHN H. ROSS and ROBERT I. KRIEGER, Department of Environmental Toxicology, University of California, Davis, California.

Paraquat is a widely used, nonselective herbicide whose ingestion 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 capable of producing 10:1 (lung:plasma) concentrations. Tissues such as liver, kidney, and brain did not concentrate paraquat to any great extent. Using the rat lung-slice uptake method developed by Rose *et al.* (*Biochem. Pharmacol.* **25**, 419, 1976), we attempted to find a compound capable of inhibiting (i.e., competitively) paraquat uptake. Among the compounds tested were homologs of paraquat in the 4,4'-bipyridyl series. Measured decreases in rate of [<sup>14</sup>CH<sub>3</sub>]paraquat uptake were used to determine the percentage inhibition. Test compounds were added at 0, 10<sup>-5</sup>, 10<sup>-4</sup>, and 10<sup>-3</sup> M concentrations. The concentration of paraquat used in uptake studies was 10 μM, which approximates paraquat concentrations in rat plasma for 15 hr following an oral dose of 126 mg/kg. Inhibitory 4,4'-bipyridyls are those with at least one quaternary nitrogen or the potential for generating one by ionization in aqueous medium at pH 7.4. The relative order of inhibitory potency for the 4,4'-bipyridyls in the series thus far tested are: biperidine (reduced 4,4'-bipyridyl) ≥ 4,4'-dimethyl ≥ 4,4'-diethyl > 4-methyl ≈ 4,4'-dipropyl > 4,4'-dibenzyl ≫ parent compound (4,4'-bipyridyl). This study suggests that increasing chain length on the nitrogen of the 4,4'-bipyridyls decreases ability to block paraquat uptake. (Supported in part by NIH-ES00125.)

10. *Studies on the Long-Term Toxicity of 2-Butanol.* MICHAEL A. GALLO, BERNARD L. OSER, GEORGE E. COX, and DAVID E. BAILEY, Food and Drug Research Laboratories, Inc., East Orange, New Jersey.

2-Butanol is being developed as a fat extractant for the production of fish protein concentrate. The major advantage of this alcohol over isopropanol, which is currently used, is a lower residue in the finished concentrate. Investigations on the toxicology of 2-butanol included a two-generation rat reproduction study to evaluate the toxicologic potential of the compound when administered via the drinking water. To provide a point of comparison, isopropanol was administered to one group of rats as a control. 2-Butanol was administered at three levels, 0.3, 1.0, and 2.0%, of the drinking water through the first (F<sub>0</sub>) and second generation (F<sub>1</sub>) and the effects were compared to those of 2.0% isopropanol similarly administered. The 2.0% level was selected as the highest dose after initial results with the F<sub>0</sub> generation rats showing toxicity at a level of 3.0%. All findings with 2-butanol at both 0.3 and 1% were negative in terms of both growth and reproduction efficiency. However, at 2.0%, both isopropanol and 2-butanol caused a significant depression in growth of weanling rats with evidence of retarded skeletal maturation. The 2.0% level of both alcohols resulted in several changes which represent mild toxicity and are reminiscent of stress lesions. The changes with 2-butanol were similar in all respects to those observed with isopropanol.

11. *Effects of Citrinin on Renal Transport Processes.* W. O. BERNDT and A. W. HAYES, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi.

Citrinin, a toxic metabolite produced by *Penicillium citrinum*, was reported nephrotoxic in several "field studies." The present laboratory undertaking involved measurement of *in vivo* and *in vitro* renal function in Sprague-Dawley and Fischer rats. No enhanced sensitivity of the Fischer rats to citrinin was found. For *in vivo* studies, animals were housed in metabolism cages for one 24-hr control period, after which a single ip dose of either 35 or 70 mg/kg of citrinin was given. Compared to controls, all treated animals showed significantly increased urine flows and decreased urine osmolalities over the first 3 to 4 days post-treatment with recovery by 6 to 8 days. Both groups of test animals showed increased urinary protein excretion and the higher dose animals showed increased urinary glucose excretion. Time-course studies on renal transport indicated that maximal effects were at 4 days after the nephrotoxin. With the higher dose, *p*-aminohippurate (PAH) transport was reduced significantly, as was transport of tetraethylammonium (TEA). The transport of  $\alpha$ -aminoisobutyrate (AIB) was unaffected by citrinin pretreatment. Altered renal transport also was noted after *in vitro* addition of toxin to fresh kidney cortex slices. Significant stimulation of AIB transport was observed at low concentrations. Both PAH and TEA uptake were depressed *in vitro*. No changes in tissue inorganic electrolytes or water were observed in either experimental protocol. It appears that citrinin may

produce a "classical" nephrotoxic response in Sprague-Dawley rats. Also, the dramatic effect on renal transport may indicate a specificity of citrinin for transport functions in addition to actions on glomerular function or intrarenal blood flow. (Supported by PHS Grants AM 18124 and ES 01351.)

12. *Vehicle-Mediated Differences in Iododeoxyuridine-Induced Embryotoxicity in Mice.* R. G. SKALKO, D. S. PACKARD, JR., D. A. CANIANO, and K.-F. BENITZ, Institute of Comparative and Human Toxicology, Albany Medical College, and Birth Defects Institute, New York State Health Department, Albany, New York.

The thymidine analog, 5-iodo-2'-deoxyuridine (IdU), is poorly soluble; thus, most studies have utilized suspensory vehicles. Previous studies have demonstrated that IdU, suspended in 0.5% CMC, is a potent embryotoxic agent when administered, ip, to pregnant mice (ICR). This study reports the differential effects of the same dose of IdU (500 mg/kg) administered on Day 10 of gestation in either of three vehicles: 0.5% CMC; 0.5% MC, or 50% 0.1 N NaOH-50% 0.9% NaCl. Autopsy on Day 17 showed the IdU-CMC regimen to be the most toxic, producing 92% resorption sites. The IdU-MC combination resulted in a reduction of the resorption rate to 19% with a concomitant increase in surviving fetuses with CP (73%). IdU dissolved in the alkaline-saline solution produced results comparable to the IdU-MC data (9% resorptions; 90% CP). Two of the vehicles (CMC, MC) had no effect when administered alone although the alkaline-saline vehicle was 100% lethal to pregnant females within 24 hr, producing a necrotizing peritonitis in various regions of the peritoneal cavity. The kinetics of a single dose of [6-<sup>3</sup>H]IdU (500 mg/kg) in either CMC or MC was studied. Qualitative differences in distribution of radioactivity in the acid-soluble fractions were observed, most particularly in the placenta and embryo, but there were no distinct quantitative alterations. Incorporation into DNA was comparable with both vehicles up to 2 hr, after which MC values reached a plateau. At 4 hr, MC values were less than those for CMC in maternal spleen (40% decrease), placenta (66% decrease), and embryo (32% decrease). These data are consistent with the interpretation that lower incorporation into the DNA of the embryo results in reduced embryotoxicity. (Supported by a grant from the National Foundation March of Dimes and Research Grant 2-P01-ES00226-10 from NIEHS, NIH.)

13. *The Human Placental Cholinergic System: A Model for Examining its Role in Placental Function.* M. FANT and R. D. HARBISON, Center in Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

We have previously demonstrated the presence of components of the cholinergic system in the placenta of several mammalian species. It has been difficult, however, to determine the exact tissue of origin of these components using placental homogenates. We have been able to overcome this problem by identifying and characterizing the cholinergic system in an isolated syncytiotrophoblast plasma membrane preparation. Plasma membranes were prepared by ultracentrifugation. Electron microscopy demonstrated that the membrane preparation consisted primarily of membrane vesicles (0.1-0.2  $\mu$ m in diameter) stainable with iron hydroxide. Assays for subcellular enzyme markers indicated a 19- to 21-fold enrichment of the plasma membrane, as demonstrated by 5'-nucleotidase activity. There was also a 20- to 21-fold increase in the ability of the membranes to hydrolyze acetyl- $\beta$ -methylcholine with respect to butyrylcholine. In both the homogenate and vesicle preparation, acetylcholine was the preferred substrate of the enzyme(s). These data support the hypothesis that the placenta contains primarily a true cholinesterase and is membrane bound. Binding sites have also been identified exhibiting high affinity for [3-<sup>3</sup>H]quinuclidinyl benzilate, a potent central muscarinic antagonist. Specific binding was saturable with respect to increasing ligand concentration and exhibited an apparent  $K_D$  of 20 nM. It has been demonstrated that these vesicles exhibit amino acid transport properties similar to those seen in intact placenta. These data suggest that the placental membrane vesicle could serve as a unique experimental model for looking at the function of the placental cholinergic system for regulation of transport processes. (Supported in part by USPHS Grants ES00112 and ES00267.)

14. *Teratogenicity Study on Hydroxyurea in the Cat*. K. S. KHERA, Bureau of Chemical Safety, Food Directorate, National Health and Welfare, Ottawa, Canada

To evaluate further the usefulness of cats for teratogenicity studies, hydroxyurea (an anti-tumor drug and a known teratogen in the rat, miniature swine, and dog), was examined for malformative potential in the cat fetus. Pregnancies were induced in cats by synchronizing gonadotropin-stimulated estrus and ovulation prior to timed copulation. Hydroxyurea, 50, 100, or 150 mg/kg, dispensed in gelatin capsules, was administered po in single daily doses on Days 9 and 10, 10 and 11, 11, 11 and 12, or 13 and 14 of gestation. Appropriate controls given empty capsules were included. Cats were necropsied on Day 44 of gestation. One-half of the fetuses were processed for visceral and the remainder for skeletal examination. Test group dosed with hydroxyurea on Days 13 and 14 had a significantly high incidence of anomalies, characterized by fleshy heart, hydrocephalus, exencephaly, and umbilical hernia. From the present and previously reported studies on thalidomide, methylmercury, acetylsalicylic acid, methotrexate, and amaranth, the cat appears to be a suitable species for teratogenicity assessment of chemicals.

15. *Dipterex Teratogenicity in the Rat, Hamster, and Mouse When Given by Gavage*. R. E. STAPLES and E. H. GOULDING, Environmental Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Previous study in this laboratory showed that Dipterex, an extensively used pesticide, was teratogenic when added to the diet of rats but not when administered by gavage once daily for the same period of gestation. This study was undertaken to determine whether the teratogenic potential of Dipterex could be detected by gavage through administration three times daily rather than once daily to the rat and through three times daily administration to additional species. Dipterex was found to be teratogenic in the rat if given three times daily at 480 mg/kg/day from Days 6 through 15 of gestation but not if given only on Days 8 or 10 of gestation. The malformations occurred in all of the 19 litters exposed to Dipterex (an average of 76.4% of fetuses in each litter was malformed) and consisted mostly of generalized edema, various types of herniation of the brain and cerebrospinal fluid through the skull, micrognathia, severely shortened radius and ulna, hypophalangism, syndactyly, fusions of ribs and sternbrae, and hematomas. A similar positive teratogenic response also occurred in the hamster after administration of Dipterex from Days 7 through 11 of gestation at 400 mg/kg/day; the apparent "no effect" level for the criteria studied was 200 mg/kg/day. Embryotoxicity, but not teratogenicity, occurred after 400 mg/kg/day given on Day 8 of gestation. In both species, the teratogenicity seen was not merely due to reduced maternal food consumption. The mouse was less susceptible to Dipterex than were the rat and hamster, but a significant increase in the incidence of cleft palates resulted from exposure on Days 10 through 14 or on Days 12 through 14 of gestation.

16. *Fetotoxicity of Dimethadione in Rats*. H. S. BUTTAR, I. DUPUIS, AND K. S. KHERA, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Dimethadione (DM), a major metabolite of a suspected human teratogen trimethadione which is used for epileptic treatment, was investigated for its teratogenic potential in Wistar rats. DM in aqueous solution was administered po in single daily doses of 0, 54, 216, 433, or 541 mg/kg on Days 1 through 21 or 6 through 15 of gestation. No maternal toxicity was apparent at any of the dosages tested. Administration of DM at doses of 541 mg/kg on Days 1-21 or 6-15 of gestation produced 100 and 8.6% fetal death, respectively. Fetal effects at the remaining three lower dose levels appeared to be similar for the two treatment durations examined and were dose dependent. These consisted of umbilical hernia, subcutaneous edema, taillessness, increased incidence of extra rib bent radius or ulna, bent tibia and fibula, retarded ossification of skull bones, and a variety of sternal defects.



17. *Pharmacodynamics of Mirex in Goats: Effect of Reproduction and Lactation.* JOHN A. LIDDLE, ANN L. SMREK, SAMUEL R. ADAMS, and RENATE D. KIMBROUGH, Center for Disease Control, Atlanta, Georgia.

Mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta(c,d)pentalene) has been used as an insecticide, particularly to combat fire ants in the southeastern United States, and has also been marketed as a flame retardant. It is a very stable compound similar to such halogenated aromatic substances as hexabromobiphenyl, octabromobiphenyl, hexa-, hepta-, and decachlorobiphenyl, and halogenated terphenyls. Some of these compounds have industrial uses, or have been suggested as flame retardants. Mirex was selected as a representative of this group of very stable compounds to be used in gaining additional information on the pharmacodynamics of these chemicals in mammals. Groups of five male and five female goats were given 0 or 1 mg of Mirex/kg daily for 61 weeks. An additional group of female goats was given 1 mg/kg daily for 18 weeks. Both groups of female goats were given daily doses of 10 mg/kg for 4 weeks after the respective exposures to 1 mg/kg for 61 and 18 weeks. The goats exposed for 61 weeks were bred twice and those exposed for 18 weeks were bred once. Plasma, milk, and adipose tissue were analyzed for Mirex at intervals. Adipose tissue concentrations of Mirex were lower in female than in male goats and were not noticeably affected by pregnancy. A steady Mirex concentration was not reached in adipose tissue, whereas the plasma concentrations became stationary after several months of exposure. The concentration of Mirex in milk fat was highest in the immediate postpartum period and then declined in spite of continued dosing. The dose of 1 mg/kg of Mirex did not affect reproduction.

18. *Chronic Toxicity and Reproduction Studies in Rats Given Pentachlorophenol by the Dietary Route.* B. A. SCHWETZ, J. F. QUAST, P. A. KEELER, C. G. HUMISTON, and R. J. KOCIBA, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

Groups of male and female Sprague-Dawley rats were maintained on diets containing sufficient technical pentachlorophenol, characterized by a low content of nonphenolic impurities, to provide dose levels of 0, 1, 3, 10, or 30 mg/kg/day for up to 24 months. Pentachlorophenol was not found to be carcinogenic when administered to rats in their diet on a chronic basis at dose levels sufficiently high to cause mild signs of toxicity. Parameters affected at the high dose level included decreased body weight (females), increased SGPT activity (males and females), and increased urine specific gravity (females). An accumulation of pigment was observed in the liver and kidneys of females at 30 and 10 mg/kg/day and males at 30 mg/kg/day. No significant toxicologic effects were associated with ingestion of 3 mg/kg/day or less by females and 10 mg/kg/day or less by males. In the reproduction study, Sprague-Dawley rats were maintained on diets containing sufficient pentachlorophenol to provide dose levels of 0, 3, or 30 mg/kg/day for 62 days prior to mating, during 15 days of mating, and subsequently throughout gestation and lactation. A reduction in the mean body weight was observed among the adult rats at the highest dose level. Except for a significant decrease in neonatal survival and growth among litters of females ingesting 30 mg/kg/day, measures of reproductive capacity were unaffected at both dose levels of pentachlorophenol. Ingestion of 3 mg of pentachlorophenol/kg/day had no effect on reproduction or neonatal growth, survival, or development.

19. *Fetotoxicity and Teratogenicity of Zearanol in Mice.* G. J. DAVIS, J. A. McLACHLAN, and G. W. LUCIER, Endocrine Laboratories of Madison, Inc., Madison, Wisconsin; and Environmental Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (P. O. Nees)

Fetotoxicity, teratogenicity, and other effects on fecundity associated with the subcutaneous administration of zearanol were evaluated. In two separate experiments, eight groups of pregnant CD-1 mice were injected subcutaneously on Days 10 through 16 of gestation with one of seven log doses of zearanol between 10 and 10,000  $\mu\text{g}/\text{kg}/\text{day}$  or vehicle. In one experiment, 10 dams from each treatment group of 20 were allowed to deliver their young at term and the litters from the remaining 10 dams were taken by caesarean section and fetuses examined for

visceral and skeletal abnormalities. In the second experiment, the eight groups of 30 pregnant mice were allowed to deliver their pups and the pups were observed for gross anomalies, growth rate, and general behavior through 6 weeks of age. Zearanol administered at doses of 1000  $\mu\text{g}/\text{kg}/\text{day}$  and above resulted in a reduction in the number of liver litters (numbers of females inseminated/number of live births) from 81% in the controls to less than 35% in the treated groups, and a decrease in the average number of live young per litter from 10.2 pups in the controls to 5.5 pups or less in the 1000  $\mu\text{g}/\text{kg}/\text{day}$  and above groups. Zearanol (300)  $\mu\text{g}/\text{kg}/\text{day}$  increased the number of stillborn litters and thus reduced the number of live litters by more than 30% compared to control groups; there was no effect on litter size. Doses of 100  $\mu\text{g}/\text{kg}/\text{day}$  or less of zearanol had no significant effect on fecundity. In limited gross observations there was increased medullary trabeculae in bones of treated animals. Fetal examinations to date indicate no significant teratogenicity at any level. (Supported by NIEHS Contract N01-ES-2111.)

20. *Teratogenic Effects of the Plant Hormone Indole-3-Acetic Acid in Mice and Rats.* J. A. JOHN, C. D. BLOGG, F. J. MURRAY, B. A. SCHWETZ, and P. J. GEHRING, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical, U.S.A., Midland, Michigan.

Indole-3-acetic acid (IAA), a naturally occurring compound, is found in higher plants as a regulator of growth and in the urine of humans as a product of the metabolic degradation of tryptophan. These studies evaluated the teratogenic potential of IAA in mice and rats. Pregnant CF-1 mice and Sprague-Dawley rats were given IAA by gavage on Days 7-15 of gestation. Groups of 25 to 30 mice were given 5, 50, 200, or 500 mg/kg/day of IAA as a cottonseed oil suspension. Groups of 25 to 30 rats were given 50, 200, or 500 mg/kg/day of IAA. Control animals were given cottonseed oil without the test material. Mice and rats were sacrificed on Days 18 and 21 of gestation, respectively, and the fetuses were removed by cesarean section. IAA was teratogenic in mice and rats at 500 mg/kg/day; cleft palate was induced in both species at this dose level. In addition, exencephaly, ablepharia, polydactyl, and crooked tail were observed among litters of mice given 500 mg of IAA/kg/day. Few major malformations were observed among litters of mice or rats given lower dose levels of IAA during gestation. Other than a decrease in the weight gained among mice at the high dose level, little maternal toxicity was observed in either species. Parameters which were unaffected by dosage with IAA include food consumption, water consumption, and the incidence and distribution of resorptions in mice and rats, and the weight gained in rats. Indole-3-acetic acid is, therefore, a teratogen in mice and rats at a dose level which did not alter the incidence of resorptions in either species, was not maternally toxic in rats, and in mice, caused only a decrease in weight gain.

21. *Toxicity and Reproduction Studies with Patulin in the Rat.* MICHAEL A. GALLO, DAVID E. BAILEY, JOHN G. BABISH, JEAN M. TAYLOR, and ROBERT E. DAILY, Food and Drug Research Laboratories, Inc., East Orange, New Jersey, and Food and Drug Administration, DHEW, Washington, D.C.

Toxicity and effects on reproductive performance were investigated in Wistar derived rats gavaged with 0.0, 0.1, 0.5, and 1.5 mg/kg of Patulin three times weekly. A preliminary study indicated that 3.0 mg/kg was not tolerated. The  $F_0$  generation consisted of 50 males and 50 females per group with a control group of 70 males and 70 females. The  $F_1$  animals were observed through growth and maturation and two males and two females from each dose group, three per sex for controls, were randomly selected for the lifetime study. A dose-related decrease in growth and maturation was suggested in the  $F_1A$  generation, but after 6 months of exposure little or no effect was observed. (Supported by FDA Contract No. 223-75-2180.)

22. *Subtle Postnatal Effects of Locoweed in Rats.* B. K. NELSON, L. F. JAMES, R. P. SHARMA, and C. D. CHENEY, USDA, ARS Poisonous Plant Research Laboratory and Toxicology Program and Psychology Department, Utah State University, Logan, Utah.

Locoweed, a well-known teratogenic plant affecting livestock, is prevalent in mountain regions of the Western United States. To common species (*Astragalus lentiginosus* and

*A. wootoni*), administered to pregnant rats, produced behavioral deviations in the offspring. Treated mothers consumed less feed and gained less weight during gestation than controls when given locoweed by gavage at the rate of 1 g of whole plant per day on Days 7 through 17 of gestation. *A. lentiginosus* reduced pup weight at birth (13%) and the weight remained reduced for the next 4 weeks. *A. lentiginosus* also reduced number of live born (34.9%) and number that survived until weaning (86.4%). No gross malformations were observed in the test offspring, however, behavioral changes were noticed beginning 30 days of age. There was considerable difference among the treatment groups when tested with the activity wheel. Water-intubated controls did not differ from nonfed controls, but the two loco-treated groups differed in opposite directions from controls. *A. lentiginosus* offspring were more active (26.5% overall) than other groups and had an abnormal pattern of activity in the day to evening to night activity totals. *A. wootoni* offspring were less active (33%) than other groups in the activity wheel. Significant differences among groups were also observed in the open-field test. The trend was toward decreased activity ( $p = 0.027$ ; 31 and 43%) and increased number of fecal boluses ( $p = 0.06$ ; 45.5 and 19.3%) in the loco-treated offspring. There were no significant differences in avoidance conditioning in a two-way shuttle box among the groups, though the loco offspring made fewer avoidance responses than controls. The results indicate that the locoweed produces behavioral deviations in the offspring of rats without inducing any apparent gross malformations. It appears that the diencephalic centers involved in activity patterns and locomotion may be more susceptible to the effects of locoweed than the cerebral centers involved in cognition.

23. *Lead-Induced Developmental and Behavioral Changes in the Mouse*. L. EARL GRAY, JR, and LAWRENCE REITER, U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina, and Duke University, Department of Physiology and Pharmacology, Durham, North Carolina. (Stata Norton)

The effects of lead exposure during early development on locomotor activity and behavior remain unclear. The present study was undertaken in an attempt to replicate and expand the previously reported findings of lead-induced hyperactivity and increased aggressiveness in mice (Charles River, CD-1). Three exposure regimens to lead acetate were employed: (1) 5 mg/ml in the drinking water from parturition until 60 days of age, (2) 5 mg/ml in drinking water of dams throughout gestation and continued until 18 days postpartum, and (3) a single injection on Day 1 postpartum (1.0 mg/pup sc). In experiments where lead administration produced undernutrition, appropriate pair-fed controls were tested. Lead-treated females from the first regimen showed a 4-day delay in sexual maturation and hypoactivity in a residential maze at 30 and 50 days of age. Aggressive behavior was measured by introducing an adult intruder into the home cage of an individual experimental male. Control males on the average wounded the intruder 85 times during a 14-hr test period, while intruders to lead-treated and pair-fed male cages had means of only 32 and 35 wounds, respectively. Lead-treated male and female mice under the second lead regimen showed a 20-25% reduction in residential maze activity when tested at 130 days of age. Following testing, males were killed and the number of wounds received from fighting with cage-mates was determined. Sixty-two percent of the males from control cages were scarred while only 25% of the lead-treated males were wounded. Finally, 30-day-old females from the third lead regimen were hypoactive when tested in a 3 x 3-ft open field. These findings of lead-induced hypoactivity and reduced aggressiveness in mice are in disagreement with previously published reports.

24. *Effects of Drugs Given Postnatally to Rats*. FREDERIC R. ALLEVA, TIBOR BALAZS, DOWELL A. DAVIS, WILLIAM S. MONLUX, and CHANG S. LAO, Food and Drug Administration, Washington, D.C.

Drugs of three pharmacological classes were tested for morphologic and functional development of newborn rats in three separate experiments. The neuroleptic drugs, penfluridol (P, 4 mg/kg), trifluoperazine (T, 4 mg/kg), and 6-hydroxydopamine (6-OHDA, 50 mg/kg), were tested in the first experiment, while the antineoplastic drugs, cytosine arabinoside (CA, 1

mg/kg), 6-mercaptopurine (6-MP, 2 mg/kg), methotrexate (MTX, 0.015 mg/kg), and cyclophosphamide (C, 3 mg/kg) were tested in the second experiment. A third experiment with antibiotics is being completed. Doses were selected from results of a dose-finding experiment and were given sc daily from 2 to 22 days of age to four litters of eight pups (four males and four females) per litter (32 pups per dose). Controls received vehicle. Three additional litters (large litter controls) of 12 pups per litter served as controls for the effect of drug-induced malnutrition. At 23 days of age, half the pups in each litter were sacrificed for pathologic and hematologic examinations. Remaining pups were used for behavioral and reproductive tests. Effects observed were reduced body weight (6-OHDA, 6-MP, C), permanent ptosis (6-OHDA), reduced ear size (C), keratitis (C), estrous cycle irregularity (6-OHDA, P), pancytopenia and reduction of bone marrow lymphocytes (6-MP), and leucocytosis (P). No adverse effects were observed in T, CA, or MTX-treated groups. Functional tests revealed no adverse effects for any group. Pathologic examinations revealed no definitive lesions. In the dose-finding test for the third experiment with antibiotics, streptomycin (400 mg/kg) given as above was found to induce rapid backward movement with intermittent circling and head bobbing. These signs persisted long after dosing was stopped. In addition, these rats, as well as rats treated with tobramycin (200 mg/kg), became deaf. These subacute tests in postnatal rats may be useful in the preclinical safety evaluation of new drugs intended for use in pediatrics.

25. *Lithium Chloride-Induced Hypophagia and Geophagia: Two Behavioral Assays of Toxicosis Compared.* D. MITCHELL, Environmental Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. R. Fouts)

One mission of the program in Behavioral Toxicology at NIEHS is to evaluate the utility and reliability of different behavioral assays of toxicosis. In the present paper, changes in two different consummatory behaviors following LiCl poisoning in the rat are compared. Decreased food consumption (hypophagia) and increased clay consumption (geophagia) occurred in rats given a single ip injection of 0.45 M lithium chloride (381.6 mg/kg). Moreover, clay consumption had no effect on food consumption, indicating that these two responses to poisoning are complimentary but independent behaviors. Further differences in hypophagia and geophagia were revealed when rats were given 10 repeated ip injections of 0.15 M lithium chloride (127.2 mg/kg/trial) spaced 5 days apart. Whereas the characteristic deficit in food consumption was remarkably stable across the 10 trials (averaging approximately 6 g/rat/trial), clay consumption increased from a low of 1.5 g on the first trial to a high of 15.3 g on the fifth trial. After the fifth trial, geophagia showed the same stability as hypophagia across the subsequent trials. Additional experiments confirmed that the two behaviors are controlled by two different neurophysiological mechanisms. It is suggested that knowledge of the separate neurophysiological correlates of these behaviors will greatly enhance their utility as behavioral assays of toxicosis.

26. *The Toxicity of Paraquat in Mature and Immature Rats.* LEWIS L. SMITH and MICHAEL S. ROSE, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Biochemical Mechanisms Unit, Alderley Park, Nr. Macclesfield, Cheshire, United Kingdom.

It has previously been shown that the lungs of immature rats are more resistant to the toxic effects of paraquat than the lungs of mature rats. It is also known that the lungs of immature rats are more resistant to the toxic effects of high concentrations of oxygen. These two observations have contributed to the suggestion that the two phenomena are related and hence that the mechanism of oxygen and paraquat toxicity may be similar. We have confirmed that immature, male rats (50 g) are considerably more resistant to paraquat dosed orally (680  $\mu$ mol/kg) than mature, male rats (180 g). We have investigated this further by comparing plasma and lung concentrations of paraquat after dosing. The concentration of paraquat in the plasma of 180-g rats was maintained at approximately 10 nmol/ml for 24 hr and then started to rise, reaching 25 nmol/ml by 48 hr, in contrast to that of 50-g rats which fell to 2 nmol/ml by 48 hr. The concentration of paraquat in the lungs of the two groups of animals reflected this difference in plasma concentrations. The 50-g rats were also more resistant to paraquat when it was administered by sc injection (105  $\mu$ mol/kg). Both the peak plasma and lung concentrations of paraquat

were significantly less than in 180-g rats. We have, therefore, concluded that immature rats are less sensitive to paraquat than mature rats because less paraquat reaches the lung for a given dose. Paraquat concentrations in the lung are lower as a consequence of reduced plasma concentrations, which reflects a difference in the rate of clearance of paraquat by the kidneys of mature and immature rats. It is, therefore, unlikely that the difference in sensitivity of mature and immature rats to paraquat is related to the difference in sensitivity of these animals to high concentrations of oxygen.

27. *Effect of Amphetamine and Alcohol on Lipid Synthesis and Transfer in the Rat.* C. DI FONZO, W. C. BRECKENRIDGE, and G. FEUER, Department of Clinical Biochemistry and Lipid Clinic, University of Toronto, Toronto, Canada.

Cholesterol (Chol), triglyceride (TG), and phospholipid (PL) concentrations and lipoprotein pattern in serum were studied in rats given amphetamine or methamphetamine (0.1 mmol/kg, four daily doses) alone, or in combination with alcohol (10% in the drinking water for 2 to 3 weeks). The *de novo* synthesis and transfer of PL in liver and brain using various precursors and intermediates ([2-<sup>14</sup>C]acetate, L-[Me-<sup>14</sup>C]methionine, S-adenosyl-L-[Me-<sup>14</sup>C]methionine, Me-<sup>14</sup>C-labeled hepatic PL) were also investigated. Both drugs, given alone, significantly reduced serum Chol and TG. Amphetamine decreased VLDL and LDL; HDL was moderately reduced. Methamphetamine diminished VLDL and LDL to a lesser extent; HDL was unaltered; total PL content of the brain and liver showed no change. Following amphetamine treatment the incorporation of L-[Me-<sup>14</sup>C]methionine or [<sup>14</sup>C]acetate into liver or brain PL was unaltered or slightly increased, S-adenosyl-L-[Me-<sup>14</sup>C]methionine was significantly raised; the uptake of Me-<sup>14</sup>C groups from Me-<sup>14</sup>C-labeled hepatic PL into the brain reached the highest level. In addition, alcohol treatment showed an antagonism to amphetamine in several parameters measured. Since the brain has a very low S-adenosine-L-methionine:phosphatidylethanolamine methyl transferase activity it accumulates PL from newly formed hepatic PL by a process of exchange diffusion of lipoproteins. Our studies revealed that although amphetamine significantly reduced the synthesis of several lipids, it raised the exchange diffusion of PL into the brain.

28. *Neurotoxicity of Kepone in Perinatal Rats following in Utero Exposure.* LAURENCE ROSENSTEIN, ANN BRICE, NEDRA ROGERS, and SUSAN LAWRENCE, Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina. (W. F. Durham)

A recent incident in a plant manufacturing Kepone has given rise to many questions and few answers involving exposure to this highly toxic insecticide. A review of the available literature would suggest that the problem of perinatal neurotoxicity following low level exposure during gestation and through weaning has not been adequately addressed. It was thus the purpose of this investigation to evaluate possible neurotoxic effects in perinatal rats. In this study, pregnant females (Sprague-Dawley) received 1, 2, or 4 mg/kg/day of Kepone by gastric intubation beginning on Day 2 of gestation and terminating at weaning. The pesticide was administered using corn oil as the vehicle. Controls, receiving only corn oil, were used throughout the study. At parturition, all animals in the control and 1.0-mg/kg/day groups delivered healthy pups. Of the pregnant females receiving 2.0 mg/kg/day, only one-third delivered healthy pups. Pregnancies in the remaining animals resulted in stillbirths and abortions. At the highest dose level, the treatment was fetotoxic to all dams. Maternal body weights were obtained directly after birth and compared to dam weights on Day 3 of gestation. For the two highest treatment groups, the weight gains were significantly ( $p < 0.05$ ) lower than the controls. On Day 15 postpartum a statistical difference ( $p < 0.05$ ) also was observed in pups derived from dams treated with 1.0 and 2.0 mg/kg/day of Kepone. At 24 days of age, the litters were divided into males and females and electroencephalograms and visual-evoked responses (VER) were obtained. The data indicated that the brain electrical activity of Kepone-treated perinates was significantly different from that of controls. This difference was not quite as evident in the dams. The perinatal VER, however, appeared normal. From the information just presented it would appear that Kepone,

given at low levels, was relatively toxic to the gravid females and resulted in a high degree of fetotoxicity with strong indications of CNS impairment in the perinatal rat.

29. *Comparative Behavioral Toxicology of Mirex and Kepone in the Rat.* LAWRENCE REITER, KAREN KIDD, GAIL LEDBETTER, L. EARL GRAY, JR., and NEIL CHERNOFF, U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina. (John Doull)

The acute toxicity of Kepone (K) is reportedly three times higher than Mirex (M), its completely chlorinated analog. The present study compared the relative behavioral toxicity of these compounds during an 8-week exposure period. A total of 117 male, Sprague-Dawley rats were assigned to one of five treatment groups at 114 days of age. The pesticides were administered in ground Purina lab chow at levels of 0, 40 ppm M, 80 ppm M, 40 ppm K, and 80 ppm K. Within 2 weeks of treatment, K-exposed rats showed an exaggerated startle response as indicated by an increased amplitude and delayed habituation. The number of startle responses to a loud click (presented at 5-sec intervals), increased to 290 and 480% of control for 40 and 80 ppm K, respectively. The amplitude of this response was decreased in M-treated animals although no differences in habituation were observed. Ambulation in open field was significantly depressed in both treatment groups, falling to 66 and 58% of control for 80 ppm K and 80 ppm M, respectively. Interestingly, ambulation in a residential maze was also depressed by 80 ppm M (83% of control) but showed a dose-related increase in the K-treated groups (130% of control at 80 ppm K). Finally, latency to emerge from the home cage was increased in K-treated animals (340% of control for 80 ppm K) and decreased in M-treated animals (50% of control for 80 ppm M). These results indicate that although M and K have similar chemical structures, they produce dissimilar behavioral effects in the rat. K treatment led to hyperreactivity as indicated by the exaggerated startle response, decreased ambulation in an open field, and delayed emergence time from the home cage. M treatment, on the other hand, produced a hypo-reactivity with attenuated startle response, increased emergence time, and decreased ambulation.

30. *A Comparative Study of Lead- and Rubidium-Induced Hyperactivity in the Rat.* C. CHOW and H. CORNISH, Interdepartmental Toxicology Program and Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan.

Rats, receiving 5, 13, or 20 mM lead acetate solution as drinking water for periods of 4 to 6 weeks and whose mothers were also placed on similar lead intake at parturition had significantly increased levels of spontaneous motor activity when compared to control animals. Rats (150 g) given 50 mM rubidium chloride as drinking water for 30 days had similarly elevated levels of spontaneous activity. It was the purpose of this study to examine biological parameters that might relate to this hyperactive state. Lead-induced hyperactivity was not associated with an elevation of electrolyte concentrations in the plasma or in the brain of treated rats. In addition, the steady-state cyclic AMP concentrations in the brain of lead-treated rats were comparable to controls. The major effect of 50 mM rubidium chloride ingestion for 30 days was a marked depression of potassium concentrations in all tissues measured and essentially a replacement of the potassium loss by rubidium. Thus, the combined potassium-rubidium tissue concentrations were comparable to the normal potassium concentrations in untreated controls. It is hypothesized that the resting membrane potential in rubidium-treated rats becomes less negative, leading to a higher neuronal firing rate and elevated concentrations of catecholamines at nerve endings. Catecholamine stimulation of adenylyl cyclase activity could in turn be responsible for the elevated concentration of cyclic AMP which was measured in the hindbrain of rubidium treated rats. (Supported by EPA Grant No. R802762.)

31. *Postnatal CNS Effects of Intrauterine Exposure to Ethylenethiourea in Rats.* L. TRYPHONAS and K. S. KHERA, Bureau of Chemical Safety, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada.

Ethylenethiourea (ETU), a degradation product of the ethylenebisthiocarbamate group of fungicides, is a potent rat teratogen. A wide spectrum of dose-dependent congenital deformi-

ties has been reported. A single oral dose of ETU, 30 mg/kg, given to Wistar rats on Day 14, 15, or 18 of gestation had no adverse effect on external morphology, weight, or number of pups at birth, but produced hydrocephalus and microphthalmia in 90% of offspring (0% in controls) dying until 9 weeks old. The anomaly was recognized on Days 6-9 of postnatal life, increased thereafter in severity and incidence, and was fatal. Cross-fostering tests revealed that milk from treated dams was without any etiologic significance and that the anomaly might have been initiated prior to cross-suckling. Twenty-four percent of the offspring from dams treated on Day 15 that survived after 9 weeks had, in addition, motor impairment highlighted by a hopping gait associated with gross and histologic progressive degenerative lesions in the neuraxis. Both gross and histopathologic findings correlated well with the neurologic deficit and their slow rate of development accounted for the delayed appearance of the neurologic impairment.

32. *Carbon Monoxide-Induced Decrease in Brain Dopamine Metabolism: Age-Related and Persistent Effects.* M. B. NEWBY, R. J. ROBERTS, and R. K. BHATNAGAR. The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, Iowa.

Carbon monoxide (CO) is known to produce neurological sequelae, including a Parkinson's-like state. We have found that exposure to 1500 ppm CO will decrease the synthesis and turnover of dopamine (DA) in the caudate nuclei of the adult rat brain (*Pharmacologist* 18, 170, 1976). The effects of CO on the immature brain have not been reported and may have implications in cases of CO poisonings of children. The acute toxicity of a 3 to 5 hr of exposure to 1500 ppm CO was examined in 8- to 21-day-old and adult rats by determining the decline of DA concentrations in the caudate nucleus after blocking catecholamine synthesis with  $\alpha$ -methyl-*para*-tyrosine (AMPT). The drug was administered (250 mg/kg) 1 to 2 hr before the start of CO exposure. Compared to controls, the rate of DA depletion was lower in CO-treated animals, regardless of age. DA concentrations after 3 hr of exposure (4 hr following AMPT) were higher than controls by 64% in adults and 59 and 56% in 13- and 8-day-old rats, respectively, indicating a decrease in DA turnover. In addition, the basal concentrations of DA in the caudate were increased in the various aged rats exposed to 1500 ppm CO for 2 to 5 hr (no AMPT given) when compared to controls. Average increases in basal values after a 5-hr CO exposure were 17 and 27% for 15- and 21-day-old rats. Eight-day-old rats treated with CO (1500 ppm, 5 hr) showed a persistence of the effect on DA turnover when examined at 23 and 42 days of age. Although a cause and effect relationship remains to be established, these data are consistent with the known neurological toxicity of CO. (Supported by Grants NIH GM-12675 and PHSST81-GM00141-18.)

33. *Neurotoxicity: A Contemporary Morphological Assay.* PETER S. SPENCER, MONICA C. BISCHOFF, and HERBERT H. SCHAUMBURG, Neurotoxicology Unit, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, New York.

Sensitive techniques have been employed routinely to detect and characterize pathological changes in the central (CNS) and peripheral (PNS) nervous systems of experimental animals undergoing chronic exposure to neurotoxic chemicals such as acrylamide, methyl *n*-butyl ketone, and *n*-hexane. The evolution of neuropathology is determined by examining tissues removed at various stages of disease: (1) by repeated biopsy of vulnerable areas of the PNS such as hindlimb pacinian corpuscles (cat), calf muscles, and their nerve supply, and (2) by extensive sampling of the PNS and CNS following systemic perfusion with fixatives. For this purpose, animals were anesthetized with sodium barbital containing heparin; the chest, pericardium, right atrium, and ventricles were cut open, a cannula was clamped into the aortic arch, and a perfusate consisting of 4% paraformaldehyde followed by 5% glutaraldehyde (each in phosphate buffer pH 7.4) was pumped into the animal at a pressure of 100-200 mm Hg for 20 sec and 15 min, respectively. Thin transverse slices of the cerebellar vermis, medulla oblongata, and spinal cord, and segments of the optic, sciatic, tibial, plantar nerves, their associated spinal ganglia, roots, and muscles, were postfixed in 2% chrome osmium, dehydrated stepwise, immersed in propylene oxide, and infiltrated with epoxy resin. Single nerve fibers, teased apart in liquid resin from distal parts of peripheral nerves were examined by bright-field and

Nomarski microscopy. After hardening blocks of tissue, 1- $\mu$ m sections were stained with 1% toluidine blue and examined with bright-field microscopy. Thin sections of selected areas, stained with uranyl acetate and lead citrate, were examined by electron microscopy. These methods permitted determination of (1) subclinical neurotoxic damage, (2) spatial-temporal distribution and pattern of change, and (3) relative involvement of CNS and PNS tissue components.

34. *Ultrastructural Morphometric and Biochemical Studies of Chronic Arsenic Exposure on Hepatocyte Mitochondria of Rats and Mice.* B. A. FOWLER, J. S. WOODS, and C. M. SCHILLER, Environmental Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

This investigation was undertaken to delineate the subcellular manifestations of arsenic toxicity following chronic exposure using combined ultrastructural morphometric and biochemical techniques. Male rats and mice were given access to dionized drinking water solutions containing 0, 20, 40, or 85 mg/liter arsenic as arsenate ( $\text{As}^{5+}$ ) for 6 weeks. Increased mitochondrial volume density was observed in hepatocytes located near the periphery of liver lobules. Respiration studies indicated decreased state 3 respiration and respiratory control ratios (RCR) for pyruvate mediated respiration at all dose levels. Rat mitochondria were more markedly affected than those of mice. Specific activity of monoamine oxidase which is localized on the outer mitochondrial membrane showed dose-related increases of 120–150% of control for both species. Cytochrome oxidase and  $\text{Mg}^{2+}$  ATPase, which are present on the inner mitochondrial membrane, showed increases in specific activity of 150–220% for rat liver mitochondria at the dose levels used while remaining unchanged in mice. Malate dehydrogenase activity, which is localized in the mitochondrial matrix, was unchanged at any dose level for either species. These studies suggest that mitochondrial damage following chronic exposure to arsenate is not uniformly distributed within the liver lobule and that decreased mitochondrial respiration is only one aspect of arsenic toxicity to this organelle. Arsenic-mediated changes in important enzyme systems localized in mitochondria which participate in the regulation of respiration and other metabolic functions are hitherto unstudied aspects of toxicity from this agent following *in vivo* exposure. The pattern of these changes does appear to show some species variation.

35. *Relationship between Plasma Cadmium-Thionein and Cadmium-Induced Nephropathy.* M. G. CHERIAN, R. A. GOYER, and L. DELAQUERRIERE-RICHARDSON, Department of Pathology, University of Western Ontario, London, Ontario, Canada.

Cadmium-induced nephropathy occurs only a long time after exposure to cadmium chloride ( $\text{CdCl}_2$ ). The reason for this long lag period is not yet understood clearly. A group of male rats were injected ip with 0.6 mg of Cd/kg as  $\text{CdCl}_2$  daily, 5 days per week for a period of 8 weeks. The results suggested that exposure to  $\text{CdCl}_2$  had two phases. The early phase I was characterized by complete tolerance to cadmium exposure. This tolerant phase persisted for about the first 4 weeks of  $\text{CdCl}_2$  injection during which synthesis of metallothionein occurred and cadmium-thionein complex accumulated within renal cortex and in hepatic cell. The concentrations of cadmium in liver and kidney at the end of 4 weeks were 114.3 and 74.7  $\mu\text{g/g}$ , respectively. Cadmium-thionein was not detected in rat plasma at this experimental period. No increase in the urinary cadmium occurred during this phase nor was there any detectable functional or morphological damage to renal cells. However, there were increased numbers of microbodies with prominent circular profile in renal tubular lining cells. In the phase II of  $\text{CdCl}_2$  exposure the toxic signs of cadmium appeared. This pathological phase was characterized by renal tubular dysfunction (glycosuria), acute renal tubular cell damage, increase in urinary cadmium, and the presence of cadmium-thionein in the plasma. Phase II appeared between 6 and 8 weeks of  $\text{CdCl}_2$  injection and the concentration of cadmium in liver and kidney increased to 245.7 and 197.2  $\mu\text{g/g}$ , respectively. Since similar acute renal tubular cell damage can be produced by injection of cadmium-thionein complex (*Toxicol. Appl. Pharmacol.* **37**, 167, 1976), it is suggested that phase II or the toxic phase of cadmium exposure reflected extracell-



ular presence of cadmium-thionein complex. (Supported in part by grants from the Medical Research Council of Canada and the International Lead and Zinc Research Organisation.)

36. *Acute Hepatotoxicity of Ethylene, Vinyl Fluoride, Vinyl Chloride, and Vinyl Bromide after Aroclor 1254 Pretreatment.* R. B. CONOLLY, R. J. JAEGER, and S. SZABO, Department of Physiology, Harvard School of Public Health and Departments of Pathology, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts.

Male rats were pretreated by gavage with the polychlorinated biphenyl mixture (PCB) Aroclor 1254 (300  $\mu$ mol of PCB/kg) for 3 consecutive days. On Day 4 these rats were exposed, by inhalation (4 hr), to various concentrations of ethane, ethylene, vinyl fluoride monomer (VFM), vinyl chloride monomer (VCM), or vinyl bromide monomer (VBM). Animals were sacrificed 24 hr after exposure and acute hepatic responses were estimated by measurement of serum alanine- $\alpha$ -ketoglutarate transaminase (SAKT) and by light microscopy. All of the compounds tested, except ethane, caused significant elevations of SAKT and produced severe degeneration and necrosis of the liver. Typical SAKT elevations (mg pyruvate/ml serum-hr) were: unpretreated controls— $0.17 \pm 0.01$ ; PCB pretreated, not exposed— $0.19 \pm 0.02$ ; PCB and ethane at 50,000 ppm— $0.11 \pm 0.02$ ; PCB and ethylene at 25,000 ppm— $1.98 \pm 0.61$ ; PCB and VFM at 10,000 ppm— $6.29 \pm 0.99$ ; PCB and VCM at 24,000 ppm— $2.58 \pm 1.25$ ; and PCB and VBM at 33,000 ppm— $3.78 \pm 2.28$ . The relative potencies of these compounds in elevating SAKT were similar and the spectrum of morphologic changes (e.g., midzonal ballooning of hepatocytes and centrilobular hemorrhagic necrosis) was the same. The acute hepatotoxic response to ethylene, the similarity of this response to that seen after exposure to the halogenated analogs, and the lack of a response after ethane suggest the importance of the double bond in the effects seen. The similar acute toxicities in PCB-pretreated rats of the compounds tested, in light of the carcinogenicity of VCM, indicates the importance of the complete toxicological evaluation of ethylene, VFM, and VBM for carcinogenic effects. (Supported by ES-00002, OH-00315, and ES-00045.)

37. *Carbon Tetrachloride-Induced Plasma Retention of Sulfobromophthalein (BSP) in the Rainbow Trout (*Salmo gairdneri*).* W. H. GINGERICH, L. J. WEBER, and R. E. LARSON. Departments of Fisheries and Wildlife, and Pharmacology and Toxicology, Oregon State University, Corvallis, Oregon.

As in mammals, intoxication of the rainbow trout with  $\text{CCl}_4$  (2.0 ml/kg ip) results in necrosis of hepatic parenchymal cells surrounding central veins and impaired plasma clearance of BSP. Results of experiments to determine the effect of  $\text{CCl}_4$  on the distribution of BSP between plasma and liver indicated that, following a single dose of the dye (10 mg/kg iv), the rate of hepatic BSP accumulation was reduced in animals receiving  $\text{CCl}_4$  despite higher plasma concentrations of the dye. To determine if hepatic excretory function had been impaired by  $\text{CCl}_4$  intoxication, BSP was administered by graded infusion to bile duct-cannulated trout over three 4-hr periods (20, 40, 60  $\mu$ g/kg/min). Bile flows, bile BSP concentrations, and the relative percentage of metabolized BSP appearing in the bile were similar in treated and control animals, suggesting that, unlike mammals, components of the hepatic excretory process are not significantly impaired 24 hr after  $\text{CCl}_4$  treatment. It is concluded that the processes of hepatic uptake and accumulation of BSP rather than those of hepatic excretion are most affected by  $\text{CCl}_4$  intoxication and are responsible in large part for plasma BSP retention.

38. *Differences in Phenobarbital- or 3-Methylcholanthrene-Induced Alterations in Intestinal and Hepatic Drug-Metabolizing Enzymes of Various Animal Species.* C. L. MIRANDA and R. S. CHHABRA, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina.

A previous report from this laboratory has demonstrated mainly quantitative differences in activities of intestinal microsomal drug-metabolizing enzymes (DME) among various laboratory animal species (Chhabra *et al.*, *Drug Metab. Disp.* 2, 443, 1974). The present study was

undertaken to determine if there were differences in inducibility between intestinal and hepatic microsomal DME by phenobarbital (PB) or 3-methylcholanthrene (3-MC) given po or ip in mouse, rat, guinea pig, and rabbit. The microsomal DME studied were ethylmorphine demethylase (EMD), aniline hydroxylase (ANH), aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin deethylase (ECD), and cytochrome *P*-450 content. Doses of PB and 3-MC varied with the route of administration and animal species. Differences in the inducibility of EMD, AHH, and ECD due to the route of administration of PB were observed among guinea pig, mice, and rat small intestines. There was no difference between PB, po, and PB, ip, in the induction of hepatic DME, but differential effects of po- and ip-administered 3-MC on hepatic AHH and cytochrome *P*-450 were noted in rat and guinea pig. The induction of intestinal DME varied with the animal species and the type of drug substrate used. None of the rabbit intestinal DME were induced by PB or 3-MC even in rabbits fed semipurified diet. However, the rabbit intestinal DME were not totally resistant to environmental insults since starvation significantly reduced their activities. All hepatic DME studied were induced by PB except AHH in rat. 3-MC induced hepatic ECD in mouse and rat but inhibited it in rabbit. These results suggest that there are quantitative and qualitative differences between small intestine and liver. Unlike in liver, the inducibility of intestinal DME activities by 3-MC or PB depended on the type of drug substrate and animal species used.

39. *Stimulation of Biliary Function following Polybrominated Biphenyls*. S. Z. CAGEN and J. E. GIBSON, Department of Pharmacology, Michigan State University, East Lansing, Michigan.

Polybrominated biphenyls (PBBs) stimulate drug metabolism in the liver and produce a marked elevation in liver weight. The purpose of this investigation was to determine the effect of PBBs on biliary function in rats and mice. Animals were fed ground diet containing 0 to 200 ppm PBBs for 2 weeks. Biliary function was assessed in mice by determining the disappearance of indocyanine green (ICG) and ouabain from plasma following a single iv dose of these drugs (ICG, 40 mg/kg; ouabain, 0.1 mg/kg). Biliary function was assessed in rats by determining the biliary excretion and plasma disappearance of sulfobromophthalein (BSP; 120 mg/kg). In mice, treatment with PBBs resulted in enhanced disappearance of ICG from plasma with plasma ICG concentrations significantly lower than control values 10 and 15 min following injection of ICG in mice fed 100, 150, and 200 ppm PBBs. Similarly, in mice, dietary doses of 100 and 200 ppm PBBs resulted in significantly lower plasma ouabain concentrations relative to controls 60 min following administration of the glycoside. Relative to controls, rats fed 100 ppm PBBs had significantly lower plasma BSP concentrations 3, 10, 20, 30, and 40 min following BSP injection. Biliary excretion of BSP in control rats was 0.13, 0.62, 0.66, and 0.76 mg/min/kg 10, 20, 30, and 40 min following BSP injection, respectively, and was significantly increased to 0.56, 1.48, 1.80, and 1.76 mg/min/kg (10, 20, 30 and 40 min, respectively) in rats fed 100 ppm PBBs. The PBB-induced increase in BSP excretion was mainly due to a higher rate of bile flow. These results demonstrate that PBBs stimulate biliary function in rats and mice.

40. *The Pattern of Induction of Hepatic and Renal Mixed Function Oxidase in Young Rats by Polybrominated Biphenyls*. J. G. DENT, K. M. McCORMACK, S. Z. CAGEN, and J. E. GIBSON. Department of Pharmacology, Michigan State University, East Lansing, Michigan.

Polybrominated biphenyls (PBBs) are potent and persistent inducers of the hepatic mixed function oxidases in adult rats. The purpose of this investigation was to determine the pattern of induction of the renal and hepatic mixed function oxidases in young rats after exposure to PBBs. Seven-day-old male and female rats were injected ip with PBBs, 0 or 150 mg/kg (Firemaster BP6) and 1, 2, 3, 7, 14, and 28 days afterward animals were sacrificed and liver microsomes and kidney postmitochondrial supernatants were prepared. Hexobarbital oxidation and the 2- and 4-hydroxylation of biphenyl were not detectable in kidney, but were significantly elevated in the liver 1 day after PBBs and were maximally induced at 7, 14, and 28 days after treatment, to 60, 10, and 19 nmol/g of liver/min (from control values of 3, 0.3, and 4 nmol of product/g of liver/min) respectively. Glutathione-*S*-aryl transferase, with bromosulfophthalein (BSP) as substrate, was also induced in the liver 7, 14, and 28 days after PBBs. Control values

of 300, 800, and 1300, were increased by PBBs to 600, 1100, and 1900 nmol of BSP conjugated/g of liver/min. Ethoxyresorufin-*O*-deethylase (EROD) was significantly elevated in the liver by PBBs 1 day after treatment and was maximally induced 7 days after PBBs from 9 to 800 nmol of product/g of liver/min. EROD was also significantly induced in the kidney 7 days after PBBs. Twenty-eight days after PBBs, the EROD induction persisted in the liver. Arylhydrocarbon hydroxylase (AHH) activity was increased in the kidney from 4 to 24 units of fluorescence/g of kidney/min 28 days after a single exposure to PBBs. In the liver AHH was elevated by PBBs 1 day after treatment and was maximally increased from 24 to 251 units of fluorescence/g of liver/min 14 days after treatment. Epoxide hydratase in the kidney was not induced by PBBs, but in liver was significantly elevated 2, 14, and 28 days after treatment from 16, 35, and 185 to 38, 250, and 788 nmol of product/g of liver/min, respectively. It is concluded that the PBBs are potent and long-lasting inducers of the mixed function oxidase enzymes, having specificity for either cytochrome *P*-450 or cytochrome *P*<sub>1</sub>-450 dependent enzymes, and that in young rats PBBs significantly modify the normal development pattern of these enzymes.

41. *Enhancement of 1,1-Dichloroethylene Hepatotoxicity by Pretreatment with Low Molecular Weight Epoxides.* M. E. ANDERSEN and L. J. JENKINS JR., Naval Medical Research Institute, Bethesda, Maryland.

The expression of the mammalian hepatotoxicity and the bacterial mutagenicity of 1,1-dichloroethylene (DCE) appears to require metabolic activation. It has been suggested that the ultimate toxic agent is the unstable, reactive epoxide intermediate. In this study we determined the effect of pretreatment with various low molecular weight epoxides on the hepatotoxicity of orally administered DCE in the mature, male rat. Rats were dosed with either 2,3-epoxypropan-1-ol (EP), butadiene monoxide (BMO), 1,1,1-trichloropropane-2,3-oxide (TCPO), styrene oxide (SO), cyclohexene oxide (CHO), or diethylmaleate (DEM) 1 hr before oral administration of 25 mg of DCE/kg. All materials were dissolved in corn oil, except EP, which was in H<sub>2</sub>O. Increased plasma alanine transaminase (AIT) and aspartate transaminase (AsT) at 24 hr after DCE challenge were taken as a measure of hepatotoxicity. (A dose of 25 mg of DCE/kg in the naive, mature male rat does not produce increases in AIT or AsT.) After pretreatment with doses of EP as low as 34 mg/kg, plasma AsT and AIT were increased following 25 mg of DCE/kg. After 278 mg of EP/kg, doses as low as 6 mg of DCE/kg caused significant increases at 24 hr. The acute oral LD<sub>50</sub> of DCE was reduced by a factor of 5 (to 40 mg of DCE/kg) in rats pretreated with 278 mg of EP/kg. Expressed on a molar basis the acute ip toxicities of the five epoxides were related as follows: TCPO > BMO > SO > (CHO:EP). Their abilities to potentiate DCE hepatotoxicity were: TCPO > EP > SO > BMO > CHO. Pretreatment with DEM which reduces liver sulfhydryl concentration is known to increase the hepatotoxicity of inhaled DCE (Jaeger *et al.*, *Exp. Mol. Path.* **20**, 187, 1974) and it enhanced DCE hepatotoxicity after oral administration but ranked, on a molar basis, between SO and BMO. The efficacy of these epoxides in increasing DCE hepatotoxicity did not appear to be related to their acute toxicity or to their previously published rates of reaction with either glutathione epoxide transferase or epoxide hydratase. (Supported by Naval Medical Research and Development Command Work Unit 51.524.023-4003.)

42. *Renal Proximal Tubular Transport after Acute and Short-Term Chronic Administration of 2,4,5-Trichlorophenoxyacetic Acid.* F. J. KOSCHIER and W. O. BERNDT, Department of Physiology, SUNY, Buffalo, New York, and Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi. (A. Wallace Hayes)

The organic acid herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), has been shown to alter the proximal tubular transport of organic acids and bases. This renal impairment appears to be competitive in nature, affecting the renal slice uptake of both acids and bases in a comparable manner. 2,4,5-T excretion is reduced after initial high doses of the herbicide, but improved with chronic (8-16 days) administration. In the present study male, Sprague-Dawley rats were pretreated with 2,4,5-T in an alcohol vehicle *sc*. The slice transport studies were performed with conventional techniques. Eight days of 2,4,5-T pretreatment (90 mg/kg) failed to

alter  $\alpha$ -aminoisobutyrate (AIB) transport in a slice experiment on Day 9. However, the transport of 2,4-dichlorophenoxyacetate (2,4-D) was depressed approximately 66% and that of tetraethylammonium (TEA) depressed about 20%. Both changes were statistically significant. Pretreatment doses of 20 mg of 2,4,5-T/kg had no effects on renal transport of organic acids or bases, including 2,4,5-T itself. Renal cortex homogenate binding of 2,4,5-T was not altered by 5 days of pretreatment with 90 mg of 2,4,5-T/kg. Taken together, these data indicate that the altered excretory pattern for 2,4,5-T observed with chronic administration was not related to increased renal transport or alterations in nonspecific renal cortex binding. (Supported by NSF Grant No. P4B3159 and PHS Grant AM 18124.)

43. *Toxic Products Produced by Ultraviolet Irradiation of Halothane.* D. B. MENZEL, J. H. KARIS, M. B. ABOU-DONIA, and P. B. BENNETT, Departments of Physiology and Pharmacology, Anesthesiology, and Medicine, Duke University Medical Center, Durham, North Carolina.

Ultraviolet lights are used in some operating rooms to reduce the potential of wound infection. While these uv lights produce negligible amounts of ozone, they represent a potential source of high-energy catalysis of reactive compounds. Halothane was rapidly decomposed to a toxic product(s) when a flowing stream was irradiated. About 2% conversion could be obtained which was not dependent on the oxygen concentration or the presence of free radical scavengers. Exposure of female mice to 0.2, 1.0, or 1.7% uv-irradiated halothane for 1 hr caused a marked weight loss of up to 25% in the highest concentration exposed group. Exposure to 3% irradiated halothane produced 80% mortality compared to none with unirradiated halothane. Lungs of exposed mice showed marked perivascular edema. While no remarkable pathology was found in the livers of animals exposed to 1% irradiated halothane, the pentobarbital sleeping time was elongated twofold. The elongation of sleeping time was proportional to the dose of irradiated halothane and the time and frequency of exposure. The data suggest that halothane is converted by a photochemical reaction to toxic product(s) having both hepato- and pulmonary toxicity. Several potentially toxic compounds have been found on gas chromatography.

44. *Subacute Toxicity of SCE-963, a New Cephalosporin Antibiotics, in Rats and Dogs.* M. NOMURA, T. ABE, and Y. MURATA, Central Research Division, Takeda Chemical Industries, Ltd., Osaka, Japan. (K. Takano)

SCE-963 ( $7\beta$ -[2-(2-imino-4-thiazolin-4-yl)acetamido]-3-[1-[2-(*N,N*-dimethylamino)ethyl]-1*H*-tetrazol-5-yl]thiomethyl-3-cephem-4-carboxylic acid) is a new cephalosporin with a broad antibacterial spectrum and its activity against gram-negative bacteria is more potent than other cephalosporin antibiotics such as cephazolin, cephalothin, and cephaloridine. Wistar rats and beagle dogs were treated im with SCE-963 at 100, 300, or 1000 mg/kg/day for 1 month. All the rats and dogs survived the treatment, showing no marked changes in hematology, blood chemistry, or urinalysis. Histological examinations revealed a dose-related deposition of brown granules in the proximal tubular epithelium of the kidney in both rats and dogs, while no other visceral organs showed any pathological changes. By histochemical and electron microscopical examination, these granules were identified as somewhat enlarged lysosomes. No degenerative or necrotic sign was seen in any of these epithelial cells. A similar change was induced in the rats after treatment with cephalothin in a separate experiment. In any case, the size and number of lysosomes returned to normal level 1 month after the treatment. It has been suggested that the above figure indicates an excretion process of the agents and/or their metabolites through the renal tubular epithelium.

45. *Heavy Metals and Lymphocytes: A Possible Site of Immunosuppression by Chemicals.* C. L. GAWORSKI and R. P. SHARMA, Toxicology Program, Utah State University, Logan, Utah.

Heavy metals have been shown to be immunosuppressive, a characteristic that may intimately involve lymphocytes. In the present study the effects of lead, cadmium, mercury, and zinc were determined on the response of lymphocyte cell cultures to stimulation by mitogens

and membrane ATPase activity. Mice were exposed to various concentrations of these metals in drinking water for 30 days. Splenic lymphocytes were cultured at 15 and 30 days to determine the response to phytohemagglutinin (PHA) and pokeweed mitogen (PWM) by measuring the uptake of [ $^3\text{H}$ ]thymidine. *In vitro* response was measured by adding the metals directly to the cell cultures. *In vivo* exposures to cadmium (160 ppm) and mercury (10 ppm) significantly ( $p < 0.05$ ) decreased the response to both mitogens after 15 days, while lead (2000 ppm) and zinc (2000 ppm) decreased these responses after 30 days. Cadmium and mercury produced dose-dependent inhibition *in vitro* with a 50% reduction in the response to PHA at 6 and 5  $\mu\text{M}$  metal concentrations, respectively. An increase in PHA response, 4.5 times that of the control, was produced by 1.0 mM lead. A concentration of 75  $\mu\text{M}$  zinc significantly increased the PHA response, but inhibited PWM response. About 95% of the intact lymphocyte total ATPase activity was ouabain-insensitive  $\text{Mg}^{2+}$ -ATPase ( $K_m = 1.4 \times 10^{-4} \text{ M}$ ). Lead, cadmium, and zinc showed noncompetitive enzyme inhibition, while mercury was uncompetitive. The results suggest that these metals produce an effect directly on the lymphocyte cells and that one site of interaction may be related to the ATPase enzyme located on the membrane.

46. *Effects of Metallic Compounds on Chick Dorsal Root Ganglia Cultures*. E. J. OBERSTEINER and R. P. SHARMA, Utah State University, Logan, Utah.

Metallic compounds have been shown to be environmental pollutants and may affect many cell and organ systems, notably the nervous system, thereby causing a multiplicity of neuropathies. To investigate their cytotoxic properties in an isolated cell system, 11-day-old chick embryo dorsal root ganglia were cultured in a microslide assembly containing appropriate nutrient media and variable concentrations of metallic compounds. Following a 72-hr incubation at 37°C, phase contrast and light microscopic observations after staining were made on cellular growth, density of nerve fibers, neuroglia, and neurons, and any abnormalities thereof. Results indicated that all cell growth was dose dependent, with its magnitude ranging from severely toxic (concentration to produce half-maximal effect,  $10^{-6} \text{ M}$  and below) for  $\text{Hg}^{2+}$ ,  $\text{As}^{3+}$ ,  $\text{Cd}^{2+}$ , Vendex-Sn; moderately toxic ( $10^{-4}$  to  $10^{-6} \text{ M}$ ) for  $\text{As}^{5+}$ ,  $\text{Tl}^+$ ,  $\text{Cu}^{2+}$ ; and least toxic ( $10^{-4} \text{ M}$  or above) for  $\text{Pb}^{2+}$ , As-oxide,  $\text{Se}^{2+}$ , and  $\text{Sn}^{2+}$ . Nerve fiber growth stimulation was apparent at low concentration ( $10^{-6} \text{ M}$  or less) for  $\text{Cd}^{2+}$ ,  $\text{Tl}^+$ ,  $\text{Pb}^{2+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Se}^{2+}$ , and Vendex-Sn, but not for  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{As}^{3+}$ , and  $\text{As}^{5+}$ . Similarly, vacuolization of glial cells occurred by  $\text{Cd}^{2+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Tl}^+$ ,  $\text{Pb}^{2+}$ , and Vendex-Sn, but not for  $\text{As}^{3+}$ ,  $\text{As}^{5+}$ ,  $\text{Hg}^{2+}$ , and  $\text{Se}^{2+}$ . In all cases nerve fibers were more sensitive to the metallic compounds than neuroglia. Other abnormalities included increased glial varicosities, nerve fiber swelling as well as cellular degeneration at the toxic levels. The effects of metallic compounds *in vitro* do not demonstrate nerve cell specificities as seen *in vivo*, hence a variety of mechanisms may be implicated in the toxic actions.

47. *Residues of Lead in Edible Tissues or Products of Cattle and Swine after Low-Level Exposures*. R. P. SHARMA, J. C. STREET, J. L. SHUPE, and D. J. WAGSTAFF, Utah State University, Logan, Utah, and Bureau of Veterinary Medicine, Food and Drug Administration, Rockville, Maryland.

Possible contamination of human food through increased levels of heavy metals in animal feeds is of major concern in environmental toxicology. Dairy cows and growing swine were treated with lead acetate (added to the diet at levels approximating 5 and 25 ppm lead above the normal dietary intake) for 3 months. After this continuous exposure, some of the animals were maintained on control feed to allow depletion of any accumulated metal. Residues for lead were determined in skeletal muscle, liver, and bone of both species and in milk from cows, using flameless atomic absorption spectrophotometry. No increase in the lead concentration of milk was noticed in cows treated with 5 ppm added lead in the diet. With the cows treated at the higher level, the lead in milk increased significantly (from 10 ppb for controls to an average of 60 ppb). Such elevated values were still lower than those often reported in processed milk. Skeletal muscle did not show any rise in lead values in either species (mean values ranged from 20 to 60 ppb). In the case of liver, lead concentrations showed a dose-dependent increase

but fell rapidly during the 3-month depletion phase. Bone, however, accumulated high amounts of lead (8–10 ppm in whole bone of the high-level treatment group after 3 months exposure) and retained it long after the exposure had ceased. The results suggest that the muscle of animals does not accumulate lead on low-level exposure to lead. Although the amount of this metal is likely to increase in milk, liver, and bone of animals in cases of environmental lead pollution, the results obtained with dietary treatments approximating 25 ppm suggest that this may not lead to a substantial increase of the human dietary intake of lead. (Supported by Contract FDA-223-74-7195.)

48. *Diminution of Regional Acetylcholine Content in Mouse Brain During Chronic Ingestion of Lead.* ARVIND T. MODAK, ROBERT H. PURDY, and WILLIAM B. STAVINOHA, Division of Toxicology, The University of Texas Health Science Center at San Antonio, and Southwest Foundation for Research and Education, San Antonio, Texas.

We have previously shown that administration of 0.25, 0.5, and 1% lead acetate solution for 21 or 30 days caused a significant decrease in acetylcholine content of the mouse brain. To study the response in specific brain regions, mice were given a concentration of 0.25, 0.5, or 1% lead acetate in drinking water before and after weaning and were compared to control mice which received tap water. Lead concentrations in the cerebellum (ce), medulla pons (mp), midbrain (mb), diencephalon (di), hippocampus (hi), corpus striatum (cs), cerebral cortex (cx), and blood were determined by atomic absorption spectrometry after 30 days of treatment. Prior to determination of acetylcholine (ACH) in brain regions, mice were exposed to 200 msec of microwave irradiation to concurrently sacrifice the mouse and to inactivate brain cholinesterase and cholineacetyltransferase. Acetylcholine concentrations were measured by pyrolysis gas chromatography. In general, the lead content of various brain regions following 30 days of treatment was dose dependent. After 30 days of treatment with 0.5 or 1% lead acetate solution, the blood concentrations were 178 and 194  $\mu\text{g}/100\text{ ml}$ , respectively, while the blood concentrations with 0.25% lead acetate solution treatment was found to be 70  $\mu\text{g}/100\text{ ml}$ . The consumption of 0.25 and 0.5% lead acetate solution had no significant effect on the growth and development of mice, while consumption of 1% of lead acetate solution caused a significant decrease in body weight gain of mice. The ACH concentration in the corpus striatum in the control mice given in nanomoles per gram  $\pm\text{SD}$  was: tap water,  $81.9 \pm 9.97$ . After 30 days of consumption of lead acetate solutions of 0.25, 0.5, and 1%, the ACH concentrations in cs were:  $67.4 \pm 5.28$ ,  $64.6 \pm 12.94$ , and  $40.4 \pm 3.91$  respectively. The other brain regions which showed a less pronounced effect on ACH content were cer, mp, mb, di, and cx. Lead consumption caused a significant decrease in the acetylcholine content of mouse brain regions. The major decrease occurred in the corpus striatum. (Supported by Grant No. R01-MH25168-01 and NIH 5-S01 RR 05654-07.)

49. *The Influence of Low-Level Lead Exposures on Free Erythrocytic Porphyrin and Blood and Liver  $\delta$ -aminolevulinic Acid Dehydratase in Three Species of Food-Producing Animals.* J. C. STREET, M. H. RAGHIB, R. P. SHARMA, and D. J. WAGSTAFF, Utah State University, Logan, Utah, and Bureau of Veterinary Medicine, Food and Drug Administration, Rockville, Maryland.

Toxicologic effects of low-level exposures of dietary lead were investigated in dairy cows, growing swine, and laying hens. Treating these animals with 5 (low) and 25 ppm (high) lead (as added lead acetate in the diet above the normal background dietary intake) failed to produce any effect on growth rate, production, feed consumption, and hematocrit in any species. Free erythrocytic porphyrin (FEP) was not affected in any species by low lead treatment. With the high lead treatment, FEP values were increased in cattle but not in swine and chicken. FEP elevation was seen after 4 weeks of treatment, continued to increase until the final (12th week) of treatment, and remained elevated as long as 3 months afterward. Blood aminolevulinic acid dehydratase (ALAD) decreased in all species in relation to the treatment level. The decrease was apparent by the second week of treatment. ALAD values slowly returned to normal after

the treatments ceased. An increase of liver ALAD activity was noticed in cows exposed to lead, but there was no consistent effect of lead exposure on liver ALAD in swine. The increased activity of liver ALAD observed in cattle may indicate some physiological adaptation. Lead acetate and lead oxide (fed as equivalent amounts of lead) were equally effective in inhibiting blood ALAD in chickens. Supplementation of the chicken diet with excess calcium resulted in less ALAD inhibition than observed in those birds receiving the normal calcium diet. The results show that low-level lead exposure may produce an increase in FEP and inhibition of blood ALAD without otherwise producing any chemical or toxicological effects. (Supported in part by Contract FDA-223-74-7195.)

50. *An Interaction between Chronic Lead Exposure and Acute Phenylhydrazine Administration in the Rat.* BENJAMIN B. GELMAN and JAMES S. BUS, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio. (P. B. Hammond)

To determine if moderately elevated blood lead (Pb-B) levels cause increased susceptibility to drug-induced oxidative hemolysis, an interaction between Pb and the erythrocyte (RBC) oxidant drug phenylhydrazine (PHZ) was investigated. Male Sprague-Dawley rats were treated for 40 days with Pb at 4 doses: 0 (control); 500 ppm in drinking water; 1000 ppm in drinking water plus seven equally spaced ip injections of either 0.75 or 2.0 mg/kg Pb (as the acetate). Pb-B concentrations (determined on Day 27 of treatment) for the four groups were (mean  $\pm$  SD)  $2.3 \pm 1.0$ ,  $33 \pm 4$ ,  $67 \pm 13$ , and  $104 \pm 17$   $\mu\text{g}/100$  ml, respectively. On Day 27 after initiation of Pb exposure, either saline or PHZ-HCl, 45 mg/kg sc was administered to half of the rats in each Pb treatment group. Hemoglobin (Hb) and hematocrit (Hct) determinations were performed on tail blood drawn 1, 2, 7, and 13 days after PHZ challenge, after which the rats were killed and the organs were weighed. In the acute hemolytic phase after PHZ (Days 1 and 2), both Pb alone and PHZ alone significantly reduced these hematologic parameters by a maximum of 4 and 15%, respectively. However, there was no significant interaction between these two agents in reducing these parameters at any dose. In contrast, a significant interaction effect occurred with increasing doses of lead (10% at the highest dose) during the recovery phase (Days 7 and 13 after PHZ), probably due to the known property of lead to inhibit hematopoiesis. Spleen weight was significantly increased by both lead alone and PHZ alone by maximums of 7 and 26%, respectively. A 4% interaction between joint lead and PHZ administration at the maximal doses for spleen enlargement was also observed, but was not significant at these dose levels. Dose-response curves were produced which suggested a threshold Pb-B of about 50  $\mu\text{g}/100$  ml for the anemic effects of Pb. Therefore, the *in vivo* interaction of Pb and PHZ was due primarily to the ability of lead to inhibit compensatory hematopoiesis after an acute hemolytic episode. However, a direct hemolytic interaction by these two agents on the red cell should not be dismissed since Hb and Hct determinations do not measure hemolysis per se, and therefore, may not detect more subtle RBC alterations. These data support the conclusion that Pb-B concentrations up to 100  $\mu\text{g}/100$  ml in rats do not enhance the potential of acute PHZ to produce hemolytic anemia, but that compensatory hematopoiesis is indeed compromised.

51. *Effects of Cd and Ni on Antibody-Mediated Rosette Formation by Rabbit Alveolar Macrophages.* J. G. HADLEY, D. E. GARDNER, D. L. COFFIN, and D. B. MENZEL, Departments of Physiology and Pharmacology and Medicine, Duke University Medical Center, Durham, North Carolina, and Health Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, North Carolina.

Pulmonary macrophages (PM) serve a vital role in the defense against pulmonary infection. Airborne toxicants may impair the ability of the PM to recognize, ingest, and kill infectious agents leading to a secondary toxic effect. Immunoglobulin G (IgG) serves as the prime recognition system of the PM for bacterial and viral pathogens. The Fc portion of IgG is attached to the PM plasma membrane by a specific receptor. Using the inhibition of IgG-mediated rosette formation as an index of receptor alteration,  $\text{NaNO}_2$ ,  $\text{NaIO}_4$ , and PCMB were active at concentrations ranging from 1 to 10 mM for 30 min. Inhibition of IgG-rosette formation occurred

at low concentrations of  $\text{CdCl}_2$  ( $2.2 \times 10^{-5}$  M) and  $\text{NiCl}_2$  ( $1.0 \times 10^{-4}$  M). A dose-response relationship was observed with both salts. Rosette formation was also inhibited by thiol combining reagents. These data suggest that IgG-mediated rosette formation by PM is a sensitive indicator of potential toxicity by both oxidizing agents and heavy metals. Inhibition was observed after 30 min compared to prior reports which required 20 hr for evidence of intoxication of PMs *in vitro*. The IgG-rosette formation may be useful as a new rapid, sensitive screen for heavy metal intoxication.

52. *Dose Dependence of Cadmium Kinetics in the Rat Liver.* JOHN M. FRAZIER, Department of Environmental Medicine, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland. (R. J. Rubin)

The objective of these experiments was to investigate the kinetics of Cd uptake and redistribution within the rat liver over a period of 6 hr following iv administration. Male Wistar rats ( $520 \pm 80$  g) were injected with total doses of 20, 200, and 1000  $\mu\text{g}$  of  $\text{Cd}^{2+}$  as the chloride. The highest dose is equal to the LD50 (24 hr). Livers were removed at various times following injection and the percentage of the total dose taken up by the liver was determined. At the low dose, the uptake was  $76 \pm 12\%$ ; at the intermediate dose,  $69 \pm 14\%$ ; at the highest dose,  $45 \pm 14\%$ . These occurred at 3 hr with no further net accumulation at 6 hr. These results indicate a saturation of the ability of the liver cells to take up Cd. The livers were further fractionated into a  $6 \times 10^6$  g-min pellet and supernatant. At all times, uptake into the pellet was linear as a function of dose while the uptake into the supernatant fraction was saturated. Gel permeation chromatography of supernatant indicated that this was due to the saturation of the binding capacity of the high molecular weight cytoplasmic macromolecules (HMWM). Although uptake into HMWM is complete by 3 hr, incorporation of Cd into low molecular weight (MW  $\sim 10,000$ ) cadmium binding proteins (Cd-BP) continues linearly throughout the 6 hr. However, the rate of incorporation shows saturation kinetics with respect to dose. Cd exposure up to 6 hr had no effect on total liver Zn concentration; however, there are alternations in the distribution of Zn with significant proportions appearing in Cd-BP fractions at 6 hr following the 200- $\mu\text{g}$  dose but not following the 1000- $\mu\text{g}$  dose. These results indicate the presence of excess metal binding sites in Cd-BP at the lower dose. Summarizing, the dose dependence of cadmium uptake by the liver is accounted for by binding to HMWM while the dose dependence of intracellular redistribution is explained by incorporation of Cd in Cd-BP.

53. *The Excretion of Cadmium and Mercury in Saliva.* L. W. SMITH and J. F. BORZELLECA, Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Although the presence of heavy metals in saliva has been reported (Joselow *et al.*, *Arch. Environ. Health* 17, 35, 1970; Dreizen and Levy, *Caries Res.* 4, 193, 1970), the mechanisms involved have not been elucidated. This study was undertaken to investigate these mechanisms and the relationships between the concentrations of  $\text{Cd}^{2+}$  and  $\text{Hg}^{2+}$  in saliva and in blood (whole blood, plasma, filtrate). Urethane-anesthetized rats received single iv injections of either  $^{109}\text{CdCl}_2$  or  $^{203}\text{HgCl}_2$  (0.1 or 1.0 mg divalent cation/kg). Pilocarpine (20 mg/kg, ip) was used to stimulate salivation. Blood and submaxillary saliva were collected over a 5-hr period. A distinct and persistent hypertension (15–25 mm Hg) was seen with both metals at both doses (Perry *et al.*, *Amer. J. Physiol.* 219, 755, 1970). All doses produced a statistically significant increase (19–26%) in the weight of the submaxillary glands and an increase in salivary flow rate compared to control. The ratios of metal concentrations in saliva (S) and submaxillary gland tissue (T) relative to concentrations in blood (B), plasma (P), and filtrate (F) were determined.  $\text{Cd}^{2+}$  and  $\text{Hg}^{2+}$  were detected in S and T at both doses. The following relative order was observed for both metals:  $S/F > S/P > S/B$ . The S/F ratios (for both metals) were consistently greater than 1.0, suggesting a concentrating effect by the salivary gland. The S/B and S/P ratios for  $\text{Hg}^{2+}$  were dose dependent (higher at the larger dose). In contrast, the S/B and S/P ratios for  $\text{Cd}^{2+}$  exhibited a decrease with increasing dose. Similar dose-dependent relationships were evident in the T/B and T/P ratios (ml/g) for both metals. These *in vivo* data suggest a saturable



transport system for  $\text{Cd}^{2+}$  but not for  $\text{Hg}^{2+}$ , in agreement with previous work from this laboratory using (*in vitro*) slices of submaxillary gland. (Supported in part by EPA Grant No. R-804318010.)

54. *Renal Porphyrin following Chronic Methyl Mercury Exposure in the Rat.* JAMES S. WOODS and BRUCE A. FOWLER, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina.

Previous reports have demonstrated the occurrence of elevated heme precursors in humans chronically exposed to methyl mercury (MM) in the diet or to organomercurial diuretic drugs. In the present study the etiology of mercury-related porphyria was investigated in adult male rats exposed to 0, 3, 5, or 10 ppm MM in drinking water for 6 weeks. Activities of heme biosynthetic pathway enzymes in livers of MM-exposed rats were not substantially different from those of 0 ppm controls. In kidney, however, both uroporphyrinogen I synthetase (US) and ferrochelatase (FC) were depressed at all dose levels, with dose-related decreases to a maximum of 58 and 69% of control levels, respectively. In contrast, renal  $\delta$ -aminolevulinic acid synthetase (ALAS), the rate-limiting enzyme in heme biosynthesis, was elevated at all dose levels with a maximal increase to over 2.5 times control values observed. This alteration of renal heme biosynthetic pathway enzymes in 6-week MM-exposed rats was accompanied by dose-related increases in urinary uroporphyrin and coproporphyrin concentrations, which reached 12 and 21 times control values, respectively, at 10 ppm. Acute exposure studies (0.7 or 1.4 mg/kg given by intraperitoneal injection for 2 days) revealed that depression of FC and US precedes elevation of ALAS activities in rat kidney. These studies suggest that MM acts primarily to depress FC and US activities, followed by a secondary induction of renal ALAS and an increase in urinary porphyrin concentrations. These results may have utility in the design of clinical tests for diagnosing pretoxic biologic responses to MM in human populations.

55. *The Effect of Thiocolesterol on the Distribution and Toxicity of Mercuric Chloride.* J. J. BURGUN and R. B. FORNEY, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

Previous studies have shown that thiocolesterol administration can protect mice from the lethal effects of acutely administered  $\text{HgCl}_2$ . The purpose of this study was to determine the effect of thiocolesterol on the tissue distribution and excretion of  $^{103}\text{Hg}$  and to evaluate the effect of thiocolesterol treatment on  $\text{HgCl}_2$ -induced renal damage. Male Wistar rats weighing 180–220 g were used in all experiments. Rats were injected via tail vein with 5  $\mu\text{Ci}$  of  $^{203}\text{HgCl}_2$  or given a single oral dose of 1 g of thiocolesterol/kg 2 hr prior to the Hg injection. Animals were sacrificed at intervals up to 14 days after treatment and tissues analyzed for Hg. Two additional groups of rats were dosed in the same manner and housed in metabolism cages for 14 days to determine the urinary and fecal excretion of Hg. To evaluate kidney function, groups of rats received either 1 mg of  $\text{HgCl}_2/\text{kg}$ , iv, or 1 g of thiocolesterol/kg, po, 2 hr before the injection of  $\text{HgCl}_2$ . Rats were housed in metabolism cages 24 hr prior to sacrificing, which took place 2 or 4 days after treatment. The 24-hr urine volume was measured and determinations made of total protein, osmolality, urine creatinine, serum creatinine, and creatinine clearance. One kidney was removed from each rat for histological examination. The results of the histological studies and function studies indicated that thiocolesterol significantly reduced the renal damage caused by  $\text{HgCl}_2$ . Thiocolesterol treatment reduced the amount of Hg in the liver, but not the kidneys, nor did it consistently alter Hg concentrations in the other tissues examined. Thiocolesterol increased the fecal excretion and decreased the urinary excretion of Hg. (Supported in part by PHS GM 1089.)

56. *Morphologic Characteristics of Hepatic Nodules Induced by Mirex and Dieldrin in Mice.* K.-F. BENITZ, R. N. ROTH, and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The purpose of the present experiment was to study the sequence of hepatic changes in mice during dietary administration of dieldrin (not less than 85% of 1,2,3,4,10,10-hexachloro-exo-

6,7-epoxy-1,4,4,5,6,7,8,8-octahydro-1,4-endo-eso-5,8-dimethanonaphthalene) and Mirex (dodecachlorooctahydro-1,3,4-methano-2*H*-cyclobutyl(*cd*)pentalene). Nine groups, each consisting of 100 male and 100 female mice (Charles River CD-1 strain), were fed dieldrin and Mirex in the diet in concentrations ranging from 0.15 to 15 ppm. Six hundred males and 600 females were used as controls. Groups of animals were sacrificed at various time intervals ranging from 2 to 25 months. At autopsy, the entire lung and representative samples of liver were fixed in 10% buffered formalin, and paraffin sections from this material were stained with hematoxylin-eosin, Masson's trichrome method, and Manuel's silver impregnation method. Initially, microscopic hepatic changes consisted of various degrees of centrilobular and pericentral hypertrophy. These changes were later associated with the appearance of hepatic nodules and their occurrence was time and dose related. These nodules consisted of hypertrophic hepatocytes, which in a few instances were mixed with hyperplastic cells. Various degrees of loss and distortion of lobular architecture were seen within these nodules; however, anaplastic changes were absent. Lung metastases were observed in three animals which had hypertrophic hepatic nodules (15 ppm dieldrin for 25 months), indicating that the dieldrin-induced nodules have to be regarded as hepatocellular carcinomas. These metastases also consisted of hypertrophic hepatocytes. No metastases were seen in the Mirex animals which had hepatic nodules. The morphology of the hepatic nodules induced by dieldrin and Mirex did not allow a differentiation between those that metastasized and those that did not. (Supported in part by Research Grant 2-P01-ES00226-10 from the National Institutes of Environmental Health Sciences, NIH and by the NIH Training Grant 2-T01-ES00103-10.)

57. *Carcinogenic Activity of Hexachlorobenzene in Hamsters.* J. R. P. CABRAL, P. SHUBIK, T. MOLLNER, and F. RAITANO, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska.

Hexachlorobenzene (HCB) has been used as a fungicide in agriculture. In 1971 it was estimated that 13,800 lb of HCB were used in the United States for agricultural purposes. HCB is also a by-product in the manufacture of many chlorinated hydrocarbons and in 1975 it was estimated that at least 2 million lbs of HCB are produced every year in the U.S. Long-term studies, designed to evaluate the chronic toxicity of this compound, are not available. The present experiment seeks to determine whether or not HCB is carcinogenic. Syrian golden hamsters were given HCB mixed into the diet at the dose levels of 50 ppm (30 females and 30 males), 100 ppm (30 females and 30 males), and 200 ppm (60 females and 60 males) for the entire life span. A control group of 40 females and 40 males was also provided. Results at 80 weeks of age are presented. At this stage exposure to HCB resulted in a significant increase of hepatomas and liver hemangioendotheliomas. The capacity of HCB to induce hepatomas and their incidence (low dose: females, 37.5%, males, 27.2%; medium dose: females, 42%, males, 75%; high dose: females, 81%, males, 87%) and latency appear related to the dose administered. No metastases were found. No hepatomas occurred in the control group. The incidence of liver hemangioendotheliomas in hamsters receiving 200 ppm was 9% in females and 34% in males. Three of the hemangioendotheliomas occurring in these males gave metastases. In view of the significantly greater number of tumors in male and female hamsters fed HCB in the diet for their entire life spans the results are considered as evidence of carcinogenicity of HCB.

58. *Sequential Alkaline Phosphatase Changes in Hyperplastic and Neoplastic Urothelium of Mice Induced by 2-Acetylaminofluorene.* B. HIGHMAN, C. H. FRITH, and N. A. LITTLEFIELD, University of Arkansas College of Medicine, Little Rock, Arkansas, and the National Center for Toxicological Research, Jefferson, Arkansas.

As shown previously (Highman *et al.*, *J. Natl. Cancer Inst.* **54**, 257, 1975) mice fed 100-500 ppm 2-acetylaminofluorene (2-AAF) for 7-19 months often develop hyperplasia and neoplasia of the bladder urothelium with marked depletion of alkaline phosphatase. To determine the effect of early and late withdrawal of 2-AAF, over 450 BALB/c mice were fed stock diets containing 0, 100, or 500 ppm 2-AAF. At 12 weeks, nearly all mice on the high (500 ppm) 2-AAF diet showed bladder epithelial hyperplasia with epithelial decrease and stromal increase in

alkaline phosphatase. The superficial polyploid and upper intermediate epithelial cell layers frequently contained many cytoplasmic vacuoles with eosinophilic inclusions often staining like alkaline phosphatase. After withdrawing 2-AAF at 12 weeks, such changes decreased, but 1 of 4 females and 2 of 4 males sacrificed at 51 weeks had invasive carcinomas. Mice on 100 ppm 2-AAF developed similar changes more slowly. At 57 weeks, epithelial and stromal changes were marked in nearly all males and decreased only slightly at 81 weeks, 27 weeks after withdrawal of 2-AAF. In females, changes were milder and virtually disappeared at 67 weeks, 13 weeks after withdrawal of 2-AAF. At 57 to 103 weeks, no females on the low 2-AAF diet developed carcinomas, whereas nearly all males, even after withdrawing 2-AAF at 54 weeks, developed papillary carcinomas. These findings indicate that even a relatively brief period on a high 2-AAF diet can induce irreversible premalignant changes, not recognizable by light microscopy, in some mice. It is suggested that the relative number of cytoplasmic vacuoles with inclusions may indicate the relative severity of urothelial damage and may correlate with later carcinomatous development.

59. *The Role of Naphthalenes as Cocarcinogens.* I. SCHMELTZ, J. HILFRICH, J. TOSK, D. HOFFMANN, and E. L. WYNDER, American Health Foundation, Valhalla, New York.

The abundance of naphthalenes in the environment, their presence in admixtures with carcinogenic aromatic hydrocarbons (respiratory pollutants, combustion products, and coal tar), and earlier reports of their tumor-promoting activity led us to conduct mouse skin bioassays on several naphthalenes in the presence and in the absence of benzo(*a*)pyrene (BaP). The assay for cocarcinogenic activity consisted of concurrent application of 250  $\mu$ g of naphthalene or one of several alkyl-naphthalenes with 3  $\mu$ g of BaP in acetone three times weekly over a period of 80 weeks to groups of 30 mice each. In addition, acetone solutions of cigarette smoke "tars," including one rich in naphthalenes, were tested together with BaP. Within 6 months up to 20% of the mice in a number of test groups developed lymphomas. When applied together with BaP, 1,5-dimethyl-, 1,4,6,7-tetramethyl-, and 2-ethylnaphthalene were the most active ones. Application of BaP alone did not produce lymphomas. The naphthalene-rich fraction of cigarette smoke induced lymphomas when applied concurrently with BaP. Several naphthalenes also enhanced the skin tumorigenicity of BaP. The most active among these were 1,4,6,7-tetramethylnaphthalene and 2,3,6-trimethylnaphthalene. There appears to be some correlation between the degree of alkylation and cocarcinogenic activity. Biochemical studies on the underlying mechanisms are in progress. (Supported by National Cancer Institute Contract No. 1-CP-55666.)

60. *Enhancement of Urethan Tumorigenesis in Mouse Lung following Multiple Injections of Butylated Hydroxytoluene.* H. P. WITSCHI and S. LOCK, Département de Pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Canada.

Intraperitoneal injection of large doses of butylated hydroxytoluene (BHT) produces, in mouse lung, necrosis of type I alveolar cells, followed by a proliferation of type II alveolar cells. Administration of urethan to mice will produce pulmonary adenomata consisting of type II alveolar cells. It was examined whether repeated proliferation of the target cells of urethan would influence the number of tumors formed. Male Swiss-Webster mice were injected ip with one single dose of urethan (1 mg/g). Beginning 7 days later, the animals received weekly injections of BHT (300 mg/kg) or corn oil (0.1 ml/10 g). Thirteen weeks later, urethan + BHT-treated animals had  $12.6 \pm 1.8$  tumors/lung ( $n = 24$ ), whereas urethan + corn oil-treated ones had  $3.7 \pm 0.7$  tumors/lung ( $n = 23$ ;  $p < 0.05$ ). Animals injected for the first 7 weeks following urethan with BHT had  $8.6 \pm 1.5$  tumors per lung ( $n = 21$ ); animals treated with corn oil had  $3.5 \pm 0.7$  tumors per lung ( $n = 20$ ;  $p < 0.05$ ). In a third group of animals, weekly BHT injections were begun 8 weeks after urethan only. Seven weeks later, there was no significant difference in the number of tumors per lung in the BHT-treated animals ( $4.2 \pm 0.9$ ;  $n = 22$ ) compared to their controls ( $3.7 \pm 0.4$ ,  $b = 24$ ). BHT alone did not induce any lung adenomata. Repeated proliferation of type II alveolar cells in mouse lung may thus enhance carcinogen-initiated adenoma formation. (Supported by the MRC.)

61. *The Putative Role of Dietary Fiber in Dimethylhydrazine-Induced Colon Carcinogenesis.* T. BARBOLT, N. RINGWOOD, and R. ABRAHAM, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The protective role of bran was investigated in dimethylhydrazine (DMH)-induced colonic neoplasia, keeping in mind the controversy that surrounds the incorporation of dietary fibers in foods. For this purpose, four groups of 10 male Sprague-Dawley albino rats each were used: Group I, control; Group II, 30.0 mg/kg of DMH given orally once a week for 10 weeks; Group III, 20% wheat bran in standard rat diet; Group IV, wheat bran and DMH. The animals were killed at 21 weeks. Initial transit times, using carmine dye as a marker, were decreased in Groups III and IV when compared to Groups I and II (11 vs 18 hr). Fecal output, measured as wet weight/24 hr, was increased in Groups III and IV by 46%. Colonic or duodenal polyps were not observed in Groups I and III. In Group II, all 10 rats had grossly visible colonic polyps (6.4/rat), whereas in Group IV, only 6 of 9 rats had polyps (2.7/rat). The size (average, 3.3 mm diam; range, 1-20 mm) and distribution (average, 10.4 cm proximal to anorectal junction) of these polyps were similar in both groups. The number (1.8/rat), size (average, 1.8 mm diam; range, 1-14 mm), and distribution (average, 2.3 cm distal to pyloric junction) of duodenal polyps were similar in Groups II and IV. The histopathology of polyps in Groups II and IV was similar to that already reported in the literature. An interesting observation was the infiltration of mononuclear cells into the lamina propria of the polyps containing intense reaction for lysosomal acid phosphatase and  $\beta$ -glucuronidase. Decreased activity of these same enzymes was observed in the neoplastic epithelium. Autoradiography, with [ $^3$ H]thymidine revealed heavy nuclear labeling in the periphery of the polyps, but upper crypt and surface labeling of morphologically normal epithelial cells adjacent to the neoplastic tissue was not observed. These preliminary data suggest that the incorporation of wheat bran in the diet of rats does not alter the evolution of DMH-induced colonic polyps, but rather it decreases the incidence of grossly observable polyps. (Supported in part by Research Grant 2-P01-ES00226-10 from NIEHS, NIH, and NIH Training Grant 3-T01-ES00103-10, Research Career Development Award 1-K04-ES70608-04.)

62. *Dominant Lethal Studies in Mice with Dietary Feeding of Metepa and MMS.* D. W. ARNOLD, C. M. SALAMON, S. H. SMITH, G. L. KENNEDY, JR., M. L. KEPLINGER, and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Groups of 15 male albino mice each were fed either Metepa at dietary levels of 100 or 300 ppm or methylmethane sulfonate (MMS) at dietary levels of 300 or 1000 ppm for 8 weeks prior to mating. Six consecutive weekly mating trials were conducted after treatment in which each male was housed with three untreated females. The females were replaced weekly with fresh females and those removed were sacrificed 8 days later and their uterine contents examined. The induction of dominant lethals was measured by increases in the numbers of early embryonic deaths in utero. Males fed Metepa at 300 ppm showed reduced fertility throughout the mating period, while those fed 100 ppm showed slightly reduced fertility for the first 2 weeks. Early embryonic deaths were not increased at either level of Metepa; however, this does not rule out the possibility of a dominant lethal effect being masked by the lowered fertility. A dominant lethal effect was produced among MMS-treated males at 1000 ppm, but was not detected at 300 ppm. Although dietary concentrations of the test chemicals were not directly analyzed, fresh diets were prepared weekly, refrigerated, and fed daily to minimize the potential loss due to instability.

63. *Induction of Micronuclei by Carcinogens.* MARVIN A. FRIEDMAN, WALTER H. CARTER, JACK STAUB, and ANTHONY SEGRETI, Department of Pharmacology and Biostatistics, Medical College of Virginia and MCV/VCU Cancer Center, Richmond, Virginia.

Four carcinogens, dimethylnitrosamine (DMN), acetylaminofluorene (AAF), aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), and 3-methylcholanthrene (3-MC), were tested acutely and chronically for induction of micronuclei in the bone marrow of both mice and hamsters. In subacute studies with hamsters, 3-MC (20 mg/kg, ip, three times a week) was active at 1 week but not at 8 weeks, while

DMN (2 mg/kg, ip, three times a week) was active at 8 and 12 weeks and AFB<sub>1</sub> (1.5 mg/kg, ip, three times a week) was active at 12 weeks but not at 8 weeks. In acute hamster studies, AFB<sub>1</sub> was inactive at the highest dose tested (3 mg/kg). All the other compounds increased the incidence of micronuclei with the order of potency being DMN > 3-MC > AAF. In acute studies using ICR mice, all compounds increased the relative frequency of micronuclei when compared to controls. The frequency of micronuclei was dose dependent and tended to tail off as bone-marrow suppression was observed. The order of potency in these tests was DMN > AFB<sub>1</sub> > 3-MC > AAF. These data were then subjected to model fitting using a Poisson distribution and a linear dose-response model. In mice, acetylaminofluorene was the weakest mutagen with AFB<sub>1</sub>, 3-MC, and DMN being 66, 14, and 140 times as potent, respectively. In hamsters, 3-MC and DMN were 21 and 246 times as potent as acetylaminofluorene. (Supported by NIH Grant ES00701.)

64. *The Induction of DNA Repair in Primary Rat Liver Cultures as a Screen for Chemical Carcinogens.* GARY M. WILLIAMS, Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York. (J. H. Weisburger)

A new short-term screening procedure for the assessment of potential carcinogenic effects of chemicals has been developed. This system utilizes as its end point the induction of DNA repair which has previously been recommended for carcinogen screening because: (1) many carcinogens generate electrophilic reactants which interact with DNA, thereby provoking repair; (2) all agents which induce DNA repair, thus far, have proven to be carcinogens; and (3) the mechanism by which chemicals are carcinogenic seems to be through their interaction with DNA. The new system employs primary cultures of adult rat hepatocytes. These cultures have two advantages: (1) they are highly active metabolically; and (2) they are nonreplicative, thereby permitting measurement of DNA repair as unscheduled DNA synthesis. A number of direct-acting carcinogens and procarcinogens have been studied in this system. The direct-acting carcinogens as well as carcinogenic analogs of aflatoxins, arylamines, polycyclic aromatic hydrocarbons, nitrosamines, and aminoazodyes were positive. Only one week carcinogen has been negative thus far, while one noncarcinogen has been positive. This assay, therefore, has demonstrated substantial sensitivity to carcinogens and offers an important means by which to study their effects on the genetic material of mammalian cells in which metabolic activation takes place.

65. *Induction of DNA Repair in Human Leukocytes by Fecal Metabolites of Dimethylhydrazine.* CAROLYN T. MILLER and J. A. RUDDICK, Health Protection Branch, Ottawa, Canada. (K. S. Khesa)

The colon carcinogen 1,2-dimethylhydrazine (DMH) was used as model compound for development of a procedure to detect potentially carcinogenic metabolites of fecal bacteria. DMH was added to a suspension of fresh rat or human feces in a dialysis sac submerged in a culture of human leukocytes preincubated with hydroxyurea ( $10^{-2}$  M) to block semiconservative DNA synthesis. Tritiated thymidine was added to the culture, and after incubation in 5% CO<sub>2</sub> at 37°C for up to 20 hr, DNA was extracted from the leukocytes. DMH metabolites induced a dose-dependent response in unscheduled DNA synthesis, with maximum response at 50 µg of DMH/ml of fecal suspension. No unscheduled DNA synthesis was induced by DMH if buffer, culture medium, or rat liver microsomal enzyme fraction was substituted for feces or if the feces themselves were preincubated in hydroxyurea. Quantitative DNA recovery and viability of leukocytes as indicated by Trypan blue exclusion were not affected by the fecal suspension.

66. *Extrapolation of Polychlorinated Biphenyl Pharmacokinetics from the Rat to the Mouse.* D. B. TUEY and H. B. MATTHEWS, Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina, and National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. R. Fouts)

One of the major goals in environmental toxicology is the accurate extrapolation of animal data to man. As part of a continuing effort to develop methods which will facilitate such extra-

polations, we have constructed a mathematical model of the disposition of polychlorinated biphenyls (PCBs) by the rat. Having achieved reasonable accuracy in the rat, the next step is the application of these techniques to other species. The initial effort has been directed toward extrapolation to the mouse. The model was adjusted for tissue volumes, perfusion rates, gut transport time, etc., and used to simulate the pharmacokinetics of four PCBs and their metabolites in the mouse. Predicted PCB disposition was compared with experimental data obtained at selected time points for 4-chloro-, 4,4'-dichloro-, 2,4,5,2',5'-pentachloro-, and 2,4,5,2',4',5'-hexachlorobiphenyl (1-, 2-, 5-, and 6-CB, respectively). Tissue and excreta concentration-time profiles obtained from the experimental data generally followed the distribution pattern predicted by the mathematical model. The disposition of these PCBs in mice was similar to that observed in rats. Peak contents in fat were about 10, 15, 40, and 75% of the dose for 1-, 2-, 5-, and 6-CB, respectively. Total excretion was inversely correlated with the degree of chlorination. Excretion was inversely correlated with the degree of chlorination. Excretion in urine was significant for 1- and 2-CB (71 and 57%) but not for 5- or 6-CB. The results suggest that mathematical models based on physiological concepts can provide an operational framework for interspecies extrapolation of pharmacokinetic profiles.

67. *Effect of the Route of Administration on Microsomal Enzyme Induction following Repeated Administration of Methadone in the Mouse.* J. C. KAPEGHIAN, G. A. BURDOCK, and L. W. MASTEN, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi. (W. Marvin Davis)

The degree of microsomal enzyme induction by methadone appears to be dependent on the route of administration employed in male ICR mice (25–30 g) receiving six daily doses of this narcotic. Following 3.13, 6.25, 12.5, and 25 mg/kg/day methadone hydrochloride via the sc, ip, and po routes, respectively, liver methadone *N*-demethylase, aminopyrine *N*-demethylase, and aniline hydroxylase activities were determined 24 hr after the final dose of methadone or an equivalent volume of water (PO) or saline (IP, SC). These activities were estimated in the 12,000g supernatant fraction using the cofactors and general metabolic procedures as outlined by Fouts (*Toxicol. Appl. Pharmacol.* 16, 48, 1970). In the groups receiving the highest dose of methadone, the activities of methadone *N*-demethylase, aminopyrine *N*-demethylase, and aniline hydroxylase were elevated by 72, 48, and 20%, respectively, in the ip group and by 72, 44, and 81%, respectively, in the po group. All were significantly elevated ( $p < 0.05$ ) with the exception of aniline hydroxylase activity in the ip group. None of the enzyme activities were changed in the sc group. Thus, on the basis of this *in vitro* work the potency of induction is: po  $\cong$  ip > sc. Pentobarbital sleeping time, an *in vivo* parameter of microsomal enzyme activity, demonstrated a similar ranking of these routes of administration. These major differences with respect to microsomal enzyme induction by methadone as a function of the route of administration may account for the apparent lack of information on this phenomenon until the present time. (Supported in part by NIDA Grant DA 01331-02, NIH Training Grant GM 07099-02, and by the University of Mississippi Research Institute of Pharmaceutical Sciences.)

68. *Acetylation of Isoniazid by Rabbit Liver and Lung N-Acetyltransferases in Vitro.* S. D. HARRISON, JR. and D. A. POTTER, Southern Research Institute, Birmingham, Alabama. (E. P. Denine)

Isoniazid (INH) is converted to an hepatotoxic acylating metabolite(s) by mammalian liver enzymes. Since some species are capable of extrahepatic xenobiotic metabolism, biotransformation of INH by lung tissue may have important toxicologic consequences. Female New Zealand rabbits (2–3 kg) served as tissue donors for these *in vitro* studies. The rabbits received either no pretreatment or phenobarbital (60 mg/kg, ip, daily for 3 days prior to sacrifice). Liver and lung *N*-acetyltransferases were prepared by the method of Gram *et al.* (*Drug Metab. Disp.* 2, 254, 1974). [ $^3\text{H}$ ]INH (Amersham/Searle) was diluted to a specific activity of 1.0  $\mu\text{Ci}/\mu\text{mol}$ . Incubation mixtures contained varying concentrations of [ $^3\text{H}$ ]INH, 0.5  $\mu\text{mol}$  of *S*-acetyl-

coenzyme A, 1 mg of liver or lung protein, and potassium tetraborate buffer (90  $\mu$ mol, pH 9.0) in a total volume of 1.0 ml. Incubations were carried out at 37°C in the presence of atmospheric oxygen. Reactions were terminated by freezing at -76°C. Acetyl-INH was separated by thin-layer chromatography, identified by comparison to an authentic sample, and quantitated by liquid scintillation spectrometry. Apparent  $V_{\max}$  values are essentially identical for liver and lung regardless of pretreatment ( $V_{\max}$  for liver =  $244 \pm 27$  nmol/min/mg of protein). This demonstrates an important potential capacity of lung tissue for contributing to the total biotransformation of INH *in vivo*. The  $K_m$  of the rabbit lung enzyme(s) appears to exceed that of liver ( $6.16 \pm 5.82$  and  $1.18 \pm 0.54$  mM, respectively). Consequently, the lung's influence probably is less important under the usual physiologic circumstances of INH administration. (Supported by Grant No. HL 17185, National Heart, Lung, and Blood Institute, NIH.)

69. *Metabolism of the Dyestuff Intermediate, 2,4-Diaminoanisole, in the Rat.* PRESTON H. GRANTHAM, LETITIA C. MOHAN, TIMOTHY T. BENJAMIN, PETER P. ROLLER, and ELIZABETH K. WEISBURGER, National Cancer Institute, Bethesda, Maryland.

2,4-Diaminoanisole, an ingredient of hair dye formulations, is a potent mutagen in the Ames bacterial test system. Animals were injected with  $^{14}$ C-labeled 2,4-diaminoanisole ip at a dose of 50 mg/kg. Urine and feces were collected at intervals and assayed for  $^{14}$ C. The animals excreted 80.9% of the dose (urine, 78.8%; feces, 2.1%) in 24 hr. By 48 hr, 93.7% of the dose was excreted (urine, 84.7%; feces, 9.0%). Urinary activity of the 24-hr urine was present as 23.1% unconjugated metabolites, 10.3% glucuronide conjugates, and 6.2% sulfate conjugates. The water-soluble nonextractable material was 34.9% of the dose. The major metabolites in the free fraction were identified as 4-acetylamino-2-aminoanisole and 2,4-diacetylaminoanisole. A small amount of diacetylaminophenol was also found. The glucuronide fraction contained 2,4-diacetylaminophenol, two ring hydroxylated derivatives of 2,4-diacetylaminoanisole, and a small amount of 4-acetylamino-2-aminoanisole.

70. *Studies on the Bioorganic Mechanism of the Metabolism of Dihalomethanes to Formaldehyde and Inorganic Halide.* A. E. AHMED and M. W. ANDERS, Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota.

Previous reports from this laboratory have described the metabolism of dihalomethanes (DHM) to carbon monoxide and formaldehyde ( $\text{CH}_2\text{O}$ ) (*Drug Metab. Disp.* 3, 104, 1975; 4, 357, 1976). The studies described herein were conducted to elucidate the bioorganic mechanism of the metabolism of DHM to  $\text{CH}_2\text{O}$  and halide. Glutathione (GSH) is a known cofactor in this reaction. The first step in the reaction appears to be a nucleophilic attack of GSH on the DHM. The resulting *S*-halomethyl glutathione derivative ( $\text{GSCH}_2\text{X}$ ) is highly reactive and undergoes rapid hydrolysis to yield *S*-hydroxymethyl glutathione ( $\text{GSCH}_2\text{OH}$ ). This compound is the hemimercaptal of  $\text{CH}_2\text{O}$  and GSH and provides a mechanism for the formation of  $\text{CH}_2\text{O}$  from DHM. As required by this mechanism, neither GSH disappearance nor an isotope effect was observed when  $\text{d}_2$ -DHM was used. *S*-Hydroxymethyl glutathione is the substrate for hepatic formaldehyde dehydrogenase (FDH) (*J. Biol. Chem.* 249, 7653, 1974). This  $\text{NAD}^+$ -dependent enzyme converts  $\text{GSCH}_2\text{OH}$  to *S*-formylglutathione ( $\text{GS-CHO}$ ) which is enzymatically hydrolyzed to formic acid and GSH. Thus, addition of  $\text{NAD}^+$  was found to decrease  $\text{CH}_2\text{O}$  yields with a concomitant increase in formate production. Enzyme purification studies further substantiate the role of FDH in this biotransformation. In summary, these results suggest that GSH transferase catalyzes the formation of  $\text{GSCH}_2\text{X}$  which is hydrolyzed to yield  $\text{GSCH}_2\text{OH}$ . The latter dissociates to yield  $\text{CH}_2\text{O}$  and GSH. In the presence of  $\text{NAD}^+$ , FDH converts  $\text{GSCH}_2\text{OH}$  to  $\text{GS-CHO}$  with subsequent enzymic hydrolysis to formate ( $\text{HCCO}^-$ ) and GSH. While the products of DHM metabolism ( $\text{CH}_2\text{O}$ ,  $\text{HCOOH}$ ) are of a low order of toxicity, the  $\text{GS-CH}_2\text{X}$  intermediate is a highly reactive species capable of covalent interaction with cellular nucleophiles. This may partially explain the irreversible binding that has been observed following treatments of rats with [ $^{14}$ C]dichloromethane. (Supported by USPHS Grant No. ES 01082.)

71. *Serum Glutathione S-Transferase Activity: A Leak of Enzyme from Liver?* HASAN MUKHTAR and JOHN R. BEND, Pharmacology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. R. Fouts)

Glutathione *S*-transferase activity was determined in rat, rabbit, and guinea pig serum using styrene 7,8-oxide (SO) and benzo(a)pyrene 4,5-oxide (4,5-BPO) as substrates. Of the species tested, rat had the highest transferase activity (62.5 and 3.2 nmol/min/ml of serum for SO and 4,5-BPO, respectively) and rabbit had the lowest activity. The relative specific serum activities did not parallel hepatic activities in the three species (reported previously from this laboratory). Glutathione *S*-transferase activity was 60% higher in serum from male rats than in female rats. In rats, serum enzyme specific activities (nmol/min/mg of protein) were less than 1% of the hepatic enzyme activities with SO and 4,5-BPO. Transferase activity was also determined in rat serum during perinatal development. Serum from minus 2-, 1-, and 4-day-old rats had barely detectable glutathione *S*-transferase activity. Activity increased with age and reached a maximum in 140-day-old animals. The ip administration of diethyl maleate (0.8 ml/kg) or methionine sulfoximine (360 mg/kg) to male rats had no effect on serum glutathione *S*-transferase activities 2 hr after dosing. Treatment with carbon tetrachloride (1 ml/kg) caused a 3.7-fold increase in serum transferase activity, however. This elevation in serum glutathione *S*-transferase activity subsequent to CCl<sub>4</sub> treatment appears to be related to liver damage.

72. *Kinetics of Diphenylhydantoin Metabolism in the Isolated Perfused Liver from Pregnant and Nonpregnant Rats.* M. VORE and J. BAUER, Department of Pharmacology and Toxicology, University of California Medical Center, San Francisco, California. (Harold C. Hodge)

Decreased levels of drug metabolism during pregnancy have been noted previously in the rat and other species including humans in *in vitro* studies using liver microsomes and *in vivo*. Since the isolated perfused liver (IPL) is the nearest *in vitro* system comparable to the intact liver for studying drug metabolism, we compared the metabolism of diphenylhydantoin (DPH) in the IPL from timed pregnant (15, 17, 19, and 21 days pregnant) and nonpregnant rats. Livers were perfused via the portal vein with 10% male rat donor blood in Krebs-bicarbonate equilibrated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 37°C at a constant flow of 10 ml/min. Bile was collected via a cannula in the bile duct. [<sup>14</sup>C]DPH was added to the perfusate (100 ml) to give an initial concentration of 38 nmol/ml. Samples of perfusate and bile were collected at 0, 10, 30, 60, 90, and 120 min after addition of [<sup>14</sup>C]DPH for kinetic analysis. The  $\beta$  half-life of [<sup>14</sup>C]DPH in the perfusate in nonpregnant and 15-, 17-, 19-, and 21-day pregnant rats was 37, 26, 64, 79, and 138 min, respectively, whereas the cumulative excretion of <sup>14</sup>C in the bile at 120 min was 1291, 1178, 472, 228, and 143 nmol, respectively. These data indicate that the ability of the liver to metabolize DPH is markedly decreased during the latter stages of pregnancy with important implications regarding toxicity to both the pregnant female and the fetuses. (Supported by Basil O'Connor Research Starter Grant, National Foundation, March of Dimes.)

73. *Effect of Dietary Fiber on Lindane Metabolism.* R. W. CHADWICK, M. F. COPELAND, and C. J. CHADWICK, Environmental Protection Agency, Health Effects Research Laboratory Research Triangle Park, North Carolina.

Since dietary fiber has been shown to provide a protective effect against many toxicants, the increased ingestion of drugs, chemicals, and food additives accompanied by the relatively low dietary fiber intake could constitute a health hazard in the western world. Marked differences in the physical, chemical, and metabolic properties of various dietary fibers have been reported. The effect of various dietary fibers on the metabolism of the organochlorine insecticide lindane was investigated in this study. Thirty weanling, female Sprague-Dawley rats randomly assigned to one of five treatment groups were fed either a synthetic low residue diet (LRD), LRD + 10% pectin, LRD + 10% agar, LRD + 10% cellulose, or commercial lab chow for 1 month. The animals were then dosed with 2.87 mg of lindane (containing 1.66  $\mu$ Ci of [<sup>14</sup>C]lindane) and were sacrificed 24 hr later. Urine, feces, fat, liver, kidney, and blood samples were taken for analysis. Although neither body nor kidney weights differed significantly, the livers of the rats on lab



chow were significantly larger than those of other rats. Total excretion of radioactivity by rats on both lab chow and LRD + 10% pectin was significantly higher than that of the other animals. However, while the rats fed LRD + 10% pectin excreted a significantly higher level of radioactivity per gram of feces, the rats on lab chow excreted significantly more radioactivity in the urine. While the rats fed lab chow excreted significantly more conjugated chlorophenols than all other animals, those fed LRD + 10% pectin excreted significantly more conjugated chlorophenols than the rats fed LRD. Although there were no significant differences in microsomal protein, the hepatic microsomal cytochrome *P*-450 content of the rats fed the lab chow was significantly higher than all rats but those receiving LRD + 10% pectin. *In vitro* enzyme activity for the hydroxylation of chlorobenzenes and both the dehydrogenation and dechlorination of lindane was significantly higher in rats fed lab chow. All rats fed diets supplemented with fiber had higher dechlorinase activity than the animals receiving LRD only. Results indicate that some dietary fibers can exert a significant effect on the metabolism and excretion of lindane and its metabolites in mammals.

74. *Modification of Cytochrome P-450 by the Fungicide Metabolite Ethylene Thiuram Monosulfide*. T. YOSHIDA and R. A. NEAL, Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

Ethylene thiuram monosulfide (ETM) and ethylene thiourea (ETU) are major breakdown products of the ethylene-bis-dithiocarbamate fungicides. Recent work in this and other laboratories has indicated that a number of compounds, including ETU, which contain a thiono-sulfur group, are capable of strongly inhibiting the cytochrome *P*-450-containing mixed-function oxidase enzyme systems when administered *in vivo* or incubated with these enzyme systems *in vitro*. Since ETM also contains a thiono-sulfur group, it was of interest to examine the effect of ETM on hepatic mixed-function oxidase activity. Male Sprague-Dawley rats, weighing 200–250 g, were used. ETM was administered orally, as a suspension in olive oil, and rats were sacrificed at the appropriate times. ETM at doses of 1, 2, and 5 mmol/kg significantly inhibited both benzphetamine demethylation and aniline hydroxylation measured 24 hr after its administration. The administration of ETM also decreased the concentration of cytochrome *P*-450 and protoheme in microsomes, suggesting that the inhibition of mixed-function oxidase activity by ETM was due to a decrease in the concentration of cytochrome *P*-450. To examine its mode of action, ETM was incubated *in vitro* with microsomes in the presence and absence of an NADPH-generating system. An inhibition of mixed-function oxidase activity by ETM ( $1 \times 10^{-3}$  M) was observed in both the presence and absence of NADPH. This inhibition seen *in vitro* was mainly due to a conversion of cytochrome *P*-450 to *P*-420. The extent of the conversion depended on both incubation time and the concentration of ETM. This conversion of cytochrome *P*-450 to *P*-420 was prevented by the addition of reduced glutathione to the incubation prior to ETM but could not be completely reversed if reduced glutathione was added after ETM. The present findings demonstrate that ETM, like ETU, acts as an inhibitor of the hepatic mixed-function oxidase enzyme system both *in vivo* and *in vitro*. However, the mode of action of ETM *in vitro* differs from that of other thiono-sulfur-containing compounds so far examined, including ETU.

75. *The Fate of Silvex following Oral Administration to Humans*. M. W. SAUERHOFF, M. B. CHENOWETH, H. L. GORDON, W. H. BRAUN, G. E. BLAU, and P. J. GEHRING, Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan.

The fate of silvex, 2-(2,4,5-trichlorophenoxy)propanoic acid, was defined in seven men and one woman following oral administration of 1 mg/kg. No adverse effects were observed. Samples of blood plasma, urine, and feces were collected at designated time intervals through 168 hr. Plasma samples were analyzed for silvex only while samples of urine and feces were analyzed for silvex, silvex conjugate(s), 2,4,5-trichlorophenol, and 2,4,5-trichlorophenol conjugate(s). Apparent first-order kinetics described the biphasic clearance of silvex from plasma and excretion in urine. The half-life values for clearance of silvex from plasma were

4.0 ± 1.9 and 16.5 ± 7.3 hr for the initial and terminal phases, respectively. Peak plasma concentrations of silvex occurred within 2 to 4 hr after dosage. Within 24 hr following administration, 65% of the administered dose had been excreted in urine. Silvex was excreted in urine as silvex and a silvex conjugate(s). The half-life values for excretion of silvex per se in urine were 5.0 ± 1.8 and 25.9 ± 6.3 hr for the two phases, respectively. Small amounts (3.2% or less) of silvex and/or silvex conjugate(s) were eliminated in feces. Recovery of silvex and its conjugate(s) in urine and feces through 168 hr ranged from 66.6 to 95.1% of the administered dose with a mean value and SD of 80.3 ± 10.5%. In man, silvex is readily absorbed following ingestion and subsequently readily excreted predominantly via the urine.

76. *Species-Dependent Pharmacokinetic Profile and Toxicity of a Thiourea Derivative in Rats and Monkeys.* J. D. YOUNG, S. J. GORZINSKI, J. E. LEBEAU, R. W. SLAUTER, and W. H. BRAUN, Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, Michigan. (P. J. Gehring)

The pharmacokinetics of 1-(2-hydroxyethyl)-2-thio-3-(2,4-xylyl) urea (HXTU) has been studied in rats and monkeys using the compound <sup>14</sup>C-labeled in either the hydroxyethyl or the ring of the xylyl group. In toxicology studies, pulmonary edema was observed in rats but not monkeys upon chronic ingestion. The pharmacokinetic profile of [<sup>14</sup>C-xylyl]HXTU was essentially the same in both species when given either iv or po at dosages of 2 or 20 mg/kg. Radioactivity was rapidly eliminated in the urine of monkeys and in the urine and feces of rats. All of the radioactivity in excreta of both species was associated with metabolites. The radio-labeled metabolites of [<sup>14</sup>C-xylyl]HXTU in the urine of rats and monkeys were identified by GC/MS as 2,4-dimethylaniline and *N*-acetyl-2,4-dimethylaniline. When the compound labeled in the hydroxyethyl group was administered to rats and monkeys at dosages of 2 or 20 mg/kg, pronounced species differences in the metabolic fate were observed. The urinary metabolites, separated by thin-layer chromatography, were different in the two species; radioactivity accumulated in the lungs and red blood cells of rats but not monkeys; plasma concentrations of radioactivity were consistently higher in rats than in monkeys; dose dependency and a pronounced first pass effect were observed in rats but not in monkeys. These results suggest that the toxicity of HXTU was due to species-dependent biotransformation of the compound to a toxic metabolite associated with the hydroxyethyl portion of HXTU. The results of these experiments also demonstrate the utility of pharmacokinetic data to provide a rational basis for interpreting the results of toxicological studies and demonstrate the importance of <sup>14</sup>C label position to the interpretation of pharmacokinetic data.

77. *Phenolic Antioxidants and the Inhibition of Dimethylamine-Nitrite-Induced Hepatotoxicity in the Rat.* B. D. ASTILL and T. MULLIGAN, Health, Safety, and Human Factors Laboratory, Eastman Kodak Company, Rochester, New York, and Hazleton Laboratories, America Inc., Vienna, Virginia.

Nitrosamine (NOA) formation from nitrite and secondary amines is mediated *in vitro* by promoters (e.g., thiocyanate) and inhibitors (e.g., ascorbate, ascorbyl esters). Phenols can act as promoters (e.g., 4-methylcatechol) or inhibitors (e.g., gallic acid). Acute oral administration of nitrite and dimethylamine (DMA) to rats produces a hepatotoxicity which can be completely suppressed by concomitant dosing with ascorbate. We therefore studied the effect of propyl gallate (PG), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and *t*-butylhydroquinone (TBHQ), widely used edible fat stabilizers, on a similar nitrosamine forming system (NOAFs). Male Sprague-Dawley rats (240-296 g), 10 per test group, received an NOAFs of 1.0 g/kg DMA immediately followed by 125 mg/kg of NaNO<sub>2</sub> by oral intubation in aqueous solution. Test compounds were given immediately afterward in doses of 25, 75, or 225 mg/kg in corn oil. Controls were the NOAFs alone or followed by 200 mg/kg of sodium ascorbate and corn oil or 225 mg/kg of test compound without the NOAFs. Indices of hepatotoxicity, established at sacrifice 48 hr after treatment, were serum GOT, GPT, and OCT activities, and the graded extent of hepatic necrosis. In the absence of the NOAFs, no changes

occurred in any index. The NOAFs produced extensive hepatic necrosis, and 24-, 19-, and 4-fold increases in SGOT, SGPT, and SOCT, respectively, all completely suppressed by ascorbate. PG completely protected against hepatic necrosis and enzyme activity elevation at 225 mg/kg, and to a lesser extent at 75 mg/kg. TBHQ gave a 60% protection against necrosis and markedly suppressed serum enzyme elevations at 225 mg/kg. PG at 25 mg/kg, TBHQ at 25 and 75 mg/kg, and BHT and BHA at all dose levels had little or no protective activity. It is concluded that PG and TBHQ are effective inhibitors of NOA formation in the rat stomach, and that BHA and BHT do not produce any detectable enhancement.

78. *Long-Term Toxicity Study with Orthotoluenesulfonamide and Saccharin*. D. L. ARNOLD, S. M. CHARBONNEAU, C. A. MOODIE, and I. C. MUNRO, Toxicology Research Division, Bureau of Chemical Safety, Health Protection Branch, Ottawa, Canada.

The possible significance of *in utero* exposure to *ortho*-toluenesulfonamide (*o*-TS) and saccharin in the production of bladder tumors in laboratory rats was investigated. In a lifetime F<sub>0</sub>-F<sub>1</sub> study, 50 male and 50 female weanling Sprague-Dawley rats were included in the following groups: (A) control, (B) 2.5 mg *o*-TS/kg/day, (C) 25 mg *o*-TS/kg/day, (D) 250 mg *o*-TS/kg/day, (E) 250 mg *o*-TS/kg/day with 1% NH<sub>4</sub>Cl in the drinking water, and (F) 5% sodium saccharin. Saccharin and *o*-TS were administered in the diet. After 3 months, the F<sub>0</sub> rats were bred and 50 pups of both sexes from each group were weaned onto the parental diets which both generations received for lifetime. The *o*-TS was more than 99% pure. Saccharin contained less than 0.2 ppm *o*-TS. Rats given diet D, E, or F showed reduced growth from weeks 10 to 12. There was no treatment related mortality, reproductive anomalies, or urinary stones/calculi. Water consumption and urine volume was approximately doubled for group F and the urine was hypotonic. Group E had a significant decrease in urinary pH. The animals were free of the bladder parasite *T. crassicauda*. The incidence of bladder or other tumors in the F<sub>0</sub> rats was similar to controls confirming previous observations (Munro *et al.*, *Toxicol. Appl. Pharmacol.* **32**, 513, 1975). Gross necropsy findings with the F<sub>1</sub> generation suggest that *in utero* exposure may increase the incidence of some tumors.

79. *Six-Year Study of Saccharin in Rhesus Monkeys*. E. W. MCCHESENEY, F. COULSTON, and K.-F. BENITZ, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Rhesus monkeys began to receive soluble saccharin orally, 6 days per week, early in 1969. The groups, composed of equal numbers of each sex, were divided as follows: controls and 500 mg/kg/day, each six animals; 20 and 100 mg/kg/day, each four animals. Growth rates were essentially unaffected by the treatment, as were standard serum biochemical and whole blood hematological parameters (these parameters being determined at about 6-month intervals). Metabolic studies conducted on several occasions during the test (cf. Byard and Golberg, *Fd. Cosmet. Toxicol.* **11**, 391, 1973; Coulston *et al.*, *Fd. Cosmet. Toxicol.* **13**, 297, 1975) indicated rapid, almost exclusively, urinary excretion of saccharin, in unchanged form. The experiment was terminated after 79 months, prior to which time there had been two deaths in the controls, and one in each of the experimental groups, but with none of the latter in any way attributable to the treatment. On sacrifice of the surviving animals, no pathology was detected grossly in any organ or tissue. Absolute and relative weights of kidneys and testes, and diameters of the latter, were normal. Kidneys, testes, and urinary bladders were examined microscopically (livers by EM); no deviations from normal morphology were detected in any of these tissues. (Supported in part by FDA Contract No. 69-7, by Canadian Food and Drug Directorate Contract No. TB 705029, and by NIEHS Grant No. 5 P01-ES00226.)

80. *Eight-year Study of Cyclamate in Rhesus Monkeys*. F. COULSTON, E. W. MCCHESENEY, and K.-F. BENITZ, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Five rhesus monkeys began to receive cyclamate orally at 200 mg/kg, 6 days per week, early in 1968. Two females continued on this dosage regimen for 94 months, one male for 91 months,

another for 40 months, and a third for 36 months. There were no deaths attributable to the treatment. Growth rates were normal throughout, as were standard serum biochemical and whole blood hematological parameters. Metabolic studies conducted on four occasions ranging from the 56th to the 356th test weeks indicated little change in the extent of absorption, and that the ingested cyclamate was being excreted more than 99.5% in unchanged form. The principal degradation product was cyclohexylamine which was, in turn, being converted to cyclohexanone and cyclohexanol to the extent of about 1-2%. When the three animals which remained on test for 90+ months were sacrificed, no pathology was detectable grossly in any organ or tissue. Liver, kidney, urinary bladder, and testes were examined histologically (the liver also by EM); no deviations from normal morphology were detected in any of these tissues. (Supported in part by NIEHS Grant No. 5P01-ES-00226 and by FDA Contract No. 69-7.)

81. *Biogenesis of Lung-Toxic Furans Produced during Microbial Infection of Sweet Potatoes (Ipomoea batatas)*. L. T. BURKA, L. KUHNERT, B. J. WILSON, and T. M. HARRIS, Center in Environmental Toxicology and Department of Chemistry, Vanderbilt University, Nashville, Tennessee.

Fundal contamination of sweet potatoes often results in the production of stress metabolites including the well-known hepatotoxin, ipomeamarone. In addition, potent lung toxins, 4-ipomeanol, 1-ipomeanol, ipomeanine, and 1,4-ipomeadiol are sometimes produced. Recent studies in our laboratories have centered on the biogenesis of the latter four compounds. Certain pathogenic fungi are able to convert a furanosesquiterpene stress metabolite of the sweet potato, 4-hydroxymyoporone, to the lung toxins. Isolation of radioactive ipomeanine and 4-ipomeanol from incubation of [4-<sup>14</sup>C]hydroxymyoporone with mycelial mats of *Fusarium solani* demonstrated that the sesquiterpene can serve as a precursor to the lung toxins. A fungus that is closely related to *F. solani*, *F. oxysporum*, was also able to carry out the conversion but less efficiently. Incubation with *Ceratocystis fimbriata*, another common fungal contaminant of sweet potatoes, resulted in no reaction. It was also found that [<sup>14</sup>C]ipomeamarone is not efficiently converted to the lung toxins by *F. solani*. The biogenesis of the lung edemagenic agents is a complex process. Chemical and physical damage, as well as many invading organisms, can stimulate the sweet potato to elaborate stress metabolites but the lung toxins are formed only when certain fungi are present. These toxins are not produced by the sweet potato but are the result of fungal catabolism of a stress metabolite formed by the host plant.

82. *Effect of Patulin on Krebs Cycle Intermediate-Stimulated Oxygen Consumption*. A. W. HAYES, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi.

Patulin, 4-hydroxy-4-H-furo(3,2c)pyran-2(6H)-one, is a carcinogenic lactone metabolite produced by several species of *Aspergillus* and *Penicillium*. Its biochemical effects in mammalian systems, however, have not been extensively investigated. The *in vitro* effect of patulin on the rate of oxygen uptake was examined using whole tissue homogenates from various animal species. At a concentration of 1.3 mM, patulin inhibited, in mice, the *in vitro* Krebs cycle intermediate-stimulated oxygen uptake in liver homogenates. Succinate, citrate, and  $\beta$ -hydroxybutyrate were inhibited 52, 66, and 52%, respectively. Inhibition (10-12%) of oxygen uptake in liver homogenates with succinate as the substrate was observed with concentrations as low as 0.033 mM patulin. Inhibition of oxygen uptake in heart (69%) and muscle (53%) homogenates was greater than in liver homogenates when succinate was the substrate. No sex difference was observed with mice. Preincubation of the toxin for 45 min with the liver homogenate increased the degree of inhibition of oxygen uptake. Patulin inhibited succinic acid dehydrogenase in mouse liver homogenates competitively. The toxin did not effect P/O ratios in liver homogenates from male rats using succinate as substrate. A varied species susceptibility to *in vitro* poisoning by patulin also was observed with the golden hamster being the most susceptible species and the male weanling rat being the least. *In vivo* effects of patulin on the rate of oxygen uptake were not as pronounced as were the *in vitro* effects. (Supported by USPHS Grants ES 01351 and ES 01352.)

83. *Aflatoxicosis in Rats Fed Low Levels of Aflatoxin Either Chemically Pure or as a Natural Contaminant.* MERRILL O. TISDEL, PAUL O. NEES, DONALD L. HARRIS, and GODFREY E. MANN, WARF Institute, Inc., Maddison, Wisconsin, and Southern Regional Research Center, U.S.D.A., New Orleans, Louisiana.

The present study was initiated to clarify the conflicting reports that ground roasted peanuts in the diet provide some dietary protective action against aflatoxin, a potent hepatic carcinogen. Aflatoxin at 0, 5, 15, 50, and 200 ppb in a semisynthetic basal diet, a diet containing peanut butter, or a diet containing cottonseed meal was fed to Fischer strain rats for up to 104 weeks. The sources of aflatoxin were a chemically pure supply and naturally contaminated peanuts and cottonseed meal. The presence of chemically pure aflatoxin in the basal diet or diet containing aflatoxin-free peanut butter resulted in a dose-related aflatoxicosis including carcinomas and/or hepatomas in animals receiving as little as 5 ppb. Aflatoxin from naturally contaminated peanuts or cottonseed meal resulted in dose-related effects comparable to those produced from chemical aflatoxin. Tumor incidence was equal or greater at all aflatoxin levels in groups receiving naturally contaminated peanuts. The presence of peanuts in the diet did not significantly alter the dose related effect. The data from this study indicate that a dose-related aflatoxicosis was due to the presence of aflatoxin in the total diet. Aflatoxicosis was not related to the source of aflatoxin, and there was no protective action against aflatoxicosis from the presence of peanuts or cottonseed meal.

84. *Effect of Dietary Sodium Selenite upon Lesions Induced by Repeated Small Doses of Aflatoxin B<sub>1</sub>.* KENNETH E. GRANT, MICHAEL W. CONNER, and PAUL M. NEWBERNE, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Previous research in this laboratory has demonstrated that dietary sodium selenite protects rats from the effects of an acute dose of aflatoxin B<sub>1</sub>. In this experiment, 140 male Sprague-Dawley rats were fed diets containing 0.03, 0.10, 0.50, 1.00, 2.50, or 5.00 ppm selenium as sodium selenite in a normal or marginally lipotrope-deficient semisynthetic basal diet. Each animal was given 25 µg of aflatoxin B<sub>1</sub> orally 5 days/week for 4 weeks. After 17 months all surviving animals were sacrificed. Histopathology revealed a very low incidence (20%) of hepatocarcinomas in animals receiving 1.00 ppm selenium in the normal basal diet and 0.10 and 0.50 ppm in the deficient basal diet. No other tumors were observed, and grading of the hepatic lesions indicated no significant difference among dietary selenium groups. Renal lesions were more striking. Large bizarre tubule cells were noted in relatively normal tubules surrounding areas of nephrosis. Nephrosis increased in severity with increasing dietary selenium concentration. Tissue selenium concentrations and erythrocyte glutathione peroxidase activities varied with dietary selenium but were lower in lipotrope-deficient animals than in normal basal diet animals. Dietary sodium selenite and repeated doses of aflatoxin B<sub>1</sub> interact to produce large bizarre cells in the renal tubules. This lesion occurs in the region of the kidney where severe necrosis was noted previously in sodium selenite-fed animals given an acute dose of aflatoxin B<sub>1</sub>. There was no significant change in aflatoxin-induced hepatic lesions.

85. *Studies on the Absorption, Distribution, Metabolism, Excretion, [<sup>14</sup>C]WR-180,409 · H<sub>3</sub>PO<sub>4</sub> in Rhesus Monkeys.* W. R. MCCONNELL and R. L. FURNER, Southern Research Institute Birmingham, Alabama. (E. P. Denine)

In the search for new, more-effective antimalarial compounds, we have undertaken studies on WR-180,409 · H<sub>3</sub>PO<sub>4</sub> [threo-α-(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridinemethanol phosphate]. Drug-equivalents in the blood of rhesus monkeys reached a maximum between 12 and 24 hr after oral dosing; the parent compound comprised about 45% of the total. In those animals dosed iv, elimination of drug-equivalents from blood was triphasic with half-lives of 1.2 hr, 2.9 days, and 14.9 days. Elimination of drug-equivalents in feces (59%) and urine (24%) followed first-order processes with half-lives of 1.9 and 1.7 days, respectively. Extracts of urine, feces, and tissues contained six products in addition to the

parent compound. The parent compound represented no more than 9% of the drug-equivalents excreted per day in urine, and no more than 50% in feces. High concentrations of drug-equivalents were found in liver (75  $\mu\text{g/g}$ ), kidneys (52  $\mu\text{g/g}$ ), lungs (175  $\mu\text{g/g}$ ), and eyes (30  $\mu\text{g/g}$ ); whole blood contained 2  $\mu\text{g/g}$ . [ $^{14}\text{C}$ ]WR-180,409 $\cdot\text{H}_3\text{PO}_4$  and its products were readily absorbed from the gut, rapidly distributed into various body compartments, and relatively slowly excreted. (Supported by Contract DAMD 17-75-C-5065, U.S. Army Medical Research and Development Command, and by the Southern Research Institute.)

86. *Effect of Salicylate on the Fate of [ $^{14}\text{C}$ ]Warfarin in the Guinea Pig.* L. T. WONG, G. SOLOMONRAJ, and B. H. THOMAS, Drug Research Laboratories, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada. (B. B. Coldwell)

The effect of sodium salicylate (177.8 mg/kg, po) on the fate of [ $^{14}\text{C}$ ]warfarin (1 mg/kg, ip) was studied in guinea pigs. Salicylate decreased the blood concentration of radioactivity during the initial 6 hr after administration and reduced the total area under the blood profile curve. At 1 hr, salicylate increased the distribution of radioactivity in the liver and brain, but decreased it in the kidney. Biliary excretion studies showed that a significant portion of the radioactivity was excreted into the bile, but was subject to enterohepatic circulation in the intact animal. Salicylate increased the biliary excretion of radioactivity during the first 5 hr after drug administration, but did not change the proportion of unchanged [ $^{14}\text{C}$ ]warfarin to its metabolites. An *in vitro* plasma protein binding study showed that salicylate displaced bound [ $^{14}\text{C}$ ]warfarin. It is concluded that (1) salicylate displaces warfarin from protein binding sites, thus facilitating its uptake into the liver, and (2) salicylate increases the excretion of warfarin and its metabolites from the liver into the bile causing a lower concentration in the blood.

87. *Biotransformation of Some Alkaloids by Liver Microsomes.* A. PEEPLES and R. R. DALVI, Department of Physiology and Pharmacology, School of Veterinary Medicine, Tuskegee Institute, Tuskegee, Alabama. (R. A. Neal)

Toxic alkaloids are of importance as hazards to farm animals and to man. They give rise to various toxic effects ranging from neurotoxicity to carcinogenicity. In most cases it remains unknown whether the parent alkaloid or its biologically transformed derivative is responsible for at least some of the various actions of the alkaloid. These studies were therefore carried out to examine the metabolism of certain alkaloids by the hepatic drug metabolizing enzyme system. *In vitro* incubation of colchicine, nicotine, or scopolamine with rat liver microsomes and NADPH resulted in demethylation of the alkaloids while that of boldine, brucine, emetine, reserpine, sanguinarine, or solanine showed little or no demethylation. Studies on the binding spectra of the alkaloids with microsomes revealed that brucine, scopolamine, and strychnine are type I compounds whereas boldine, emetine, nicotine, reserpine, and sanguinarine show type II binding. Colchicine and solanine failed to produce normal binding spectra. Studies on the *in vivo* effect of the alkaloids on components of the drug metabolizing enzyme system indicate that treatment of rats with sublethal dosage levels of emetine and sanguinarine significantly decreased the aniline hydroxylase activity and the concentration of cytochrome P-450. On the other hand, administration of nicotine, reserpine, and scopolamine enhanced the activity of aniline hydroxylase with concomitant increase in cytochrome P-450 content. These results suggest that the alkaloids studied herein can both be metabolized by and serve as competitive inhibitors for the hepatic drug metabolizing enzyme system. (Supported by Research Grant HES75-09294 from National Science Foundation.)

88. *The Antagonism of Ethanol Depression by 2[(3,4-Dichlorophenoxy)methyl]-2-imidazoline Hydrochloride.* L. C. GRIFFIS and R. B. FORNEY, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana.

2[(3,4-Dichlorophenoxy)methyl]-2-imidazoline hydrochloride (DL-524) is a possible antagonist to some CNS depressants. The purpose of this investigation was to determine the effect of DL-524 in combination with ethanol. Groups of mice were administered 0, 5, 10, or 20 mg/kg

DL-524 ip 10 min before an hypnotic dose of ethanol (4.5 g/kg, ip). The duration of the loss of righting reflex (sleeping time) was determined. Each animal was sacrificed when it regained the righting reflex and the brain removed for the determination of ethanol concentration. DL-524 significantly decreased the duration of ethanol-induced sleeping time in a dose-related fashion. The brain ethanol concentrations of DL-524-treated animals was higher than controls indicating a site of action in the CNS. The effect of the above doses of DL-524 on spontaneous locomotor activity was also determined. Sixteen groups of mice were tested in four activity cages on four consecutive days. The four doses were assigned to the cages according to a complete latin square. Activity was monitored every 30 min for 3 hr. DL-524 had no significant effect on activity. (Supported in part by PHS GM 1089.)

89. *Changes in Plasma Proteins of Ducklings from Dietary Fusarotoxins.* D. S. SAWHNEY, Cornell University Ithaca, New York. (J. M. Taylor)

Fusarotoxins, heat-stable mycometabolite(s), were produced on rice by culturing with *Fusarium roseum* and incubating at  $22 \pm 2^\circ\text{C}$  for 7 days. The rice was fed to 1-day-old Pekin ducklings at levels of 0, 5, 10, and 20% of the diet. The toxicity of dietary fusarotoxins was characterized by a reduction in body weight and an increase in food consumption. A decrease in total plasma proteins with other biochemical changes was observed at 4, 7, and 11 days of treatment in the ducklings (*Toxicol. Appl. Pharmacol.* **37**, 141, 1976). A dose-related decrease in protein values was observed. At the 20% level a marked decrease was evident at 4 days. Alterations in the electrophoretic pattern of plasma proteins were determined using 7% polyacrylamide gel electrophoresed in Tris-borate buffer of pH 8.4. These changes were further investigated by ion-exchange chromatography-diethylaminoethyl (DEAE) column. A linear gradient of NaCl 0 to 0.5 M Tris buffer at pH 7.3 was used. Proteins were fractionated at a flow rate of 3.5 ml/hr. Electrophoresis and chromatographic patterns of the affected animals showed that hypoproteinemia was due to a simultaneous reduction in all plasma protein fractions. The reduction in albumins and adenosine-5'-triphosphate (ATP) is consistent with the lesions of liver and other tissues seen histopathologically. This suggests that protein synthesis in general is severely depressed by fusarotoxins, with essential membrane disturbances causing a reduced rate of ATP synthesis. Globulins were also decreased after 11 days of fusarotoxicosis. This is in contrast to the usual findings that the globulin fraction increases with a decrease in albumins in hepatic lesions. A dangerous sequel to the suppression of globulin synthesis would be an increased susceptibility to bacterial and other infectious diseases. The determination of plasma protein spectrum may prove to be a valuable diagnostic and mechanistic aid in fusarotoxicosis.

90. *Alterations of SH and NH<sub>2</sub> Groups in Membranes in Relation to Their Interactions with Sulfated Polysaccharides (SPS).* R. JONES, E. P. PITZ, D. ROURKE, and I. ROSENBLUM, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The potential toxicity of SPS in the diet is related to their ability to become systemic and to interact subsequently with membranes. *In vitro* studies show that SPS interact with erythrocytes (RBC) via electrostatic surface adsorption (Pitz *et al.*, *Life Sci.* **17**, 969, 1975). Studies were conducted to determine the specific cell surface groups involved in SPS-RBC interactions by establishing a model system consisting of the incubation of chemically modified RBCs with degraded iota carrageenan (a SPS) of number average molecular weight ( $M_n$ ) 145,000 (ICG). Photomicroscopy and erythrocyte sedimentation rate were used as indicators of SPS-RBC interaction (i.e. ICG induced RBC rouleaux formation). Neither the NH<sub>2</sub> reagents, 4-acetamido, 4'-isothiocyano,2,2'-stilbene disulfonic acid or 2,4,6-trinitrobenzene sulfonic acid, nor the SH reagents, iodoacetic acid or *p*-chloromercuribenzenesulfonic acid, significantly inhibited the ICG induced rouleaux formations. Competitive binding studies between ICG and Triton X-100 indicated a low affinity of ICG for RBC ghost membrane proteins; therefore RBC membrane proteins are not considered to be of primary importance in SPS-RBC

interactions. The reagents glutaraldehyde, methyl mercury chloride, and various anions (salicylate<sup>-</sup> > I<sup>-</sup> > Cl<sup>-</sup>) were found to inhibit ICG-induced rouleaux formations. Monomeric phosphatidyl choline(PC) was observed to precipitate ICG, while microelectrophoretic studies indicate no increase in mobility of PC liposomes incubated in ICG. It is hypothesized that the SPS-cell surface adsorption phenomenon is dependent upon the polarizability of specific cell surface groups by SPS. (Supported by Research Grant 2-P01-ES00226-10 from the National Institute of Environmental Health Sciences, N.I.H., and by National Institute of Health Training Grant 2-T01-ES00103-10.)

91. *Increased Acid Phosphatase Activity in Hens following Long-Term Low-Level Oral Administration of Leptophos* M. B. ABOU-DONIA, Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina.

Leptophos [*O*-(4-bromo-2,5-dichlorophenyl)*O*-methyl phenylphosphonothioate] is a new organophosphorus insecticide that produces neurological deficits in hens similar to that caused by tri-*o*-cresyl phosphate (Abou-Donia and Preissig, *Toxicol. Appl. Pharmacol.* **35**, 269, 1976). This study reports the possible role for hydrolytic enzymes in the mechanism of organophosphorus ester-induced delayed neurotoxicity (OPIDN). Six groups of laying hens were given daily a single oral dose of 0.5, 1, 2.5, 5, 10, and 20 mg/kg in gelatin capsules for 72, 156, 128, 128, 128 and 36 days, respectively. All hens fed 1, 2.5, 5, 10, and 20 mg/kg developed ataxia after 62-68, 59-70, 48-59, 34-39, and 31 days. Hens given 0.5 mg/kg daily showed only weakness of the legs. The condition of hens given 20 mg/kg progressed to paralysis (33-40 days). The "latent period" and "total dose" before onset of ataxia depended on the daily dose. Spinal cord and sciatic nerves of ataxic hens exhibited axon and myelin degeneration. There was a dose-dependent inhibition of plasma cholinesterase; 20 mg/kg produced 58% depression of enzyme activity at time of sacrifice (130 days). Plasma acid phosphatase in all treatment groups significantly increased. The increase over the control ranged between 36 and 190% for hens receiving daily oral doses of 0.5 and 20 mg/kg of leptophos. The increased activity of acid phosphatase correlates with the clinical signs following the oral administration of the insecticide. It is suggested that the increase in plasma acid phosphatase activity accompanied by a decrease in plasma cholinesterase activity may be used as a biochemical index for an early diagnosis of OPIDN. (Supported in part by NIEHS Fellowship No. IF22ES01723 and EPA Contract No. 68-02-2452.)

92. *Pesticide Toxicity in Cultured Mammalian Cells*. D. P. DEVORE, T. H. HUTSON, M. A. SHERIDAN, and J. S. PITMAN, Battelle, Columbus Laboratories, Columbus, Ohio. (R. I. Freudenthal)

Suspension cultures of HeLa cells were used to expand previous methodologies (*J. Agr. Food Chem.* **21**, 362, 1973; *Pharmacologist*, **18**, 245, 1976) for the measurement of pesticide toxicity based on biochemical responses. Experiments were conducted to relate pesticide toxicity in *in vitro* cell systems to the inhibition of cellular biochemical processes. Cell toxicity was measured using the prescreen confluence assay. Pesticide effects on biochemical functions related to cell growth were examined by monitoring processes related to synthesis of cell macromolecules and by measuring cellular concentrations of cyclic AMP (cAMP). Aldrin, dieldrin, and parathion at maximal soluble concentrations of approximately 86, 10, and 13 13  $\mu\text{g/ml}$ , respectively, increased cellular concentrations of cAMP. Aldrin and parathion at maximum soluble concentrations completely inhibited cellular utilization of <sup>3</sup>H-labeled amino acids after 15 min. Paraoxon at a soluble concentration of 100  $\mu\text{g/ml}$  had little effect on either cellular uptake or macromolecular incorporation of <sup>3</sup>H-labeled amino acids. Prescreen confluency assays showed similar trends. Concentrations of aldrin and parathion yielding 50% confluency were approximately 45 and 10  $\mu\text{g/ml}$ , respectively. Paraoxon at concentrations as high as 100  $\mu\text{g/ml}$  had only slight effects on monolayer growth. Inhibitory effects of parathion and aldrin on [<sup>3</sup>H]thymidine utilization by synchronized HeLa cells were also shown. In preliminary studies to examine toxic mechanisms, aldrin effects on cellular ATP concentrations



were determined with ATP concentrations being grossly affected and decreased following aldrin incubation. These results relating pesticide inhibition of cell growth to inhibitory effects on basic cellular processes provide basic information required for the development of an accurate-predictive cell culture screen for pesticides. (Supported by USPHS, NIEHS Grant ES00865.)

93. *Similar Toxic and Cardiovascular Actions of the Pesticide Chlordimeform and Local Anesthetics.* G. K. W. YIM, V. NOLAN, and A. LUND, Departments of Pharmacology and Toxicology, and Entomology, Purdue University, West Lafayette, Indiana. (T. S. Miya)

Since chlordimeform (CDM) was shown to have local anesthetic activity ( $0.6 \times$  procaine) on isolated frog sciatic nerves (*Fed. Proc.* 35, 729, 1976), other actions of CDM were examined for possible similarities to those of local anesthetics. As with local anesthetics, diazepam and pentobarbital reduced the incidence of convulsions and death following CDM (100 mg/kg ip in mice), whereas phenytoin, tridione, and mephenesin were ineffective. In pentobarbital anesthetized dogs, CDM (3–30 mg/kg) reversibly lowered blood pressure by direct myocardial depression and peripheral vasodilation. This depressor response was unaffected by autonomic and histaminic blockade, and was followed by a centrally mediated pressor response that could be blocked by diazepam. At higher doses (50–75 mg/kg), CDM caused simultaneous respiratory arrest and irreversible hypotension that could not be prevented by artificial respiration. Comparable effects were observed following lidocaine (1–20 mg/kg). These results provide additional evidence that CDM toxicity in mammals is due largely to actions that are shared by local anesthetics. (Supported in part grant No. R-803965 from the EPA and a grant from the Purdue Research Foundation.)

94. *The Influence of Respiratory Stress on Blood Pressure Dose-Response to Isoproterenol in Rats.* MEI-CHWEN M. TSENG, DAVID P. HENRY, and DANIEL J. BROWN, Department of Toxicology and Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana.

Male Wistar rats, weighing 220–300 g, were anesthetized with urethane (900 mg/kg, ip) and bilaterally vagotomized, and a carotid artery was cannulated for blood pressure recording. Different volumes of isoproterenol ( $5 \times 10^{-11}$  M/ $\mu$ l/kg) were administered as a single bolus via microburet through the jugular vein cannulae. Groups of five rats were exposed to room air during control blood pressure dose-response determination. Following a 30-min stabilizing period of experimental gas (10% O<sub>2</sub> in N<sub>2</sub>, 1550 ppm CO in air, or 6.8% CO<sub>2</sub> in air), the blood pressure dose-response was redetermined. A shift in dose-response was expressed as the difference of the log doses calculated for half-maximal decrease in blood pressure (D50). Increases in D50 occurred after CO<sub>2</sub> and CO exposure. In previous experiments, increases in the dose for half-maximal increase in blood pressure of norepinephrine occurred after CO<sub>2</sub> (5.2, 5.9, and 6.8%), CO (1210 and 1550 ppm), and hypoxic hypoxia (10% O<sub>2</sub>) exposure. These results demonstrate a partial dissociation of the effect of respiratory stress upon the cardiovascular  $\alpha$ - and  $\beta$ -catecholaminergic responses.

95. *Toxicity of Free Fatty Acids upon Cultured Rat Heart Muscle and Endothelial Cells.* D. G. WENZEL and T. W. HALE, Department of Pharmacology and Toxicology, University of Kansas, Lawrence, Kansas.

Free fatty acids (FFA) have been implicated in the genesis of atherosclerosis, myocardial arrhythmias, and thrombosis. In this study, primary monolayer cultures of rat heart muscle and endothelial cells were used to evaluate the toxicities of major medium and long chain FFA. Indices of toxicity employed were viability and the latency of enzymes in lysosomes and mitochondria. Enzyme latency may represent impermeability of organelle membranes to a substrate, or the intraorganelle binding or sequestration of the enzyme. Mitochondrial latency was determined from changes in the rate of nitroblue tetrazolium conversion to formazan by

succinic dehydrogenase. Lysosomal latency was determined from changes in the rate of  $^{210}\text{Pb}$ -phosphate production in the Gomori lead method for acid phosphatase. Viability was measured by determining the release of  $^{51}\text{Cr}$  after injury. Concentrations of  $5 \times 10^{-4}$  to  $1 \times 10^{-8}$  M FFA (6:1 FFA/albumin ratio) were employed. FFA tested were: capric ( $\text{C}_{10:0}$ ), lauric ( $\text{C}_{12:0}$ ), myristic ( $\text{C}_{14:0}$ ), palmitic ( $\text{C}_{16:0}$ ), stearic ( $\text{C}_{18:0}$ ), oleic ( $\text{C}_{18:1}$ ), linoleic ( $\text{C}_{18:2}$ ), linolenic ( $\text{C}_{18:3}$ ), arachidic ( $\text{C}_{20:0}$ ), and arachidonic ( $\text{C}_{20:4}$ ). Four-hour treatments of heart muscle cells at  $5 \times 10^{-4}$  M FFA produced minimal cell death except for linolenic (22%) and arachidonic (82%) acids. After 24 hr, stearic, linolenic, and arachidonic acids produced, respectively, 79, 89, and 93% losses of viability. Medium chain length saturated FFA were essentially nontoxic. Endothelial cells were less susceptible to injury. Only arachidonic acid produced significant cell death (65%) after 24 hr. Relatively little effect was produced on the lysosomes. Mitochondria were markedly affected by some FFA. Stearic acid produced a 25% increase in mitochondrial lability at  $5 \times 10^{-5}$  M for 2 hr, whereas arachidonic acid increased the reaction rate by 71% after a 30-min treatment at  $1 \times 10^{-8}$  M. Results indicate a greater toxicity of polyunsaturated than saturated FFA. (Supported by the National Livestock and Meat Board, and USPHS GRS Grant 5-S01-RR05606-07.)

96. *Enzymatic and Physiological Responses of Rat Lungs to Acute Cadmium Chloride ( $\text{CdCl}_2$ ) Inhalation.* JAMES S. BUS, ALLEN VINEGAR, and STUART M. BROOKS, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio. (C. C. Smith)

The purpose of this study was to determine changes in pulmonary physiological measurements and enzymatic activities in lungs of rats after acute exposure to  $\text{CdCl}_2$  aerosol. Male Sprague-Dawley rats, 300–350 g, were exposed for 1 hr to a 0.5% aerosol of  $\text{CdCl}_2$  (10.7 mg  $\text{CdCl}_2/\text{m}^3$ , mass median particle size of 1.1  $\mu\text{m}$ ). At 1, 5, and 11 days after  $\text{CdCl}_2$  exposure, respectively, exposed rats compared to controls (percentage of control values) had lung to body weight ratios of 192, 174, and 140%; tidal volumes of 62, 63, and 89%; respiratory frequencies of 170, 145, and 108%; lung weight specific static deflation volumes at 30 cm of water of 30, 13, and 31%; lung glucose 6-phosphate dehydrogenase activity of 97, 153, and 115%; glutathione peroxidase activity of 45, 64, and 114%; and superoxide dismutase activity of 48, 61, and 105%. Lung cadmium concentrations were 3.2, 2.6, and 2.8  $\mu\text{g}/\text{g}$  wet weight on Days 1, 5, and 11, respectively. Light microscopic examination of lung tissue revealed initial pulmonary edema on Day 1 which progressed to interstitial pneumonitis on Day 5 with some recovery by Day 11 after exposure. Physiological, biochemical, and histopathological alterations were observed in rat lungs by 24 hr after  $\text{CdCl}_2$  exposure. Although the biochemical and some physiological parameters returned to control values after 11 days, static deflation volumes and histopathology remained altered at 11 days. The inhibition of glutathione peroxidase and superoxide dismutase activity by  $\text{CdCl}_2$  may indicate oxidant damage to lung tissue with cadmium exposure, since these enzymes are critical in preventing tissue oxidant injury. The return of glutathione peroxidase and superoxide dismutase activities to control values by 11 days after exposure, despite little decrease in tissue cadmium concentrations, may indicate a shift in the subcellular distribution of cadmium.

97. *Oxygen Toxicity: Comparison of Lung Biochemical Responses of Newborn and Adult Rats.* JOHN YAM, LEE FRANK, and ROBERT J. ROBERTS, Division of Pediatric Clinical Pharmacology, The Toxicology Center, Departments of Pediatrics and Pharmacology, The University of Iowa, Iowa City, Iowa.

Although newborn animals have long been known to be less susceptible to oxygen toxicity than adults, the basic mechanism to explain this phenomenon remains unresolved. Two potential protective mechanisms have recently been suggested in preventing oxidant injury to the lung: the glutathione system in reducing toxic lipid peroxides and the superoxide dismutase system in eliminating superoxide anion. Experiments were carried out to determine whether these lung defense systems respond differently in newborn and adult rats exposed to toxic concentrations of oxygen. Newborn rats (1 to 7 days old) and adult rats (250–350 g) were continuously exposed either to 96–98% oxygen or room air in stainless steel exposure chambers.

Sixty-five percent of the adult rats died within 3 days of oxygen exposure and all the oxygen-exposed adults at this time showed extensive pulmonary edema. Newborn rats, however, all survived up to 5 days of oxygen exposure without gross evidence of lung edema. Animals were sacrificed daily for biochemical analysis. Glutathione (GSH) and superoxide dismutase (SOD) concentrations were determined in lung homogenate, and glutathione peroxidase (GP), glutathione reductase (GR), and glucose 6-phosphate dehydrogenase (G6PDH) activities were determined in the 15,000g lung supernatant. Results were expressed on a per lung basis as percentage of control values. Seventy-two hours after oxygen exposure, newborn rat lungs showed elevations of GSH(185%), SOD(112%), GP(147%), GR(125%), and G6PDH(155%). Adult rats, however, failed to increase GSH, SOD, GR, and GP activities in the lung. G6PDH was elevated (190%) in oxygen-exposed adult rats. It is concluded from this study that the ability of newborn rats to rapidly increase their lung biochemical protective capabilities may contribute to their resistance to oxygen toxicity compared to the adults. (Supported in part by Research Grant NIGMS 12675.)

98. *The Effect of Two Hallucinogens on in Vivo Concentrations of Cyclic AMP in Rat Brain.* M. RAPPOPORT and H. CORNISH, Interdepartmental Toxicology Program and Department of Environmental and Industrial Health, The University of Michigan, Ann Arbor, Michigan.

The hypothesis that cyclic AMP (cAMP) may play an important role in the function of the central nervous system and may act as a mediator of psychotropic drug effects is based upon a large body of evidence. It has been reported that hallucinogens including *d*-LSD and dimethyltryptamine (DMT) cause significant increases in concentrations of cAMP in rat brain stem slices and *in vivo* as measured following decapitation and rapid dissection and freezing of cerebrum, brain stem, and cerebellum. The presently accepted method of sacrifice of rats prior to cyclic nucleotide assay, i.e., microwave irradiation (1.2 kW) focused on the head of the restrained animal, was used in the present studies. Cyclic AMP was measured either by a modified Gilman assay or by radioimmunoassay. Both methods gave comparable values for cAMP. Following ip administration of 3,4-methylenedioxyamphetamine (MDA) and DMT (0.1 mmol/kg), no significant differences in concentrations of cAMP were found between vehicle control and hallucinogen-injected animals when killed at 2, 5, or 15 min postinjection. After pretreatment with several potent inhibitors of cyclic nucleotide phosphodiesterase there were still no differences in cAMP concentrations between control and hallucinogen-treated rats. Representative cAMP concentrations in Sprague-Dawley rats were as follows (pmol/mg of protein): cerebellum, 21.8; brain stem, 16.5; cortex, 13.7; whole forebrain, 10.5. These basal values are relatively high when compared to brain cAMP concentrations reported in rats killed in a 5-kW microwave oven. Thus, there appears to be an inverse relationship between the rate of enzyme inactivation and post mortem concentrations of cAMP. (Supported by USPHS Grant ES00138.)

99. *Use of Videotape to Monitor Clinical Expression of Neurotoxicity in Beagle Dogs.* D. R. SAUNDERS, G. R. STEFFEN, M. T. CASE, and R. A. NELSON, Riker Laboratories, Inc., 3M Company, 3M Center, St. Paul, Minnesota.

In a chronic toxicity study in beagle dogs, videotape segments of the behavior of individual dogs in an open runway were used to define and document both the development of clinical signs of neurotoxicity and recovery from the neurotoxicity after cessation of dosing. While early clinical signs (subtle gait changes) could be discerned from daily observation, signs of neurotoxicity were more easily detected and defined by comparing videotape segments of the dogs' behavior made at 3- to 5-week intervals. Clinical signs of the neurotoxicity began with intermittent favoring of one hind leg and repositioning of the feet and progressed to delayed placing of hind feet, increasing stiffness of gait, knuckling of hind digits and to incoordination of hind limbs, with eventual inability to support the hind quarters. After paresis developed, half of the dogs were killed to confirm the presence of a spinal lesion, while dosing of the other half ceased and they were allowed to recover. The recovery period continued until their gait

matched that on videotape segments made before signs developed. Complete clinical recovery occurred and the behavior of the treated dogs in final videotape segments was indistinguishable from that of the control dogs.

100. *Histopathology of Low Chlorinated Biphenyls and Hexachlorobenzene in Female Rhesus Monkeys.* M. J. IATROPOULOS, R. FELT, F. COULSTON, and F. KORTE, International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico, and Institut fuer Oekologische Chemie, Gesellschaft fuer Strahlen und Umweltforschung, mbH, Muenchen, Germany.

The toxicology of Clophen A-30, a mixture of low chlorinated biphenyls used in Germany, has been investigated. The morphological effects of Clophen A-30, 2,5,4'-trichlorobiphenyl (TCB), and hexachlorobenzene (HCB) were compared in adult female rhesus monkeys. Clophen was given daily by stomach tube for 28 days in amounts of 4, 16, and 64 mg/kg. Radiolabeled TCB was given for 84 days in the diet at a dose of 0.25 mg/kg; half of these animals were sacrificed at 85 days and half were allowed to recover for 28 days prior to sacrifice. HCB was administered daily by gavage for 60 days in amounts of 8, 32, 64, and 128 mg/kg. Complete histopathological examination revealed dose-dependent degenerative changes in the liver, kidney, and central nervous system (CNS) of the animals receiving Clophen A-30. The CNS changes included a decrease in the amount of stainable myelin in the fiber tracts. TCB-reversible changes were observed involving the endothelial cells of small vessels of most organs, with secondary hypoxia and degenerative changes in the CNS, liver, and kidney. Finally, examination of HCB animals showed thymic cortical atrophy, degenerative ovarian changes, and degenerative changes in the liver and kidneys.

101. *Lactation-Transfer Changes Caused by Clophen A-30 and Hexachlorobenzene in Male and Female Infant Rhesus Monkeys.* M. J. IATROPOULOS, W. HOBSON, F. COULSTON, and F. KORTE, International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico, and Institut fuer Oekologische Chemie, Gesellschaft fuer Strahlen und Umweltforschung, mbH, Muenchen, Germany.

The effects of Clophen A-30 (mixture of low chlorinated biphenyls) and hexachlorobenzene (HCB) were compared in infant monkeys through administration to their lactating mothers. Clophen was given by gavage daily during lactation in the amount of 16 mg/kg. Two received Clophen for 22 days and their infants were sacrificed at 66 and 147 days postpartum. A third mother received Clophen for 28 days and her infant was sacrificed at 110 days postpartum. HCB (64 mg/kg) was given daily by stomach tube for 22, 38, and 60 days. The infants were sacrificed at 37, 122, and 81 days postpartum, respectively. The variation in the time of sacrifice of the infants was dictated by the condition of their mothers. The histopathological examination of the Clophen infants revealed degenerative changes of slight to moderate degree of severity in the liver, kidney, and central nervous system. The severity of changes increased with age. HCB infants showed only mild degenerative changes in the liver and kidney. These changes did not show any correlation to the age of the infant.

102. *The Distribution and Elimination of [2,5,2',5'-<sup>14</sup>C]Tetrachlorobiphenyl in Rainbow Trout (Salmo Gairdneri).* P. D. GUINEY, R. E. PETERSON, M. J. MELANCON, JR., and J. J. LECH, School of Pharmacy, University of Wisconsin, Madison, Wisconsin, and Department of Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin.

Polychlorinated biphenyls (PCBs) have been found to be persistent environmental pollutants. These compounds are taken up readily from water and the diet by a number of fish species and are released much more slowly, especially by salmonids. To examine this phenomenon in detail we studied the tissue distribution and washout of [2,5,2',5'-<sup>14</sup>C]tetrachlorobiphenyl (TCB) following exposure of 125-g rainbow trout to an initial level of 0.5 ppm aqueous [<sup>14</sup>C]TCB for 36 hr. No effort was made to identify the <sup>14</sup>C compounds in the fish but in previous studies with

rainbow trout metabolism, they were found to be minimal. During the exposure, [ $^{14}\text{C}$ ]TCB was accumulated to a whole body concentration of 4.2 ppm. Five fish were sacrificed immediately following exposure and at 1, 4, 7, 14, 28, and 56 days after transfer to a raceway. Initially, carcass, muscle, skin, lower GI tract, and fat contained most of the [ $^{14}\text{C}$ ]TCB, a total of 88% of the radioactivity being located in these tissues. Adipose tissue, carcass, and eyes acted as translocation depots. The bile and blood initially contained relatively high concentrations of [ $^{14}\text{C}$ ]TCB, but elimination from these sites was rapid and nearly complete. During the first 2 weeks following exposure there was some redistribution of [ $^{14}\text{C}$ ]TCB within the fish and a loss of approximately 25–30% of accumulated total body [ $^{14}\text{C}$ ]TCB. The most striking feature of the early redistribution of TCB was an increase in concentrations of [ $^{14}\text{C}$ ]TCB in adipose tissue. Following this period of rapid loss of [ $^{14}\text{C}$ ]TCB there was a much slower rate of release, accounting for only 1% body burden over the next 6 weeks. Over the duration of this study the body weight of the fish increased by about 50% and calculations of [ $^{14}\text{C}$ ]TCB elimination based on tissue concentrations gave artificially high values for loss because of dilution by growth. When corrected for growth, the half-life for [ $^{14}\text{C}$ ]TCB on a whole body basis, calculated from the slope of the second phase of elimination was approximately 2.66 years. (Supported by NIH Grant ES01080 and EPA Grant R803971010, and by the University of Wisconsin Sea Grant Program.)

103. *2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD)-Induced Depression in Biliary Excretion of Polychlorinated Biphenyls (PCBs) in Rats.* K. H. YANG, P. D. GUINEY, J. L. SEYMOUR, and R. E. PETERSON, School of Pharmacy and Regional Primate Research Center, The University of Wisconsin, Madison, Wisconsin.

Chlorinated dibenzo-*p*-dioxins occur as contaminants in certain commercial chlorophenols. TCDD, the most toxic member of this class of compounds was given as a single oral dose, 10 or 25  $\mu\text{g}/\text{kg}$ , to male rats (230–340 g); control animals received an equal volume of acetone/corn oil (0.5/9.5, v/v). Ten days post-treatment the rats were anesthetized, the common bile duct, femoral artery, and vein were cannulated, and 6 mg/kg of [2,5,2',5'- $^3\text{H}$ ]tetrachlorobiphenyl (4-CB) or [2,4,5,2',4',5'- $^3\text{H}$ ]hexachlorobiphenyl (6-CB) administered iv. Plasma disappearance of  $^3\text{H}$  was similar in control and TCDD-treated rats for both PCBs. Total hepatic content of 4-CB- and 6-CB-derived  $^3\text{H}$  was significantly greater in TCDD than control animals throughout the 4-hr duration of the experiment. Hepatic concentration of 4-CB and 6-CB while similar in all groups 15 min and 1 hr after administration, tended to be higher in the TCDD-treated groups after 2, 3, and 4 hr. In addition to higher hepatic content and concentration of 4-CB and 6-CB at 4 hr, both doses of TCDD produced an increase in liver weight, a decrease in bile flow, and a decrease in biliary excretion of 4-CB and 6-CB. Percentage recovery in bile in 4 hr for control and 10- $\mu\text{g}/\text{kg}$  and 25- $\mu\text{g}/\text{kg}$  TCDD treatment groups, respectively, administered 4-CB was:  $11.0 \pm 0.9$ ,  $5.9 \pm 0.7$ , and  $3.7 \pm 0.5\%$ ; and for 6-CB:  $1.2 \pm 0.2$ ,  $0.8 \pm 0.2$ , and  $0.1 \pm 0.03\%$  (mean  $\pm$  SE). Content of 4-CB- and 6-CB-derived MH in perirenal fat, foreleg skeletal muscle and urine 4 hr after administration tended to be similar in TCDD-treated and control rats, but skin concentration of both PCBs was less in the TCDD treatment groups. The mechanism by which TCDD increases hepatic content and concentration of 4-CB and 6-CB while at the same time decreasing their excretion into bile remains to be determined. (Supported by NIH Grant ES 01332.)

104. *Comparison of the Fate of Vinyl Chloride following Single and Repeated Exposure in Rats.* P. G. WATANABE, J. A. ZEMPEL, and P. J. GEHRING, Toxicology Research Laboratory, The Dow Chemical Company, Midland, Michigan.

Rats were exposed to 5000 ppm nonlabeled vinyl chloride (VC) 6 hr/day, 5 days/week for 7 weeks. On the last day of exposure  $^{14}\text{C}$ -labeled VC was used and the fate of the [ $^{14}\text{C}$ ]VC was followed for 72 hr and compared with the fate in rats subjected to a single 6-hr exposure to 5000 ppm [ $^{14}\text{C}$ ]VC. The routes and rates of excretion of  $^{14}\text{C}$  activity were the same for the

two experimental groups. The activity of microsomal enzymes, as reflected by aniline hydroxylase and *p*-nitroanisole *O*-demethylase of 9000g liver supernatants were essentially the same in rats exposed once or repeatedly or in nonexposed control rats. Covalent binding to hepatic macromolecules was greater in rats repeatedly exposed when compared to those subjected to a single exposure. The hepatic nonprotein sulfhydryl concentration of the repeated and single exposed groups immediately following exposure was 79 and 39% of control, respectively. These results indicate that repeated exposure to VC does not induce its biotransformation. However, the increase in hepatic macromolecular binding indicates that repeated exposure augments the reaction of electrophilic metabolites with macromolecules, and this may be expected to enhance potential toxicity including carcinogenicity.

105. *Consequences of Vinyl Bromide Debromination in the Rat.* E. W. VANSTEE, J. M. PATEL, B. N. GUPTA, and R. T. DREW, NIEHS, Research Triangle Park, North Carolina.

Male rats (130–150 g) were exposed to 2% vinyl bromide (VB), 5 hr/day, once, twice, or for 5 or 10 consecutive days. They were either treated or not treated with 1.0 g/liter of sodium phenobarbital or 0.19–0.75 g/liter of KBr in the drinking water. Intermittent daily exposure of rats to 2% VB caused weight loss during the first few days of exposure. The weight loss was increased by the inclusion of phenobarbital in the drinking water. Results of paired feeding studies suggested that most of the weight loss could be attributed to decreased food intake. The reason for the decreased food intake was determined to be the consequence of a combination of anesthetization by the VB plus an additive increment of CNS depression associated with an elevated serum bromide concentration. The purely bromide components were quantitatively similar whether the bromide was derived from the debromination of VB or from inorganic bromide included in the drinking water. The phenobarbital effect was attributed to induced acceleration of VB debromination with the subsequent liberation of increased amounts of bromide. Toxic injury to the liver observed during the first 2 days of exposure resolved by Day 5 of exposure. An additional weight loss in the VB plus phenobarbital group, not attributable to reduced food intake, corresponded to the period during which hepatotoxicity was histopathologically definable. Recovery of weight gains paralleled recovery from hepatotoxic injury.

106. *Effect of Two Halogenated Hydrocarbons on Hepatic Microsomal Enzyme Activity.* J. P. MURPHY, E. W. VANSTEE, and K. C. BACK, 6570 Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

The halogenated alkanes bromochlorodifluoromethane (F-1211) and bromochloromethane (F-1011) are of interest to the United States Air Force as fire control agents. Preliminary studies indicated that short duration exposure of one of these agents might result in hepatotoxicity. This study was designed to verify whether or not there was hepatotoxicity, and if so, to what extent the hepatotoxicity might be related to biotransformation of the compounds. Inhalation of F-1011 at a concentration of 0.25%, 5 hr daily for 3 days, caused hexobarbital sleeping time to increase by 140–250% of controls in mice by Day 4. The decreased rate of metabolism of hexobarbital and ethylmorphine by the 9000g microsomal supernatant fraction prepared from mice exposed to F-1011 (0.25%, 5 hr daily for 3 days) was accompanied by an increase in serum bromide concentrations. Treatment of mice with 2,4-dichloro-6-phenyl-phenoxyethyl-diethylamine (SKF 525-A) (25 mg/kg) during the F-1011 exposure period increased metabolism of hexobarbital and ethylmorphine with no significant change in the elevated serum bromide concentrations. SKF 525-A reduced hexobarbital sleep time for both control and F-1011-exposed mice. Mice treated with sodium phenobarbital (80 mg/kg) during a similar exposure period also showed an increase in the metabolism of hexobarbital and ethylmorphine (decreased sleep time for both control and F-1011-exposed mice) with no significant change in the elevated serum bromide concentrations. Histopathological changes were not evident in either exposed or control groups. Conclusions were that F-1011 was easily debrominated while F-1211 was relatively resistant to debromination. Neither the parent compound nor the products of biotransformation caused hepatotoxicity after 3 days of intermittent exposure of up to 0.4%.

107. *Subacute Effects of Brominated Benzenes on Xenobiotic Metabolism.* G. P. CARLSON and R. G. TARDIFF, Department of Pharmacology and Toxicology, Purdue University, West Lafayette, Indiana, and U.S. Environmental Protection Agency, Cincinnati, Ohio.

Previous studies indicated that several brominated benzenes, particularly 1,4-dibromobenzene and 1,2,4-tribromobenzene, when administered for 14 days caused increases in xenobiotic metabolism, and longer studies with chlorinated benzenes demonstrated that after 90 days of administration, activity remained elevated for at least 30 days. In view of this, male rats were administered 5, 10, or 20 mg/kg/day of 1,4-dibromobenzene or 2.5, 5, or 10 mg/kg/day of 1,2,4-tribromobenzene po for 45 and 90 days. Other groups were similarly treated for 90 days, but a 30-day recovery period was interposed before measurements were made. 1,4-Dibromobenzene at dose levels of 10 and 20 mg/kg/day and 1,2,4-tribromobenzene at dose levels from 2.5 to 10 mg/kg/day caused increases in the liver to body weight ratio, EPN detoxification, azoreductase, benzpyrene hydroxylase, cytochrome P-450, and cytochrome c reductase. However, when measurements were made following the 30-day recovery period, almost all the parameters returned to control values even at the highest dose levels. Compared to the chlorinated benzenes, when administered for 90 days, the brominated benzenes are more potent inducers of xenobiotic metabolism but their effects are not as prolonged. In an additional study, 1,2,4-trichlorobenzene (20 mg/kg/day) or 1,2,4-tribromobenzene (10 mg/kg/day) was administered for 14 days with or without sodium phenobarbital (50 mg/kg/day for 2-4 days); no increases were observed beyond those of phenobarbital alone, indicating lack of additivity in their ability to alter xenobiotic metabolism. (Supported by EPA Grant R804328.)

108. *Pesticide-Induced impairment of hepatobiliary function.* H. M. MEHENDALE, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, Mississippi. (A. W. Hayes)

The effect of preexposure to hexachlorobenzene (HCB), DDT (both at 100 ppm, in diet, 8 days), and toxaphene (TX) (20 mg/kg, po, 5 days) on the biliary excretory function was investigated using isolated perfused liver (IPL) preparations obtained from treated and control rats. Preexposure to HCB caused an increase (28%) in the biliary excretion of endogenously formed metabolites of imipramine (IMP) while DDT and TX pretreatment caused no significant effect. However, biliary excretion of otherwise readily excretable, exogenously provided, polar metabolites of IMP was suppressed after preexposure to all three pesticides. These results are consistent with the hypothesis that liver maintains two pools of metabolites, viz., one representing a synthetic phase and the other representing postsynthetic phase of metabolite synthesis. Moreover, modifying agents affect biliary excretion of metabolites from the two pools via discrete mechanisms. Biliary excretion of an acid nonmetabolizable transport model compound, chlorothiazide, was unaffected by HCB, while biliary excretion of a neutral transport model compound, ouabain, was enhanced after preexposure to HCB. These results suggest that although impairment of hepatobiliary function may be apparent as a generalized effect, use of model transport substrates would be useful in dissecting discretely distinguishable manifestations exerted by toxic agents. (Supported by University of Mississippi Medical Center Account No. 26007.)

109. *Bioavailability in Rats of Bound and Conjugated Plant Residues of Carbamate Insecticides.* T. C. MARSHALL and H. W. DOROUGH, Department of Entomology and Graduate Program in Toxicology, University of Kentucky, Lexington, Kentucky.

Bound and conjugated forms of pesticides and/or their metabolites may occur as major terminal residues in plants destined for human consumption. The potential bioavailability of such residues in mammals was investigated by administering bound or conjugated plant metabolites of four <sup>14</sup>C-labeled methylcarbamate insecticides (carbaryl, carbofuran, aldicarb, and Croneton; the latter is an experimental material, 2-ethylthiomethylphenyl methylcarbamate) orally to female Sprague-Dawley rats. Bound <sup>14</sup>C residues (those remaining in the plant matrix after thorough solvent extraction) from carbaryl- and carbofuran-treated bean plants were voided by the rats predominately via the feces (99 and 79% of dose), with 1 and 8% detected in the urine. Bile duct cannulation experiments showed that a maximum of 1.5% of the

doses was present in the bile. Similar results were obtained using bound residues of Croneton formed by sorghum plants. Quantities of bound residues of aldicarb were insufficient for testing. Conjugated, or water-soluble, residues of each insecticide were rapidly eliminated in the urine; generally, greater than 90% of a dose was voided in the 0- to 24-hr urine. These data demonstrate that bound residues of the test compounds were very poorly absorbed from the gastrointestinal tract of rats, while the conjugated ones were readily absorbed. Thus, the latter appear to be of greater significance as residues in plant or animal products consumed by man.

110. *In Vivo and in Vitro Effects of di-n-Alkyl 2,2-Dichlorovinyl Phosphoric Acid Esters on Hen Brain Neurotoxic Esterase*. J. R. ALBERT, S. M. STEARNS, and H. J. MERSMANN, Shell Development Company, Biological Sciences Research Center, Modesto, California.

The delayed neurotoxic effects of a series of bis and mixed *n*-alkyl 2,2-dichlorovinyl phosphoric acid esters in the hen were reported previously (*Tox. Appl. Pharmacol.* **29**, 136, 1974). A number of these esters have been assayed for their *in vivo* and *in vitro* effects on hen brain neurotoxic esterase. After single oral dosage of high (lethal or neurotoxic) and low doses to groups of five hens, the degree of inhibition of brain neurotoxic esterase was determined at 3 and 24 hr and at 22 days. Essentially the technique of Johnson (*Crit. Rev. Tox.*, 1975) was used for the *in vitro* studies: a pooled hen brain homogenate with phenyl phenylacetate as a substrate and a 10-min incubation period at 25°C. *In vivo*, at neurotoxic doses, TOTP, mipafox, and the bis C<sub>4</sub> ester homolog of dichlorvos resulted in 88, 86, and 92% inhibition. The non-neurotoxic compounds, dichlorvos and paraoxon, at lethal doses (atropine pretreatment) caused 36 and 42% inhibition, respectively. *In vitro* the IC<sub>50</sub> decreased with increasing chain length (dichlorvos IC<sub>50</sub>, 104 μM; C<sub>4</sub> analog IC<sub>50</sub>, 0.08 μM). Longer alkyl chains were less potent. The inhibitory potency of mixed methyl, *n*-alkyl esters of varying chain length paralleled the effects of the bis series. The most potent ester *in vitro* was the bis chloroethyl ester with an IC<sub>50</sub> of 0.03 μM. Overall, the IC<sub>50</sub> relationships parallel the grossly observable delayed neurotoxic ED<sub>50</sub> values determined previously and in general support Johnson's hypothesis that when the degree of inhibition of the enzyme exceeds 80% *in vivo*, it is indicative of a neurotoxic effect.

111. *Reproductive Dysfunction in Nonhuman Primates Exposed to Dioxins*. J. R. ALLEN, D. A. BARSOTTI, and J. P. VAN MILLER, Department of Pathology and Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), one of the most lethal environmental contaminants known, produces intoxication in adult female rhesus monkeys within 6 months. They showed a loss of hair about the head and shoulders, dermatitis, swelling of the eyelids, loss of eyelashes, and irregularities in the menstrual cycles when TCDD was added to their diets at a level of 500 parts per trillion. Radioimmunoassays of the serum estrogen and progesterone revealed a decrease in, and prolongation of, the progesterone peak during the luteal phase of the cycle. Repeated breedings of the 8 females following 6 months of TCDD exposure (mean total intake, 11.7 μg) have resulted in two pregnancies, one of which was aborted on Day 46. After 9 months on the TCDD diet, all of the animals experienced an anemia and leukopenia, and the dermatitis and loss of hair were more extensive. Although the food intake of the animals did not decrease, the majority of them experienced a gradual weight loss. One of the eight animals died, after 8 months on the experimental diet, from a severe nonresponsive aplastic anemia. These data indicate that continuous exposure of nonhuman primates to low levels of TCDD is capable of producing widespread deleterious effects, particularly on the reproductive and hematopoietic systems. (Supported in part by USPHS Grants ES-00472, GM-00130, and RR-00167 from NIH and the University of Wisconsin Sea Grant Program.)

112. *Mortality in 1973 and 1974 from Pesticides*. W. J. HAYES, JR., Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee.

Mortality in the United States associated with pesticides was studied for the years 1973 and 1974 to learn whether there had been any important change as compared to years in which



earlier studies were made. The work, which depended on cooperation of the National Center for Health Statistics and of all state departments of health, involved review of all death certificates in seven main categories in which deaths associated with pesticides sometimes are reported. There were 1591 such certificates in 1973 and 1561 in 1974. For each death in which a pesticide was even suspected, a letter was written to the signer of the certificate, to the hospital where death occurred, or to both. Most accidental deaths from pesticides were classified properly, but some still were reported in categories other than the one (E865) specifically established for this class of poisons. As in the past, most poisonings in 1973 and 1974 were nonoccupational and involved gross carelessness. However, the number of accidental deaths associated with pesticides was greatly reduced. In 1961 and earlier, there were over 100 such deaths per year in the United States, but the number reported was 56 in 1969, 32 in 1973, and 35 in 1974.

113. *Effects of Pesticides on Steroid Hormone Binding in Cytoplasm of Rodent Tissues.* LONNIE SCHEIN, J. A. THOMAS, and M. P. DONOVAN, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.

Specific androgen and estrogen binding proteins have been prepared from several steroid target and nontarget organs of castrate mice. Using a dextran-coated charcoal separation procedure to distinguish bound steroid from unbound steroid provided a screening procedure to assess the potential of certain pesticides (viz., methoxychlor, heptachlor, DDT, dieldrin, diaznon, parathion, carbaryl, or carbofuran) to compete with male or female sex hormone for the steroidophilic molecules. Parathion ( $10^{-5}$  M) was capable of interfering with the binding of [ $^3$ H]dihydrotestosterone ([ $^3$ H]DHT) to specific cytosol receptors in the accessory sex organs, the kidney, and the liver. Similar *in vitro* concentrations of methoxychlor, heptachlor, DDT, dieldrin, diaznon, carbaryl, or carbofuran were unable to interfere with the binding of [ $^3$ H]DHT to its cytosol receptor. There was little tendency for any of the pesticides studied to interfere with the binding of [ $^3$ H]estradiol ([ $^3$ H]E<sub>2</sub>) to its specific cytosol receptor. However, in the liver most pesticides caused marked elevations in the binding of [ $^3$ H]E<sub>2</sub> to estrophilic molecules. These preliminary studies would seem to indicate that organophosphates (viz., parathion) can affect androphil-[ $^3$ H]DHT interactions, and that estrophil-[ $^3$ H]E<sub>2</sub> interactions can be increased by the several chemical classes of pesticides. (Supported in part by EPA Grant R803578.)

114. *Pathologic Changes in Mice Induced by Diethylstilbestrol and 17 $\beta$ -Estradiol.* B. HIGHMAN, M. J. NORVELL, and T. E. SHELLENBERGER, University of Arkansas College of Medicine, Little Rock, Arkansas, and the National Center for Toxicological Research, Jefferson, Arkansas.

To study the long-term effects of estrogen administration, virgin female C3H/HeJ mice are being fed graded levels (10, 100, 500 ppb) of diethylstilbestrol (DES) and 17 $\beta$ -estradiol (100, 1000, 5000 ppb) continuously from 6 to 104 weeks of age. A comparable population of C3HeB/FeJ mice are being fed DES at 10, 100, and 500 ppb. Pathological studies were made on over 700 mice that were sacrificed or died during the first 15 months. After prolonged high dosage, the cervix of both populations often showed stromal mucoid changes and adenosis associated with foci of low stratified or columnar epithelium lining the cervical canal. Numerous secretory glands of the endometrial type, frequently showing epidermidization, were often seen, particularly in the upper cervix. The uterine horns showed marked hyperplasia of the glands, which often penetrated the muscularis. Minute secretory glandular inclusions or sprouts were noted focally in the tall epithelium lining the endometrial surface and glands. The ovaries showed atrophy with absence of corpora lutea. Ceroid deposits were increased in the ovaries and adrenal. The sternum showed an increase in bony trabeculae. Hyperplastic alveolar nodules and mammary tumors, mainly type B (Dunn's classification), were frequent in the C3H/HeJ but rare in the C3HeB/FeJ population. To date, a total of 12 adenocarcinomas involving both populations have been observed, variably involving the uterine horns, corpus and cervix. Some appeared to arise from endometrial glands and some from areas of cervical adenosis.

Some additional mice showed marked glandular atypia suggesting premalignant uterine lesions. In mice on a lower dosage, the findings were similar, but less frequent and severe. These findings indicate that mammary tumor virus (MTV) is required for DES or estradiol-induced mammary tumorigenesis in C3H mice, but that other DES and estradiol-induced carcinogenic and precarcinogenic lesions of the reproductive tract are independent of the MTV.

115. *Mammary Tumorigenesis in Female C3H Mice Fed Diets Containing Diethylstilbestrol or 17 $\beta$ -Estradiol.* M. J. NORVELL and T. E. SHELLENBERGER, Molecular Biology Division, National Center for Toxicological Research, Jefferson, Arkansas.

The present study was undertaken to examine further the effects of mouse mammary tumor virus factor, diethylstilbestrol (DES), and 17 $\beta$ -estradiol in estrogen-induced mammary tumorigenesis in female CeH mice obtained from Jackson Laboratories. Beginning at 6 weeks of age, high (C3H/HeJ) or low (C3HeB/FeJ) virus expressor female mice were fed diets containing 0, 10, 100, or 500 ppm added DES and C3H/HeJ female mice were fed levels of 100, 1000, or 5000 pp bestradiol. Each group consisted of 288 animals. At 12 months, the cumulative total number of tumor-bearing C3H/HeJ mice fed dietary levels of DES were 8 at 0 ppb DES (control), 12 at 10 ppb, 22 at 100 ppb, and 37 at 500 ppb. The number of tumor-bearing C3H/HeJ mice fed dietary levels of estradiol were 7 at 100 ppb, 17 at 1000 ppb, and 20 at 5000 ppb. At 18 months, dose-related mammary tumorigenesis in DES-fed mice persisted but was less pronounced in the estradiol-fed animals. In the C3HeB/FeJ animals, feeding dietary levels of DES at a level as high as 500 ppb for as long as 18 months did not increase the mammary tumor incidence over that observed in the controls. Results reaffirm the roles of DES and the mouse mammary tumor virus factor in tumorigenesis in female C3H mice and demonstrate that a naturally occurring chemical, 17 $\beta$ -estradiol, also stimulates mammary tumorigenesis in this animal model system.

116. *Functional and Structural Alterations in the Testes of Mice Exposed in Utero to Diethylstilbestrol.* J. A. McLACHLAN and R. R. NEWBOLD, Environmental Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (R. L. Dixon)

Preliminary studies from our laboratory have shown that prenatal exposure to the synthetic estrogen, diethylstilbestrol (DES), reduces the fertility of male mice (McLACHLAN *et al.*, *Science* **190**, 991, 1975). Recently, similar reproductive tract abnormalities have been reported in young men whose mothers had been treated with DES (Gill *et al.*, *J. Reprod. Med.* **16**, 147, 1976). In the present study, groups of 15 pregnant CD-1 mice were treated subcutaneously with DES (100  $\mu$ g/kg) from Days 10 to 16 of gestation. When the male offspring were 3 months old, their reproductive capacities were evaluated. All the control males were fertile and had descended testes; abnormal sperm in the vas deferens ranged from 1.5 to 13.9%. Conversely, when 17 mice exposed prenatally to DES were evaluated, all were sterile; 10 of these mice had cryptorchid testes with sperm in the vas absent or abnormal. The seven males with descended testes also had a higher incidence of abnormal sperm (range, 2.6 to 40%) than the corresponding controls. Histological examination of the testes in these mice suggests that prenatal exposure to DES may alter germ cell maturation and subsequent reproductive function.

117. *Renal Tubular Regeneration following Experimentally Induced Hyperuricemic Nephropathy in the Rat.* B. STAVRIC and E. A. NERA, Toxicology Research Division, Bureau of Chemical Safety, Health Protection Branch, Health & Welfare Canada, Ottawa, Canada. (I. C. Munro)

Hyperuricemic nephropathy (HN) or gouty kidney is a well-recognized complication of hyperuricemia and gout. Histopathologic features of gouty nephropathy have been obtained mainly on the investigation of human subjects with well-advanced stages of the disease. Very few experimental data have been reported on the initiation, progression, and regression of the HN and even less about the regeneration of the kidney tubules damaged by HN. Employing

the hyperuricemic rat (Johnson *et al.*, *Proc. Soc. Exp. Biol. Med.* **131**, 8, 1969; Stavric *et al.*, *Proc. Soc. Exp. Biol. Med.* **130**, 512, 1969) it became possible to study the pathogenesis of uric acid (UA) induced nephrotoxicity, and the regeneration of the kidney tissue impaired by HN. The HN was induced by dietary supplement and ip injection of both UA and potassium oxonate (a uricase inhibitor). After 2 days of treatment, epithelial cell degeneration with resultant blockage of the tubular lumen from desquamated cellular debris was observed. Each additional day of treatment produced more severe and extensive lesions. After 9 days of treatment, hyperuricemia and HN were so acute that half of the animals had died (average serum UA, 17 mg/100 ml vs 2 mg/100 ml in controls). At this point all treatment was terminated, and the surviving rats were used to investigate the possibility of spontaneous regeneration of the nephrotic kidney, after normalizing serum urates. Seventeen days after termination of treatment, complete regeneration of the epithelial cells of the tubules was observed.

118. *The Influence of Fasting on the Diuretic Response of Rats to Furosemide.* SAROJ CHAKRABARTI and JULES BRODEUR, Département de Pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Québec, Canada.

Previous studies in this laboratory (Laliberté *et al.*, *J. Pharmacol. Exp. Therap.* **196**, 194, 1976; *ibid.*, in press) have shown that fasting enhances the anticoagulant response to warfarin and provided evidence that this might be due to an interference of endogenous free fatty acids (FFA) with binding of warfarin to plasma proteins. The present investigation was therefore undertaken to assess the influence of fasting on the response of adult male Sprague-Dawley rats to furosemide (F), a diuretic drug strongly bound to plasma proteins. Fasting consisted in withholding solid food, but not water, for a period of 16 hr before administering F (10 mg/kg, sc) or a saline vehicle. Normally fed animals also received F or the vehicle. Fasting did not modify the diuretic or natriuretic effect (per 100 g) of F. In isotonic saline-loaded rats (5%, po), fasting moderately reduced the diuretic and natriuretic effects of F, but mild antidiuretic and antinatriuretic effects were also observed in fasted rats given only the vehicle. The distribution of total F in plasma or tissues was not affected by fasting. On the other hand, the concentration of unbound F was higher in the plasma, but not in the kidney nor in the liver of fasted rats. Fasting had no effect on the urinary excretion of unchanged F, although it increased the latter in animals given a saline load. The *in vitro* binding constant of F to undiluted plasma proteins was decreased from the control value by a factor of 5 as a result of fasting. Finally, incubation of kidney cortex tissue slices with F both in the presence and absence of FFA indicated an inhibition of the uptake of the diuretic in a manner that closely paralleled inhibition by probenecid. These results suggest that the failure to observe a more pronounced diuretic and natriuretic effect after F in fasted rats, in spite of higher concentrations of unbound F in the plasma, might be due to the inhibitory effect of endogenous FFA and/or other endogenous substances on the uptake of F by renal tubular cells. In conclusion, the present results indicate that *in vivo* displacement of a drug from its plasma binding sites by endogenous substances does not necessarily always lead to an increased pharmacological or toxicological response, because the disposition of the free drug from plasma into the target organ could be affected adversely by certain other physiopathological processes. (Supported by an MRC Group Grant in Drug Toxicology.)

119. *Sensory Irritation to Formaldehyde during Single and Repeated Exposures in Mice.* L. E. KANE and T. ALARIE, Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

Formaldehyde is present in both the environmental and occupational atmospheres. It is one of the oxidants associated with photochemical smog. It is introduced into the industrial environment because of its use in resins in the chemical, paper, and textile industries, as well as in histologic preparations. The immediate reaction to exposure to formaldehyde is one of sensory irritation that is characterized by a burning of the eyes, nose, and throat. Sensory irritation also causes a reflex decrease in respiratory rate. This research uses the degree of decrease in respiratory rate in mice as the measure of sensory irritation. A dose-response curve

was developed using Swiss-Webster male mice exposed to a given formaldehyde concentration for 10 min. The concentration associated with a 50% decrease in respiratory rate was 3.1 ppm. When mice were pretreated for 3 hr/day for 3 consecutive days to a concentration of 0.3 ppm, the dose-response curve was unchanged. When mice were exposed for 3 hr/day for 4 consecutive days to 3 ppm of formaldehyde the maximum percentage decrease increased each day (48, 63, 68, and 74%). A similar pattern of sensitization (defined as increasing response to the same stimulus) was seen when mice were exposed to 1.3 ppm of formaldehyde for 3 hr/day for 3 consecutive days (18, 33, and 35% decreases in respiratory rate). Thus, it has been shown that mice can be sensitized to formaldehyde within 24 hr by inhalation. (Supported by NIEHS Grant 5-R01-ES00872-02.)

120. *Respiratory Anaphylaxis to Aerosolized Chemicals in Guinea Pigs.* M. KAROL, S. OKA, H. JOSET, S. YOO, and Y. ALARIE, Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

A number of low-molecular-weight industrial chemicals are reported to cause allergic pulmonary hypersensitivity in exposed workers. Currently, there is no method to predict which industrial chemicals would be most allergenic for man. Pulmonary hypersensitivity in several animal species following exposure via the respiratory route has previously been achieved. Both complex naturally occurring allergens (pollens, flour grains, pigeon droppings) and purified proteins (bacterial amylase) have successfully induced the respiratory anaphylactic response in animal models. However, there has been only one report of experimental asthma in which the hypersensitivity was directed toward a low-molecular-weight molecule. The sensitizing allergen in that instance was picryl-amylase, and the sensitivity was specific for the picryl group. We have experimentally extended the ability to induce hapten-specific pulmonary hypersensitivity in guinea pigs. Two chemicals, picryl sulfonate and *p*-amino arsanilic acid, each covalently linked to a carrier protein (ovalbumin) were selected as haptens. After repeated exposure to an aerosol of the conjugated protein, guinea pigs displayed pulmonary hypersensitivity upon challenge with an aerosol of the conjugate. The degree of hypersensitivity was quantified by measurement of pulmonary function. Challenge with the chemical (hapten) linked to a heterologous carrier revealed pulmonary sensitivity to the haptenic portion of the antigen. Serological examination of the sensitized guinea pigs disclosed the presence of specific cytophilic antibodies. The titer of this group of antibodies did not correlate with the time of appearance of maximal respiratory hypersensitivity. (Supported by NIOSH Grant R01-OH00367-05.)

121. *Effects of some Cardioactive Drugs on Isolated Rat and Guinea Pig Lungs.* SAMIR NAJJAR and FREDERICK SPERLING, Department of Pharmacology, Howard University College of Medicine, Washington, D.C.

Pulmonary and pulmonary vascular species differences after cardioactive drugs were studied in isolated lungs. Single doses of such drugs were injected into the pulmonary artery of excised, continuously perfused, guinea pig and rat lungs kept in a Delaunoy machine. Digoxin, quinidine, and diphenylhydantoin reversibly reduced tidal volume only in guinea pig lungs, as expected, by bronchiolar action. The degree of reduction was dose related for the two former compounds, but not for the latter. Duration of effect was dose related for each of the three compounds. Theophylline and nitrite had no effect on tidal volume in either species. The degree and duration of reversible pulmonary vascular constriction by digoxin, in both species, was dose related. The degree and duration of the pulmonary vascular dilatation induced by the other four compounds in both species was also dose related, with the exception of the highest of three nitrite doses. Only digoxin had constrictive effects: on the guinea pig bronchioles and on the pulmonary vasculature of both species. The other four drugs dilated the pulmonary vasculature in both species, even though quinidine and diphenylhydantoin constricted guinea pig bronchioles. The correlation between dose and duration of effect in both species would indicate that with the possible exception of nitrite in the guinea pig lung, there did not seem to be

any significant binding of the drugs. In the instances in which both respiratory and vascular effects were seen, the duration of effect on the two systems were equal and simultaneous.

122. *Pulmonary Mechanical Response to Acute Low-Level Chemical Inhalation.* ANTHONY A. HERRMANN, LEE E. OSTRANDER, EDWARD D. BARETTA, RICARDO J. SOTO, DONALD P. SCHLUETER, and RICHARD D. STEWART, S. C. Johnson & Son, Inc., Racine, Wisconsin.

Lung function changes related to chemical inhalation have been examined using a partial forced expiratory flow-volume method. This technique was previously reported by others as a sensitive method for detecting small airway changes and has been suggested as an effective screening tool for assessing the irritant potential of products, such as hair sprays, prior to release for consumer use. Forty-five healthy, trained male and female volunteers ranging in age from 19 to 56 were studied using a battery of tests including partial and maximum forced expiratory maneuvers. Testing was done immediately pre- and post-chemical exposure. Exposures were to nine hair sprays, multiple room air freshener fragrances, cold,  $\text{SO}_2$ , and methacholine. Matching controls were performed. Variation in test results compared favorably with those reported in the literature. For most acute exposures, no significant changes in expiratory flow rates were noted. Exceptions were  $\text{SO}_2$  and methacholine, which showed decreased flow in both the partial flow volume and the more commonly used maximum flow-volume curves. These physiologic changes were accompanied by readily discernible respiratory symptoms. The partial forced expiratory maneuver followed immediately by maximum inspiration and a maximum expiratory maneuver produced fatigue and symptoms of airway and musculoskeletal irritation in several test subjects. Contrary to previously published data, the partial flow-volume technique of assessing small airway function following low-level inhalation of chemical irritants does not appear to offer any advantages over maximum flow-volume methods.

123. *Pulmonary Changes following Chronic Exposures to Aluminum Chlorhydrate.* FINIS L. CAVENDER, WILLIAM H. STEINHAGEN, and WILLIAM M. BUSEY, Becton, Dickinson Research Center, Research Triangle Park, North Carolina, and Experimental Pathology Laboratories, Inc., Herndon, Virginia.

In order to determine the inhalation toxicity resulting from chronic exposures to aluminum chlorhydrate, a 2-year chronic toxicity study was initiated at NIEHS in which rats and guinea pigs were exposed to 0.25, 2.5, and 25  $\text{mg}/\text{m}^3$  of aluminum chlorhydrate. The aluminum chlorhydrate was generated as a particulate dust using a Wright dust feed. The animals were exposed for 6 hr/day, 5 days/week. Measurements of body weight were recorded weekly and observations for clinical signs were conducted daily. Following 6 and 12 months of exposure, animals were sacrificed and necropsied. Peripheral blood was collected for hematological determination and arterial blood was collected for clinical chemistry. The lungs, heart, liver, kidney, spleen, and brain were weighed and organ-body weight and organ-brain weight ratios were calculated. In some animals, the lungs were lavaged for macrophage studies and tissue aluminum concentrations were determined in lungs, heart, brain, kidney, spleen, liver, peribronchial lymph nodes, and serum. Significant differences between control and exposed animals were seen in the numbers of macrophages, lung weights, lung weight-body weight ratios and tissue aluminum concentrations.

124. *Morphologic Changes following Chronic Inhalation of Aluminum Chlorhydrate in the Rat and Guinea Pig.* WILLIAM M. BUSEY, BEVERLY Y. COCKRELL, and FINIS L. CAVENDER, Experimental Pathology Laboratories, Inc. Herndon, Virginia, and Becton, Dickinson Research Center, Research Triangle Park, North Carolina.

Groups of 10 rats and 10 guinea pigs were exposed to 0.25, 2.5, and 25  $\text{mg}/\text{m}^3$  aluminum chlorhydrate (ACH) for 6 months in inhalation chambers to assess pulmonary effects of a common component of aerosol antiperspirants. Similar groups of animals of both species ex-

posed to room air served as controls. At necropsy, trachea, lungs, and nasal cavity were removed and perfused with fixative. Tissues were dispersed in a systematic manner for light microscopy (LM) and scanning (SEM) and transmission (TEM) electron microscopy by using alternate 5-mm sections. By this procedure, exposure-related lesions observed in H & E sections by LM could be used to identify lesions in adjacent tissues for SEM or TEM. At 0.25 mg/m<sup>3</sup> ACH, LM revealed focal proliferations of alveolar macrophages in the lungs of rats. Exposure-related respiratory lesions were observed in all animals at 2.5 mg/m<sup>3</sup> ACH. The lesions consisted of multiple foci of large alveolar macrophages, approaching 20 μm surrounding ends of airways. Cellular debris and, in the rat, lymphoid cells accompanied the focal macrophagic reaction. Exposure to 25 mg/m<sup>3</sup> ACH resulted in a multifocal granulomatous pneumonia characterized by a macrophagic and mononuclear cell reaction in alveoli near the termination of airways. SEM of exposed lungs confirmed the distribution of lesions around the terminal bronchioles while the morphology of phagocytosed particles was defined by TEM.

125. *Effect of Particle Size on the Inhalation Toxicity of Naled Aerosols.* PETER E. BERTEAU, WALLACE A. DEEN, and ROBERT L. DIMMICK, University of California, School of Public Health, Naval Biosciences Laboratory, Naval Supply Center, Oakland, California. (W. F. Durham)

Ultra-low-volume (ULV) dispersal of pesticide aerosols in the field is increasing. These aerosols contain more particles in the respirable range than do aerosols generated from conventional foggers. To determine if one pesticide, dispersed as particles in this range, was more toxic to mammals than when dispersed as aerosols where most particles were in a larger range, we exposed groups of eight female rats, constrained in head-only exposure units, either to aerosols of naled concentrate (mass median diameter, MMD, 2.1 μm), generated from Wells-type refluxing atomizers, or to larger particle aerosols (MMD, 13–20 μm) of the same insecticide formulation generated from an electrically driven spinning disk. Aerosol concentration was about 1.3 mg/liter in both cases. Exposure was continued until there was evidence of mortality, but never longer than 65 min. Dosage was calculated as LD50 (mg/kg) from the insecticide concentration (mg/liter of air), exposure time (min) respiratory minute volume (ml/min/g), and the estimated deposition (%) of the inhaled material. The LD50 value was 3.1 mg/kg for the small-particle aerosol but was greater than 12.4 mg/kg (25% mortality at this level) for the large-particle aerosol. Thus, naled aerosols, as respirable particles, were at least four times more toxic than the larger particles. Inhalation toxicity decreased to about half (LD50, 7.7 mg/kg), when the concentrate was diluted with vegetable oil, but was still about 20 times greater than the oral toxicity (LD50, 160 mg/kg). We believe that ULV spraying of naled may present a greater hazard to the poorly protected worker than conventional sprays. (Sponsored by the U.S. Army Research and Development Command and the Office of Naval Research.)

126. *Inhibition of Pulmonary Mixed-Function Oxidations by Paraquat and Ozone.* MARK R. MONTGOMERY and GERALD A. MORTENSON, Veterans Administration Hospital and University of Minnesota, Minneapolis, Minnesota.

The activities of two pulmonary, mixed-function oxidations have been evaluated following *in vivo* and *in vitro* treatment of rats and lung microsomal preparations with paraquat (PQ) and ozone. Benzphetamine *N*-demethylase activity (an indicator of cytochrome *P*-450 activity) was unchanged from control values when measured 24 hr after PQ administration (20 mg/kg, ip). However, this enzymatic activity was significantly depressed by 50% at 4 and 7 days post-administration. At 14 days the pulmonary activity returned to normal values in the animals (80%) that survived this time span. The liver benzphetamine *N*-demethylase activity was unchanged throughout the 2-week period. *In vivo* exposure to 1 ppm of ozone (O<sub>3</sub>) for 24 hr resulted in a more rapidly developed inhibition of benzphetamine *N*-demethylase activity, which also returned to control values by 14 days postexposure. While inhibition of stearyl-CoA desaturase activity (an indicator of cytochrome *b*<sub>5</sub> activity) was also inhibited acutely following

PQ or O<sub>3</sub> treatment, these results were complicated by the strong anorexic effects of these agents, which makes difficult an accurate assessment of the possible direct inhibition of cytochrome b<sub>5</sub> function. *In vitro*, both PQ (1 mM) and O<sub>3</sub> (3 ppm) inhibited benzphetamine *N*-demethylase activity; an effect which was simply additive when both were present together (1 mM PQ plus 3 ppm O<sub>3</sub>). A similar, additive interaction was observed with PQ- and O<sub>3</sub>-mediated enhancement of pulmonary, microsomal lipid peroxidation. These results suggest that significant biochemical lesions may be detected in pulmonary mixed-function oxidations prior to the development of the histopathological lesion and that these biochemical alterations actually return to control values at the time when the oxidant-induced pulmonary fibrosis has fully developed.

127. *The Effects of Diethyl Maleate and Diethyl Fumarate on Ozone Toxicity in Mice.* M. C. BRUCE and S. D. MURPHY, Department of Physiology, Harvard University School of Public Health, Boston, Massachusetts.

Several reports have suggested that maintenance of adequate tissue glutathione (GSH) concentrations is necessary to protect tissues against oxidant injury. This study was undertaken to evaluate the effect of GSH depletion on the development of pulmonary edema in response to ozone (O<sub>3</sub>) exposure. Treatment of male mice with 1 mg/kg diethyl maleate (DEM) ip resulted in lung GSH contents that were 21, 51, 50, 73.5, 126.5, and 113.2% of control values at 1, 4, 6, 16, 24, and 48 hr, respectively. Treatment with diethyl fumarate (DEF), the *trans*-isomer of diethyl maleate, reduced lung GSH concentrations to 48, 62, 65, and 89% of control values at 1, 4, 6, and 16 hr, respectively. Mice were injected with DEM or DEF 30–60 min prior to exposure to 10 ppm O<sub>3</sub> for 4 hr. The mice were sacrificed at regular intervals during a 12-hr period following O<sub>3</sub> exposure, and lungs were removed, weighed, and analyzed for GSH content. Means  $\pm$  SE for lung weights (mg) were: 169.17  $\pm$  4.48 for saline controls, 328.60  $\pm$  18.35 for saline-pretreated O<sub>3</sub>-exposed mice, 231.55  $\pm$  15.64 for DEM-pretreated O<sub>3</sub>-exposed mice, and 420.05  $\pm$  13.71 for DEF-pretreated O<sub>3</sub>-exposed mice. These values were all significantly different from each other ( $p < 0.05$ ). Significant protection against the development of pulmonary edema in response to exposure to 10 ppm O<sub>3</sub> for 4 hr was also seen in mice pretreated with DEM either 1 or 2 days prior to O<sub>3</sub> exposure. Pretreatment with DEM 3, 5, or 7 days prior to O<sub>3</sub> exposure did not protect to a significant degree. Paradoxically, pretreatment with DEM protected while pretreatment with DEF potentiated O<sub>3</sub> toxicity under conditions in which lung GSH was depleted by both pretreatment compounds. Increased lung GSH concentrations at 24 and 48 hr after DEM could explain the protection against ozone toxicity at these times. (Supported by Research Grants OH 00315 from the National Institute of Occupational Safety and Health and ES 00002 from the National Institute of Environmental Health Sciences.)

128. *Alterations in the Fatty Acid Composition of Lung Phospholipids and Triglycerides following Age-Dependent Lipid Peroxidation in Neonatal Rat Lungs.* JAMES P. KEHRER and ANNE P. AUTOR, The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, Iowa.

We have previously demonstrated the presence of an age-dependent lipid peroxidizing activity in neonatal rat lung homogenates. This activity, assessed by measuring the formation of malondialdehyde following incubation at 37°C for 90 min, occurs without the addition of any initiating factor. The activity appears 2 days following birth, reaches a maximum at 5 days of age and gradually diminishes to undetectable levels by 20 days after birth. Gas chromatographic analysis of the fatty acid composition of rat lung phospholipids revealed no differences in tissue composition for newborn, 5-day, 12-day, or adult animals. In the triglyceride fraction, however, arachidonate (C-20:4) was found to decrease from 11% of the total fatty acid content in 5-day-old rats to 3% in adult rats while docosahexaenoate (C-22:6) decreased from 17 to 2%. To determine changes induced in the fatty acid composition of lung phospholipids and triglycerides by this lipid peroxidizing activity, 900g supernatant fractions of lung homo-

genates were analyzed both before and after incubation at 37°C for 90 min. The changes observed in this system occurred primarily in the triglyceride fraction isolated from 5-day-old rat lungs. These changes consisted of a decrease in the triglyceride content of arachidonate and docosahexaenoate. This decrease was not observed in either newborn or adult lung fractions after similar analysis. The phospholipid fraction from 5-day-old rat lung homogenates showed only a slight decrease in arachidonate and docosahexaenoate content after incubation while the phospholipids from newborn and adult rat lungs showed no alterations. No other fatty acids in either the phospholipid or triglyceride fractions showed any appreciable changes following incubation. These data indicate that the triglyceride fraction of neonatal rat lungs contain increased amounts of polyunsaturated fatty acids and it is these fatty acids which appear to be susceptible to lipid peroxidation dependent upon age. (Supported in part by USPHS Grant No. NIGMS 12675.)

129. *Influence of Pretreatment on B(a)P Metabolism in the Isolated Perfused Lung.* R. NIEMEIER, D. WARSHAWSKY, and E. BINGHAM, University of Cincinnati College of Medicine, Department of Environmental Health, Cincinnati, Ohio.

An isolated perfused rabbit lung preparation (IPL) was used to study the metabolism of benzo(a)pyrene (B(a)P), a ubiquitous potent carcinogen that has been associated with the increased incidence of human bronchiogenic carcinoma in occupational and urban populations. [<sup>14</sup>C]B(a)P was administered intratracheally to the IPL after pretreatment with either phenobarbital, 3-methylcholanthrene (3-MC), B(a)P, or corn oil ip or B(a)P intratracheally. Metabolites were isolated from serial blood samples up to 3 hr after administration of the [<sup>14</sup>C]B(a)P to the IPL. Metabolic patterns and amounts were determined following solvent extraction, thin-layer chromatography, and liquid scintillation counting. The identity of metabolites separated from thin-layer procedures was substantiated by high-pressure liquid chromatography. Total metabolic rate of B(a)P in ip-treated rabbits was approximately four times the rate of the corn oil controls and about nine times the rate of the nonpretreated controls. 3-MC pretreatment resulted in a slightly higher metabolic rate compared with corn oil control and was four times the rate of the nonpretreated control. In addition to the increase in total metabolic rates (ng/hr/g of lung), B(a)P and 3-MC pretreatment resulted in an increase in dihydrodiol formation relative to other metabolites. (Supported by a NCI Grant CA 15344-02, and EPA Contract EPA 68-02-1678, and is part of the Center of the Study for the Human Environment No. ES 00159.)

130. *Alanine Aminotransferase Activity of Rat Tissues following the Administration of Cefazolin.* G. FEUER, T. BALAZS, T. FARBER, M. LIPIEN, M. S. ILYAS, and R. ILSE, Food and Drug Administration, Washington, D.C., and Department of Clinical Biochemistry, University of Toronto, Toronto, Canada.

A few drugs, e.g., isoniazid, phenothiazine derivatives, and cefazolin, have occasionally been shown to produce low serum transaminase activity. We have been investigating the mechanism of the reduced activity. Cefazolin given to male rats in daily oral doses of 500–2000 mg/kg significantly decreased alanine aminotransferase activity in the serum, liver, kidney, heart, and brain 2 to 4 weeks after the beginning of the treatment. Serum aspartate transaminase activity was also reduced, but alkaline phosphatase activity remained unaltered. The effect of cefazolin at low concentrations was partly reversed by the simultaneous administration of pyridoxal *in vivo*. The low transaminase activities were elevated by the subsequent addition of pyridoxal phosphate *in vitro*. Similar results were obtained when rats were treated with isoniazid at daily oral doses of 10 mg/kg. In the case of this drug, concurrent administration of pyridoxal completely restored the enzyme activity to normal within 2 weeks. Cefazolin was found to be partly metabolized. A major metabolite detected in the urine of rats also formed *in vitro* when cefazolin was exposed to hepatic microsomes and the mixed-function oxidase system. This metabolite probably possesses a hydrazine group since it gave a positive reaction with dimethylbenzaldehyde and fast blue B salt. The mechanism of alanine aminotransferase reduction by cefazolin or



isoniazid might be related to a direct action. In the presence of the hydrazine groups, serum and tissue pyridoxal is trapped and therefore its availability as the functional cofactor required by transaminase enzyme systems is greatly reduced.

131. *Evidence for a Highly Specific Interaction between  $\Delta^8$ -Tetrahydrocannabinol and Hydrocortisone in the Liver Tyrosine-Aminotransferase System.* JACQUELINE M. WRENN and MARVIN A. FRIEDMAN, Department of Pharmacology, Medical College of Virginia, and MCV/VCU Cancer Center, Richmond, Virginia.

$\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC) suppresses the hydrocortisone induction of mouse and rat liver tyrosine aminotransferase activity (TAT). It is the purpose of the present work to compare the effects on TAT of  $\Delta^8$ -THC with those of  $\Delta^9$ -THC.  $\Delta^8$ -THC was dissolved in Emulphor-ethanol and injected ip. When ICR mice were given a single injection of  $\Delta^8$ -THC and killed 12 hr later, there was a dose-dependent stimulation of liver TAT activity ranging from 56% at 12.5 mg/kg to 235% at 200 mg/kg. When mice were injected with 150 mg/kg hydrocortisone acetate and killed 2 hr later, the induction was 400%. In mice pretreated 12 hr previously with 12.5, 25, and 50 mg/kg  $\Delta^8$ -THC the steroid inductions were only 220, 180, and 50%, respectively. The effects of  $\Delta^8$ -THC on tryptophan (600 mg/kg, ip, 4 hr prior to sacrifice) induction of TAT activity was determined in adrenalectomized Sprague-Dawley rats.  $\Delta^8$ -THC (200 mg/kg, ip, 6 hr prior to sacrifice) induced a marked increase in liver TAT activity but had no effect on the tryptophan induction. It then became important to know whether other types of induction were affected by  $\Delta^8$ -THC. Therefore, groups of mice were injected with 1.5 mg/kg glucagon either alone or in combination with 200 mg/kg  $\Delta^8$ -THC and killed 3 hr after glucagon (6 hr after  $\Delta^8$ -THC). Glucagon induced a 250 and 440% increase in TAT activity in control and  $\Delta^8$ -THC-treated mice, respectively. It is apparent that there is a highly specific interaction between  $\Delta^8$ -THC and hydrocortisone, the nature of which is currently being investigated. (Research supported by NIH Grants DA01248, DA00490, and NIH Predoctoral Training Grant DA07027.)

132. *The Influence of Esterase Inhibition on the Hepatotoxicity of Esters of Allyl Alcohol in Rats.* EILEEN H. SILVER and SHELDON D. MURPHY, Department of Physiology, Harvard University School of Public Health, Boston, Massachusetts.

Modification of carboxylesterase activity was used to determine the importance of enzymatically mediated hydrolysis in the production of liver injury by esters of allyl alcohol. Triorthotolyl phosphate (TOTP, 125 mg/kg), 0.5, 1, 2, 4, 6, or 10 mg/kg of the defoliant *S,S,S*-tributyl-phosphorotrithioate (DEF), or corn oil (1 ml/kg) was administered ip to male rats. Eighteen hours later the esters allyl acetate, allyl cinnamate, or allyl phenoxyacetate were administered po to the pretreated, fasted animals. TOTP pretreatment significantly inhibited the rise in plasma alanine- $\alpha$ -ketoglutarate (AKT) activity at 8 hr after treatment with allyl acetate (60, 90, 120, and 150 mg/kg). This protection by TOTP was also observed at 16 and 24 hr after allyl acetate. TOTP also prevented changes in liver morphology produced by allyl acetate. Pretreatment with 0.5 mg/kg DEF inhibited liver esterase 28% and reduced the elevation in plasma AKT activity caused by 60 mg/kg allyl acetate. DEF (4 mg/kg), which produced 85% inhibition of liver esterase, completely protected against the hepatic injury produced by 60 mg/kg allyl acetate. TOTP prevented the elevation in plasma AKT activity induced by 250, 400, and 600 mg/kg allyl cinnamate. TOTP pretreatment prevented liver injury produced by 200 mg/kg, but not that produced by 250 or 400 mg/kg of allyl phenoxyacetate. Plasma AKT activity, however, was significantly lower in TOTP-pretreated animals compared with corn oil-pretreated animals that received 250 mg/kg allyl phenoxyacetate. The hydrolysis of all three esters of allyl alcohol *in vitro* was greatly inhibited when liver homogenates from TOTP-pretreated rats were used. The results indicate that enzymatic hydrolysis is a necessary step in the activation of the esters allyl acetate and allyl cinnamate to hepatotoxins. Since esterase inhibition was only weakly protective against the hepatotoxic effect of allyl phenoxyacetate, other mechanisms may be involved in the toxic action of this ester. (Supported by Grants

ES-00002, ES-00084, and ES-00045 from the National Institute of Environmental Health Sciences and a grant from Procter & Gamble Co.)

133. *Interaction of Steroids with the Hepatic Drug-Metabolizing Enzyme System in the Rat.*

R. KARDISH, R. FARKAS, and G. FEUER, Department of Clinical Biochemistry, University of Toronto, Toronto, Canada.

The *in vivo* action of various progesterone derivatives (2.5–10 mg/kg, 7 daily oral doses) on the properties of the hepatic drug-metabolizing enzyme system was studied in the female rat. Reduced derivatives (5 $\alpha$ - or 5 $\beta$ -pregnane-3 $\beta$ -ol-20-one, 5 $\alpha$ - or 5 $\beta$ -pregnane-3 $\beta$ -20 $\beta$ -diol) caused significant reduction in the activities of drug-metabolizing enzymes (aminopyrine *N*-demethylase, coumarin 3-hydroxylase) and glucose 6-phosphatase; inosine diphosphatase activity was slightly raised. Pregnanolone and pregnanediol also decreased microsomal phospholipid synthesis (*de novo* incorporation of L-[Me-<sup>14</sup>C]methionine into phospholipids, S-adenosyl-L-methionine:microsomalphosphatidylethanolamine methyl transferase activity). Phospholipid changes mainly manifested in phosphatidylcholine (PC)-ethanolamine (PE), and lysophosphatidylcholine fractions; the PC:PE ratio was significantly reduced. In contrast, 16 $\alpha$ -hydroxyprogesterone and pregnenolone-16 $\alpha$ -carbonitrile increased drug metabolism and slightly elevated glucose 6-phosphatase. These compounds also enhanced the *de novo* synthesis of microsomal phospholipids, methyltransferase activity, and PC:PE ratio. Treatment of rats with reduced progesterone derivatives decreased microsomal progesterone hydroxylation (16 $\alpha$ - and 6 $\beta$ -hydroxylase) and increased progesterone  $\Delta^4$ -5 $\alpha$ -dehydrogenase activity *in vitro*, whereas 16 $\alpha$ -hydroxyprogesterone and pregnenolone-16 $\alpha$ -carbonitrile raised progesterone hydroxylation. These data suggest that progesterone metabolism may play a role in the induction of drug metabolism in the liver of the female rat.

134. *Results of 90-Day Toxicity Studies in Rats Given Vinylidene Chloride in Their Drinking*

*Water or Exposed to VDC Vapors by Inhalation.* J. F. QUAST, C. G. HUMISTON, B. A. SCHWETZ, M. F. BALMER, L. W. RAMPY, J. M. NORRIS and P. J. GEHRING, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

Groups of male and female Sprague-Dawley rats ingested vinylidene chloride (VDC) in their drinking water at concentrations of 0, 60, 100, or 200 ppm for 90 days. These concentrations were equivalent to the respective average doses of 0, 6, 10, or 19 mg of VDC/kg/day for male rats; and 0, 8, 13, or 26 mg of VDC/kg/day for female rats. In the inhalation study, groups of male and female Sprague-Dawley rats were exposed to VDC vapors by inhalation 6 hr/day, 5 days/week. Rats were exposed to 0, 25, or 75 ppm VDC vapors 60 times during 90 days. In both of these studies, the following parameters revealed no toxicologically significant changes: body weight, hematology, urinalyses, blood urea nitrogen, serum alkaline phosphatase activity, serum glutamic pyruvic transaminase activity, gross pathology, organ weights, and organ/body weight ratios of liver, kidneys, heart, testes, and brain. Microscopic evaluation of tissues from both of these studies revealed a minimal degree of hepatocellular cytoplasmic vacuolation in several rats ingesting VDC in their drinking water at 200 ppm, and in several rats exposed to VDC vapors at 25 and 75 ppm. These minimal hepatocellular changes are interpreted to be of a reversible character.

135. *Hepatotoxicity of 1,1-Dichloroethylene: A Proposed Mechanism.* R. J. JAEGER and

L. COFFMAN, Kresge Center for Environmental Health, Harvard School of Public Health, Boston, Massachusetts.

1,1-Dichloroethylene (vinylidene chloride, 1,1-DCE) is hepatotoxic to 18-hr fasted rats after inhalation exposure (exposed at 200 ppm  $\times$  4 hr; killed at 6 hr); fed rats are resistant to liver damage as measured by serum enzyme elevation and histological change. In both groups of rats, liver glutathione (nonprotein sulfhydryl) concentrations decrease with exposure but the decline is greatest in fasted rats; mitochondrial glutathione presents the largest fed-fasted decrease. This organelle was severely injured at early times of 1,1-DCE exposure (2 hr), oxygen uptake ability decreased, and intramitochondrial enzymes appeared in the serum. We propose

that 1,1-DCE toxicity is mediated by a specific mitochondrial toxin. 1,1-DCE metabolism has yet to be elucidated but biotransformation by the mixed function oxygenase system (MFOS) could lead to an epoxide intermediate which spontaneously rearranges to chloroacetylchloride and subsequently to chloroacetic acid. Previously, we reported that induction of the MFOS decreased 1,1-DCE toxicity; in contrast, trichloropropane epoxide (0.1 ml/kg) an epoxide hydrolase inhibitor given po before exposure, enhanced the elevation of serum enzyme activity indicative of injury. If chloroacetic acid results from 1,1-DCE metabolism, lethal synthesis in mitochondria could produce chlorocitric acid which might inhibit aconitase and cause citric acid to accumulate. In fasted rats exposed to 1,1-DCE, liver citric acid was 416% of control (fasted but not exposed) while exposed fed rats were slightly above control. Experiments were done *in vitro* using washed mitochondria from fasted rats. Both chloro- and fluoroacetic acids (2 and 5 mmol) significantly inhibited mitochondrial oxygen uptake in the presence of malic and pyruvic acids and ADP (state 3). The potencies of the halo acids were similar. These experiments support the hypothesis that lethal synthesis of chlorocitric acid from chloroacetic acid, a possible metabolite of 1,1-DCE, could lead to mitochondrial injury and may be the cause of the acute damage seen in rats. (Supported by NIEHS Center Grant ES-00002 and OH-00315.)

136. *Hepatic Effects of 2-Chloroethanol in the Rat.* L. FRIEDMAN, J. SCALERA, T. BALAZS, L. KASZA, J. E. KEYS, E. L. PETERS, and C. H. GRAHAM, Divisions of Toxicology, Pathology, and Drug Biology, Food and Drug Administration, Washington, D.C.

2-Chloroethanol (CE) is a commercially used solvent and is also formed in the presence of chloride ion in ethylene oxide-sterilized materials. The effects of CE on liver composition, RNA and protein synthesis (measured with the use of radiolabeled precursors), and histology were investigated following single oral doses of this compound to young adult rats. A dose of 50 mg/kg produced a 66% decrease in liver glutathione and a 57% decrease *in vivo* protein synthesis 3 hr after administration; RBC glutathione and *in vivo* RNA synthesis were unchanged. The lowest effective dose range for these effects was 10–20 mg/kg. After rats were given CE at 30 mg/kg, liver glutathione and protein synthesis were maximally reduced at 1.5 and 2.5–3.5 hr, respectively. By 7 hr these parameters were no longer affected but a 25% increase in total liver lipids and a 33% increase in RNA synthesis were seen at this time. As was reported earlier for diethylmaleate (*Fed. Proc.* 33, 247, 1974), which is also a depletor of liver glutathione, CE at a dose of 30 mg/kg enhanced the inhibition of RNA synthesis induced by aflatoxin at a dose of 0.33 mg/kg (44 vs 66%). Inhibition of protein synthesis was also demonstrated *in vitro* by using liver slices with concentrations of CE as low as 2.5 mg/ml. Twenty-four hours after rats were given CE at 40 mg/kg, livers stained with Oil Red O indicated the presence of an increased amount of fat although no other apparent histological changes could be demonstrated. A significant reduction in serum total protein and an increase in albumin/globulin ratio were also seen in female rats following treatment at 40 mg/kg. CE at sublethal levels can produce definitive biochemical and histological effects in the livers of orally dosed rats.

137. *Lysosomal Mechanisms in Intestinal Transport of Sulfated Macromolecules in the Neonatal Rat and Guinea Pig.* G. ESTEL, R. MANKES, T. BARBOLT, and R. ABRAHAM, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

This study was conducted to investigate lysosomal mechanisms involved in transport of macromolecules during maturation of gut epithelial cells. Cortisone acetate, a lysosomal stabilizer, was utilized to manipulate lysosomal activity. The Hartley-strain guinea pig, born with a mature intestinal epithelium, and Sprague-Dawley rats in which the gut matures 3 weeks postpartum were used. They were divided into four groups: Group I, control; Group II, dextran sulfate (500 mg/kg) daily by gavage; Group III, cortisone acetate ip (12–18 mg/100 g) for 3 consecutive days; Group IV, cortisone acetate and dextran sulfate. Rats were killed when 12 or 18 days old and guinea pigs when 12 days old. The ileum was processed for histochemistry, autoradiography, histology, and electron microscopy. In 12-day-old rats, Groups I and II showed the presence of supranuclear vacuoles and in Group II there was an increase in metachromasia (dextran sulfate) within these vacuoles accompanied by an increase in  $\beta$ -glucuro-

nidase. There was also an increase in Gomori's and Barka and Anderson acid phosphatase. Groups III and IV showed no epithelial supranuclear vacuoles. This coincided with decreased uptake of dextran sulfate in Group IV, as demonstrated by an absence of metachromasia and a total loss of  $\beta$ -glucuronidase activity. Both supranuclear vacuoles and metachromasia in the gut epithelium were absent in guinea pigs. Dextran sulfate and/or cortisone acetate had no effect on the lysosomal enzymes of the epithelium. Metachromasia, however, was seen in the lamina propria macrophages. Eighteen-day-old rats in all groups lacked supranuclear vacuoles. A slight increase in metachromasia was seen only in Group II accompanied by an increase in Barka and Anderson acid phosphatase activity. Group IV had only slight increases in enzymes over controls. Similar to 12-day-old guinea pig, metachromasia was seen in lamina propria macrophages. There was no difference in the incorporation of [ $^3$ H]thymidine in the crypts of epithelial cells in any of the groups. These results indicate that as epithelial cells mature the means of intracellular transport and degradation of polysulfated macromolecules shifts in favor of intercellular transport. On the other hand, cortisone acetate, by stabilizing the lysosomes, shuts off the intracellular route even in immature intestine but does not block the intercellular mechanisms of transport. (Supported in part by Research Grant 2-P01-ES00226-10 from NIEHS, NIH, NIH Training Grant 3-T01-ES00103-10, Research Career Development Award 1-K04-ES70608-04, and a Cystic Fibrosis Foundation grant.)

138. *Alterations of Hepatic Nuclear Ploidy in Rodents by PCB and Mirex.* B. MAYES, R. ABRAHAM, and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Previous studies from this laboratory indicated that combining two chlorinated hydrocarbons causes hyperplasia in mammalian livers in contrast to hypertrophy induced by administering the compounds separately. Studies were therefore undertaken to determine the classes of liver cells involved in hypertrophy and hyperplasia by employing quantitative and qualitative techniques for detecting such changes. Two chlorinated hydrocarbons, polychlorinated biphenyls (PCB) (Aroclor 1254) and Mirex were given to 6- to 7-week-old mice (Charles River CD-1) by gavage for up to 7 days: Group I, corn oil; Group II, 25 mg/kg PCB; Group III, 4.5 mg/kg Mirex; Group IV, 25 mg/kg PCB and 4.5 mg/kg Mirex. Isolated liver nuclei were counted and the volumes were measured on an electronic Coulter counter. After 4 days, in Group II, only diploid cells were decreased in frequency with little change in volume; in Group III the tetra- and octoploid cells were affected predominantly with a marginal increase in nuclear volume, whereas in Group IV a further increase in tetra- and octoploid volumes were noted as well as an increase in the relative frequency of octoploid cells. At 7 days in Group III the octoploid nuclear volumes and in Group IV the tetraploid nuclear volumes were increased. In addition, in Group IV the frequency of diploids was increased. Autoradiographic analysis revealed a 2- and 3-fold increase in labeled hepatocytes and endothelial cells in periportal and midzonal regions of Group II, a 4-fold increase of similar cells in midzonal regions in Group III, and a 10- and 6-fold increase midzonally in Group IV. These studies reveal that chemicals effect different classes of hepatocytes and that the type of hepatocyte so affected determines whether the liver responds by hypertrophy or hyperplasia. These short-term, high-dose studies, by employing the present techniques, might provide good indicators of the long-range effects of environmental chemicals. (Supported in part by Research Grant 2-P01-ES00226-10 from NIEHS, NIH, NIH Training Grant 3-T01-ES00103-10, and Research Career Development Award 1-K04-ES70608-04.)

139. *Comparative Toxicity of Chlordiazepoxide, Phenobarbital, Phenylbutazone and Thioridazine in Normal and Acute Liver-Damaged Mice.* M. F. MATAS, P. J. DEBAECKE, A. J. GEORGE, and R. E. CIMPRICH, Biomedical Research Department, Stuart Pharmaceuticals, Division of ICI United States Inc., Wilmington, Delaware. (L. E. Gongwer)

This investigation was designed to develop an animal model to aid in evaluation of the potential for adverse drug reactions. The potentiation of oral toxicity of selected compounds in a liver-damaged animal model was studied.  $\text{CCl}_4$  was given po at 0.5 ml/kg and liver damage was

confirmed by gross and histological examination. Lethality curves and sleep times were examined in normal and acute CCl<sub>4</sub> liver damaged male mice dosed with either chlordiazepoxide, phenobarbital, phenylbutazone, or thioridazine. Single-dose LD50 values in mice pretreated with CCl<sub>4</sub> were 24% less than in normal mice for phenobarbital and phenylbutazone (173 vs 226 mg/kg and 600 vs 790 mg/kg, respectively), 34% less for chlordiazepoxide (640 vs 970 mg/kg), and 72% (113 vs 400 mg/kg) less for thioridazine. Sodium pentobarbital sleep times were also compared. Mice were treated po with either H<sub>2</sub>O or CCl<sub>4</sub> 24 hr prior to receiving therapeutic levels of a test compound. Sodium pentobarbital at 35 mg/kg was then administered ip 1 hr after the test compound, and the sleep times determined by loss and subsequent recovery of righting reflex. A positive control (H<sub>2</sub>O pretreatment plus test compound plus sodium pentobarbital), and a negative control (CCl<sub>4</sub> pretreatment plus no test compound plus sodium pentobarbital) were employed for comparison. At termination, animals were sacrificed and the livers were examined grossly and histologically. No difference in the liver was seen by light microscopy between those mice pretreated with CCl<sub>4</sub>, with or without test compound. In all cases, there was a significant difference (Student's *t* test, *p* = 0.05) in duration of sleep time for damaged animals compared to normal for each compound. These results indicate that differences in toxicity of test compounds, even at therapeutic levels can be detected in damaged models.

140. *Primary Hepatocyte Cultures for the Investigation of the Fate and Mechanism of Action of Environmental Chemicals.* K. K. DOUGHERTY, S. D. SPILMAN, C. E. GREEN, A. R. STEWARD, and J. L. BYARD, Department of Environmental Toxicology, University of California, Davis, California.

A primary hepatocyte culture was developed as a model system to investigate the fate and mechanism of action of environmental chemicals. Hepatocytes were prepared from adult male Charles River CD-1 mice and adult male Sprague-Dawley rats by *in situ* perfusion of the liver with 5 mM Ca<sup>2+</sup> and 0.05% collagenase. The digested liver was dispersed, and hepatocytes were isolated by filtration and differential centrifugation yielding  $8 \times 10^7$  hepatocytes per mouse liver and  $2 \times 10^8$  hepatocytes per rat liver. About 90% of the hepatocytes excluded trypan blue. Hepatocytes were prepared aseptically, plated on petri dishes coated with rodent tail collagen (50–100  $\mu$ g of collagen/plate), and cultured in serum-free modified Waymouth's 752/liter media. Within 4 hr the hepatocytes attached to the collagen, and by 24 hr they had flattened and formed a confluent monolayer of  $2 \times 10^6$  cells (2 mg of cell protein)/60-mm petri dish. The hepatocytes have been maintained in culture for up to 4 days. A nonmetabolized alanine analog,  $\alpha$ -aminoisobutyric acid (AIB), was accumulated in mouse hepatocytes to a concentration nine times that in the media (0.5 mM AIB), with peak incorporation occurring at 24 hr. Mouse hepatocytes, after 12 hr in culture, incorporated leucine into protein (1.8 fmol of leucine/cell/hr, 45% of which was secreted into the medium); this synthesis was inhibited by cycloheximide. Mouse hepatocytes, maintained in culture for 24 hr, contained cytochrome P-450 (1.1 fmol/cell), and were able to *N*-demethylate *p*-chloromethylaniline (4% of 3  $\mu$ mol) incubated with the culture for 1 hr. Mouse hepatocytes ( $2 \times 10^7$  cells) metabolically activated parathion to paraoxon (3% of 1.6 mg in 24 hr). Cultured hepatocytes also metabolically activated 2-acetylaminofluorene (0.5  $\mu$ g/ $2 \times 10^6$  cells) resulting in 0.4% covalent binding to macromolecules in 2 hr. A higher level of sulfate-dependent covalent binding was found in hepatocyte cultures from the rat as compared to the mouse, correlating with the species susceptibility to liver carcinogenesis by this chemical. In conclusion, mouse and rat hepatocytes can be maintained in culture, and the cultures have been shown to carry out characteristic hepatic metabolic functions which afford a basis for comparative studies of the fate and mechanism of action of environmental chemicals.

141. *Effects on the Rat Testis of Sodium Fluoroacetate in Drinking Water.* JUDY L. SULLIVAN, FRANK A. SMITH, and ROBERT H. GARMAN, University of Rochester School of Medicine and Dentistry, Rochester, New York.

This study correlates biochemical and histological responses in testes of rats receiving NaFAc in the drinking water. Groups of animals receiving 20, 6, or 2 ppm NaFAc were sacrificed

daily through 7 days of treatment and at more widely spaced intervals in the succeeding 21 days. Testis weights and ATP concentrations in rats receiving 20 or 6 ppm were decreased, citrate concentrations were elevated in all three groups, and morphological damage was seen in testes from all groups. Initial cellular changes common to all three dosage levels included altered appearance of the spermatids, decreased numbers of spermatids, giant cell formation of spermatids and spermatocytes, and slight seminiferous tubular degeneration. At the two higher concentrations, damage progressed to marked atrophy, but this stage did not develop at 2 ppm. Regeneration of the damage in the 2-ppm group was complete by 7 days post-treatment. At the higher concentrations regeneration was not complete by post-treatment Day 21. Spermatogenesis was abnormal in some instances during regeneration after the two higher doses. These findings are consistent with impaired carbohydrate metabolism through the Embden-Meyerhof pathway and impaired energy production via the Krebs cycle. (Supported by USPHS-NIH Toxicology Training Grant 5-T01-GM-01781, NIEHS Grant ES-00779, and the U.S. Energy Research and Development Administration at the University of Rochester Biomedical and Environmental Research Project and has been assigned Report No. UR-3490-997.)

142. *The Reactivation of Plasma Cholinesterase in Human Fetus and Adult following in Vitro Exposure to the Organophosphate, Dichlorvos.* JOHN U. BELL, Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia. (John A. Thomas)

Reactivation of organophosphate inhibited plasma cholinesterase (EC 3.1.1.8) may occur spontaneously or in response to treatment with specific chemical compounds (e.g., pralidoxime chloride). In this project, both spontaneous and chemically induced reactivation were studied using as an enzyme source plasma obtained from nonpregnant females, pregnant females at term, and their respective neonates, sampled immediately following delivery. Aliquots of plasma were incubated with dichlorvos ( $1 \times 10^{-6}$  M) for 5 min at 37°C resulting in a 96% inhibition of cholinesterase in all three groups. Following the 5-min exposure to the organophosphate, either pralidoxime chloride ( $1 \times 10^{-3}$  M) or an equivalent volume of saline was added to the reaction flask and the restoration of cholinesterase activity was monitored over the next 120 min. Pralidoxime mediated cholinesterase reactivation in "nonpregnancy" plasma was significantly greater than that observed in either "maternal" or "fetal" plasma; however, no significant difference was noted in reactivation rates for these latter two groups. Significant differences were also observed in the rates of spontaneous reactivation and these were found to correlate fairly well ( $r = 0.75$ ) with plasma arylesterase (EC 3.1.1.2) activities. However, after correcting for this spontaneous reactivation, there were still significant differences in the rates of pralidoxime mediated reactivation (nonpregnant > pregnant > fetal).

143. *A Comparison of the Effects of Model Irritants on Anesthetized and Nonanesthetized Rabbit Eyes.* A. G. ULSAMER, P. L. WRIGHT, and R. E. OSTERBERG, Consumer Product Safety Commission, Bethesda, Maryland.

Present Federal Hazardous Substances Act Regulations make no provisions for the use of anesthesia in eye irritation testing. In order to evaluate the use of local anesthesia on the severity of the eye irritation response, the effects of model irritations on anesthetized and nonanesthetized rabbit eyes were compared. The appropriate dose (0.1 ml) of the following irritants were instilled into the right eye of New Zealand white rabbits: 5 or 10% acetic acid, 5% phenol, 10% H<sub>2</sub>SO<sub>4</sub>, 1 or 10% ammonia, or 3% NaOH. Anesthetized animals received 0.1 ml of butacaine sulfate (2%) topically in the right eye (excess removed) prior to instillation of the irritant. Opacity, iritis, and redness responses were determined according to 16 CFR 1500.42 at 1, 2, 7, 14, or 21 days postexposure. Animals were then sacrificed by sodium pentobarbital injection and the corneas removed at the above times. Corneal water content, dry weight, and disc gel electrophoretic protein patterns were determined as described previously (15th Annual Meeting of the Society of Toxicology). Significant differences (10% acetic acid and 10% ammonia only) in mean corneal opacity scores were noted in four of 29 comparisons among all irritants at

the selected times postexposure. In each instance, where there was a difference, the nonanesthetized eye had a lower opacity score than did the anesthetized eye. With regard to mean moisture content, three significant differences (5% acetic acid, 1% ammonia, and 3% NaOH) were found in 24 comparisons. The nonanesthetized corneas had a lower water content with 5% acetic acid and 1% ammonia and a greater water content with 3% NaOH. No significant differences in dry weight or in corneal electrophoretic protein patterns were noted with any irritants tested. Overall, neither opacity scores nor moisture contents produced a clear-cut pattern of statistical significant differences for anesthetized vs nonanesthetized corneas. Based on these data, the use of a local anesthetic, e.g., butacaine SO<sub>4</sub>, should be considered when doing eye irritation tests.

144. *Preclinical Toxicological Evaluation of Bruceantin (NSC-165563) in Mice, Dogs, and Monkeys.* T. R. CASTLES, R. L. BRIDGES, J. C. BHANDARI, and C. C. LEE, Pharmaceutical Toxicology Section, Midwest Research Institute, Kansas City, Missouri.

Bruceantin, a quassinoid isolated from the plant *Brucea antidysenterica*, has been reported to have potent antileukemic activity on mice. Its iv LD<sub>50</sub> values in male and female mice (BDF/1 strain) were 1.95 and 2.58 mg/kg, respectively. In beagle dogs, single iv doses of 1.0 or 0.5 mg/kg caused death within 12 to 18 hr. Immediate toxic signs included emesis, salivation, rhinorrhea, and a persistent erythema (up to 3 hr). Toxic signs (edema, diarrhea, and hematochezia) after 5 hr suggested an effect on peripheral vasculature. Hemorrhages were found in several tissues at necropsy. In dogs, daily doses of 0.25 mg/kg/day produced similar signs and death within 3 days. Daily doses of 0.416 mg/kg/day in rhesus monkeys produced toxicity similar to that seen in dogs, and death occurred between Days 4 and 6. Toxic doses in dogs produced changes in hematology (anemia, granulocytosis, granulocytopenia, reticulocytopenia, and/or thrombocytopenia) and blood chemistry (increased BUN, BSP retention, alkaline phosphatase, SGOT, SGPT, serum creatinine, and prothrombin time). Except for the absence of anemia, hematologic, and blood chemistry changes in monkeys were similar to those observed in dogs. Major pathology in both species was found in the liver, kidneys, hematopoietic, and lymphoid tissues. Toxicity appeared to be reversible in both species. In dogs, bruceantin toxicity was not cumulative if 7 to 9 days of rest were allowed between single or multiple dose regimens. (Supported by a subcontract from the Battelle Columbus Laboratories, under Contract No. N01-CM-43746 from the Division of Cancer Treatment, National Cancer Institute, NIH.)

145. *Effect of Monomethylhydrazine on Plasma Glucose, Plasma Insulin, and Liver Glycogen.* M. E. GEORGE and K. C. BACK, 6570th Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Exposure to monomethylhydrazine (MMH) is known to affect glucose metabolism in rats although the exact mechanism is not known and reports in the literature reflect a wide disparity in experimental results. The variety of experimental conditions that were utilized could explain, at least in part, the differences in the results reported. Some of the factors that could affect glucose response to MMH were the mode of exposure, length of exposure, anesthesia used, the convulsigenic action of MMH, and the amount of liver glycogen stores prior to exposure. There have also been some data reported indicating that a decrease in insulin release following MMH may be a causative factor. The series of experiments reported here were performed to define the effect of MMH on plasma glucose, plasma insulin, and liver glycogen concentrations and to determine the influence of various experimental parameters on the glucose response. Anesthesia was not required and all MMH exposures were below convulsigenic levels, canceling the effects of these two parameters. MMH was given in a single ip injection or by iv infusion over a period of time, to rats that had been fasted, allowed food *ad libitum*, or given exogenous glucose. Rats that had been fed or fasted to deplete liver glycogen showed a hyperglycemic response to MMH exposure although the degree of response differed. Both methods of exposure, ip and iv, produced hyperglycemia but the response occurred at lower exposure

levels when MMH was continuously infused. There was no effect on insulin concentrations. MMH also appeared to interfere with glycogenesis in rats given exogenous glucose. These data indicate the hyperglycemia response to MMH exposure is not related to decreased plasma insulin concentrations or an increased rate of glycogenolysis.

146. *Detection of Membrane Protein Modifications by Toxicants Using <sup>14</sup>C Chemical Probes and SDS Polyacrylamide Gel Electrophoresis (SDSPAGE)*. D. ROURKE, E. P. PITZ, I. ROSENBLUM, R. JONES, and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Methods are being developed to (a) detect subtle changes in cellular enzymes (proteins) due to xenobiotics, (b) identify specific enzymatic pathways effected by toxicants, and (c) relate these changes to toxicity. The model system consists of *in vitro* incubation of erythrocytes (RBCs) with MeHgCl or <sup>14</sup>C chemical probes or a combination of these. RBC ghosts and hemolyzates are then prepared for analysis (Dodge *et al.*, *Arch Biochem. Biophys.* **100**, 119, 1963). The analysis consists of (a) scintillation counting of solubilized (Soluene-350) ghosts after reaction of [<sup>14</sup>C]iodoacetic acid (IAC) or [<sup>14</sup>C]iodoacetamide (IAM) with RBCs in the presence and absence of MeHgCl, (b) SDSPAGE of host and hemolyzate preparations after treatment with IAC or IAM in the presence and absence of MeHgCl. MeHgCl (0.025 mmol) inhibited interaction of [<sup>14</sup>C]IAC with ghosts (-46%), while it enhanced the interaction of ghosts with IAM (+36%). SDSPAGE of ghost preparations shows that MeHgCl and IAC act summatively to cause the disappearance of band III (Fairbanks *et al.*, *Biochemistry* **10**, 2106 1971), the RBC anion transport protein. MeHgCl, IAC or IAM alone, or IAM and MeHgCl in combination do not cause the effect. These results implicate MeHgCl in subtle effects on the anion transport system. MeHgCl affects the disappearance of two bands from the SDSPAGE patterns of hemolyzates. IAM or IAC alone affect greater changes in the lower molecular weight bands of RBC ghosts than does MeHgCl. These results are discussed in light of methods being developed to use SDSPAGE to determine specific enzymes associated with toxic effects. (Supported by Research Grant 2-P01-ES00226-10 from the National Institutes of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant 2-T01-ES00103-10.)

147. *A Method for Comparative Testing of Smoke Toxicity*. W. J. POTTS and T. S. LEDERER, Toxicology Laboratory, The Dow Chemical Co., Midland, Michigan. (E. S. Harris)

Improved methods for testing the inhalation toxicity of smoke have been developed. These methods represent a significant improvement over previous methods in that they: (1) recognize the importance of and make provision for testing pyrolysis and combustion as strictly separate phenomena, which gives more reproducible data and presents a sounder rationale on which to extrapolate laboratory tests to large fires; (2) have solved the problem of ensuring that the smoke is not fractionated in the testing process, while at the same time not overheating the air in the test chamber; and (3) allow dose-response data to be obtained, thus making it possible to obtain comparative data among materials. This report gives inhalation toxicological data for the smoke produced from the pyrolysis and combustion of 10 materials.

148. *Automatic Processing of Clinical Laboratory Data in a Small Laboratory*. JAMES J. BARE, RONALD D. PLATT, and BARRIE M. PHILLIPS, Toxicology and Management Sciences Departments, Miles Laboratories, Inc., Elkhart, Indiana.

Large amounts of clinical laboratory data are generated over a prolonged period of drug administration in toxicity studies. It is often difficult to conduct a systematic and thorough data evaluation without computer assistance. Various approaches to data analysis by computer have been formulated (Drew *et al.*, *Tox. Appl. Pharm.* **22**, 284, 1972; Small *et al.*, *Drug Inform. J.*, January/June, **27**, 1973; and Shirasu *et al.*, *Tox. Appl. Pharm.*, **25**, 495, 1973) that utilize automated instruments on-line with computer systems. This report describes a computerized data



analysis system for a smaller laboratory not having analytical instruments on-line. The system can be implemented on the typical in-house batch processing computer. Features of the system are: (1) production status, resulting in a 12–24-hr turnaround time without specialized analyst intervention; (2) daily laboratory record sheets, which are computer source documents, and hence require no recopying or coding of data; (3) comprehensive statistical evaluation to indicate drug effects; (4) modular system amenable to addition of algorithms for other types of studies; (5) interim reports can be generated any time during a study; (6) a normal value file, by species, sex, and parameter, is updated with each new set of data; (7) all data are retained in a history file for future reference; (8) computer output suitable for incorporation into internal formal reports and for submission to government regulatory agencies without revision.

149. *Reproductive Toxicity of Cadmium.* C. DWIVEDI, D. N. SINGH, E. P. CRUMP, and R. D. HARRISON, Department of Pediatrics, Meharry Medical College, Nashville, Tennessee, and Center in Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Cadmium is a recognized health hazard and an etiological factor in various pathological processes, including testicular necrosis. It produces sterility when administered to rats at a dosage of 10  $\mu\text{mol/kg}$ . It has been shown that the inhibition of spermatozoa choline acetyltransferase activity impairs spermatozoa function and produces sterility. Choline acetyltransferase activity in spermatozoa from various segments of the epididymis was measured by the formation of [ $^{14}\text{C}$ ]acetylcholine from [ $^{14}\text{C}$ ]acetyl-CoA and choline. Cadmium at a dosage of 5  $\mu\text{mol/kg}$  ip produced 16% inhibition in corpus and 44% inhibition in cauda spermatozoa. Cadmium, however, at a dosage of 10  $\mu\text{mol/kg}$  ip produced 27% inhibition in corpus and 80% inhibition in cauda spermatozoa choline acetyltransferase activity 72 hr after treatment. At these dosages, the caput was devoid of spermatozoa. Cadmium at a dosage of 10  $\mu\text{mol/kg}$  also depressed spermatogenesis about 50%. Degeneration of sex vesicles, precocious segregation of bivalents and increased production of tetraploid cells was noted. No significant effects were observed at lower dosages; however, a chronic dosage of 1  $\mu\text{mol/kg}$  for 1 month produced similar effects. These results indicate that cadmium affects the activity of spermatozoa by alteration of spermatozoa choline acetyltransferase as well as depressing spermatogenesis. (Supported by USPHS Grant No. 440 from Health Services and Mental Health Administration and USPHS Grant No. ES00267.)

150. *A Simple Behavioral Assay for Use with Avian Species.* HUGH L. EVANS and VICTOR G. LATIES, Environmental Health Sciences Center and Department of Radiation Biology and Biophysics, University of Rochester, Rochester, New York. (Thomas W. Clarkson)

Avian species have proven useful in several areas of behavioural research, notably in sensory and pharmacologic studies. A similar contribution to toxicology can be expected to accompany the growth of behavior research in that field. Traditional methods for birds provide for complex and detailed evaluation, but are time-consuming and costly. These sophisticated techniques could be of greater utility to the toxicologist if there also were simpler techniques for rapid preliminary screening, as are available for rodents. We now describe a new technique measuring the pigeon's rate and accuracy of pecking grain, that is, reliable, yet brief, simple, and inexpensive. Male white Carneaux pigeons (*Columba livia*) were presented, in their home cages, with a fixed number of grains (usually milo and hemp) in a clear glass dish. Dimensions of 8.5 cm diameter and 5.5 cm depth have proven suitable. An observer records, with stopwatch and tally counter, the time and number of pecks required to consume the grain. The test could be automated. High correlations between two independent observers were obtained. Methylmercury was administered, po, Monday through Friday, until clear behavioral effects were observed (usually 6–8 weeks). Acute doses of ethanol (po) and scopolamine (im) also were studied. Results, in terms of response rate and accuracy, suggest two independent features of behavioral performance. In all cases, the new test proved sensitive to doses similar to those required to alter behavior with conventional operant techniques.

151. *Teratogenic and Lethal Effects in the Rat Fetus following Absorption of Hexafluoroacetone from Maternal Skin.* MAVIS R. BRITTELLI, RUDOLF CULIK, and O. LOUIS DASHIELL, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware. (J. J. Clary)

Hexafluoroacetone (HFA) was applied daily to the skin of pregnant rats from Day 6 through 16 of gestation at doses of 2.3–90 mg/kg/day in a range-finding study and 1, 5 and 25 mg/kg/day in the main study. All rats were sacrificed on Day 21 of gestation. Uterine weights, fetal survival, number of resorptions, live fetal weights plus crown–rump lengths, and gross abnormalities by external examination were determined at necropsy. The fetuses were processed for examination of visceral/neural and skeletal abnormalities. At 40 mg/kg and above all but one of 36 fetuses were resorbed. At 90 mg/kg signs of maternal toxicity were observed. At 25 mg/kg there were 58 live fetuses, 6 dead, and 66 resorptions. At 5 and 25 mg/kg the mean fetus weights and crown–rump lengths were significantly less than the controls. Maternal weight gain was adversely affected at 5 and 25 mg/kg. At all nonlethal doses, teratogenic effects were observed. HFA has an adverse effect on maternal weight gain due to smaller fetuses and fewer live fetuses at 5 and 25 mg/kg. Embryo toxicity, measured by number of resorptions and liver fetuses per litter, is evident at 25 mg/kg. At 40 mg/kg HFA is embryolethal. At lower doses HFA is a potent teratogen. Teratogenic effects consist of gross external and internal soft tissue abnormalities at 1 to 25 mg/kg and skeletal abnormalities at 5 to 25 mg/kg.

152. *Accumulation of Lower Chlorinated PCBs in Fetal Intestine.* G. W. LUCIER and H. B. MATTHEWS, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (R. L. Dixon)

Although much is known about the fate of specific PCB isomers in adult animals, little information is available concerning the pharmacology of these isomers in perinatal systems. Therefore, we have studied specific fetal and newborn tissue distribution of several <sup>14</sup>C-labeled chlorinated biphenyls: 4-chloro-(1-CB); 4,4'-dichloro-(2-CB); 3,4-3'4'-tetrachloro (4-CB); 2,3,6-2',3',6'-hexachloro-(6-CB'); and 2,4,5-2',4',5'-hexachloro-(6-CB). Pregnant rats were administered the labeled PCBs on Day 18 of gestation and the radioactivity was measured in maternal blood, liver, intestine, fat, skin, muscle, fetal blood, liver, lung, intestine, kidney, brain, skin, reproductive tract, and placenta. PCBs that do not accumulate significantly in adipose tissue do accumulate dramatically in fetal intestine. Fetal intestine to blood radioactivity ratios on Day 21 of gestation (3 days after treatment) were as follows; 1-CB(41), 2-CB(12), 6-CB'(18), 4-CB(3), 6-CB(1). Values for 1-CB on Days 20 and 19 of gestation were 20 and 7, respectively. By 5 days after birth, intestine to blood ratios were approximately 1 for all PCBs. When 1-CB was administered on Day 15 of gestation, intestinal accumulation still did not occur until Day 19 of gestation, corresponding with the developmental onset of hepatic glucuronyl transferase. Approximately 8% of the fetal intestinal radioactivity following 1-CB treatment was due to the parent compound, 10% 4'-hydroxy-4-chlorobiphenyl, and most of the remainder represented conjugates. 6-CB, which does not accumulate in the fetal intestine, is secreted in the milk although significant newborn tissue concentrations of this isomer also result from placental transfer. Newborn skin and fat contained the highest concentrations of 6-CB after gestational and/or milk exposure.

153. *Effects of Food Restriction on the Teratologic Response of CD-1 Mice.* R. D. HEMM, L. ARSLANOGLU, and J. J. POLLOCK, Ayerst Research Laboratories, Drug Safety Section, Chazy, New York.

Following food restriction of pregnant CD-1 mice, the maternal serum corticosterone concentrations and incidence of cleft palate in the progeny were determined. Dams from the groups receiving restricted food on gestation Days 6–15 had increased resorptions, and the progeny had delayed ossification of the skeleton with cleft palate accompanied by delayed ossification of the presphenoid bone. The incidence and intensity of these changes were generally correlated with the level of food restriction. The incidence of cleft palate for dams receiving 4.0 to 2.5 g of

feed per day ranged from 6.3 to 100.0%, respectively. Serum corticosterone concentrations of pregnant control and food-restricted dams were similar on gestation Day 10, but the concentrations of food-restricted dams were 6 to 10 times greater than controls on gestation Day 15. The cleft palate induced by food restriction in the mouse may thus be secondary to stress and the elevation of endogenous serum corticosteroid concentrations. Care should be taken in interpreting teratology data to differentiate potential secondary drug effects on food consumption from direct effects on embryogenesis.

154. *Inhalation Teratology Studies of Five Chemicals in Rats.* J. V. DILLEY, N. CHERNOFF, D. KAY, N. WINSLOW, and G. W. NEWELL, Stanford Research Institute, Menlo Park, California, and Environmental Protection Agency, Research Triangle Park, North Carolina.

Pregnant rats were exposed daily to chloroform vapors or aerosols of ethylenethiourea, Thimet, Simazine, or Bromacil during Days 7 through 14 of gestation. The highest concentrations for each exposure were: chloroform,  $20.1 \pm 1.2$  g/m<sup>3</sup>; ethylenethiourea,  $120.4 \pm 8.0$  mg/m<sup>3</sup>; Thimet,  $1.94 \pm 0.48$  mg/m<sup>3</sup>; Simazine,  $317 \pm 89$  mg/m<sup>3</sup>; and Bromacil,  $165 \pm 6$  mg/m<sup>3</sup>. Two lower concentrations of each compound were used simultaneously with the highest dose, with all vapor/aerosol exposures generated from a single source for each compound. All animals were sacrificed on Day 20 of gestation, and the dams and fetuses were examined for gross changes. Fetuses were fixed in Bouin's solution or alcohol and examined later for teratology. Chloroform, ethylenethiourea, and Thimet caused increased fetal mortality and decreased fetal weight gain. Simazine and Bromacil did not cause any prenatal changes. None of the compounds was teratogenic at the concentrations used and under these experimental conditions. (Supported by the Environmental Protection Agency under Contract No. 68-02-1751.)

155. *In Vitro Mutagenic Activity of Fourteen Pesticides.* D. C. POOLE, V. F. SIMMON, and G. W. NEWELL, Stanford Research Institute, Menlo Park, California.

As part of the EPA's substitute pesticide program, mutagenic studies are being conducted on many pesticides. In the current study, 14 pesticides were evaluated for their mutagenic potential in five microbial assay systems. The 14 pesticides tested were: Aspon, Acephate, Carbofuran, Chloropyrifos, Crotoxyphos, Diazinon, Dicamba, Demeton, Disulfoton, Fensulfothion, Fonofos, Trichlorophon, Siduron, and Methoxychlor. The microbial assay systems used were the histidine reverse mutation system in five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, and TA100), the tryptophan mutation system in *Escherichia coli* WP2, the mitotic recombination assay in *Saccharomyces cerevisiae* D3, and relative toxicity assays in *Escherichia coli* and *Bacillus subtilis*. A mammalian metabolic activation system using the liver of rats that had been treated with Aroclor 1254 was used in all assays except the *E. coli* and *B. subtilis* relative toxicity assays. Demeton increased the mutation frequency in *S. typhimurium* TA1535 and TA100 and in *E. coli* WP2 and increased mitotic recombination in *S. cerevisiae* D3. Trichlorophon and acephate increased the mutation frequency in *S. typhimurium* TA100 and *E. coli* WP2 and the mitotic recombination frequency in *S. cerevisiae* D3. Crotoxyphos increased the mitotic recombination frequency in *S. cerevisiae* D3. None of the other pesticides was mutagenic. The relative toxicity assays have not yet been completed. (Supported by EPA Contract 68-01-2458.)

156. *Study of the Mutagenic Potential of bis(2-Chloroethyl) and bis(2-Chloroisopropyl) Ethers in Mice by the Heritable Translocation Test.* T. A. JORGENSEN, C. J. RUSHBROOK, G. W. NEWELL, and R. G. TARDIFF, Stanford Research Institute, Menlo Park, California, and EPA/HERL, Cincinnati, Ohio.

Among the various chemicals identified by the EPA in drinking water are bis(2-chloroethyl) and bis(2-chloroisopropyl) ethers. Heritable translocation tests in mice were conducted to determine the mutagenicity of these two compounds. Adult male mice were treated by gavage daily for 8 weeks at three dose levels per compound. Reference controls were included as well

as a positive control group receiving TEM in the drinking water for 4 weeks. After treatment, each male was mated to two virgin females to produce an F<sub>1</sub> generation, the males of which were raised to maturity. One hundred F<sub>1</sub> males per treatment group then were selected and bred to three virgin females each. The pregnant females were evaluated against predetermined selection criteria to identify suspect F<sub>1</sub> males. These suspect F<sub>1</sub> males then were rebred to three additional virgin females each. Presumptive F<sub>1</sub> males after two breedings then were examined cytogenetically. All breeding data were evaluated and correlated with cytogenetic examinations. No positive reciprocal translocations were observed in the control of bis(2-chloroethyl) ether-treated groups. Preliminary evaluation of the data suggests that heritable translocations did not occur in either bis/chloro ether compound. (Supported by EPA Contract 68-03-2203.)

157. *Munitions Wastewater Recycling: Do Bacteriocidal Treatments Produce Microbial Mutagens?* V. F. SIMMON, S. L. ECKFORD, A. F. GRIFFIN, R. SPANGGORD, and G. W. NEWELL, Stanford Research Institute, Menlo Park, California.

A number of compounds present in wastewater from munitions plants were examined before and after bacteriocidal treatments (ozonation or chlorination) to determine whether they were mutagenic before treatment and/or whether the mutagenic activity, if any, was decreased, increased, or unaltered by treatment. Several photolytic or metabolic products of TNT were also examined for mutagenic activity. Test compounds included TNT, TNT condensate water, and components of condensate water (1,3-dinitrobenzene, 2,4-dinitrotoluene, 3,5-dinitrotoluene), RDX, HMX, photolysed TNT (pink water), PETN, and trinitroresorcinol. Two reagents used to determine chlorine concentration, Syringaldazine and the sulfate and oxalate salts of *N,N*-dimethyl-*p*-phenylenediamine, were also tested for mutagenicity. The *in vitro* mutagenic assays used were histidine reverse mutation in five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, and TA100) and mitotic recombination in the yeast *Saccharomyces cerevisiae*. A mammalian metabolic activation system using the liver of rats that had been pretreated with Aroclor 1254 was used in each assay. The bacteriocidal treatments in most cases did not affect mutagenicity. Some of the products of TNT were mutagenic. (Supported by USAMRDC, Department of the Army, Contract No. DAMD-17-17-C-6013).

158. *Possible Mutagenic Effects of 2,4-Dinitrotoluene: A Dominant Lethal Study in the Rat.*

GLENN S. SIMON, ROBERT G. TARDIFF, and JOSEPH F. BORZELLECA, Department of Pharmacology, Division of Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia, and Health Effects Laboratory, Environmental Protection Agency, Cincinnati, Ohio.

The discharge of 2,4-dinitrotoluene (2,4-DNT) into rivers and streams from munitions plants raises concern about the potential adverse health effects in exposed humans. Of particular interest are the effects upon reproductive performance and mutagenesis. Forty male Sprague-Dawley rats, weighing 330–390 g, were divided into four equal groups. Two groups received either 10 or 40 mg/kg/day of 2,4-DNT dissolved in corn oil. One group received corn oil. The fourth group (positive control) received 0.5 mg/kg/day triethylenemelamine (TEM) dissolved in corn oil. Test materials were administered po for 10 consecutive days. Following a 2-day rest, each male was mated with two naive females per week for 14 weeks. Copulation was determined by the presence of sperm in the vagina. Females were sacrificed on Day 14 of gestation. TEM showed statistically significant ( $p \leq 0.05$ ) decreases in the proportion of pregnant females, and the proportion of viable to nonviable fetuses during the first 4 weeks of the experiment. There was a significant decrease in the numbers of corpora lutea the first week, and in the numbers of implantation sites during the first 3 weeks. At the lower dose, 2,4-DNT caused significant decreases in the numbers of implantation sites during Weeks 6 and 7. A significant increase in pre-implantation losses, and a significant decrease in the fetal index were seen during Week 7. The high dose of 2,4-DNT caused significant decreases in the numbers of implantation sites and the fetal index during Week 7. During Weeks 8, 9, and 13, there were significant increases in the proportions of viable to nonviable fetuses. There was a significant increase in the proportion of females bearing one or more, and two or more nonviable fetuses during Week

13. Additionally, statistical significance was approached in some of these parameters during Weeks 7 through 12. These effects were probably caused by spermatozoa that existed as stem cell spermatogonia and gonocytes when 2,4-DNT was administered. These data suggest that 2,4-DNT is not a potent mutagen as determined by dominant lethal mutations. (Supported by EPA Grant No. R-804290010.)

159. *Smoke Exposure of Rodents: Correlation between Renal Aryl Hydrocarbon Hydroxylase Activity and Mutagenesis in S. typhimurium.* M. H. BILIMORIA, J. JOHNSON, J. C. HOGG, and H. P. WITSCHI, Pathology Institute, McGill University, and Department of Pharmacology, Université de Montréal, Montreal, Canada.

Inhalation of tobacco smoke by various rodent species usually increases the activity of aryl hydrocarbon hydroxylase (AHH) in lung. Several reports also indicate that smoke exposure enhances renal AHH activity. We exposed male, random-bred guinea pigs and male Sprague-Dawley rats to the smoke from five cigarettes within a 1-hr period. In both species, renal AHH activity was significantly increased 3 hr after smoke exposure. Peak values were measured after 6 hr in rats, when AHH activity was 12 times higher than in controls. In guinea pigs, peak activity was seen after 12 hr and was four to five times higher than control values. After 24 hr, AHH activities had returned to normal. From both rat and guinea pig kidneys, 9000g supernatants were prepared and tested for their ability to convert 2-aminoanthracene to intermediates mutagenic for *S. typhimurium* TA98. It was found that 9000g supernatant from guinea pig kidneys had a greater capability to transform 2-aminoanthracene than had rat kidney preparations. Moreover, preparations from kidneys of guinea pigs exposed to tobacco smoke were two to three times more active than preparations from animals not exposed to tobacco smoke. In rats, no difference was found in the *S. typhimurium* assay between controls and smoke-exposed animals. The observations suggest that it might be possible to assess biological activity of tobacco smoke using suitable preparations obtained from kidneys of smoke-exposed animals.

160. *Experimental Human Exposures to Methyl Chloride at Industrial Environment Levels.* C. L. HAKE, R. D. STEWART, A. WU, H. V. FORSTER, and P. E. NEWTON, The Medical College of Wisconsin, Milwaukee, Wisconsin.

Adults of both sexes were exposed repetitively on a daily basis to methyl chloride gas in air concentrations of 0, 20, 100, and 150 ppm for periods of 1, 3, and 7.5 hr in a controlled-environment chamber for two purposes: (1) to develop a practical "biologic" test which would indicate the magnitude of an industrial exposure, and (2) to monitor the physiological responses of healthy adults to different vapor concentrations and durations of exposure. These studies were designed to simulate the types of exposure encountered in the industrial setting and consisted of both steady, nonfluctuating concentrations of the gas in air, as well as widely fluctuating concentrations. Physiological, neurological, behavioral, clinical, and medical studies revealed no deleterious effects of the methyl chloride exposures during these studies. Blood and breath analyses revealed that the subjects breathing an atmosphere contaminated with methyl chloride gas could be divided into two groups; the majority of subjects had methyl chloride blood and breath concentrations two to six times lower in concentration than did three of nine male and one of nine female subjects. This important phenomena should encourage the use of breath monitoring to identify the employee who consistently carries a higher body burden of methyl chloride upon exposure to this gas. Although we found no deleterious responses in any of our subjects at any magnitude of exposure that could be attributed to methyl chloride, it is possible that the physically stressed worker, who in addition carries a higher body burden than the majority, may be adversely affected by exposure to the recommended TWA concentration.

161. *Subacute Inhalation Toxicity Studies on Eight Fluorocarbons.* H. J. TROCHIMOWICZ, B. L. MOORE, and T. CHIU, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Inc., Newark, Delaware. (J. J. Clary)

Fluorocarbons containing hydrogen, considered more degradable than common commercial fluorocarbons, are being evaluated for potential use as aerosol propellants and refrigerants. However, toxicity data on these materials are limited. As a preliminary indication of inhalation

toxicity, 10 ChR-CD male rats were exposed for 6 hr/day, 5 days/week, for 2 weeks to air (control) or a sublethal level of each of 8 fluorocarbons: dichlorofluoromethane (FC-21), chlorofluoromethane (FC-31), difluoromethane (VC-32), 1,1-dichloro-2,2,2-trifluoroethane (FC-123), 2-chloro-1,1,1,2-tetrafluoroethane (FC-124), 1,2-dichloro-1,1-difluoroethane (FC-132b), 1-chloro-1,1-difluoroethane (FC-142b), and 1,1-difluoroethane (FC-152a). After the last exposure and at 14 days postexposure, five rats from each test and control group were given clinical laboratory examinations (hematology, blood chemistry, and urine analysis) and sacrificed, and tissue samples were taken for histopathological evaluation. Under these experimental conditions, exposure to FC-32 (20%, v/v), FC-123 (1%, v/v), FC-124 (10%, v/v), FC-142b (1%, v/v), and FC-152a (10%, v/v) produced no adverse changes relative to the preceding indices. However, rats exposed to FC-21 (1%, v/v) did show extensive liver damage which was not completely reversible at 14 days postexposure. FC-31 (1%) produced moderate damage to kidneys, adrenals, testes, epididymis, and hemopoietic tissues. Slight effects on liver morphology, thymus and testes were observed in rats exposed to FC-132b (1%, v/v) but appeared to be reversible. Thus, although more degradable, hydrogen-containing fluorocarbons like FC-21 and FC-31 appear to be substantially more toxic than halogenated fluorocarbons now in commercial use.

162. *Subacute Inhalation Studies of Sevoflurane and Halothane in Macaca speciosa*. DONALD C. SAWYER and RICHARD F. WALLIN, Michigan State University, East Lansing, Michigan, and Baxter Travenol Laboratories, Inc. Morton Grove, Illinois. (J. E. Gibson)

Two groups of 12 monkeys ( $6.5 \pm 0.5$  kg) were anesthetized with sevoflurane (fluoromethyl 2,2,2-trifluoro-(trifluoromethyl)ethyl ether) or halothane at 1.5 MAC, 3 hr each weekday for a total of 30 hr per animal. Eight monkeys were held 24 days after the 10th exposure and anesthetized for 5 daily 3-hr periods. Hematology and serum chemistry determinations were made before, during, and after exposure. ECG, EEG, arterial pressure,  $P_{aCO_2}$ ,  $P_{aO_2}$ ,  $pH_a$ ,  $\dot{V}_T$ , and  $\dot{V}_E$  were also recorded. Determinations were made to confirm anesthetic concentrations and to assess soda lime reactivity with sevoflurane. No significant changes were observed in hematology or serum chemistry values except in the sevoflurane group, where inorganic fluoride values were elevated from control levels. Gross and histologic examination of tissues revealed no lesions that could be related to sevoflurane, while fatty infiltration of the liver was seen in the halothane group. At equipotent concentrations, halothane was slightly more hypotensive than sevoflurane and produced mild respiratory acidosis.  $\dot{V}_E$  and  $\dot{V}_T$  were both higher in the sevoflurane group. Intermittent periods of electrographic silence were observed only during sevoflurane anesthesia. No significant ECG changes were seen in either group. The inspired concentration of the dehydrofluorination product of sevoflurane averaged  $14 \pm 4$  ppm at total gas flow of  $300 \text{ cm}^3/\text{min}$ . Based on these studies, no adverse effects were observed with either agent. Because of rapid induction and recovery, low solubility, cardiorespiratory stability, and apparent safety, sevoflurane is considered advantageous as a new volatile liquid anesthetic.

163. *Metabolic Fate of Bromodichloromethane in Rats and Rhesus Monkeys*. C. C. SMITH, W. W. WEIGEL, and G. F. WOLFE, Department of Environmental Health, University of Cincinnati, College of Medicine, Cincinnati, Ohio.

Bromodichloromethane (BDC), an analog of chloroform detected regularly in potable water supplies obtained by chlorination of surface waters, has been studied in rats and monkeys following single oral or intravenous doses. The compound was labeled with  $^{14}\text{C}$  and dissolved in Emulphor:ethanol: $\text{H}_2\text{O}$  (1:1:8, v/v/v), a solvent which worked equally well for oral or iv administration. In rats, total recovery from the urine, organs, and carcass was 27% (as  $^{14}\text{C}$ ) at 3 hr and 22% at 6 hr following iv doses of 10 mg/kg,  $\frac{1}{2}$  to  $\frac{2}{3}$  of the  $^{14}\text{C}$  being recovered in the fat. Only traces (0.1–0.3%) appeared in the urine. Following single oral doses of 20 mg/kg, total recovery from carcass and GI tract accounted for 32% at 3 hr and 41% at 6 hr. In these rats, most of the  $^{14}\text{C}$  in the body was recovered from the stomach; fat contained more than any of the other tissues and no more than 1% of the  $^{14}\text{C}$  appeared in the urine. In monkeys, BDC

had a half-life of 4 to 6 hr whether the compound was given orally or iv, and peak blood concentrations varied from 1 to 2 mg/liter (as  $^{14}\text{C}$ ). Total recovery of  $^{14}\text{C}$  in the urine varied from 2 to 3% except for one monkey, in which 6% was recovered. It is clear from these data that BDC is cleared rapidly by both rats and monkeys and is probably exhaled either as parent compound or as a metabolite such as  $\text{CO}_2$ . (Supported in part by Environmental Protection Agency Grant No. R803963.)

164. *Ninety-Day Inhalation Toxicity Studies on Two Fluorocarbons*. H. J. TROCHIMOWICZ, J. P. LYON, D. P. KELLY, and T. CHIU, Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Inc., Newark, Delaware. (J. J. Clary)

As part of a continuing testing program to evaluate hydrogen-containing fluorocarbons for potential use as aerosol propellants and refrigerants, ChR-CD rats (27/sex/level) and male beagle dogs (4/level) were exposed to 0.1 and 1.0% (v/v) 1-chloro-1,1-difluoroethane (FC-142b) or to 0.1 and 0.5% (v/v) dichlorofluoromethane (FC-21) for 6 hr/day 5 days/week for 90 days. Under these experimental conditions, no adverse clinical, hematological, blood chemistry, urine analytical, or histological changes attributable to FC-142b were observed in rats or dogs at either test level. However, rats exposed to FC-21 showed a bilateral hair loss at 0.1% (7 of 54) and 0.5% (16/54) and an excessive mortality (20 of 54 at 0.1%, 15 of 54 at 0.5%) during the study. Histopathological examinations of rats exposed to FC-21 at either test level revealed extensive liver damage. The only significant effects observed in dogs exposed to FC-21 occurred at the high test level only and included a slight weight loss during exposure and minimal morphological change in the liver. Thus, FC-21 appears to be substantially more toxic than commercial fluoromethanes containing no hydrogen.

165. *Effects of Food Deprivation in Rats Previously Exposed to Mirex*. D. C. VILLENEUVE, A. YAGMINAS, I. CHU, and I. A. MARINO, Biochemical Toxicology Section, Environmental Health Directorate, Health Protection Branch, Ottawa, Canada.

The present study was carried out as part of a general program designed to determine the effects of food deprivation on the toxicity of pesticides. Adult male rats were dosed daily with Mirex by gavage for 14 days at levels of 0, 0.1, 1.0, and 10 mg/kg. The food intake of these animals was then reduced to 25% of their normal consumption. After 8 days the animals were anesthetized and blood was removed via the abdominal aorta. Following death, organs were weighed and stored frozen pending residue analysis. Liver was analyzed for microsomal enzyme activity and a portion examined microscopically for lesions. Routine hematology was carried out on all blood samples and serum analyzed for sorbitol dehydrogenase and lactic dehydrogenase activities. Food restriction caused mobilization of the Mirex stored in fat depots and resulted in increased Mirex levels in the brain, heart, and spleen. No hematologic abnormalities were observed. Mirex was observed to induce microsomal enzyme activity at all levels tested, an effect which was not increased by food deprivation. Lactic dehydrogenase activity was increased at 1.0 and 10 mg/kg and sorbitol dehydrogenase activity increased at 10 mg/kg in animals on normal food intake. The same effects were observed in animals subjected to restricted food intake. Food deprivation did not accentuate Mirex-induced liver hypertrophy or fatty infiltration. The results from this study indicate that although the tissue distribution of Mirex was altered by food deprivation, there was only slight increase in the biological activity of this compound.

166. *Three-Generation Reproduction Study of Rats Ingesting 2,3,7,8-Tetrachlorodibenzo-p-dioxin*. F. J. MURRAY, F. A. SMITH, K. D. NITSCHKE, C. G. HUMISTON, R. J. KOCIBA, and B. A. SCHWETZ, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a highly toxic contaminant formed in the production of trichlorophenol. A three-generation reproduction study was conducted to evaluate the effects of chronic, low-level ingestion of TCDD. Sprague-Dawley rats were maintained continuously on diets providing dose levels of 0, 0.001, 0.01, or 0.1  $\mu\text{g}$  of TCDD/kg/day.

No signs of toxicity were noted in  $f_0$  rats of either sex during 90 days of TCDD ingestion prior to mating. The fertility of the  $f_0$  rats receiving 0.1  $\mu\text{g}/\text{kg}/\text{day}$  was decreased to an extent which, combined with a poor survival rate in the few litters born, precluded continuation of this dose level in subsequent generations. At 0.01  $\mu\text{g}/\text{kg}/\text{day}$ , fertility was significantly decreased in the  $f_1$  and  $f_2$  but not  $f_0$  generations. At 0.001  $\mu\text{g}/\text{kg}/\text{day}$ , no deleterious effect on fertility was seen in any generation. Indications of toxicity clearly evident at 0.01  $\mu\text{g}/\text{kg}/\text{day}$  among the  $f_2$  and  $f_3$  but not  $f_1$  litters included smaller litter size at birth, plus decreased survival and growth of neonates. Among litters of rats receiving 0.001  $\mu\text{g}/\text{kg}/\text{day}$ , no effect on litter size at birth or neonatal growth was observed in any generation; statistically significant increases and decreases in pup survival were seen at 0.001  $\mu\text{g}/\text{kg}/\text{day}$ . At 0.01  $\mu\text{g}/\text{kg}/\text{day}$ , the weights of the liver (relative to body weight) and thymus (relative and absolute) were increased and decreased, respectively, in the weanlings. At 0.001  $\mu\text{g}/\text{kg}/\text{day}$ , there was a trend toward increased relative liver weights. Microscopic examination of the liver, kidney, and thymus of weanlings at 0.001 or 0.01  $\mu\text{g}/\text{kg}/\text{day}$  revealed no evidence of toxicity. Dilated renal pelvises were seen in the three  $f_1$  rats at the high dose level which survived to adulthood. Sporadic increases in the frequency of dilated renal pelvis occurred in weanlings at lower dose levels in a manner which was not dose-related or consistent from generation to generation. In summary, the reproductive capacity of rats ingesting TCDD was clearly affected at dose levels of 0.01 and 0.1  $\mu\text{g}/\text{kg}/\text{day}$ , but not at 0.001  $\mu\text{g}/\text{kg}/\text{day}$ , through three successive generations.

167. *Polychlorinated Biphenyl Transport by Blood Components*. H. B. MATTHEWS, J. R. SURLS, J. G. CARVER, and M. W. ANDERSON, Pharmacology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. (J. R. Fouts)

We have previously shown that, following an iv dose, polychlorinated biphenyls (PCBs) are rapidly removed from the blood and initially stored in liver and muscle. Those PCBs which are not readily metabolized are then redistributed to skin and adipose tissue. However, these same PCBs were not readily extracted from blood by neutral solvents. Thus we have investigated the nature of the association between PCBs and blood components. *In vitro*, or after *in vivo* dosing, PCBs are associated with red blood cells (RBCs), albumin, and lipoproteins. The fraction associated with the RBCs is most rapidly removed from the blood by the tissues while the fractions associated with the plasma proteins are released more slowly. Gel filtration of plasma containing a PCB showed that the lipoprotein fraction contained a higher specific activity (dpm/mg of protein) than did albumin. Other methods of plasma fractionation, e.g., chromatography on glass powder columns and gradient centrifugation, have confirmed the affinity of the PCBs for the lipoproteins and have shown that the relative amounts of a given PCB associated with the lipoproteins and the albumin is related to the lipophilicity of the given PCB. The results of these studies imply that the association between PCBs and blood proteins is nonspecific and that the factors controlling the relative amounts of PCB associated with the different classes of proteins are biophysical rather than biochemical.

168. *PCB- and HCB-Induced Immunosuppression in Mice*. L. D. LOOSE, K. A. PITTMAN, K. BENITZ, and J. B. SILKWORTH, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Although the lymphoid histological effects induced by such environmental chemicals as polychlorinated biphenyls (PCB) would suggest a functional effect on immunological parameters, scant evidence is available delineating the influence of environmental chemicals on immune responses. The present study was undertaken to evaluate the effect of two organochlorines on humoral antibody and immunoglobulin formations in mice. Balb/c mice were fed diets containing 167 ppm of Aroclor 1242 (PCB) or hexachlorobenzene (HCB) for 6 weeks, at which time the mice received a primary immunization of 0.1 ml of 10% sheep erythrocytes (SRBC). The animals were maintained on their respective diets during the immunization period. An approximately twofold increase in primary and secondary splenic antibody plaque-forming



cell (PFC) formation was manifested in control mice as compared to that observed in mice exposed to PCB or HCB. Control mice elicited a peak primary response of 233 PFC/ $10^6$  spleen cells as compared to 99 PFC/ $10^6$  cells in the PCB-treated mice and 110 PFC/ $10^6$  cells in HCB-treated mice. Also, PCB- and HCB-treated mice had primary serum IgA concentrations which were consistently 40–80 mg/dl lower than control values throughout the primary immunization period. Mice immunized with SRBC prior to being placed on the PCB or HCB diets also elicited an impaired secondary PFC response and a slight decrease in serum IgA and IgM. Analysis of tissue contents of HCB demonstrated lung concentrations of 240  $\mu\text{g/g}$ ; thymus, 160  $\mu\text{g/g}$ ; liver, 70  $\mu\text{g/g}$ ; and spleen, 50  $\mu\text{g/g}$ . Tissue PCB concentrations in lung were 7.2  $\mu\text{g/g}$ ; thymus, 7.1  $\mu\text{g/g}$ ; liver, 3.5  $\mu\text{g/g}$ ; and spleen, 3.4  $\mu\text{g/g}$ . Morphometric analysis of the spleens did not reveal any change in size, number, or cellular populations of the germinal follicles in the PCB- or HCB-treated mice when compared to control values. An increased liver weight, perhaps as a result of the observed centrilobular and pericentral hypertrophy, was evident in the organochlorine-treated mice. It is suggested that in addition to the obvious impairment in immunological responsiveness induced by the PCB and HCB, the immune parameters may also be sensitive indicators of toxicity. (Supported, in part, by NIH Grant 2-P01-ES00226-10 from the National Institutes of Environmental Health Sciences.)

169. *Combined Effect of Food Deprivation and Hexachlorobenzene Feeding in Rats.* D. C. VILLENEUVE, M. J. VAN LOGTEN, E. M. DEN TONKELAAR, P. A. GREVE, J. G. VOS, G. J. A. SPEIJERS, and G. J. VAN ESCH, Biochemical Toxicology Unit, Environmental Health Directorate, Health Protection Branch, Ottawa, Canada, and Laboratory for Toxicology, National Institute of Public Health, Bilthoven, The Netherlands.

Food deprivation has been shown to alter the toxicity and tissue distribution of organohalogens in animals previously exposed to these compounds (Dale *et al.*, *Toxicol. Appl. Pharmacol.* **4**, 89, 1963; Villeneuve, *Toxicol. Appl. Pharmacol.* **31**, 313, 1975). However little information is available on the effects of administering such compounds to animals concomitantly exposed to food deprivation. Male and female adult rats were divided into six groups. Groups 1, 3, and 5 were fed standard control diet *ad libitum* for 2 weeks. For the next 4 weeks their respective diets contained 0, 20, and 100 ppm HCB. Groups 2, 4, and 6 were also fed a standard control diet for 2 weeks but at an intake of approximately 50% of those groups fed *ad libitum*. For the following 4 weeks the 50% level of food intake was maintained but the diets contained 0, 40, or 200 ppm HCB. After the 4 weeks of HCB exposure all animals were anesthetized, and their organs were excised, weighed, and stored pending residue analysis. Other parameters measured included food intake, body weight changes, microsomal enzyme activity (AH), and histopathological changes in the liver. Food deprivation was observed to increase the ability of HCB to cause liver hypertrophy and induce microsomal enzyme activity. HCB was shown to accumulate to a higher degree in the tissues of animals subjected to food deprivation. HCB induced hypertrophy of the cytoplasm of hepatocytes in the centrilobular area and was increased by food deprivation in male rats. These results indicate that food deprivation can alter the biological activity of HCB in rats.

170. *The Absence of Porphyria in Beagle Dogs Administered Hexachlorobenzene for Twelve Months.* E. J. GRALLA, R. W. FLEISCHMAN, Y. K. LUTHRA, M. HAGOPIAN, J. R. BAKER, H. ESBER, and W. MARCUS, Mason Research Institute, Worcester, Massachusetts, and Office of Toxic Substances, EPA, Washington D.C.

Hexachlorobenzene is known to cause acute hepatic porphyria in a variety of species including man. The toxic effects in dogs was not previously investigated. In the present study this chemical was administered in gelatin capsules to male and female beagles at 1000, 100, 10, and 1 mg/dog/day for 12 months. Mortality, anorexia, and weight loss occurred primarily at the highest but also to a lesser degree at the next lower level. After approximately 3 months, body weight stabilized or losses were regained. Clinical laboratory changes found immediately before

death in severely affected animals which may have been related to malnutrition, included anemia, hypoglycemia, hypocalcemia, and testicular degeneration. A dose-related neutrophilia appeared in the two highest dosage groups. No hepatic fluorescence was found at necropsy, indicating that these dogs were free of porphyria. Nevertheless, hepatic fluorescence was induced in female rats by feeding the material which was administered to the dogs. The most widespread pathological lesions were confined to the abdomen and included serositis; necrosis, fibrosis, and steatitis of the omentum; and lymphoid atrophy at the two highest dose levels. Nodular hyperplasia of gastric lymphoid tissue was found in all treated animals including those at 1/mg/day. Four severely affected animals at the highest dose level showed a generalized vasculitis and one had amyloidosis. One dog from each of the two highest levels had bile duct hyperplasia and subchronic per cholangitis. Bile and perirenal fat showed a time- and dose-related accumulation of HCB. The beagle dog appears to be resistant to the porphyrogenicity of hexachlorobenzene.

171. *Effects of Heptachlor on Broiler Chickens.* D. J. WAGSTAFF, J. R. MCDOWELL, and H. J. PAULIN, Food and Drug Administration and Litton Bionetics, Rockville, Maryland.

This study was done to determine feed levels of heptachlor which would not produce unacceptable tissue residues or adversely affect animal health or productivity. Technical grade heptachlor was fed to broiler chickens during the first 8 weeks of life at 0, 0.01, 0.03, 0.1, and 0.3 ppm of the ration. Residues of heptachlor and its epoxide (HE) were determined in abdominal fat, liver, and muscle at 1, 2, 4, 8, 10, 12, and 14 weeks. Residue levels rose rapidly for 4 weeks and then reached a plateau, e.g., abdominal fat at 8 weeks had four times more pesticide than the ration. When exposure ceased, residues decreased by half in 4 weeks. The apparent depletion was probably due mainly to dilution of pesticide in growing birds. Residues were not uniformly distributed among tissues; liver fat had four times more HE than abdominal fat. Health and productivity were not affected during the growing phase but some laying hens had egg binding. It is concluded that maintaining feed levels of heptachlor at less than 10% of a limit level provides a reasonable margin of safety for residues, but further study is needed to determine whether reproduction is impaired.

172. *Impaired Host-Defense in Mice Fed Aroclor 1242 or Hexachlorobenzene for Six Weeks.* L. D. LOOSE, J. B. SILKWORTH, and F. COULSTON, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

The impaired humoral immune response to sheep erythrocytes in mice fed 167 ppm of PCB or HCB for 6 weeks has been suggested to be a result of macrophage dysfunction. Further studies were initiated to determine the extent of the PCB- and HCB-induced macrophage dysfunction relative to macrophage-mediated impaired host defense. Male Balb/c mice (18-20 g) were maintained on diets containing 167 ppm of Aroclor 1242 or hexachlorobenzene for 6 weeks at which time their sensitivity to gram-negative endotoxin was evaluated. Endotoxin (*Salmonella typhosa*, Difco) was suspended in saline and was administered intraperitoneally and mortality was recorded at 24 hr. The LD50 for endotoxin was determined by log probit analysis. Control mice were demonstrated to have an endotoxin LD50 of 1000  $\mu$ g, whereas mice maintained on a diet containing 167 ppm of PCB for 6 weeks manifested an LD50 of 200  $\mu$ g and HCB-treated mice had an LD50 of 50  $\mu$ g of endotoxin. Since endotoxin detoxification has been reported to be a macrophage-mediated event, it would suggest an impairment in endotoxin inactivation as a result of the exposure to PCB or HCB. A similar increase in sensitivity to the lethal effects of a *Plasmodium berghei* (NYU-2) infection was observed in mice maintained on the diets containing 167 ppm of PCB or HCB. Inoculation of the mice fed PCB or HCB with the malarial parasite resulted in a markedly enhanced peripheral blood parasitemia and an approximate 50% reduction in mean survival time. The enhanced sensitivity to the lethal effects of the endotoxin and the malarial parasite suggest a significantly impaired host-defense in environmental chemical exposed animals. (Supported in part by NIH Grant 2-P01-ES00226-10 from the National Institutes of Environmental Health Sciences.)

173. *Results of a Two-Year Chronic Toxicity Study with Hexachlorobutadiene (HCB) in Rats.*

R. J. KOCIBA, D. G. KEYES, G. C. JERSEY, J. J. BALLARD, D. A. DITTENBER, J. F. QUAST, C. E. WADE, C. G. HUMISTON, and B. A. SCHWETZ, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

Male and female Sprague-Dawley rats were maintained on diets supplying 20, 2.0, 0.2, or 0 mg/kg/day of hexachlorobutadiene (HCB) for up to 2 years. Rats ingesting 0.2 mg/kg/day had no discernible ill effects that could be attributed to this dose level. Ingestion of the intermediate dose level of 2.0 mg/kg/day caused some degree of toxicity, affecting primarily the kidney in which increased renal tubular epithelial hyperplasia was noted. Urinary excretion of coproporphyrin was also increased at this dose level. Ingestion of the highest dose level of 20 mg/kg/day caused a greater degree of toxicity. Effects included decreased body weight gain and length of survival, increased urinary excretion of coproporphyrin, increased weights of kidneys, and renal tubular adenomas and adenocarcinomas, some of which metastasized to the lung. In this study irreversible toxicological effects, such as the development of neoplasms, occurred only at a dose level which caused significant tissue injury and other manifestations of toxicity. No neoplasms resulted with dose levels which caused no injury or only mild, reversible injury.

174. *Effects of Chronic Arsenic Exposure on Pyruvate Dehydrogenase in Rat Liver and Intestine.*

C. M. SCHILLER, B. A. FOWLER, and J. S. WOODS, Environmental Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

A metabolic consequence of chronic exposure to arsenic is altered utilization of pyruvate. Both the state 3 respiration and the respiratory control ratios are lower in liver mitochondria isolated from arsenic-exposed animals when pyruvate/L-malate oxidation is compared to that of succinate. Early studies suggested an inhibitory effect of arsenicals on the lipoic acid and dithiol moieties of two enzymes in the pyruvate dehydrogenase complex. Recent reports have demonstrated the regulation of the pyruvate dehydrogenase complex by a phosphorylation/dephosphorylation mechanism which suggested an additional site of action for arsenic. We measured pyruvate dehydrogenase (PDH) activity before (basal) and after (total) Mg activation by the stoichiometric release of CO<sub>2</sub> from [1-<sup>14</sup>C]pyruvate. Male rats were given access to deionized drinking water solutions containing 0, 20, 40, and 85 ppm arsenic as arsenate (As<sup>5+</sup>) for 3 and 6 weeks. After 3 weeks exposure, the specific activity (nmol/min-mg) of liver basal PDH was 2.84, 2.27, 2.20, and 1.49 at 0, 20, 40, and 85 ppm, respectively. The liver total PDH was 5.03, 4.34, 4.26, and 3.64 at these same doses. The ratio of the difference between the total and basal activities to the basal activity varies from 0.82 for the control animals to 1.80 for the highest dosed animals. After 6 weeks, the rat liver PDH was markedly inhibited at 20 and 40 ppm, but not at 85 ppm. In the rat intestine, PDH was inhibited after 3 weeks exposure to values of 23, 29, and 37% for the basal activity and 20, 27, and 32% for the total activity at these doses. The results indicate a decrease with increasing dose of both basal and total activity and also an increasing ease of activation with increasing dose. The lack of inhibition of PDH after 6 weeks of exposure at the highest dose may be a reflection of mitochondrial regeneration at this time and dose.

175. *Chronic Oral Toxicity of Beryllium in the Rat.* KENNETH MORGAREIDGE, GEORGE E. COX, DAVID E. BAILEY, and MICHAEL A. GALLO, Food and Drug Research Laboratories, Inc., East Orange, New Jersey. (B. L. Oser)

The incorporation of beryllium sulfate in the diet of rats at concentrations of 5, 50, and 500 mg/kg over a 2-year period had no effect on the incidence of tumorigenesis in the Wistar-derived albino rat employed in the study. This is in contrast with the known carcinogenicity of the element when administered via the respiratory tract or by injection in the same species. Beryllium sulfate by the oral route is not highly toxic to the rat. At the highest dosage rate, mild weight depression was the only overt sign noted, although analyses demonstrated urinary

excretion and bone storage in proportion to intake. Concurrent observations also showed no effect on reproduction, or lactation, nor was the compound teratogenic or mutagenic. The highest dietary level employed is estimated to represent a 10,000-fold exaggeration over that normally present in the average human diet. No clear explanation for the lack of a carcinogenic response to beryllium in the diet is apparent, but it may be inferred that this route permits some detoxication mechanism to act in a more efficient manner.

176. *Fetal Toxicity and Transplacental Transport of Ni(II) in Rats.* F. W. SUNDERMAN, JR., S. SHEN, J. MITCHELL, P. ALLPASS, and I. DAMJANOV, University of Connecticut School of Medicine, Farmington, Connecticut.

NiCl<sub>2</sub> was administered im to three groups of pregnant Fischer rats on Day 8 of gestation in dosages of 8 mg of Ni/kg (Group A, *N* = 12); 12 mg of Ni/kg (Group B, *N* = 11); and 16 mg of Ni/kg (Group C, *N* = 12). A control group (*N* = 13) of pregnant rats received an im injection of 0.4 ml of the vehicle (NaCl solution, 145 mmol/liter). The dams were killed on Day 20 of gestation. The number of live fetuses per dam (mean ± SD) was 9.7 ± 1.1 (controls); 8.9 ± 2.0 (Group A); 7.7 ± 1.9 (Group B, *p* < 0.001); and 7.0 ± 3.9 (Group C, *p* < 0.01). The ratio of dead fetuses to conceptuses was 2/128 (1.6%, controls); 6/113 (5.3%, Group A, *p* < 0.05); 8/93 (8.3%, Group B, *p* < 0.001); and 19/103 (18.3%, Group C, *p* < 0.001). No fetal malformations were found. These findings show that im administration of Ni(II) to pregnant rats caused fetal mortality at dosages that did not cause maternal mortality. <sup>63</sup>NiCl<sub>2</sub> was administered im (12 mg of Ni/kg) to three groups of four Fischer rats: (a) nonpregnant females; (b) pregnant rats on Day 8 of gestation; and (c) pregnant rats on Day 18 of gestation. The rats were killed 24 hr after injection of <sup>63</sup>Ni(II). The relative concentrations of <sup>63</sup>Ni (μg/g wet wt) in organs and tissues of the nonpregnant and pregnant rats were: kidney > serum > adrenal ≈ lung ≈ ovary > spleen ≈ heart ≈ liver > skeletal muscle. The mean concentrations of <sup>63</sup>Ni in products of conception (placentas and fetuses) on Days 9 and 19 of gestation were equivalent to <sup>63</sup>Ni concentrations in adrenal, lung, and ovary of these rats. These findings demonstrate that <sup>63</sup>Ni(II) crosses the rat placenta and becomes localized in fetuses. (Supported by ERDA Contract No. E(11-1)3140.)

177. *Biliary Excretion of Cadmium in the Rat, Rabbit, and Dog.* CURTIS D. KLAASSEN and FRANK N. KOTSONIS, University of Kansas Medical Center, Kansas City, Kansas.

Fecal elimination of cadmium is more important than urinary elimination. Within 1 week after iv administration of cadmium to the rat (1 mg/kg), 17% is excreted into the feces and less than 0.5% into the urine. However, of that excreted into the feces in 1 week, 85% is excreted within 2 days. The disappearance of <sup>109</sup>Cd from the plasma and its excretion into bile were measured for 2 hr after the iv administration of 0.1, 0.3, 1.0, and 3.0 mg/kg of cadmium to rats. The bile/plasma concentration ratio of cadmium was highly dose dependent; at the lowest dose it was 2.6, and at the highest dose it was 133. The bile/plasma ratio was greater than one because the concentration of cadmium in the liver was 100 to 700 times higher than in the plasma. However, the bile concentration of cadmium was equal to or much less than that in the liver. The dependence of dose of cadmium on its biliary excretion was also reflected in the percentage of cadmium excreted into the bile within 2 hr, which ranged from 0.23 to 9% as the dose was increased from 0.1 to 3 mg/kg. The biliary excretion of cadmium was increased approximately four times as the temperature of the rat was increased from 30 to 40°C. The effect of 4 days of pretreatment with phenobarbital, spironolactone, pregnenolone-16α-carbonitrile, or 3-methylcholanthrene on the biliary excretion of cadmium was measured; only phenobarbital significantly increased its excretion. Marked species variation in the biliary excretion was observed. Rabbits excreted cadmium at a rate about 1/6 and dogs excreted cadmium at a rate about 1/300 of that observed in the rat. These results suggest that while biliary excretion is the main route for elimination of cadmium, the rate at which it is excreted appears to be highly dependent on the time after administration, the dose, and the species employed. This rate is not as responsive to alteration in the temperature of the animal or by administration of microsomal enzyme inducers as is that of some other metals. (Supported by USPHS Grant ES-01142.)

178. *Toxicology and Distribution of Cadmium at Nonlethal Doses.* FRANK N. KOTSONIS and CURTIS D. KLAASSEN, University of Kansas Medical Center, Kansas City, Kansas.

Male Sprague-Dawley rats were given a single oral dose of cadmium chloride (0, 25, 50, 100, and 150 mg Cd/kg) mixed with  $^{109}\text{Cd}$  and sacrificed 2 or 14 days after dosing. Blood glucose, blood hemoglobin, and hematocrit values in dosed animals were the same as control values. Hepatic cytochrome *P*-450 concentrations, aniline hydroxylase activity, and hexobarbital oxidase activity in dosed animals were not significantly different from controls; however, there was a trend toward lower hexobarbital oxidase activity at the higher doses. Testicular function was measured 14 days after dosing by a serial mating technique. A lower percentage of the females mated with the males administered the higher doses of Cd (100 and 150 mg of Cd/kg) became pregnant than controls. Systolic blood pressure, heart rate, body weight, urine protein, urine weight, and motor activity were measured daily for 14 days. Body weights for the animals given the higher doses were lower during the 14-day study period. Urine volume and total protein in dosed animals were lower than controls for 2 days after dosing, but the same as controls thereafter. Total daily motor activity, measured in a residential maze, was lower in the dosed animals (50, 100, and 150 mg of Cd/kg) than controls for 3 to 4 days following dosing. Cadmium tissue concentration after 2 days was highest in the liver, which contained most of the Cd burden, followed by the intestine, kidney, pancreas, spleen, heart, lung, testes, muscle, brain, blood, and plasma. After 2 weeks most tissue concentrations decreased 50%, with some exceptions, notably the liver, which remained unchanged, and the kidney, which showed up to a fourfold increase. The concentration of metallothionein in the liver was increased both at 2 and 14 days after administration; however, in the kidney there was an increase only at 14 days. Thus, it appears that the major toxicity of a single, sublethal dose of cadmium is testicular injury, and that the redistribution of cadmium to the kidney may be due to the longer time period for the induction of metallothionein in the kidney. (Supported by USPHS Grant ES-01142.)

179. *Cadmium-Mediated Cellular Dysfunction.* D. WARRINGTON, J. B. SILKWORTH, K. BENITZ, R. ROTH, and L. D. LOOSE, Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York.

Scant evidence exists regarding the effect of heavy metals on immunocompetent cell function. The present study was undertaken to examine the influence of cadmium on the *in vivo* antibody and immunoglobulin formation and the *in vitro* polymorphonucleated (PMN) leucocyte response to *Pseudomonas aeruginosa*. A single iv or ip injection of cadmium acetate at a concentration of either 6, 0.6, or 0.06 mg/100 g to Balb/c male mice failed to influence significantly the hemagglutinin titer or serum immunoglobulin formation to sheep erythrocytes (SRBC) administered 8 or 24 hr following the injection of cadmium. However, a dose-related decrease in viability and  $\text{O}_2$  consumption was observed in PMNs incubated with cadmium acetate or cadmium chloride prior to the addition of the bacteria *Pseudomonas aeruginosa*. The cadmium-induced cytotoxicity and inhibition of  $\text{O}_2$  consumption was more apparent with cadmium chloride than with cadmium acetate. Since the  $\text{O}_2$  burst following addition of bacteria to PMNs is a result of an increased hexose monophosphate shunt activity and is directly related to the cellular microbicidal capability, the observation that a decreased respiratory burst occurs in PMNs exposed to cadmium may possibly be extended to a decreased bactericidal capacity. This could be correlated to the increased incidence of respiratory infections as a function of cadmium toxicity. (Supported in part by NIH Grant 2-P01-ES-00226-10 from the National Institutes of Environmental Health Sciences.)

180. *The Distribution of Lead in Tissues following Oral Administration to Rats of Different Ages.* M. L. KEPLINGER, G. L. KENNEDY, JR., R. J. WINGENDER, and J. C. CALANDRA, Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois.

Albino rats either 21, 110, or 180 plus days of age, were fed lead acetate in the diet for 90 days. Dietary levels of either 250 or 2500 ppm were employed and separate groups of control

animals were tested concurrently. Following 30, 60, and 90 days of feeding, blood samples were analyzed for total lead content. At the end of 90 days of feeding, animals were sacrificed. Organs were weighed and histopathological examinations were conducted on the brain, kidneys, and liver. Sections of kidney, liver, and femoral bone were also taken for analysis of lead concentration. Normal growth, food consumption, and general behavior was observed among all lead-treated rats. Blood lead concentrations were elevated among groups fed lead acetate in a dose-related manner. Values obtained at 30, 60, and 90 days of feeding were essentially the same among the various groups. The animals fed from 21 days of age showed the highest blood lead values following 30 days of feeding. However, when the actual lead intake was considered on a milligram per kilogram basis, similar blood lead concentrations were observed among rats from all three age groups. Tissue lead concentrations were elevated independently of the age of the animal at initial feeding. Pathological changes were limited to the kidney and to animals fed 2500 ppm lead acetate.

181. *Prolonged Administration of Lead to Developing Neonatal Rats as an Experimental Model of Plumbism.* A. J. STEVENSON, S. KACEW, and R. L. SINGHAL, Department of Pharmacology, University of Ottawa, Ottawa, Canada.

Administration of lead (50  $\mu\text{g}$ ) to rat pups from birth until 3 weeks of age followed by ingestion of 80 ppm metal in the drinking water for 5 weeks was the protocol chosen in this study, to demonstrate increased motor activity in animals. In rats subjected to this treatment regimen, approximately 0.5  $\mu\text{g/g}$  lead was present in kidneys of 2-week-exposed animals which remained at this level for up to 2 months. While the amount of lead detected in 2-week-treated hepatic tissue was approximately 0.4  $\mu\text{g/g}$ , extending the exposure period to 1 or 2 months, resulted in a decrease of lead content to 0.2  $\mu\text{g/g}$ , which was still significantly greater than the values found for controls. Throughout the course of this study, only trace amounts of lead were detectable in the pulmonary tissue of plumbic rats. Even though virtually little or no lead was present in lungs of 8-week-treated animals, the capacity of this tissue to incorporate [ $^{14}\text{C}$ ]thymidine into DNA was increased almost twofold. A similar enhancement in the incorporation of [ $^{14}\text{C}$ ]thymidine into DNA was noted in the kidneys and livers of plumbic animals. In all three tissues examined, the lead-stimulated elevation in DNA synthesis was accompanied by an increase in the endogenous concentrations of cyclic AMP as well as an enhancement in the activity of hepatic and renal adenylate cyclase. In general, prolonged exposure to this heavy metal did not seem to affect polyamine concentrations in the organs examined. Evidence indicates that the lead-inflicted enhancement of tissue DNA synthesis may be associated with stimulation in the adenylate cyclase cyclic AMP system of liver, kidney, and lung and may be independent of any apparent changes in polyamines. (Supported by the Medical Research Council of Canada.)

182. *Toxic Effects of Inorganic Lead in Infant Monkeys.* R. F. WILLES, E. LOK, and J. F. TRUELOVE, Toxicology Research Division, Bureau of Chemical Safety, Health Protection Branch, Health & Welfare Canada, Ottawa, Canada. (K. S. Khera)

Data are needed from which an assessment of safe levels of lead intake, particularly from foods, can be established for children. To investigate the applicability of the infant monkey to providing these data, lead acetate was administered orally at 500  $\mu\text{g}$  of Pb/kg/day to four infant monkeys beginning at 1 day of age. A decrease was observed in  $\delta$ -amino-levulinic acid dehydrase activity after 2 to 4 weeks of treatment when the enzyme was measured at pH 7.2. After 2 to 6 weeks of treatment with lead, free erythrocyte porphyrin concentrations increased from 30 to 50  $\mu\text{g}/100$  ml of red blood cells in control infants to 200 to 300  $\mu\text{g}/100$  ml of red blood cells in lead-treated infants. Electroencephalograms were recorded from all infant monkeys every 2 weeks and analyzed by Fourier analysis techniques. The power spectra of treated infants showed a shift to higher frequencies with lead treatment. Abnormalities were detected in both hopping and placing reactions at 12 to 14 weeks of treatment with lead acetate. Blood,

urine, and fecal lead concentrations were determined on samples collected bimonthly for a 6-month period.

183. *The Effects of Triethyllead on Central Catecholamine Function in the Adult Rat.* A. M. SCHUMANN, W. L. DEWEY, and J. F. BORZELLECA, Department of Pharmacology Medical College of Virginia, Richmond, Virginia.

Organic lead is known to produce marked behavioral and biochemical effects involving the central nervous system. The present study was conducted to assess the effects of  $\text{Et}_3\text{Pb}$  on the activity of central catecholamine neurons by determining the rate of formation of the hypothesized "functional" pool of newly synthesized, preferentially released transmitter, 10 min after the iv administration of 200  $\mu\text{Ci}$  of [ $^3\text{H}$ ]tyrosine. Adult male rats received either distilled water or 0.5 or 5 mg/kg of  $\text{Et}_3\text{Pb}$ , ip, on each of 3 consecutive days. At sacrifice on Day 4, the 5-mg/kg group exhibited tremors, irritability, fighting, hind limb paralysis, and a significant decrease in body and brain weight. The mean concentrations of endogenous norepinephrine (NE) were significantly lower ( $p \leq 0.025$ ) in the 5-mg/kg group (0.2372  $\mu\text{g/g}$ ) than the control or 0.5-mg/kg groups (0.3079 and 0.3150  $\mu\text{g/g}$ , respectively), whereas the rate of formation of [ $^3\text{H}$ ]NE was significantly increased ( $p \leq 0.025$ ) in the 5-mg/kg group (0.0647  $\mu\text{g/g/10 min}$ ) compared to the control and 0.5-mg/kg groups (0.0404 and 0.0428  $\mu\text{g/g/10 min}$ , respectively). In addition, the concentrations of endogenous tyrosine were significantly greater ( $p \leq 0.01$ ) in the 5-mg/kg group compared to the control group (16.19 and 13.87  $\mu\text{g/g}$ , respectively).  $\text{Et}_3\text{Pb}$  failed to alter the endogenous concentrations of dopamine (DA) or the rate of formation of [ $^3\text{H}$ ]DA. These results suggest that repeated administration of a high dose of  $\text{Et}_3\text{Pb}$  selectively alters the noradrenergic component of the central catecholamine system. The observed behavioral and neurochemical changes lead to the speculation that  $\text{Et}_3\text{Pb}$  may promote the release of, and thus lower the brain concentrations of, endogenous NE. A compensatory increase in NE synthesis might then result from the decreased feedback inhibition of intraneuronal NE on tyrosine hydroxylase. Further study is planned to investigate additional doses and to determine whether the alterations produced are direct or indirect effects of  $\text{Et}_3\text{Pb}$ . (Supported by USPHS Grant No. GM07111 and DA00490.)

184. *Concentrations of Inorganic and Organic Mercury in Mouse Brain Associated with Varying Degrees of Neuropathology and Functional Impairment.* J. S. MACDONALD and R. D. HARBISON, Center for Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee.

This investigation was undertaken to identify the concentrations of different forms of mercury in various parts of the brain that were associated with previously observed effects of long-term, low-level exposure to methyl mercury. Adult, male mice were administered methylmercury in the drinking water at levels of 10 or 50  $\mu\text{g}$  of Hg/ml of water. Neurological signs of intoxication as well as neuropathology that closely parallel those seen in man were observed. Changes could be detected on Days 7-8 in both exposure groups; the appearance of neuropathology coincided with the appearance of clinical signs of intoxication only in the high exposure group. A technique of flameless atomic absorption spectroscopy enabled determination of total and inorganic mercury present in the same sample. Examination of various anatomical regions of the brain as well as kidney and whole blood revealed that animals accumulated mercury in a dose-related manner. Although differences in the concentration of total mercury were seen between the various tissues examined, the ratio of organic to inorganic mercury was relatively constant. There was a lack of correlation between tissue concentrations of total mercury and morphological changes in the brain though good agreement was seen between tissue total mercury concentrations and overt clinical signs. The brain concentrations of mercury were equivalent in both dose groups when neurological signs of intoxication were present though the total time of exposure in the two groups was quite different. It is suggested that neurological signs of methylmercury intoxication are not manifested until a critical concentration of the metal is reached in the brain. (Supported in part by USPHS Grants ES 00267 and ES 01018.)

185. *Effect of Gentamicin on Organic Acid and Base Accumulation by Rat Renal Cortical Slices.* WILLIAM M. KLUWE, WILLIAM R. HEWITT, and JERRY B. HOOK, Department of Pharmacology, Michigan State University, East Lansing, Michigan.

The nephrotoxic aminoglycoside antibiotic, gentamicin (G), is excreted by the kidney at a rate equal to glomerular filtration, suggesting that the drug is handled only passively by the kidney. However, renal cortical concentrations of the antibiotic exceed plasma concentration severalfold. These experiments were designed to test the hypothesis that a high tissue concentration of G reflects active accumulation and intracellular binding. If this is correct and if nephrotoxicity results from high tissue concentration, nephrotoxicity should be prevented by treatment with a second base that would compete for the transport system. Male Sprague-Dawley rats were given G, 100 mg/kg, sc, once daily for 2 or 4 days and/or quinine, 150 mg/kg ig, 90 min before G. Twenty-four hours later, thin renal cortical slices were incubated in an oxygenated phosphate-buffered medium containing *p*-aminohippurate (PAH) and *N*-methyl-nicotinamide (NMN) at 25°C for 90 min under 100% O<sub>2</sub>. Tissue and medium were analyzed for PAH and NMN and slice-to-medium ratios (S/M) were calculated. NMN S/M was reduced after 2 days of G treatment and depressed further after 4 days. PAH was not reduced. Quinine reduced NMN S/M at both 2 and 4 days with no effect on PAH S/M. Total daily urinary excretion of the lysosomal enzyme  $\beta$ -D-galactosidase was significantly increased by G at both 2 and 4 days. Quinine had no effect on urinary enzyme excretion and did not block the G-induced increase. It is concluded that 100 mg/kg/day G selectively reduced the ability of renal cortical slices to actively accumulate NMN. Quinine probably does not prevent G accumulation, as evidenced by its failure to prevent the G-induced increase in urinary  $\beta$ -D-galactosidase excretion.

186. *Toxic Interactions between Narcotic Analgesics and Catechol-O-methyltransferase Inhibitors.* W. M. DAVIS and N. HATOUM, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi.

Inhibitors of catechol-O-methyltransferase (COMT) have been considered for therapeutic uses to the extent of preliminary clinical testing (Simpson and Varga, *J. Clin. Pharmacol.* **12**, 417, 1972; Ericsson, *J. Neurol. Sci.* **14**, 193, 1971). In view of our finding of a lethal synergism between morphine and the COMT inhibitor tropolone in male rats (Davis and Khalsa, *Res. Commun. Chem. Pathol. Pharmacol.* **6**, 867, 1973), we have investigated whether such synergism would occur also with other narcotics and whether the synergism is sex-related. Adult male and female Sprague-Dawley rats were injected ip with either saline or tropolone (50 mg/kg) 10 min before the ip administration of various doses of methadone, meperidine, and levorphanol. The combination with tropolone significantly reduced the 24-hr LD<sub>50</sub> values in male rats for methadone, meperidine, and levorphanol from 24, 66, and 61 mg/kg, respectively, to 13.6, 37, and 40 mg/kg. In females, the LD<sub>50</sub> values were reduced from 26, 65, and 48 to 14, 42, and 25 mg/kg. In no case was a significant sex difference observed. The synergism of lethality for these narcotics is considerably less than that observed for morphine, but is still quite significant and important. The potentiation seen is believed to be due to the interaction of the altered brain amine metabolism with the central amine-releasing effects of narcotics. (Supported by USPHS Grant No. MH 25170 and in part by the Research Institute of Pharmaceutical Sciences of the University of Mississippi School of Pharmacy.)

187. *Effects of Aminergic and Cholinergic Blocking Agents on the Lethality of Morphine in Rats.* N. HATOUM and W. M. DAVIS, Department of Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi.

A previous study has shown that an inhibitor of catechol-O-methyltransferase (tropolone) synergized, whereas an inhibitor of tyrosine hydroxylase ( $\alpha$ -methyltyrosine) antagonized the lethality of morphine to rats (Davis and Khalsa, *Res. Commun. Chem. Pathol. Pharmacol.* **6**, 867, 1973). These data implicated catecholaminergic mechanisms in morphine toxicity. Therefore, we investigated adrenergic receptor blockers for effects on morphine lethality. Because of apparent reciprocal relationships between central cholinergic or serotonergic systems and cate-



cholaminergic systems, blockers of serotonin and acetylcholine also were investigated relative to the primary toxicity of morphine and to the synergism observed of morphine and the  $\beta$ -adrenergic blocker propranolol. Female Sprague-Dawley rats were given 75 or 100 mg/kg ip doses of morphine sulfate 15 min prior to ip doses of either phentolamine HCl (25 mg/kg), atropine sulfate (0.4 or 1.0 mg/kg), methylatropine Br (1.0 mg/kg), haloperidol HCl (1.0 mg/kg), or methysergide maleate (1.0 mg/kg), and 30 min before an ip dose of propranolol HCl (40 mg/kg). The morphine-propranolol combination also was tested in male rats. Only propranolol and phentolamine showed significant interactions with morphine; paradoxically, both produced synergism of lethality. For propranolol this was true in rats of either sex. Further study of morphine and propranolol showed that their combined effect was altered significantly only by phentolamine and atropine (0.4 mg/kg), which further enhanced lethality. Only methysergide tended to oppose the lethality. These data confirm the finding of a lethal morphine-propranolol synergism (Winter, *Arch. Int. Pharmacodyn.* **212**, 195, 1974) and extend this to an  $\alpha$ -adrenergic blocker. The results may have relevance to the clinical use of narcotics with adrenergic receptor blockers. (Supported by USPHS Grant No. MH 25170 and in part by the Research Institute of Pharmaceutical Sciences, University of Mississippi.)

188. *Evaluation of Diazepam and Pyridoxine as Antidotes for Lethal Isoniazid Intoxication.*

L. CHIN, M. L. SIEVERS, H. E. LAIRD, R. N. HERRIER, and A. L. PICCHIONI, Department of Pharmacology and Toxicology, The University of Arizona College of Pharmacy, Tucson, Arizona, and Phoenix Indian Medical Center, Phoenix, Arizona.

Isoniazid (INH) poisoning is an increasing problem, especially among American Indians in whom tuberculosis is prevalent and suicide common. Because information regarding the most effective antidote for acute INH toxicity is incomplete and controversial, diazepam and pyridoxine were investigated as antidotes, by iv injection, in male rats and dogs following oral administration of lethal doses of INH. The consistently lethal dose of INH was much larger for rats (1500 mg/kg) than for dogs (75 mg/kg); the dog more nearly approximates man's sensitivity to the toxicity of INH (80-150 mg/kg). In rats, diazepam produced a dose-related protection against convulsions, but not for survival; in dogs it did not prevent convulsions but provided dose-related protection against death. The higher doses of diazepam employed caused toxic signs in both species, including ataxia, unconsciousness, and respiratory depression. Pyridoxine failed to protect rats against INH toxicity; but in dogs it had dose-related effectiveness against convulsions, and all doses (75, 150, and 300 mg/kg) prevented lethality. Significantly, the highest dose of pyridoxine in rats (750 mg/kg) was substantially below the optimal pyridoxine to INH antidotal ratio recommended for man (a dose that at least equals the amount of INH ingested), but optimal doses of pyridoxine for rats would exceed the LD50 of pyridoxine for rats. Combined administration of nontoxic doses of diazepam with pyridoxine protected against convulsions and death in rats and dogs. Used concurrently, the two antidotes are clearly synergistic for controlling the manifestations of experimental INH overdose. These results have important implications for the management of acute INH intoxication in man.

189. *Effects of Tricyclic Antidepressant Drugs on Isoproterenol-Induced Cardiotoxicity.* L. R.

WEISS and S. KROP, Drug Pharmacology Branch, Division of Drug Biology, Food and Drug Administration, Washington, D.C.

Adverse effects of tricyclic antidepressant drugs, such as desipramine (DMI) and amitriptyline (AMT), are well documented in man and animals. These drugs produce a high incidence of cardiac abnormalities. This paper describes the interaction between DMI and AMT on cardiac stress and the lesions induced by isoproterenol (ISO) in the hamster heart. Hamsters were treated with DMI or AMT, 50 mg/kg/day, sc, for 7 days. This dose effectively blocked an unlearned behavioral response in this species and caused cardiac arrhythmias in 25% of the treated animals. Loss of body weight averaging 9 g by DMI and 18 g by AMT treatment was also noted. On Days 8 and 9, the animals were challenged with 5 or 20 mg/kg, sc, of ISO; the former is a nonlethal, lesion-inducing dose, while the latter causes death and severe lesions.

Survivors were sacrificed on Day 10 for histopathological examination of the heart. The cardiotoxicity of ISO at both doses was reduced by prior treatment with DMI and AMT. No evidence was found that these drugs augment the cardiac stress, lesion grade, mean lesion score, or lethality of ISO in hamsters. These data suggest that the stimulating actions of ISO on the heart are modified by the myocardial depressant effects of the tricyclic antidepressants and that the ischemic necrosis and myocardial damage of ISO in the hamster heart are diminished by DMI and AMT.

190. *Toxicity Studies of  $\beta$ -(3,4-Dihydroxyphenyl)-L-Alanine (Levodopa) in Sprague-Dawley and Long-Evans Rats.* JOHN P. PRYTHERCH, ROBERT A. LEVIN, F. W. SIGLER, and C. D. KING, Pathology and Toxicology Section, Pharmacometrics Division, Norwich Pharmacal Company, Norwich, New York. (Louis E. Van Petten)

Levodopa is indicated in the treatment of Parkinson's disease and syndrome. The studies being reported were conducted to establish its toxicological and pathological properties in Sprague-Dawley (SD) and Long-Evans (LE) rats over an 18-month period. Dietary levels of levodopa of 0.15 (T2), 0.5 (T3), and 1.0% were fed to 35 male and 35 female rats of both strains, 49 days of age at the initiation of the study. Interim necropsy examinations occurred at 9 months for both studies. Body weight gain was depressed for the SD male T3 and male and female T4 rats from the 3rd month to termination, whereas the female LE T4 rats were depressed throughout the 18 months and the T4 males from the 15th month. Significantly higher mortality rates occurred for the SD rats as compared to LE. Erythromicrocytosis occurred in SD and LE during the first year, but was not detected thereafter. Urea nitrogen (BUN) increased for LE T4 females and SD T3 females. Increased SGOT was noted for SD T4 males and females, but not for any LE groups. Histopathology showed cardiovascular effects (angiitis-myocarditis or polyarteritis-periarteritis) in the T3 and T4 LE rats, but these were questionable in the SD rats. Adrenocortical hyperplasia was found in the T3 and T4 male and female LE groups, but was of low incidence in SD rats. The no-effect level in LE rats was 79 mg/kg/day (0.15%), whereas each of the dietary levels of levodopa caused some effect in SD rates.

191. *One-Year Toxicity Study of  $\beta$ -(3,4-Dihydroxyphenyl)-L-Alanine (Levodopa) in Beagle Dogs.* ROBERT A. LEVIN, JOHN P. PRYTHERCH, F. W. SIGLER, and C. D. KING, Pathology and Toxicology Section, Pharmacometrics Division, Norwich Pharmacal Company, Norwich, New York. (Louis E. Van Petten)

Levodopa is indicated in the treatment of Parkinson's disease and syndrome. The studies being reported were conducted to establish its toxicological and pathological properties in dogs over a 1-year period. Dosages of 75 (T2), 150 (T3), and 300 (T4) mg/kg/day were given po in capsules to groups of four male and four female beagle dogs, 7 to 10 months old. Another group of four males and four females received 300 mg/kg/day (T5) for 92 days, when dosing was discontinued. Dose-dependent toxicological effects included emesis, inappetence, body weight loss, CNS stimulation characterized by hyperkinesia, and clonic-tonic convulsions, and death. Leucotrichia occurred at dosages of 150 mg/kg/day or more. Subnormal hemograms intermittently appeared among the T3, T4, and T5 dogs. Urinalyses showed decreases in urine volume among each treatment group and sporadically appearing black-colored urine specimens, probably caused by oxidized levodopa. Deaths attributed to visceral congestion due to shock occurred at T2 (1), T3 (1), and T4 (2) and two dogs were sacrificed when in extremis at T4 (1) and T5 (1). Mortality and morbidity appeared to be time-dosage related. After 1 year of dosing, however, the only drug-related pathological lesions detected were leucotrichia and renal melanoid granules. Severe toxic effects noted at T5 immediately abated upon the withdrawal of dosing on Day 92. The T4 dosage was reduced to 200 mg/kg/day at Day 99 and the toxic effects were ameliorated. Plasma concentrations of levodopa, determined after chronic administration, peaked 1 to 1.5 hr after dosing and were undetectable after 12 hr. The data indicate the toxicity caused by levodopa in dogs is readily reversible upon discontinuing dosing.

192. *Toxic Effects of Mexrenoate Potassium in the Rat and Dog.* M. KOKA, R. STEJSKAL, K. S. RAO, and R. G. MCCONNELL, Searle Laboratories, Chicago, Illinois.

Mexrenoate potassium (SC-26714; the 7-methyl ester, 21-potassium salt of 17-hydroxy-3-oxo-17 $\alpha$ -pregn-4-ene-7 $\alpha$ ,21-dicarboxylic acid), a steroid, is a potential therapeutic agent, exhibiting at the renal level a potent inhibiting effect upon sodium retention induced by aldosterone and deoxycorticosterone acetate in laboratory animals. Routine endocrinology screening in rodents employing lower dosages and for shorter duration of treatment did not reveal any significant estrogenic, progestational, and androgenic activity. Toxicity of this compound has been studied by oral and iv routes of administration for 4 weeks in Charles River CD rats and beagle dogs. In oral studies, the compound was administered at daily dosage levels of 0, 15, 38, and 90 mg/kg in rats and 0, 12, 30, and 75 mg/kg in dogs. In iv studies, the compound dosages given to each species were 0, 10, 24, and 72 mg/kg/day. The highest dose levels employed in rats and dogs were approximately equal to 30 and 25 times the anticipated human dosage, respectively. Three dogs and five or six rats of each sex per group were employed. Conventional physical, hematology, clinical chemistry, urinalysis, and postmortem examinations were performed. Notable increases in liver/body weight ratios were seen in both sexes of rats at all levels of compound given orally. No other signs of toxicity were seen in either species by this route of administration. Following intravenous administration, increased incidences of prostate and seminal vesicle atrophy were seen in rats at 72 mg/kg only. In intravenously treated dogs, the toxic effects seen included: (a) dose-related increased incidences of prostate and testicular atrophy; (b) non-dose-related increased incidences of mild hepatocellular degeneration and vacuolization, and pituitary cysts. These results illustrate the endocrine activity of this agent.

193. *Toxicological Profile of Octanucline in Rat, Dog, and Monkey.* R. STEJSKAL, K. S. RAO, F. RENO, and R. G. MCCONNELL, Searle Laboratories, Chicago, Illinois and Hazleton Laboratories America, Inc., Vienna, Virginia.

Octanuclin, 2-[3,3-diphenyl-3-(2-methyl-1,3,4-oxadiazol-5yl)propyl]-2-azabicyclo [2.2.2] octane, is a potential antidiarrheal agent for oral use which, in comparison to other classical opiate and antidiarrheal agents, demonstrates minimal CNS effects in pharmacology studies in animals. Thirteen-week oral toxicology studies were performed in the Charles River CD rat and rhesus monkey. High dose groups in both studies received 12.75 mg/kg daily for 6 consecutive weeks and 17 mg/kg daily (100 times the estimated human dose) for the remaining 7 weeks. In the beagle dog, dose levels up to 5.95 mg/kg/day were given for 4 consecutive weeks. Conventional physical, hematology, clinical chemistry, ophthalmic, and postmortem gross and microscopical examinations were performed in all three studies. In addition, ECG was examined in the dog and monkey. No biologically meaningful compound-related alterations were observed either ante- or postmortem in the rat and monkey. In the dog, the only treatment-related effect was an increased incidence of emesis in the high dose group. Other parameters were unremarkable. Seven-day intravenous studies at dose levels up to 0.36 mg/kg/day were also performed in rat and dog. Ante- and postmortem parameters were unremarkable with the exception again of emesis in the dog. An emetic effect occurring after both oral and iv administrations in the dog only, suggests a centrally mediated, species-specific mechanism. It is concluded that octanucline does not produce any toxic effects at the dose levels used in these studies.

194. *Subacute Oral Toxicity of a Synthetic Tetrahydropyridobenzopyran (ABBOTT-41988) in Monkeys.* C. L. YANG, G. R. THOMPSON, S. LEVIN, and B. A. BECKER, Division of Drug Safety, Abbott Laboratories, North Chicago, Illinois.

ABBOTT-41988 (5,5-dimethyl-8-[5(4-fluorophenyl)-2-pentyl]-10-hydroxy-2-(2-propenyl)-1,2,3,4-tetrahydro-5H-[1]-benzopyrano-[3,4-d]pyridine) was suspended in 0.5% methocel and administered po for 35 days to 18 male and 18 female rhesus monkeys at 2.5, 10, and 40 mg/kg/day. Food consumption and growth rates were decreased in monkeys treated with 10,

and 40 mg/kg/day. Test dosages produced bradypnea during the first 3 days of treatment; behavioral depression was characterized by inactivity, ptosis, drowsiness, and huddled posture. Tolerance gradually developed to these effects. A total of 17 monkeys exhibited a dose-related incidence of hemorrhagic diarrhea. Seven monkeys succumbed during treatment; mortality was roughly dose related. All deceased monkeys exhibited hemorrhagic diarrhea and were confirmed to be carriers of *Shigella*. Similar hemorrhagic diarrhea and deaths had been reported in rhesus monkeys treated with  $\Delta^9$ -THC. In another 5-week oral toxicity study with the dosages of 0.15, 0.6, 2.5, and 40–80 mg/kg/day in 36 rhesus monkeys free of fecal *Shigella* and *Salmonella* there were no deaths, diarrhea and lesions characteristic of shigellosis. Effects in the first study apparently resulted from exacerbation of shigellosis rather than from direct primary intestinal toxicity produced by ABBOTT-41988.

195. *In Vitro and In Vivo Testing for Reactive Metabolites of Cocaine and Procainamide*. R. W. FREEMAN, R. L. WOOSLEY, and R. D. HARBISON, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

Recent studies with cocaine have demonstrated a delayed hepatotoxicity in the mouse following induction of microsomal enzymes with phenobarbital (PB). Cobalt and pyrazol treatment did not inhibit PB-induced cocaine delayed toxicity but did shift the location of histopathological changes in the liver. Procainamide (PA), a benzoic acid amide ester used in the management of cardiac arrhythmias, showed no PB-induced delayed hepatotoxicity. However, a major side effect of chronic PA treatment in man is the induction of antinuclear antibodies (ANA) and a drug-induced lupus erythematosus (DLE).  $\beta$ -Diethylaminoethyl diphenylpropylacetate (SKF-525A) and diethyl maleate (DEM) increased PA toxicity. Glutathione concentrations were lowered by PA treatment. Mutagenesis testing of cocaine and PA in Ames' *Salmonella typhimurium* system was performed in order to examine the nature of reactive metabolites formed from these compounds. Using PB-induced mouse liver microsomes, cocaine, over a 1000-fold concentration range, did not significantly increase the number of histidine revertant colonies in strains TA98 and TA100. However when microsomes were absent, the number of colonies per plate for strain TA100 was significantly reduced from spontaneous mutation controls. Cocaine itself was cytotoxic in mutagenesis testing, but *in vivo* studies have demonstrated a reactive metabolite. PA, in the presence of PB-induced mouse liver microsomes, significantly increased the number of revertant colonies in strain TA100 but did not increase histidine reversions in either strain TA98 or TA100 when microsomes were not present. Two possible metabolites of PA, *N*-acetyl procainamide (NAPA), and *N*-hydroxy procainamide (NOHPA), were also tested for mutagenic potential. NAPA, with or without microsomes, over a 1000-fold concentration range, did not significantly alter histidine reversions in strains TA98 and TA100. However, in the presence of PB-induced microsomes, NOHPA significantly increased histidine reversions in both strains. Without microsomes, NOHPA did not increase histidine reversions in either strain. We speculate that reactive metabolites are formed from both cocaine and PA and are responsible for their toxicity. (Supported in part by USPHS Grants ES00267 and GM00581.)

196. *Use of Laboratory Models for the Evaluation of Fibrogenic Potential*. G. C. FULLER, J. K. YERMAKOFF, and D. O. FISHER, Department of Pharmacology and Toxicology, University of Rhode Island, Kingston, Rhode Island.

Fibrosis has long been recognized as one of the sequelae of inflammation when it occurs with or as a consequence of chemical-induced tissue damage. In the past decade it has become apparent that drug-induced systemic stimulation of connective tissue formation, resulting in fibrosis, may occur independently from an inflammatory state. In this investigation, the effect of several fibrogenic agents, dibutyltin dichloride (DBT), hydralazine (HYD), and methylsergide (MS), was investigated in the whole animal and in tissue culture systems, in an attempt to distinguish between fibrosis associated with direct stimulation of fibroblasts and that associated with inflammation and wound repair. Collagen, the predominant protein in fibrotic

tissue, is synthesized in a series of sequential steps consisting of assembly of a proline-rich and lysine-rich polypeptide precursor of collagen (procollagen), enzymatic hydroxylation of some of the prolyl and lysyl residues, and glycosylation of some of the hydroxylysyl residues. Since prolyl residues are not hydroxylated before they are in peptide-bound form, the conversion of isotopically labeled proline to hydroxyproline by prolyl hydroxylase (EC 1.14.11.2) can be taken as one parameter reflecting the rate of collagen formation. The daily administration of DBT (10 mg/kg) to rats produced liver histopathological changes associated with inflammation-mediated fibrosis and an early five- to eightfold increase in prolyl hydroxylase activity. However, there was no apparent increase in this enzyme when cells in culture (L929) were incubated with  $10^{-6}$  to  $10^{-7}$  M for 22 hr. Since MS has been clinically implicated in cardiovascular fibrosis, cultures of rabbit vascular smooth muscle cells were incubated with MS or HYD ( $10^{-4}$  to  $10^{-5}$  M). With these two drugs prolyl hydroxylase was increased two- or threefold, and MS was also found to increase total collagen synthesis as measured by the formation of collagenase digestible protein by the cells. Thus, the use of whole animal systems in combination with cell culture systems permits the identification of fibrogenesis mediated via inflammation (DBT) as opposed to that mediated through direct stimulation of connective tissue forming cells (MS and HYD). (Supported in part by NIH Grant AA01422.)

197. *Organophosphate Inhibition of Tissue Esterases and Interactions with Ester Drugs in the Mouse.* RICHARD E. OUELLETTE and STEVEN D. COHEN, Section of Pharmacology and Toxicology, School of Pharmacy, University of Connecticut, Storrs, Connecticut.

Previous studies in this laboratory have demonstrated that prior inhibition of mouse tissue carboxylesterases by triorthotolyl phosphate (TOTP) resulted in potentiation of the acute toxicity of subsequently administered procaine (PRO). From a dose-response standpoint, inhibition of mouse liver hydrolysis of procaine by selected doses of TOTP was closely correlated with increases in procaine-induced loss of righting ability (LRA) and increased PRO lethality. To determine if the observed potentiation might occur with other organophosphates of insecticidal importance, groups of five male mice were pretreated with 1 to 10 mg/kg of Dasanit (fensulfothion) and were sacrificed 1 hr later for determination of brain cholinesterase and liver carboxylesterase activities. A dose of 5 mg/kg did not significantly inhibit brain cholinesterase activity, yet caused greater than 50% inhibition of liver hydrolysis of diethyl succinate (DES), triacetin (TA), and PRO. A time-course study indicated that brain cholinesterase activity and liver PRO hydrolysis were maximally inhibited in 1 to 5 hr and were completely recovered within 18 hr after Dasanit (7.5 mg/kg, ip). In contrast, liver hydrolysis of DES and TA remained 30 and 50% inhibited, respectively, at that time. Thirty-minute pretreatment with 1, 2, 3, 5, or 7.5 mg/kg resulted in 10, 33, 80, 60, and 80% lethality after 175 mg/kg, ip, of PRO yet this PRO dose killed 23% of the corn oil-pretreated mice. Mean durations of LRA for the survivors were 6.4, 15.3, 12.0, 12.2 and 21.5 min, respectively, compared to 6.6 min for PRO-injected controls. Pretreatment of mice with Dasanit (7.5 mg/kg, ip) followed by challenge with PRO 0.5, 2, 10, or 18 hr later resulted in 80, 70, 40, and 15% lethality and durations of LRA in survivors of 21.5, 16.7, 14.7, and 9.9 min, respectively. These results demonstrate that doses of Dasanit which selectively inhibited carboxylesterase activity potentiated the acute toxicity of PRO in mice and suggest that commonly used insecticidal organophosphates may alter the metabolism and toxicity of ester-containing drugs. (Supported by the University of Connecticut Research Foundation.)

198. *A Comparison of Benzopyrene Metabolism by 3-Methylcholanthrene-Induced and Non-induced Liver and Lung Enzymes from the Rhesus Monkey, Evaluated by High-Pressure Liquid Chromatography.* RALPH I. FREUDENTHAL and STEPHEN G. HUNDLEY, Battelle's Columbus Laboratories, Columbus, Ohio.

At present most studies evaluating the metabolism of benzopyrene use rodent tissue as their enzyme source. That the commonly used rodent enzyme preparations are suitable models for primate enzymes is yet to be proven. In the present study, three adult male rhesus monkeys re-

ceived 2 ip injections of 3-MC (40 mg/kg) 24 hr apart, and were sacrificed 34 hr after the final injection. Three additional adult male rhesus were used as controls. Microsomal enzymes were prepared from the livers and lungs of the pretreated and control animals. The specific activity (nmol of product/mg of protein/min) was calculated for each of the primary metabolites. Far less induction occurs in rhesus liver than reported for rat liver (Yang *et al.*, *Cancer Res.* **35**, 3642, 1975; Holder *et al.*, *Arch. Biochem. Biophys.* **170**, 557, 1975). 3-MC-induced increases in liver AHH specific activity varied for each metabolite. Increases of about 7-, 2-, 2-, 3-, and 1.5-fold were obtained for the 9,10-diol, 4,5-diol, 7,8-diol, 3,6-quinone, and 9-hydroxyBP. In contrast, extensive induction of lung AHH was observed in the rhesus as a result of 3-MC pretreatment. Increases in lung AHH specific activities of about 16-, 4-, 15-, 4-, 5-, and 15-fold were obtained for the 9,10-diol, 4,5-diol, 7,8-diol, 3,6-quinone, 6,12-quinone, and 3-hydroxyBP. Substantial quantitative variations between animals were observed for both liver and lung.

199. *Pharmacokinetics and Metabolism of Pentachlorobenzene in Rhesus Monkeys.* A. PHILIP LEBER, RALPH I. FREUDENTHAL, RONALD L. BARON, and AUGUST CURLEY, Battelle, Columbus Laboratories, Columbus, Ohio, and EPA, Pesticide and Toxic Substances Laboratory, Research Triangle Park, North Carolina.

Pentachlorobenzene (PCBz) is a minor contaminant of the fungicide, Terrachlor (pentachloronitrobenzene). The purpose of this study was to examine the pharmacokinetic properties of this compound along with its metabolic fate in male rhesus monkeys. Blood, urine, and fecal concentrations of radioactivity were determined at specified time intervals following a single oral dose (100  $\mu$ Ci of [ $^{14}$ C]PCBz, 20 mg/animal) to five monkeys. Peak radioactivities appeared in the blood between 12 hr and 1 month following dosing. A maximum of 22% of the administered dose was excreted in the urine and feces during the first 6 days following dosing. The major metabolites which were identified in the urine included two isomers of tetrachlorophenol, while the major radioactive component in the feces was PCBz. The data suggest a prolonged retention of the chlorohydrocarbon in the rhesus monkey. (Supported by EPA Contract No. 68-02-1715.)

200. *Metabolism of the Pancreatic Carcinogen N-Nitroso-2,6-dimethylmorpholine in the Syrian Hamster.* R. GINGELL, D. NAGEL, and P. POUR, Eppley Institute for Research in Cancer, Omaha, Nebraska.

The pancreatic carcinogen *N*-nitrosobis(2-oxopropyl)amine (BOP) was metabolized in hamsters to *N*-nitroso(2-hydroxypropyl) (2-oxopropyl)amine (HPOP), which was proposed as the proximate pancreatic carcinogen (Gingell *et al.*, *J. Nat. Cancer Inst.*, in press). HPOP can exist in a cyclic form which is a derivative of *N*-nitrosomorpholine, namely, *N*-nitroso-2,6-dimethyl-2-hydroxymorpholine. The metabolism of *N*-nitroso-2,6-dimethylmorpholine (NDMM) was examined in hamsters to determine if it too could form the postulated pancreatic carcinogen HPOP. The 24-hr urine of male hamsters administered 100 mg/kg NDMM ip contained about 2% of the dose as HPOP and 2% as the further metabolite *N*-nitrosobis(2-hydroxypropyl)amine (BHP). NDMM has subsequently been shown to be a pancreatic carcinogen for the hamster (Mohr *et al.*, *J. Nat. Cancer Inst.*, in press). NDMM is a mixture of *cis*- and *trans*-isomers. The metabolism of each isomer was investigated to determine if any difference may lead to a different carcinogenic potency or organotropic response. The blood HPOP concentrations 1 and 2 hr after administration of *cis*-NDMM were about twice those after *trans*-NDMM. However, the HPOP concentrations in the urine at these times were about 40 times greater after *cis*-NDMM than after *trans*-NDMM. These results are probably due to a difference in enzymic hydroxylation of the NDMM isomers, since urine metabolite concentrations were greatly increased in phenobarbital-pretreated animals. Although the initial metabolism rates for these isomers differ considerably, the 24-hr urine contained similar amounts of metabolites, indicating that any anticipated difference in carcinogenic potency of these isomers would be minimal. (Supported by PHS Contract N01 CP33278 from the National Cancer Institute.)

201. *Fate of p-Nitroanisole in Mice and Studies of its O-Demethylation Using a Sensitive Radioassay.* THOMAS D. TRAUTMAN, TIANCHAI THONGSINTHUSAK, and ROBERT I. KRIEGER, Department of Environmental Toxicology, University of California, Davis, California.

*p*-Nitroanisole (PNA) has been used extensively in *in vitro* studies of the monooxygenase systems of insects and mammals. Studies in this laboratory frequently utilize primate liver biopsy specimens and colorimetric methods currently available do not meet the sensitivity requirements of low-volume systems. Adaptations of radiometric *O*-demethylase assay procedures (Matthews and Casida, *Life Sci.* 9, 989, 1970) provide the necessary sensitivity. Rapid analysis of large numbers of samples and favorable cost/time relationships were obtained. One-milliliter suspensions incubated with PNA in capped scintillation vials required NADPH and air. The reaction was stopped by addition of scintillation fluid. Using either [<sup>14</sup>C-methyl]- or [<sup>3</sup>H-methyl]PNA, water-soluble metabolites including formaldehyde remain in the aqueous phase while unmetabolized PNA partitions into the organic phase, where it is measured by liquid scintillation spectrometry. Colorimetric measurements of *p*-nitrophenol (PNP) and formaldehyde also were made. Studies on the effect of liver protein, substrate, time, pH, and cofactors were completed using radiometric and colorimetric analyses. At 30 min the rates of [<sup>14</sup>C-methyl]PNA and [<sup>3</sup>H-methyl]PNA disappearance and PNP production differ significantly ( $p < 0.10$ ). Comparative data were obtained using several vertebrate and invertebrate species as enzyme sources. *O*-Demethylation activities obtained radiometrically ranged from 3.1 to 309 nmol/mg/30 min. *In vivo* induction of *O*-demethylation was demonstrated by phenobarbital (50 mg/kg  $\times$  3 days, ip) in mice and by phenobarbital (15 mg/kg  $\times$  2  $\times$  4 days, im) and DDT (10, 100, 500, ppm in diet) in rhesus monkeys. Significant inhibition ( $p < 0.01$ ) by SKF 525-A was seen *in vitro* at  $5 \times 10^{-5}$  M (molar I50  $6 \times 10^{-4}$  M). Lilly 18947, piperonyl butoxide, and disulfiram were less inhibitory. The LD50 of PNA in male Swiss-Webster mice was  $698 \pm 1.1$  mg/kg ip. At doses higher than 400 mg/kg, loss of righting reflex is typical; however, recovery time was highly variable. Low toxicity, limited pharmacological activity, and simple first and second phase metabolic fate make PNA a useful *in vivo* indicator of metabolic capability. (Supported in part by NIH ES00125 and ES00054.)

202. *Measuring Antipyrine Plasma Half-life and Antipyrine Metabolism in Rhesus Monkeys.* CHARLES R. CLARK, JEFFREY L. MILLER, and ROBERT I. KRIEGER, Department of Environmental Toxicology, University of California, Davis, California.

*In vivo* plasma elimination of [<sup>14</sup>C]antipyrine in rhesus monkeys is being used as an indicator of alterations in hepatic microsomal monooxygenase activity. Antipyrine is not bound to plasma proteins, is metabolized by hepatic oxidative systems, and can be measured using radioassay, high-speed liquid chromatography (HSLC), or spectrophotometry. To evaluate methods for antipyrine plasma half-life (APH) determination, six male rhesus monkeys (*Macaca mulatta*, 5.2–10.8 kg) were given [<sup>14</sup>C]antipyrine (12.5 mg/kg, iv) and blood samples were taken at 30, 60, 90, and 120 min after injection. APH values obtained using spectrophotometric analysis of CHCl<sub>3</sub> extracts of plasma (66–96 min) were higher than those obtained by HSLC analysis of the same extracts (60–83 min). Radioassay of 12% isopentanol:toluene extracts of the plasma produced even higher APH values (87–123 min). In a second study, three of the animals were given [<sup>14</sup>C]-antipyrine (40 mg/kg, iv) and blood was taken at 5, 15, 30, 75, 120, 160, and 200 min after injection. Exhaustive ethylacetate extraction of plasma before and after  $\beta$ -glucuronidase/aryl sulfatase treatment was done to ensure complete extraction of radioactivity and provide a more complete profile of antipyrine metabolism. HSLC analysis for antipyrine produced APH values which agreed well with HSLC values obtained from the CHCl<sub>3</sub> extracts. HSLC analysis of these extracts for antipyrine metabolites may determine whether the larger APH values seen in the other two methods are a result of metabolite interferences. While all three methods may be sensitive enough to detect changes in hepatic monooxygenase activity, HSLC analysis is more selective and permits more complete analysis of the kinetics of antipyrine disappearance. (Partially supported by NIH ES00125 and NIH ES00054.)

203. *Long-Term Feeding Study of Hexachlorobenzene in Rhesus Monkeys.* K. ROZMAN, W. MUELLER, F. COULSTON, and F. KORTE, International Center of Environmental Safety, Albany Medical College, Holloman AFB, New Mexico, and Institut fuer Oekologische Chemie, Gesellschaft fuer Strahlen- und Umweltforschung mbH, Muenchen, Germany.

The high accumulation tendency of hexachlorobenzene in the food chain is well documented in the literature. It was therefore of interest to establish absorption and excretion patterns of HCB in the rhesus monkey with the objective of determining a possible plateau level of storage. The following biological and biochemical parameters were monitored in order to detect possible harmful effects of chronic HCB-intake: metabolism, porphyrine concentrations, hormone concentrations, blood concentrations of HCB, enzyme activities and hematology. For 15 months three male and three female rhesus monkeys obtained a daily dose of 110  $\mu\text{g}$  of [ $^{14}\text{C}$ ]-HCB orally. So far this experiment has proven the expected high accumulation tendency of HCB in the rhesus monkey. The excretion storage pattern shows a very slow approach to a saturation level. The manifold increase of the urinary excretion is remarkable. While the ratio of the major metabolites remained constant, the total amount of metabolism increased significantly. There are strong indications of enzyme induction. Porphyrine and hormone concentrations remained within the normal range.

204. *Trichloroethylene-Induced Deactivation of Liver Endoplasmic Reticulum and Glutathione Depletion.* EDWARD S. REYNOLDS, MARY TREINEN MOSLEN, PAUL J. BOOR, KATHRYN BAILEY, and SANDOR SZABO, Departments of Pathology, University of Texas Medical Branch, Galveston, Texas, and Peter Bent Brigham Hospital, Boston, Massachusetts.

We have found the hepatotoxicity of trichloroethylene (TRI) potentiated by induction of the hepatic mixed function oxidase system (MFOS), specifically of cytochrome *P*-450, with the extent of injury correlating to enhanced metabolism of TRI (Moslen *et al.*, *Fed. Proc.* **35**, 375, 1976). Additional studies were undertaken to monitor MFOS Activities and liver glutathione (GSH) contents during the development of TRI-induced injury in phenobarbital (PBT)-pretreated animals. Male Charles River rats weighing 200 g were given PBT (400  $\mu\text{mol}/\text{kg}$ ) or the administrative vehicle (1 ml of 0.1% Tween 80) by gavage for 7 days. On the morning of Day 8, after an overnight fast, animals were exposed to air or to 1% TRI for 2 hr. In the PBT-TRI group, contents of cytochromes *P*-450 and *b*<sub>5</sub> diminished by the end of the first hour of TRI exposure and NADH-cytochrome *c* reduction increased three-fold by the eighth hour. In contrast, the only change in the vehicle-TRI group by 8 hr was decreased NADPH-cytochrome *c* reduction. Hepatic GSH contents of vehicle-TRI animals were constant during TRI exposure but then rose almost twofold by 12 hr. Hepatic GSH contents of PBT-TRI animals decreased during exposure and then rebounded; decreases were most profound in the microsomal fraction. Because of this apparent involvement of GSH in TRI's biotransformation, we also exposed fed animals with approximately twofold higher hepatic GSH concentrations to TRI. While 24-hr urinary metabolite excretions were similar, the fed animals had shorter anesthesia recovery times and less liver injury. The hepatotoxicity of TRI appears to be caused by inadequate rates of detoxification of its reactive intermediates. (Supported by NIH Grants HL-06370, AM-18163, and AM-19814.)

205. *Metabolic Fate and Disposition of Carbon-14-Labeled Methyl n-Butyl Ketone in the Rat.* G. D. DIVINCENZO, M. L. HAMILTON, C. J. KAPLAN, and J. DEDINAS, Eastman Kodak Company, Rochester, New York. (B. D. Astill)

Methyl *n*-butyl ketone (MnBK) has been associated with peripheral neuropathy in laboratory animals. Previous studies have shown that 2,5-hexanedione, a metabolite of MnBK, had produced peripheral neuropathy in laboratory animals, thus suggesting that MnBK neuropathy may be produced by metabolic activation. A more complete accounting of the fate and disposition of MnBK was therefore undertaken. [ $^{14}\text{C}$ ]MnBK was given to male Charles River CD rats by gavage in doses of 20 and 200 mg/kg. [ $^{14}\text{C}$ ]MnBK was rapidly absorbed and radioactivity was eliminated in both the breath and urine. Radioactivity in breath was identified



as unchanged MnBK (6% of the dose) and respiratory CO<sub>2</sub> (38%). Urinary radioactivity accounted for 40% of the dose; fecal radioactivity 1.4%. About 14% of the radioactivity remained in the carcass after 48 hr and 8% remained after 6 days. <sup>14</sup>C was widely distributed throughout the tissues of the rat with highest concentrations in blood and liver. The half-life and clearance time of MnBK in serum were 178 min and 6 hr, respectively. MnBK metabolites detected in rat serum were 2-hexanol, 5-hydroxy-2-hexanone, and 2,5-hexanedione. Metabolites in urine were 2-hexanol, 5-hydroxy-2-hexanone, 2,5-hexanedione, 2,5-dimethylfuran,  $\gamma$ -valerolactone, norleucine, and urea. Principal metabolic pathways were the reduction of the carbonyl group, oxidation at the  $\alpha$  and  $\omega$ -1 carbon atoms and evidently the decarboxylation of metabolites possessing an  $\alpha$ -keto acid moiety. A minor metabolic pathway involves the transamination of  $\alpha$ -keto acid intermediates to amino acids. Rats were pretreated with MnBK, phenobarbital, or SKF 525A. MnBK and phenobarbital pretreatment produced no significant overall effects; however, pretreatment with SKF 525A produced a marked increase in the excretion of <sup>14</sup>CO<sub>2</sub> (50 vs 38%) and decrease in urinary radioactivity (23 vs 40%), suggesting that the  $\omega$ -1 oxidation was mediated by the microsomal mixed function oxidase system. Apparently  $\alpha$ -oxidation of MnBK to CO<sub>2</sub> is the detoxification mechanism and  $\omega$ -1 oxidation leads to metabolic activation.

206. *The Fate of [<sup>14</sup>C]Vinylidene Chloride following Inhalation Exposure and Oral Administration in Rats.* M. J. MCKENNA, J. A. ZEMPEL, E. O. MADRID, and P. J. GEHRING, Toxicology Research Laboratory, Health & Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

The purpose of this study was to characterize the fate of ingested and inhaled [<sup>14</sup>C]vinylidene chloride ([<sup>14</sup>C]VDC) in the rat. Male rats (normally fed or previously fasted for 18 hr) were given a single dose of [<sup>14</sup>C]VDC in corn oil (1 or 50 mg/kg) or exposed to 10 or 200 ppm [<sup>14</sup>C]VDC for 6 hr and the routes and rates of elimination of <sup>14</sup>C activity were then followed for 72 hr. Following a single oral dose of 1 mg/kg [<sup>14</sup>C]VDC or a 6-hr exposure to 10 ppm [<sup>14</sup>C]VDC, 97–99% of the body burden of <sup>14</sup>C activity was retained and metabolized, either to <sup>14</sup>CO<sub>2</sub> or to nonvolatile <sup>14</sup>C metabolites which were excreted primarily in urine. Fasting for 18 hr prior to VDC administration decreased hepatic glutathione (GSH) concentrations but had no effect on the fate of [<sup>14</sup>C]VDC at the 1-mg/kg or 10-ppm exposure levels. However, after a single oral dose of 50 mg/kg [<sup>14</sup>C]VDC, only 60–75% of the body burden was metabolized. Fasted rats showed a diminished ability to metabolize [<sup>14</sup>C]VDC when compared to fed rats. Similarly, after a single 6-hr inhalation exposure to 200 ppm [<sup>14</sup>C]VDC fasted rats metabolized only 92% of their body burden of <sup>14</sup>C activity, whereas fed rats metabolized 96%. Centrolobular hepatic necrosis observed only in fasted rats exposed to 200 ppm [<sup>14</sup>C]VDC was associated with an increase in covalently bound <sup>14</sup>C activity in the liver over that of fed rats. Two major urinary metabolites of VDC were identified as *N*-acetyl-*S*-(2-hydroxyethyl)cystein and thiodiglycolic acid, indicating that a major pathway for detoxification of VDC is via conjugation with GSH. The fate of VDC in the rat is dependent upon both the route of administration and the dose. The reduction in VDC metabolism in the rat with increasing dose is enhanced by fasting (i.e., hepatic GSH depletion). The data are consistent with the hypothesis that VDC-induced hepatotoxicity is associated with an increase in covalent binding of <sup>14</sup>C activity in the liver, whereas detoxification of VDC is related to liver concentrations of GSH.

207. *Pharmacokinetic and Toxicological Evaluation of Dogs Fed 1,2,4,5-Tetrachlorobenzene in the Diet for Two Years.* W. H. BRAUN, L. Y. SUNG, D. G. KEYES, and R. J. KOCIBA, Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A., Midland, Michigan.

1,2,4,5-Tetrachlorobenzene (TCB), an intermediate in several industrial processes, was administered in the diet to dogs at 5 mg/kg/day for 2 years, followed by a 20-month recovery phase. The animals were examined periodically for toxicity; the concentration of TCB in the plasma and fat was measured during the 2 years of exposure and 20 months of recovery. After

18 months of dosage, all clinical chemistry parameters were normal; however, after 24 months, serum alkaline phosphatase activity and total bilirubin concentrations were elevated in the dogs dosed with TCB. Both clinical chemistry parameters returned to normal values within 3 months of the cessation of exposure. After the 20-month recovery, gross and histopathological examination of tissues revealed no morphological changes considered related to the ingestion of TCB. At the end of 2 years of exposure, TCB had reached 98 and 97% of the calculated steady-state concentrations in fat and plasma, respectively. TCB was eliminated from the fat and plasma with half-life values of 111 and 104 days, respectively. These small differences in the approach to steady state and the rate of elimination of TCB from fat and plasma and dramatic changes in the fat to plasma ratio of TCB were observed throughout the entire study.

208. *The Effect of Diquat on the Gastrointestinal Tract of Rats.* HELEN C. CRABTREE, EDWARD A. LOCK, and MICHAEL S. ROSE, Imperial Chemical Industries Limited, Central Toxicology Laboratory, Biochemical Mechanisms Unit, Alderley Park, Nr. Macclesfield, Cheshire, United Kingdom.

The herbicide diquat (*N,N'*-ethylene-2,2'-bipyridilium) is structurally and chemically related to paraquat (*N,N'*-dimethyl-4,4'-bipyridilium). However, despite similar herbicidal activity these compounds have different toxic actions in animals. Histological examinations of tissues from rats given an oral LD50 dose of diquat have failed to demonstrate significant damage to any major organs. At postmortem the main effect appears to be distention of the gastrointestinal tract. Studies have therefore been carried out to quantitate the effects of diquat on the gastrointestinal tract and to assess the relevance of this to mortality. After an oral administration of 900  $\mu$ mol of diquat/kg (LD50 dose) to male rats, there was a rapid accumulation of fluid in the gastrointestinal tract. This reached a maximum 24 hr after dosing, the excess fluid being about 14 ml. Similar changes were not seen after other diquaternary cations such as hexamethonium dibromide at the same osmolality as diquat. Following diquat other tissues, especially blood, were severely dehydrated. Twenty-four hours after dosing, the degree of hemoconcentration and the amount of excess fluid in the gastrointestinal tract were related to the amount of diquat administered. Following oral administration of diquat to rats, deaths occurred over 9 days with about 50% of these deaths occurring by 3 days. Rats which lost body weight over the first 24 hr died within the first 3 days and had a significantly higher gastrointestinal fluid content than remaining animals which gained weight. It is concluded that an LD50 dose of diquat causes a rapid, massive shift of fluid from the tissues into the lumen of the gastrointestinal tract following oral administration and that early death (occurring within the first 3 days) are related to this phenomenon.

209. *Relative Toxicity of Thermal Decomposition Products from Various Polymers.* CRAIG S. BARROW, HELEN LUCIA, MARYANNE F. STOCK, and YVES ALARIE, Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

The relative hazards of the thermal decomposition products of a plasticized polyvinyl chloride formulation, 60% homopolymer (PVC-A); a nonplasticized, nonfire retarded polyvinyl chloride formulation, 90% homopolymer (PVC-C); polytetrafluoroethylene (PTFE); flexible polyurethane foam (PUF); Douglas Fir; fiberglass reinforced polyester (FGPE), and a copper-coated copper wire with mineral insulation (CW) were evaluated. Utilizing male Swiss Webster mice, a Sensory Irritation Stress Index based upon the percentage decrease in respiratory rate during a 10-min exposure and 5-min postexposure period was developed as the response parameter. This permitted a wide range of exposure concentrations to be evaluated. Included on each dose-response curve were pathological changes and acute lethality as well as the degree of upper respiratory tract irritation to the various thermal decomposition products. Based on these results a hazard rating index was formulated. The conclusions showed PTFE > PVC-A, PVC-C > PUF, Douglas Fir > FGPE > CW. The thermal decomposition products of the two polyvinyl chloride formulations were found to be nearly equal in their hazard rating. (Supported under NBS Grant No. 5-9005.)

210. *The Subacute Oral Toxicity of Trinitrotoluene in Rats, Mice, and Dogs.* J. V. DILLEY, G. W. NEWELL, S. SCHOBERT, D. PALMER, J. DACRE, and R. SHIOTSUKA, Stanford Research Institute, Menlo Park, California, and U.S. Army Biomedical Engineering Laboratories, Fort Detrick, Frederick, Maryland.

Dogs were dosed daily for 90 days with 0, 0.2, 2.0, or 20 mg/kg of trinitrotoluene (TNT) by capsule. For 90 days, rats received 0, 0.002, 0.01, 0.05, or 0.25% TNT in their diet, and mice received 0, 0.001, 0.005, 0.025, or 0.125% TNT in their diet. All three species receiving the high dose of TNT exhibited anemia, with reduced numbers of RBCs, and reduced values for hemoglobin and hematocrit. Also observed in all species was enlargement of the liver, particularly in males. Animals administered the high dose had enlarged spleens. A marked reduction in the size of the testes was observed in both rats and mice receiving the high dose and, to a lesser degree, in those receiving the next highest dose. The primary toxicity of TNT appears to be toward the erythroid cells and the developing germinal cells of males. (Supported by U.S. Army Medical Research and Development Command, Contract No. DAMD17-76-C-6050.)

211. *A Toxicity Prediction System.* KURT ENSLEIN, DEV KHANNA, and PAUL N. CRAIG, Genesee Computer Center, Inc., Rochester, New York, and Franklin Institute Research Laboratories, Philadelphia, Pennsylvania. (H. M. Peck)

A statistical method has been developed for prediction of acute toxicity. The system, currently operational for rat LD50, permits the prediction of rat LD50 for untested chemical compounds. Only the chemical structure, partition coefficient, and molecular weight for a compound are needed for prediction purposes. The chemical structure is partitioned into substructural fragments using the CIDS fragment keys. Compounds are initially classified by the system as being highly toxic or not highly toxic. For those compounds which are initially classified as not being highly toxic, a set of regression equations are used. These permit the prediction of toxicity within approximately 0.4 log units. Potentially highly toxic compounds are identified with approximately 96% probability. This toxicity prediction system can be readily adapted to other species and to other measures of toxicity by the use of suitable design data bases. The use of this new system can materially reduce the amount of toxicological testing for new compounds. It also permits the ranking of potentially toxic compounds to allow the most likely candidates to be tested. The method can also be used to determine optimum dosages for new drugs.

212. *Halogen Substituents at the 4- and 4'-Positions of Biphenyl: Influence on Hepatic Function in the Rat.* D. J. ECOBICHON, M. M. HANSELL, and S. SAFE, Departments of Anatomy and Pharmacology, Dalhousie University, Halifax, Canada, and Department of Chemistry, University of Guelph, Guelph, Canada.

The purpose of the study was to correlate changes in hepatic drug-metabolizing enzymes in rat liver with the halogen substituent at the 4- and 4'-position on the biphenyl nucleus and the persistence of hepatic residues of the agents. Groups of male Wistar rats (80-100 g) received daily ip injections of 0.2 mmol/kg of either 4,4'-difluoro-, dichloro-, dibromo-, or diiodobiphenyl in peanut oil for 3 consecutive days, the animals being killed 4 days after the third dose. Samples of liver were fixed and embedded for light and electron microscopy. Residues were extracted from 20% (w/v) homogenates and were analyzed by gas-liquid chromatography. Hepatic *p*-nitroanisole *O*-demethylase and aniline hydroxylase were assayed on 12,000g 20-min supernatants of 20% (w/v) liver homogenates. Little change was observed in morphology or enzymatic activities after treatment with difluorobiphenyl. Marked induction of the enzymes and significant changes in hepatic morphology were observed following treatment with dichloro- and dibromo-biphenyl, the effects produced by the latter agent being somewhat more pronounced. Though the hepatic residues of 4,4'-diiodobiphenyl were approximately 10-fold higher than those of the other agents, this chemical caused minimal enzymatic and morphological changes in the liver. The size of the halogen appears to influence hepatic function but it leaves open the question of why 4,4'-diiodobiphenyl produced no observable effect.

213. *The Effect of Cadmium on Growth and Mineral Disposition in the Male Rat.* E. M. YUHAS, T. S. MIYA, and R. C. SCHNELL, Department of Pharmacology and Toxicology, Purdue University, West Lafayette, Indiana.

Male Sprague-Dawley rats were given cadmium (0, 1, 10, or 100 ppm) in their drinking water for 13 weeks to examine the effects of this metal on growth and mineral (Ca, Cd, Mg, P, Zn) disposition. A dose-dependent decrease in water consumption was observed in the animals that received 10 or 100 ppm. Food consumption was lower in the animals that received 100 ppm, and body weights of these animals were lower than controls. The weight gain/food consumption ratio was the same between all treatment groups, which suggested that growth impairment was due to decreased food intake. Cadmium, at all concentrations, inhibited serum alkaline phosphatase activity. Serum phosphorus was elevated in the animals that received 100 ppm. There were no changes in serum calcium or urea. Significant increases in bone cadmium were found at 10 and 100 ppm. The only other observed bone mineral change was a decreased zinc content at 100 ppm. There was no cadmium-induced effect on percentage bone ash, and X-ray analysis revealed no abnormalities. A dose-dependent increase in kidney concentrations of cadmium was observed, and kidney zinc concentrations were higher in the animals that received 10 or 100 ppm Cd. No histological changes were observed in the kidney. There was no cadmium-induced effect on the absorption of Ca, Mg, P, or Zn. The study indicated that cadmium, at 100 ppm, exerted profound effects on animal growth and mineral disposition. (Supported by NIEHS Grant ES-00921 and NIEHS Training Grant T01-ES-00071.)

214. *Species Comparison of the Binding of the Organic Arsenical Herbicide, Cacodylic Acid, to Erythrocytes.* JAMES T. STEVENS, JACK D. FARMER, and LARRY L. HALL, USEPA, Health Effects Research Laboratory, Environmental Toxicology Laboratory, Research Triangle Park, North Carolina.

A species-specific accumulation of inorganic arsenicals by rat erythrocytes with the formation of a stable complex with hemoglobin is well documented (Lang *et al.*, *Univ. Calif. Publ. Pharmacol.*, 1949). The percentage of the dose of radiolabeled cacodylic acid taken up by rat erythrocytes after *iv* or oral administration did not appear to be dose (150  $\mu\text{g}/\text{kg}$  to 200  $\text{mg}/\text{kg}$ ) or label dependent ( $^{14}\text{C}$  and  $^{74}\text{As}$ ). The half-lives of [ $^{14}\text{C}$ ]cacodylic acid in the rat erythrocyte were found to be approximately 90, 92, and 76 days after oral, *iv* or intratracheal administration, respectively. These values agree well with the mean life of the rat red cell, which is 95 days. The half-life of [ $^{14}\text{C}$ ]cacodylic acid administered *iv* to the rabbit was found to be 25 hr, the percentage dose associated with red cells being 7% as opposed to 16% in the rat. Despite evidence of absorption, erythrocytes taken from the guinea pig dosed orally with 200  $\text{mg}/\text{kg}$  did not accumulate amounts greater than 0.3% as opposed to 21% of the oral dose in rat. In spite of these *in vivo* differences in the concentration of the cacodylic acid by erythrocytes with these three species, red blood cells from the rat, rabbit, and human do not differ in their rate of uptake. Affinity constants indicate that cacodylic acid has erythrocyte affinities as follows: rat > rabbit > human. Since the rate of uptake of cacodylic acid *in vitro* does not indicate a species difference, this *in vitro* accumulation of organic arsenical must be related to either qualitative and/or quantitative differences of receptor materials with which the arsenical complexes.

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Bill Hamm

1973 Co. 4th, a letter got a, 1/2 page 7 ungrouped.  
(New class & ungrouped Co. 1) ungrouped,  
algebra, algebra (1974), algebra, algebra (1974) general,  
1974 1974 & 3 ungrouped classes

New & ungrouped class, ungrouped for the 9 classes

First & ungrouped, algebra, ungrouped, ungrouped

Two & ungrouped class

---

As ungrouped algebra ungrouped, ungrouped.

Bill Harms

OT's on testing activities - get a, 7 pages, paragraphs.

Chem classes important to see +1 activities,  
alloylating agents (194), allylating agents (49) phenoxide,  
17C 17E + 5 other classes

Have a hierarchical testing scheme for these 9 classes

Test 1 standard, equal, resembles, human

Test 3 chronic test -

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