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Abstracts of Papers for the Tenth Annual Meeting of the Society of Toxicology, Washington, D.C. March 7–11, 1971

1. *Sex-Related Disposition of Cyclacillin (Wy-4508) and Associated Renal Changes in Rats.* W. E. TUCKER, H. W. RUELIUS, F. W. JANSSEN, and H. P. K. AGERSBORG, JR., Wyeth Laboratories, Inc., Radnor, Pennsylvania.

In chronic and subchronic dietary studies of cyclacillin [6-(1-aminocyclohexanecarboxamido)-penicillanic acid, Wy-4508] in rats, a nephrotoxic effect was observed in male, but not in female, rats. This was the only important toxicologic effect at the dietary levels used. Comparable time-dose treatment of dogs and rhesus monkeys did not result in nephrotoxicity in either sex. The principal morphologic changes in the male rat after chronic (6 mo) treatment were marked renal enlargement, extreme dilatation of convoluted tubules with cast formation and focal degeneration, and luxuriant regenerative hyperplasia of tubular epithelium. Chronic inflammation and fibrosis were also present in the more severely affected kidneys. Since the nephrotoxic effects are completely sex-related the metabolic disposition of cyclacillin was compared for both sexes. The unchanged drug and Wy-4708 (1-aminocyclohexanecarboxylic acid) were identified among the renal excretion products of cyclacillin-treated animals. A marked sex-related difference in the disposition of these two compounds was observed: the ability to excrete Wy-4708 and cyclacillin is much lower in the male rat than in the female rat. Consequently, these two compounds accumulate in the blood of the male. Wy-4708, an unnatural amino acid which is not further metabolized accumulates to a far greater extent than cyclacillin. Prolonged treatment with Wy-4708 produces renal changes less severe but qualitatively similar to those observed with the parent compound. It is considered that the renal changes probably result from the low excretion, and ensuing accumulation, of cyclacillin and Wy-4708. These two compounds are possibly reabsorbed by the renal tubules and, therefore, recirculated. No sex difference in the disposition of cyclacillin was observed in dog, monkey, and man. In view of these observations, the male rat appears to be an inappropriate model for the safety assessment of cyclacillin.

2. *Chromomycin A_3 (NSC-58 514): Pharmacologic Evaluation in Dogs and Monkeys.* U. SCHAEPPI, G. R. THOMPSON, R. W. FLEISCHMAN, V. ILIEVSKI, and H. ROSENKRANTZ, Mason Research Institute, Worcester, Massachusetts; D. A. COONEY and R. D. DAVIS, National Cancer Institute, Bethesda, Maryland.

Chromomycin A_3 (Toyomycin), an antibiotic from *Streptomyces griseus* with a molecular weight of 1183 has potent antitumor activity against Ehrlich carcinoma, Yoshida sarcoma, and leukemias L-1210 and P388. A pharmacologic evaluation was carried out in 14 dogs, 3 monkeys, and 120 mice. The LD50, for 5 daily consecutive iv injections in mice, was 0.6 mg/kg. Seven dogs injected iv with 400 or 200 μg/kg (single dose) or with 100 μg/kg (5 consecutive daily treatments or 1 treatment on days 1 and 5) became acutely moribund or died. Histopathologic evaluation revealed focal necrotic areas in lymphoid tissues (6/7), bone marrow (5/7), liver (1/7), renal tubules (1/7), pancreas (1/7), salivary glands (2/7), epithelium of the urinary bladder (1/7), cervix of the uterus (1/7) and esophagus (1/7) and necrosis of the intestinal plasma cells in the lamina propria (3/7). The intestines exhibited submucosal hemorrhages without important epithelial changes (6/7). Other generalized scattered hemorrhages and pulmonary edema occurred in 2/7 dogs. Prior to death, the dogs exhibited vomiting, hemorrhagic diarrhea, fever, weakness, and shock. Laboratory findings included leukocytosis, with neutrophilia and shift to the left, and elevation of alkaline phosphatase, transaminases, and α_2-globulins, all changes indicating an acute inflammatory process. One dog reacted with severe hypocalcemia. Dogs treated with a single dose of 100 μg/kg or 50 μg/kg or with 5 daily doses of 75, 50, or 25 μg/kg

survived without appearance of important toxicity. Monkeys injected with a single dose of 330 μg/kg iv died or became moribund (2/2). One monkey treated with 165 μg/kg survived. Monkeys exhibited the same toxicity as dogs, but without hemorrhagic enterocolitis. The present observations generally agree with earlier work by Aramaki *et al.* Nonlethal doses failed to induce characteristic toxicity that could be used as a therapeutic guide in clinical cancer therapy. In the dog, hemorrhagic enterocolitis was possibly a symptom of shock. (Supported by Contract NIH-70-2055 with the Chemotherapy Program, National Cancer Institute, USPHS.)

3. *Comparison of the Behavioral Effects of Imipramine in Monkeys through Continuous Avoidance Operant Schedules.* S. FIELDING, M. L. MILLER, T. MCGREEVY, and M. CORNFELDT, CIBA Pharmaceutical Company, Summit, New Jersey. (H. Lal.)

A comparison of the behavioral effects of imipramine, given in various doses, was made between the shock avoidance components of 2 operant behavioral tests. Four squirrel monkeys were trained to operate a behavioral multiple schedule of reinforcement consisting of a shock avoidance RS-20, SS-20 schedule, a positively rewarding fixed ratio 15:1 schedule, and a conflicting positively and negatively (foot shock) rewarding 15:1 schedule. Eight other squirrel monkeys were trained on a continuous shock avoidance RS-20, SS-20 procedure without any other positive rewarding behavioral contingencies. The results indicate that in the multiple schedule, imipramine po at doses of 5–20 mg/kg produced rate-increasing effects in avoidance, while fixed ratio responding to the neutral stimulus was markedly disrupted, indicating specific behavioral toxicity. These doses also had no effect on conflict behavior. By way of contrast, the marked increase in shock avoidance behavior observed during the multiple schedule, was absent when imipramine was tested without the 2 additional fixed-ratio contingencies. When imipramine was tested without the fixed-ratio contingencies, marked depression of avoidance behavior with a concomitant increase in shocks received by the monkeys was observed. The behavioral multiple schedule was needed to bring out the stimulatory properties of imipramine. The results also illustrate the application of behavioral techniques to assess the behavioral toxicity of imipramine when administered at low doses, while still maintaining a desired psychotropic effect.

4. *Interactions of the Tranquilizer Molindone with Other Drugs in Toxicity Studies.* HAROLD BLUMBERG and HOWARD S. ROWE, Endo Laboratories, Garden City, New York.

Molindone hydrochloride (EN-1733A or 3-ethyl-6,7-dihydro-2-methyl-5-(morpholinomethyl)indol-4(5*H*)-one HCl) is a new structural type of tranquilizer which is effective in animals and man. Tranquilizers are sometimes administered in conjunction with other drugs, such as antiparkinsonian agents or sedative-hypnotics; therefore, acute oral toxicity studies were carried out in rats to determine what effect the simultaneous administration of benztropine mesylate, phenobarbital, or chloral hydrate might have upon the toxicity of molindone. When molindone HCl was given with the antiparkinsonian drug benztropine mesylate at the molindone HCl:benztropine mesylate approximate clinical ratio of 10:1, the toxicity of molindone was not increased, but instead tended to be slightly decreased. At a molindone HCl:phenobarbital ratio of 1:2, the dominant lethal toxicity was that of phenobarbital and was not affected by the addition of molindone HCl. Similarly, at a molindone HCl:chloral hydrate ratio of 1:5, the dominant lethal toxicity was that of chloral hydrate and showed little or no increase over the toxicity of chloral hydrate alone. When smaller, nonlethal doses of phenobarbital or chloral hydrate were combined with a high, convulsant dose of molindone HCl, the convulsant toxicity of the molindone HCl was markedly reduced or prevented.

5. *Effect of Nicotine on the Performance Behavior of Normal and Reserpinized Male Rats.* JASBIR M. SINGH and G. DON BYRD, Xavier University of Louisiana, College of Pharmacy, New Orleans, Louisiana.

To elucidate the effect of nicotine on the performance behavior in activity wheel cages (PBAWC) the following series of experiments was performed: Series A—(1) control, saline;

(2) nicotine, 0.057 mg/kg; (3) nicotine, 0.114 mg/kg; (4) nicotine, 0.171 mg/kg. There were 7 animals per group. After determination of the initial PBAWC for 0–15, 0–30, 0–45, and 0–60 min, the animals were allowed to rest for 1 hr, then were injected with nicotine, ip, and the PBAWC was recorded. Acute administration of nicotine increased the PBAWC in 71% of the animals. Series B—(5) control, saline; (6) nicotine, 0.057 mg/kg; (7) nicotine plus reserpine, ip, 2 mg/kg. After recording of the PBAWC on the first day, group 7 was treated with reserpine 2 mg/kg for 4 days, and on day 5 only nicotine was injected to groups 6 and 7. Nicotine did not increase the PBAWC in the reserpine-treated rats. Groups 5, 6, and 7 were allowed to recover for 5 days, and on day 6 they were again given respective treatments. Nicotine did not increase the PBAWC in reserpine-treated animals.

6. *Animal Safety Evaluation Studies on the Antipsychotic Phenothiazine, Mesoridazine.* R. J. VAN RYZIN, S. E. CARSON, H. A. HARTMAN, and J. H. TRAPOLD, SANDOZ Pharmaceuticals, Hanover, New Jersey, and Food and Drug Research Laboratories, Inc., Maspeth, New York.

Mesoridazine (SERENTIL ®) is a highly potent phenothiazine that was recently marketed as an antipsychotic and for the treatment of alcoholism. Prior to marketing, the following animal safety evaluation studies were completed: acute toxicities, subchronic (4 wk) and chronic (60 and 72 wk) oral toxicity in dogs and rats; subchronic (2 and 4 wk) parenteral toxicity in rats and dogs; reproduction studies in rats; teratology studies in rats and rabbits; and tissue irritation tests in rabbits and guinea pigs. Acute toxicity results were: mice, po cf. iv LD50 560 ± SE 62 cf 26 ± SE 0.086 mg/kg; rats, po LD50 644 ± SE 48 mg/kg; rats, im ♂ cf ♀ LD50 509 (95% CL 305–847) mg/kg cf 584 (95% CL 500–680) mg/kg; rabbit, oral minimum lethal dose 800 mg/kg; rabbit, im LD50 405 (95% CL 180–911) mg/kg; dog, oral minimum lethal dose above 800 mg/kg. In the chronic rat feeding study, psychomotor depression was noted in all dose groups (10, 40, 100–180 mg/kg), but only at the highest dose did it persist throughout the study. At the 100–180 mg/kg level food intake and body weight gain were lowered, but no other evidences of toxic effects were seen. A melanin-like pigment occurred in the kidneys of the high-dose (100–180 mg/kg) animals. As with other potent phenothiazine tranquilizers, interaction with the pituitary/endocrine axes was evidenced by changes in relative endocrine organ weights. Slight increase in liver weight accompanied by increased histologically visualized lipid was noted in the highest dose group at 52 wk but not at 72 wk. In the oral chronic dog study, central nervous system depression was evident in all dose groups (10, 20, 40–80–120 mg/kg). Toxic effects were not noted until the high-level was elevated to 120 mg/kg, after which some dogs were found dead after prolonged periods of emesis, tremors, salivation, aggressive resistance to dosing, dry thick skin, and aspiration of vomitus into the trachea. In reproduction studies, when drug was fed to rats from weaning age through to the production of two successive litters, 10 mg/kg was without effect whereas 40 mg/kg decreased reproductive performance. Teratologic studies in rats and rabbits resulted in no evidence of teratogenesis with doses as high as 100 mg/kg. Tissue irritation tests showed mesoridazine to be comparable to chlorpromazine in producing lesions in rabbit muscle. The results obtained with mesoridazine in animals indicate that toxic, adverse or undesired side effects occur only at dosages greatly in excess of the clinically useful dose in humans.

7. *Effect of Nicotine, Epinephrine, and Norepinephrine on the Enzyme Thrombin and Substrate Fibrinogen.* M. DIANE SINGH and JASBIR M. SINGH, Xavier University of Louisiana, College of Pharmacy, New Orleans, Louisiana.

Our previous work has indicated that nicotine accelerates the blood-clotting time in vitro and in vivo. In vitro, nicotine antagonizes or inactivates the anticoagulant effect of the mucopolysaccharide heparin on blood clotting. Nicotine also exerts a biphasic action on the prothrombin time. Our hypothesis that the clot-promoting activity of the enzyme thrombin is affected when nicotine, epinephrine, or norepinephrine are added to the clotting mixture (total volume 0.3 ml), was tested as follows: Thrombin solution 0.2, 1.0, or 2.0 units was mixed with

different concentrations of nicotine, epinephrine, or norepinephrine (1.95–500 μg/0.3 ml), then 0.1 ml of this mixture was added to 0.2 ml of plain plasma or plasma containing heparin, and the clotting or thrombin time was determined. In another series of experiments, 0.2 ml of thrombin-nicotine, thrombin-epinephrine, or thrombin-norepinephrine was added to 0.1 ml of purified fibrinogen and also to fibrinogen containing heparin, and the clotting or thrombin time was determined. It is proposed from our experimental results that nicotine, epinephrine, and norepinephrine affect the clot-promoting activity of the enzyme thrombin and substrate fibrinogen. When higher concentrations of nicotine, epinephrine, and norepinephrine affect the enzyme thrombin and substrate fibrinogen, the clot formation is delayed. By decreasing the concentration of nicotine, epinephrine, or norepinephrine in the clotting mixture, the clot formation is accelerated. Nicotine and heparin inactivate or neutralize the effect of each other on the enzyme thrombin and substrate fibrinogen. (Supported by Edward G. Schlieder Foundation Grant.)

8. *Rapid Forensic and Biomedical Identification of Drugs.* R. W. WARFIELD and R. P. MAICKEL, Indiana University, Bloomington, Indiana.

While much attention is being given to the problem of drug identification from the point of view of law enforcement, a related area has been relatively ignored. The rapid identification of drugs and drug metabolites in biological materials obtained from subjects admitted to hospitals under emergency circumstances is also a severe need. We are developing a multiuse system, designed for the rapid identification of organic compounds in a variety of sample materials, and applicable in a variety of laboratories: clinical, forensic, or toxicologic. The initial sample may be urine, blood, saline, powders, tablets, or liquids. Three aliquots are taken and extracted at different pH with chloroform. The chloroform extracts are evaporated on Gelman ITLC® plates. After development in suitable solvent systems and visualization, comparison with known standards yields positive identification. For example, barbiturates are extractable at pH 2, but not at pH 11, while amphetamines show reverse extraction characteristics. Thus, the combination of extraction characteristics and chromatographic Rc yields positive identification. Many drugs can be detected at levels of 5–10 μg in less than 2 hr. (Supported by USPHS Grants MH-14658 and KO2-MH-41083.)

9. *Metabolism of a New, Tritium-Labeled Phenanthrene Methanol with Pronounced Antimalarial Activity.* CARL C. SMITH and WALTER WEIGEL, University of Cincinnati College of Medicine, Ohio.

A new antimalarial drug, α-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol (WR-122,455), labeled with tritium on the methanolic bridge, has been studied in rhesus monkeys, owl monkeys, and rats after single and repeated oral doses of 5 mg/kg. Peak levels of radioactivity in the blood occurred in rats at 2 days and in monkeys at 4–5 days after administration. Excretion of radioactivity occurred primarily in the feces in all species with less than 10% of the dose generally appearing in the urine. There was prolonged excretion of the drug in urine and feces; ^{3}H could be demonstrated for at least 21 days after a single dose. Enterohepatic circulation was prominent in the rat and probably accounted at least in part for the slow excretion. The drug as ^{3}H was localized in lung, liver, adrenals, spleen, pancreas, and gonads. Data on the fractionation of radioactivity in plasma of owl monkeys will be reported. (Supported in part by Army Contract DADA17-67-C-7065.)

10. *Biosynthesis of Nitrosopiperidine from Nitrite or Nitrate and Piperidine in the Small Intestine of the Rat.* B. S. ALAM, I. B. SAPOROSCHETZ, and S. S. EPSTEIN. Children's Cancer Research Foundation, Inc., and Harvard Medical School, Boston, Massachusetts.

Nitrosamines are chemically synthesized from nitrite and secondary amines under acidic conditions. In vitro and in vivo biosynthesis of nitrosamines has also been demonstrated under acidic conditions We report here biosynthesis of the carcinogenic nitrosopiperidine (NP) from

nitrite or nitrate and piperidine at neutral pH in the isolated small intestine of the rat. Solutions of 25 mg nitrite or nitrate, and 1250 mg piperidine were infused into the isolated small intestine of the rat and incubated in vivo for 30 min. NP synthesis from nitrate and piperidine or nitrite and piperidine was quantitatively determined by gas–liquid chromatography (GLC) and its identity confirmed by both GLC and thin-layer chromatography. The NP yield from mixtures of nitrate and piperidine and mixtures of nitrite and piperidine was 21 and 159 μg, respectively. (Supported by Grants C-6516 and FR-05526 from the National Institutes of Health, and Contract PH-86-66-169 from the National Air Pollution Control Administration.)

11. *The Effect of Carbaryl on Reproduction in the Monkey (Macacca mulatta).* W. J. DOUGHERTY, L. GOLBERG, and F. COULSTON, Albany Medical College, Albany, New York.

Carbaryl (1-naphthyl-*N*-methylcarbamate) is known to affect embryonic development and reproductive function in various species. The purpose of this investigation was to examine its effect on reproduction in the nonhuman primate. Mature female rhesus monkeys (*M. mulatta*) with regular menstrual cycles were mated with males having a proved record of fertility. Subsequently, the females were treated po with Carbaryl (2 mg/kg or 20 mg/kg) in 1% aqueous gum tragacanth, daily throughout the entire gestation period. Control females received only the suspending medium. Five control monkeys conceived; 4 of these delivered normal infants, and one aborted. (The spontaneous abortion rate among untreated females in our colony is approximately 12%.) Two of 4 monkeys receiving 2 mg/kg of Carbaryl conceived and both of these aborted. Six of 10 monkeys receiving 20 mg/kg conceived; 3 of these delivered normal infants and the remaining 3 pregnancies ended in abortion. Examination of the live infants, and of some of the aborted fetuses, revealed no gross developmental abnormality in any of the groups. It appears that in the rhesus monkey, the administration of Carbaryl does not produce any teratologic effect, but is associated with a higher rate of abortion as compared with the control group. (Supported by Food and Drug Administration Contract No. FDA 67-30, by Research Grant 5PO1-ES00226-04 from the National Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant ES 00103-04.)

12. *Observations on the Alteration by Carbaryl of Smooth Muscle Preparation Responses to Norepinephrine.* J. A. SANTOLUCITO, A. HASSAN, and E. R. WHITCOMB, DHEW, Perrine Primate Research Branch, Box 490, Perrine, Florida. (W. F. Durham.)

Previous work from this laboratory (Hassan) suggested that orally administered carbaryl caused an increased synthesis and release of norepinephrine in rats. The present experiment was an attempt to determine whether carbaryl interacts directly with adrenergic mechanisms. Motility response patterns to norepinephrine (NE) in conjunction with alpha- and beta-receptor blocking agents were recorded from isolated preparations of uteri from ovariectomized rats and intestinal segments from rats and rabbits. Nonestrogenized uterus motility was inhibited by 4×10^{-6} M NE or 1×10^{-4} M carbaryl; 7×10^{-5} M phentolamine (PHEN), an alpha-blocking agent, did not abolish the inhibitory action of either compound. Estrogenized uterus motility was stimulated by NE alone and inhibited by PHEN + NE. Carbaryl did not modify this response. 4×10^{-4} M carbaryl antagonized the inhibitory effect of NE on intestinal motility, as did 7×10^{-5} M PHEN. The beta-blocking agent propranolol (PRO), 4×10^{-6} M, did not antagonize the inhibitory effect of NE. It is concluded that carbaryl can interact with adrenergic receptors behaving as a beta-sympathomimetic in uterine preparations and as an alpha-blocking agent in intestinal segments.

13. *Comparative Metabolic Profiles of Carbaryl by Kidney, Liver, and Lung of the Dog and Rat Using an Organ Maintenance Technique.* B. H. CHIN and L. J. SULLIVAN, Carnegie-Mellon University, Pittsburgh, Pennsylvania. (C. P. Carpenter.)

In order to investigate the comparative metabolism of nonhepatic tissue, a test chemical, carbaryl, was studied using a recently reported organ maintenance technique. Because this in

vitro liver technique was qualitatively and semiquantitatively related to the in vivo results established by analysis of urine of the test animal, comparative profiles of organs should illustrate metabolic activity when compared to results obtained from the liver of the same test animal. 1-Naphthyl-^{14}C and *N*-methyl-^{14}C carbaryl were applied separately to growth medium containing fragments of kidney, liver, and lung from dogs and rats. The mixture was incubated for 18 hr, and the medium was analyzed using DEAE-cellulose column chromatography and fluorometric analysis. Metabolic profiles obtained from the liver in vitro are compared with profiles of urines from the rat and the dog dosed po with the test chemical. Metabolic profiles obtained from the kidney and lung in vitro are compared with those obtained from the liver in vitro for both rats and dogs. Both the lung and the kidney from rats and dogs were found to have significant metabolic activity when compared to the liver. The metabolic products found in vitro are species and organ specific and do not depend on the addition of cofactors for the production of the correct metabolic end products. The technique can be a useful tool to study the comparative metabolism of both species and organs and, therefore, help evaluate species variations in toxicologic studies.

14. *The Metabolism of Carbaryl (1-Naphthyl N-Methylcarbamate) by Tobacco Cells in Suspension Culture.* RAYMOND K. LOCKE and RONALD L. BARON, Food and Drug Administration, Washington, D.C. (Clara H. Williams.)

The utility of plant cell culture techniques in preliminary studies of pesticide metabolism was investigated, carbaryl being used as a model compound. Within 12 days, tobacco cells incorporated 21% of the radioactivity added to the culture medium as ring-^{14}C-carbaryl. The radioactivity present in the medium (65%) and cell wash (14%) was shown to be unchanged carbaryl. The cells were homogenized in water and separated by centrifugation into supernatant and debris pellet. Forty percent of the radioactivity incorporated into the cells (8.4% of the administered radiolabel) was associated with the debris pellet. Data obtained by acid hydrolysis of the pellet followed by organic extraction and comparative thin-layer chromatography of the extract with known standards suggested conjugates of carbaryl and α-naphthol. The supernatant, containing 60% of the radioactivity incorporated into the cells, was separated by DEAE-cellulose column chromatography into a neutral fraction consisting of an unidentified unconjugated metabolite and conjugates of α-naphthol, 5,6-dihydro-5,6-dihydroxycarbaryl, and carbaryl, as well as an acidic fraction consisting of conjugates of carbaryl and α-naphthol. The neutral metabolites represented 8.8% of the administered label, and the acidic metabolites accounted for 3.8%. Utilization of cell culture techniques has proved to be a useful tool in providing preliminary information prior to more extensive studies of the metabolism of pesticides.

✓ 15. *Inhibition of Rat Tissue Carboxylesterases and Cholinesterase by Abate® (O,O,O',O'-Tetramethyl O,O'-Thiodi-p-phenylene Phosphorothioate.* SHELDON D. MURPHY and KENNETH L. CHEEVER, Harvard School of Public Health, Boston, Massachusetts.

Abate has been proposed as a purposeful additive to stored potable water for mosquito control in tropical areas. Large single doses inhibit acetylcholinesterase and potentiate the toxicity of malathion in rats. Since carboxylesterase inhibition appears to explain malathion potentiation, the present study was undertaken to determine the dose-response relationships for carboxylesterase (CaE) and cholinesterase (ChE) inhibition by single doses and repeated administration of Abate in the drinking water of male rats. Enzyme assays were performed manometrically. Liver CaE was measured with triacetin, diethyl succinate, or methyl butyrate as substrates. Brain ChE was inhibited 50% at 16 hr after po administration of 250 mg/kg of technical Abate. Liver CaE was 10–20 times more susceptible to inhibition. Administration of 3–50 ppm Abate in the drinking water for 7 days produced dose-related depression of liver CaE. ChE activity of brain, submaxillary gland, erythrocytes, or plasma was unaffected by drinking water concentrations of 30 ppm or less. Administration of drinking water containing 5 ppm for 30 days produced 40–60% reduction in liver triacetin esterase activity. Five ppm of

technical Abate in the drinking water for 7 days potentiated the in vivo anticholinesterase action of a single dose of 400 mg/kg, but not 200 mg/kg, of malathion. In vitro 10^{-7} to 10^{-6} M technical Abate was required for 50% inhibition of liver CaE, but 100–1000 times greater concentrations of a highly purified sample were required. Equivalent inhibitions of liver, brain, or plasma ChE was not achieved with either sample with concentrations as high as 2×10^{-3} M. Only about 2-fold differences for in vivo anticarboxylesterase action were noted for the technical and purified samples. The results indicate that the relatively strong anticarboxylesterase action of Abate might contribute to potentiation with high doses of malathion. (Supported by Research Grants ES 00084 and ES 00002 from the National Institute of Environmental Health Science.)

16. *The Effects of Chronic Disulfoton Treatment on the Cholinesterase Activity of the Rat.* A. MODAK, L. BARLEY, S. WEINTRAUB, and W. B. STAVINOHA, University of Texas Medical School at San Antonio, San Antonio, Texas.

The purpose of this investigation was to study the effect of chronic disulfoton treatment on acetylcholinesterase activity (AChE) and butrylcholinesterase activity (BuChE). Thirty Holtzman rats weighing 100–130 g were distributed into 3 groups of 10. Two groups received 1.5 mg/kg, ip of disulfoton for 10 days; the third group received only the vehicle. One of the 2 treated groups was allowed to recover for 7 days. Rats were sacrificed by decapitation. The AChE and BuChE activities in mmoles/g protein/hr were estimated in hypothalamus (Hy), medulla (M), hippocampus (Hi), caudate nucleus (CN), ileum (I), and gastrocnemius (G) employing labeled acetylcholine (ACh) and butyrylcholine as substrates. Control AChE activities were: M 5.2599, Hy 4.6205, Hi 3.1906, and CN 13.5607; activities in the treated group were: M 1.9546, Hy 1.8724, Hi 0.6245, and CN 2.4229; and activities in the recovery group were: M 4.0020, Hy 3.2781, Hi 1.7921, and CN 8.6547. CN had the highest activity of the components studied while Hi had the least AChE activity. Disulfoton inhibited the AChE activity in the CN 83%, Hi 81%, M 63%, and Hy 60%. The AChE activity in the ileum was: control 2.9041, poisoned 2.3775, and recovery 2.8064. The gastrocnemius AChE activity was: control 0.4817, poisoned 0.2774, recovery 0.4022. Disulfoton had little or no effect on ileum AChE; however, it inhibited AChE to 43% in gastrocnemius muscle. (Supported in part by USPHS Grant 1RO1NS09356-01 and Air Force 69-1775.)

17. *Differences in the Stereospecificities of Cholinesterases and Cholinergic Receptors.* B. V. RAMA SASTRY and H. C. CHENG, Vanderbilt University School of Medicine, Nashville, Tennessee.

Two working hypotheses have been postulated about the relationship of acetylcholinesterase (AChE) and pseudocholinesterase (ChE) to muscarinic and nicotinic receptors, respectively: (1) The active sites of AChE and ChE are identical with those of muscarinic and nicotinic receptors, respectively; (2) the active sites of AChE are different from those of the muscarinic receptors, and they may be situated on the same macromolecule at two different locations or on two different macromolecules. In spite of close similarities between them, the active sites of nicotinic receptor are different from those of ChE. In order to test the above hypotheses, we have investigated the stereospecificities of muscarinic receptors (on the guinea pig longitudinal ileal muscle) and nicotinic receptors (on the frog rectus abdominis muscle) using optical isomers of lactoylcholines (LCh) as agonists and optical isomers of tropinoyl- and mandeloylcholines (TCh, MCh) as antagonists. We have determined the stereospecificities of Electric Eel-AChE and horse serum ChE using LCh as substrate, and TCh and MCh as inhibitors of ACh hydrolysis. Muscarinic receptors were stereospecific for all 3 D-isomers (dissociation constants, K_A: D-LCh 7.3×10^{-5}, L-LCh 3.02×10^{-4} M; K_B: D-MCh 3.00×10^{-6}, L-MCh 5.22×10^{-6}, D-TCh 2.15×10^{-8}, L-TCh 3.26×10^{-7} M). However, AChE was stereospecific for the L-configuration of TCh (K_I: D-TCh 12.48×10^{-4}, L-TCh 7.8×10^{-4} M). K_I of D-MCh (3.71×10^{-4} M) was not different from that of L-MCh (4.03×10^{-4} M). K_m and V_{max} of D-LCh (6.43×10^{-4} M, 1.2 μmole/min/unit) were lower than the corresponding values for

L-LCh (8.30×10^{-4} M, 3.1 μmole/min/unit). These results indicate that there are differences in the stereospecificities of AChE and muscarinic receptors. Nicotinic receptors were stereospecific for the D-isomers of LCh and MCh. There was no significant difference between the nicotinic activities of D- and L-TCh. ChE was stereospecific for all 3 L-isomers. Therefore, there are differences in the stereospecificities and the environment of the active sites of nicotinic receptors and ChE. (Supported by USPHS Research Grant No. NS-04699.)

18. *Sulfhydryl Groups in the Vicinity of Mammalian Cholinergic Receptors and Their Implication in the Toxicity of Mercurials.* B. V. RAMA SASTRY, Vanderbilt University School of Medicine, Nashville, Tennessee.

A number of investigations have speculated on a role for sulfhydryl groups in mammalian neuromuscular transmission. The locations of these —SH groups are not known. In order to verify the presence of such reactive —SH groups at cholinergic receptors, we have studied the effects of receptor-directed alkylating agents on the rat phrenic nerve hemidiaphragm. One of these compounds is iodoacetylcholine (IACh), in which the pharmacophoric moieties of acetylcholine and iodoacetate were incorporated. The following results were obtained: IACh induced blockade of neuromuscular transmission and contracture of the muscle. The blockade preceded the contracture. The response of the muscle was not abolished for direct electrical stimulation when the muscle contractions elicited by the electrical stimulation of the nerve were blocked. The dose of IACh (0.8×10^{-3} M) which blocked neuromuscular transmission completely, inhibited only 11–30% (11% in 10 min; 30% in 20 min) of 3-PGDH in vitro. Addition of *d*-tubocurarine (2×10^{-5} M) provided partial protection against the irreversible blockade by IACh. The irreversible blockade was produced in nerve–muscle preparations only. These results indicate that an —SH group which is normally embedded by hydrogen bonding might have been unmasked during depolarization; the unmasked —SH group is easily susceptible for alkylation by IACh. The pattern of the neuromuscular blockade produced by *p*-chlormercuric benzoate (10^{-3} M) resembles that of iodoacetate (5×10^{-3} M) and IACh (0.8×10^{-3} M), which suggests that same —SH groups are partly involved in the neurotoxicity of mercurials. (Supported by USPHS Research Grant No. NS-04699.)

19. *The Toxicity of Siduron, a Substituted Urea Herbicide.* HENRY SHERMAN, E. I. du Pont de Nemours and Company, Newark, Delaware.

Siduron [1-(2-methylcyclohexyl)-3-phenylurea] is a herbicide that is used in the selective control of annual seedling grass weeds in noncrop areas, such as turf. Siduron has low acute and subacute oral toxicity for the male rat. Its approximate lethal dose is >5000 mg/kg. When administered at 3400 mg/kg/day for a total of 10 doses, siduron produced transient cytological changes in the liver, i.e., fine diffuse vacuolation. A 3-generation, 6-litter reproduction study with rats fed dietary levels of 100 and 500 ppm siduron was without adverse effects upon reproduction and lactation performance. Male and female rats have been fed nutritionally-complete diets containing 100, 500, or 2500 ppm siduron for 2 years. Except for a lower rate of weight gain among the female rats that received 2500 ppm siduron, there was no nutritional, clinical, hematologic, urinary, or biochemical evidence of toxicity in the test groups; however, there was a histologic effect upon the thyroid gland of rats that received 2500 ppm siduron in the diet. Male and female dogs, 1–2 yr of age, fed nutritionally complete diets containing 100, 500, or 2500 ppm siduron for 2 years showed no evidence of toxicity.

20. *Metabolism of a New Carcinogen Related to Benzidine.* SOUHEIL LAHAM, Department of National Health and Welfare, Ottawa, Ontario.

During biological studies on carcinogenic derivatives of benzidine, it was deemed necessary to study the influence of a methoxyl group placed in the *ortho*-position on the biological activity of biphenylamines. Furthermore, it was important to study the metabolic fate of a typical compound of this series 3-methoxybenzidine (3-MB) the carcinogenic activity of which has

been demonstrated in our laboratory (unpublished data). The purpose of this report is to describe the results obtained in these metabolic studies. Male Sprague-Dawley rats (400 g) were injected ip once a day for 4 consecutive days, with 1 ml of a corn oil solution containing 40 mg (100 mg/kg) of 3-MB, whereas the control rats received an equivalent volume of corn oil. Urine of each animal was collected in a glass container cooled with liquid nitrogen and pooled for chromatographic analysis. Freshly collected urine was thawed, spotted immediately on chromatographic plates (ANALTECH, SiO_2, 250 μ), and developed in several solvent systems. After drying, the plates were sprayed with Ehrlich reagent and viewed under ultraviolet light. Several metabolites, including an acetyl derivative of 3-MB (4-amino-4′-acetamido-3-methoxybiphenyl), were detected with this method. This compound has been isolated and identified by its R_f in several solvent systems, by the color reactions it gives with various spraying reagents, and by microanalytical and spectroscopic data. It is interesting to note that in vivo acetylation of this amine occurs in 4′-position, which leads us to conclude that the methoxyl group in the 3-position favors steric hindrance in aromatic amines of the benzidine series.

21. *Incidence of Teratologic Anomalies in Control Charles River C-D Strain Rats.* B. N. BANERJEE and R. S. DURLOO, Woodard Research Corporation, Herndon, Virginia.

In order to evaluate the occurrence of teratologic anomalies in a normal population, an experiment was conducted in Charles River C-D strain rats of Sprague-Dawley origin. A total of 2155 rat fetuses was studied; of these 1430 fetuses were examined for skeletal, and 725 for visceral, anomalies. The dams received Purina Laboratory Chow, supplemented with 1% cod liver oil NF XIII, and water ad libitum. On day 20 of gestation, the fetuses were removed from each rat by cesarean section. Some of the fetuses (725) were stored in Bouin solution for visceral examination, and the others (1430) were cleared and stained with Alizarin Red S for skeletal examination. The most common skeletal anomalies observed were incomplete ossification of interparietals (10.70%), incomplete ossification of supraoccipitals (11.05%), and incomplete ossification of centra (6.08%). No pronounced visceral anomalies were observed except hydroureter (1.38%).

22. *Pre- and Postnatal Studies on 2,4,5-T, 2,4-D, and Derivatives in Wistar Rats.* K. S. KHERA, B. L. HUSTON, and W. P. MCKINLEY, Food and Drug Directorate, Research Laboratories, Ottawa, Ontario, Canada.

Several herbicides have been evaluated in Wistar rats for developmental effects in the progeny. The compounds studied were 2,4,5-T (2,4,5-trichlorophenoxyacetic acid); 2,4,5-T butyl ester; 2,4-D (2,4-dichlorophenoxyacetic acid); and the isooctyl, butyl, dimethylamine, and butoxyethanol derivatives of 2,4-D. The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin content of the last two 2,4-D derivatives was not known. The other herbicides contained less than 0.5 ppm of this dioxin. The treatment consisted of single daily po doses of each herbicide in corn oil or 0.5% aqueous gelatin suspension during the 6th to the 15th day of gestation. The amount administered was adjusted daily to maintain a constant dosage based on body weight. Maternal treatments with 2,4,5-T and 2,4-D (up to 100 mg/kg), the butyl esters, and the 2,4-D isooctyl ester (up to 150 mg/kg) was not associated with any adverse effects detectable after 12 wk in naturally littered progeny. A non-dose-dependent low incidence of delayed opening of the eyelids, corneal opacity, and hydrocephalus was observed during initial trials. Microscopically, the abnormal offspring had cytomegalovirus inclusions in the internal lacrimal glands. The cranial defects were not reproducible when replicate experiments were conducted in an isolated area. In prenatal studies, dams were killed at term and embryos were scored for skeletal and visceral abnormalities. All specimens were coded and examined blindly. A treatment of up to 100 mg/kg of 2,4,5-T or 2,4-D had no apparent adverse effects on gravida, but was associated with depression of average litter weight and a significantly increased incidence of spontaneously occurring skeletal anomalies. The significance of the teratogenic effects of 50 mg/kg of these compounds was inconclusive whereas 25 mg/kg had no apparent malformative effects.

A preliminary study with doses of 150 mg/kg of the butyl and isooctyl esters of 2,4-D and 300 mg/kg of the dimethylamine and butoxyethanol derivatives of 2,4-D indicated teratogenic effects (skeletal malformations) while doses ⅓ as large had no effect.

23. *Mutagenic and Teratogenic Studies with Lead Acetate and Tetraethyl Lead.* G. KENNEDY, D. ARNOLD, M. L. KEPLINGER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

There has been concern regarding environmental contamination with lead and its toxicity. Mutagenic (dominant lethal) and teratogenic studies were conducted with lead acetate and tetraethyl lead. Male mice, treated with one dose of a compound, were mated with untreated females. An increase in postimplantation losses was produced by induction of dominant lethal genes. In the teratogenic study, females were treated during organogenesis, and the young were examined for structural deformities. Lead acetate was administered po to male mice for 5 consecutive days and added to the drinking water of male mice for 60 days. Tetraethyl lead was administered as a single dose to groups of male mice, po and ip. The males were mated with 4 females per wk for 6 wk; 1 wk later, the females were sacrificed at mid-pregnancy, and the numbers of implantation sites, resorption sites, pups, and corpora lutea were counted. There was no significant increase in early resorptions. Lead acetate and tetraethyl lead were administered to gravid mice during the period of embryogenesis. At levels producing toxic (lethal) effects in the female, as well as those well tolerated, the fetuses were normal. Lead acetate or tetraethyl lead was neither mutagenic nor teratogenic in the mouse.

24. *Reproduction and Teratology Studies with the Insecticide Carbofuran.* J. F. MCCARTHY, Niagara Chemical Division, FMC Corporation, Middleport, New York; and O. E. FANCHER, G. L. KENNEDY, M. L. KEPLINGER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Teratogenic studies with the insecticide Carbofuran were conducted on beagle dogs, albino rats, and albino rabbits. No adverse effects on the general health of male or female dogs, or on reproductive behavior of dogs were found at dietary levels up to 50 ppm. All progeny, delivered by cesarean section or delivered naturally, appeared normal in every respect. No evidence of teratogenic activity was found in the rats or rabbits. In the three-generation reproduction studies with rats, a dietary level of 100 ppm Carbofuran resulted in decreased weight gain of parental animals and markedly reduced survival of the young. Lower feeding levels had no adverse effects through three generations in the rat. Investigation into the causative factors for decreased survival of the young rats included studies of milk and pup tissues.

25. *Drug Effects on Spermatogenesis Studied by Velocity Sedimentation Cell Separation.* INSU P. LEE and ROBERT L. DIXON, National Cancer Institute, Bethesda, Maryland.

The velocity sedimentation technique of Lam and co-workers for spermatogenic cell separation has been used to study the incorporation of isotopically labeled thymidine, uridine, and L-leucine during spermatogenesis and to investigate the effect of antineoplastic agents on these metabolic processes. Suspensions of cells from the seminiferous tubules of mice (control and drug treated) were separated and routinely collected in 135 fractions which subsequently indicated at least 5 major distinct cell types. These cells were identified microscopically as spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa. Radioactivity present in acid-insoluble cell constituents was determined by liquid scintillation counting. Following a single ip injection of thymidine, the radioactivity appeared in the spermatogonial fractions 1 hr after treatment, and started to appear in spermatozoa 11 days after injection. One hour after uridine administration, the major portion of radioactivity was present in spermatids with lesser amounts in spermatozoa. Radioactivity was also mainly present in the spermatids and spermatozoa cell fractions 1 hr after the administration of

leucine. The percent radioactivity incorporated (controls 100%) of thymidine, uridine, and leucine, respectively, 1 hr after the treatment of individual animals with the optimal antitumor doses of the following antineoplastic agents is approximately as follows: cytosine arabinoside 0, 100, 66%; vincristine 66, 34, 100%; procarbazine 9, 21, 81%; and L-asparaginase 92, 39, 61%.

26. *Mutagenic Studies with 6-Amino Nicotinamide in Rats.* R. F. LOEHR, G. E. COX, S. CARSON, S. M. REIMER, and E. E. VOGIN, Food and Drug Research Laboratories, Inc., Maspeth, New York.

Experiments have been carried out to characterize the chromosomal karyotype of FDRL Wistar-derived rat. These studies included the determination of the optimum tissue for evaluating chromosomal aberrations in vivo. As an extension of this work, and to evaluate the susceptibility of the rat to chemical mutagens, dominant lethal tests and chromosomal analyses have been performed in rats given 6-aminonicotinamide. Based on a variety of experiments, the data indicate the following decreasing tissue order for sensitivity in visualizing and characterizing chromosomes in the FDRL Wistar-derived rats: adult bone marrow, 14-day-old whole embryo, 19-day-old fetal spleen, and 19-day-old fetal liver. The use of these has been preferred over that of culturing leukocytes because of the reduced time required to obtain preparations suitable for examination. Karyotyping has indicated the strain to have $2N = 42$ chromosomes with a 2–4% occurrence of abnormalities (aneuploidy with a few chromatid breaks) in all of the above-listed tissues. Increased effectiveness of tissue culture preparation has been attained by the prior injection of colchicine into the rat 2 hr prior to tissue sampling. This results in a more complete mitotic arrest than is achieved with the addition of colchicine to the tissue culture in vitro. A known teratogen 6-aminonicotinamide at doses of 1 mg/kg ip caused dominant lethal effects during week 1 of an 8-wk mating sequence in males. Fetuses obtained from pregnant rats given 10 mg/kg of 6-aminonicotinamide on day 13, showed a 60% increase of chromosomal abnormalities and gross evidence of teratogenesis. The data support the utility of the various tissues for chromosome karyotyping and of the FDRL Wistar-derived rat for studying chemical mutagens.

27. *The Embryotoxic and Teratogenic Effects of Various Agents in the Fetal Mouse and Rabbit.* K. T. SZABO, S. M. FREE, H. A. BIRKHEAD, Y. J. KANG, E. ALSTON, and M. HENRY, Smith Kline & French Laboratories, Philadelphia, Pennsylvania.

Six agents, an antibiotic (actinomycin D), 2 alkaloids (colchicine and vincristine), two salicylates (aspirin and methylsalicylate), and an antineoplastic agent (hydroxyurea) were evaluated for embryotoxicity and teratogenicity in randombred mice and rabbits. Various doses and routes of administration were used to establish optimal dose and time of treatment between days 5 and 14 of pregnancy. The fetuses were removed by cesarean section and examined for gross, skeletal, and visceral malformations. Approximately 550 pregnant mice and 300 pregnant rabbits were employed. All agents were relatively well tolerated by the pregnant mice, but embryonic development and survival were seriously disturbed. Colchicine and hydroxyurea were abortifacient in both species. Actinomycin D and vincristine were abortifacient only in rabbits. Actinomycin D produced malformations of virtually every organ system in the mouse embryo, but it was not teratogenic in rabbits. Colchicine, contrary to the action of other agents, was teratogenic only just before or around the time of implantation in mice and resulted in highly specific craniofacial malformations. Rabbits were less susceptible to the teratogenic effect of colchicine, and mainly skeletal and visceral defects were produced. Vincristine was less teratogenic and more embryotoxic in mice than in rabbits. Aspirin was highly embryotoxic but not teratogenic in rabbits. The abnormalities produced in mice by aspirin were mainly skeletal except a few cases of cleft palate and hydrocephalus. Methylsalicylate was embryotoxic and teratogenic in both species. The malformations comprised

cleft palate, exencephaly, hydrocephalus, omphalocele, open eyelid, and spina bifida. Treatment with hydroxyurea caused phocomelia in both species. Malformations of the central nervous system and digits were also found.

28. *Teratogenicity of a Group of Phthalate Esters in Rats.* A. R. SINGH, W. H. LAWRENCE, and J. AUTIAN, University of Tennessee Medical Units, Memphis, Tennessee.

Polyvinyl chloride is used for a large number of medical and paramedical devices. Flexible polyvinyl chloride contains one or more plasticizers, of which the phthalate esters are the most widely used. It has been demonstrated that small quantities of phthalates may be released from polyvinyl chloride items to physiological solutions. To ascertain the effect these plasticizers might have upon the fetus, a teratogenic study was undertaken in rats on 8 phthalate esters. Adult Sprague-Dawley rats received ip injections of the test agent on days 5, 10, and 15 of gestation. On day 20 each rat was sacrificed with an overdose of ether and the uterus was surgically exposed to permit removal of live or dead fetuses and counting of resorption sites and corpora lutea. Gross malformations were observed and recorded, and 30–50% of the fetuses were visualized and stained for study of skeletal structures. Six of the phthalates, whose acute ip LD50 values were below 10 ml/kg, were administered at doses of $\frac{1}{10}$, $\frac{1}{4}$, and $\frac{1}{2}$ of the acute LD50 dose at each time period, and the two relatively nontoxic compounds were injected at a level of 5 and 10 ml/kg. Distilled water and normal saline were tested at 10 ml/kg, and cottonseed oil at 5 and 10 ml/kg. Untreated animals were used for control values. The number of corpora lutea ranged from 52 to 65 per group of 5 rats, with no apparent distribution according to treatment. The extent of resorptions ranged from 0% in some groups to as high as 91%. Embryotoxic activity ranged from 0 to 50%. Fetal malformations ranged from 0 to 100% for both gross and skeletal abnormalities. Most fetuses from the phthalate-treated animals were smaller than from the untreated, water, saline, or cottonseed oil-treated animals. In most instances, a higher percentage of skeletal abnormalities were observed than noted grossly. Absence of tail, anophthalmia, and twisted hind legs were the most common gross abnormalities, while elongated and fused ribs and abnormal skull bones were the most common deformaties found in stained skeletal specimens. These data indicate that phthalate esters have the capacity to produce teratogenic effects in rats.

29. *Comparative Studies on the Absorption of ^{3}H-Thalidomide in Rabbits and Rats.* GEORGE R. KELLER and DAVID A. BLAKE, University of Maryland, School of Pharmacy, Baltimore, Maryland.

Extensive literature review revealed that equal oral doses of thalidomide produce a variable teratogenic response in rabbits. On the other hand, rats are uniformly resistant to the teratogenic effects of thalidomide. In an effort to explain these individual and species differences, a study was designed to compare the oral absorption of thalidomide-^{3}H suspension in both species under identical conditions at teratogenic dose levels (50, 100, and 200 mg/kg po). New Zealand white female virgin rabbits and Sprague-Dawley female virgin rats were employed. Unlike rats, rabbits were quite variable in absorbing thalidomide suspensions, presumably because of delayed gastric emptying time in some animals. However, the mean plasma concentrations were similar in both species during multiple daily administration of thalidomide suspension. When thalidomide was administered to rabbits in a hard gelatin capsule, it was poorly absorbed because of slow dissolution of the powdered drug in gastrointestinal fluids. Regardless of the dosage form administered, only negligible absorption occurred from the rabbit stomach. The small intestine was shown to be the site of absorption in the rabbit. These results suggest that the absorption of orally administered thalidomide suspension is so similar in rabbits and rats that it could not be responsible for the species difference in its teratogenic potency. However, the availability of thalidomide from encapsulated powder is so poor in rabbits that the positive teratogenic results recorded in the literature with this dosage form are questionable.

30. *A Comparison of the Teratogenic Properties of Sodium Salicylate, Sodium Benzoate, and Phenol*, JAN L. MINOR and B. A. BECKER, College of Medicine, University of Iowa, Iowa City, Iowa.

The following sodium chloride controls and functional analogs of sodium salicylate were compared for teratogenic properties by ip administration to Sprague-Dawley rats on gestational (copulation evidence = day 1) days 9–11 or 12–14: sodium chloride, 600, 90 mg/kg; sodium salicylate, 500, 283, 158, 50 mg/kg; sodium benzoate, 1000, 315, 100 mg/kg; and phenol, 200, 63, 20 mg/kg. On days 12–14, fetal body weight was reduced from high control dose by high doses of all three drugs: 5.25 ± 0.17; 4.22 ± 0.06; 4.56 ± 0.05; 4.64 ± 0.08 g (respective to listing above). Gross anomalies were not observed. In utero deaths were increased by both sodium salicylate and sodium benzoate: 1, 26, 12, 1% (respective to listing above). On days 9–11 fetal body weight was reduced from control by sodium salicylate, $\geqq 158$ mg/kg, and sodium benzoate, 1000 mg/kg, but not by phenol: 5.10 ± 0.03, 3.98 ± 0.05, 4.75 ± 0.04, 4.12 ± 0.07, 5.09 ± 0.04 g, respectively. In utero deaths were increased by high doses of all of the sodium salts, but not by phenol; 15, 5, 99, 16, 12, 4%. Gross anomalies were observed with sodium salicylate, 500, 283, and sodium benzoate 1000 mg/kg. In conclusion, at equitoxic doses the teratogenicity appears to be associated with carboxyl moiety. The hydroxy group may contribute to, but is not necessary for, teratogenicity. (Supported by NIH Grant Nos. GM-12,675 and GM0141.)

31. *Effects of Barbiturates on Ethanol Elimination in the Rat.* G. S. WIBERG, J. M. SAMSON, B. B. COLDWELL, and H. L. TRENHOLM, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Earlier work from this laboratory indicated that phenobarbital had little effect on blood and brain ethanol decay curves in the rat. The effect of pentobarbital on ethanol metabolism is more complex. At the molecular level, with the use of a purified rat liver alcohol dehydrogenase preparation, pentobarbital not only stimulated the conversion of ethanol to acetaldehyde, but also the reverse reaction: the conversion of acetaldehyde to ethanol. However, studies at the cellular level using rat liver slices, showed that pentobarbital inhibited the metabolism of ethanol but increased the accumulation of acetaldehyde. In the intact animal, increasing doses of pentobarbital (0, 20, 40 mg/kg ip) prolonged the pulmonary elimination of ethanol in rats dosed ip with 1.25 g/kg EtOH but not when the dose was 2.5 g/kg EtOH. In the absence of pentobarbital, increasing the dose of ethanol from 1.25 to 2.5 g/kg produced a more than 2-fold increase in pulmonary elimination of ethanol; pentobarbital abolished this response. These changes can be correlated in part to altered levels of blood ethanol, acetaldehyde, and acetone; these in turn, reflect not only immediate enzyme-substrate responses, but also changes in blood pO_2 and pCO_2 levels coincidental with altered physiologic function following the simultaneous administration of pentobarbital and ethanol.

32. *Effect of Ethanol on the Distribution of Phenobarbital and Pentobarbital in the Rat.* B. B. COLDWELL, H. L. TRENHOLM, B. H. THOMAS, and G. S. WIBERG, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada. (J. F. Borzelleca.)

Previous work in our laboratory has indicated that the brain concentrations of barbital and phenobarbital are enhanced by the simultaneous administration of ethanol. It was of interest to determine whether this effect was specific for brain tissue. Groups of young, adult, male Wistar rats were administered ^{14}C-labeled phenobarbital (50 mg/kg, ip) or pentobarbital alone (30 mg/kg, ip) and with ethanol (3 g/kg, ip). The parameters measured were the total radioactivity in 9 tissues and blood at timed intervals after dosing and the rate of urine excretion. The results showed that (1) barbiturate levels in all tissues were elevated in the presence of ethanol, particularly at the later time periods; (2) the rate of barbiturate elimination from the body was reduced by ethanol; (3) the combination of barbiturate with ethanol markedly

reduced urine excretion during the first 2 hr after dosing; and (4) qualitatively, the effect of ethanol on the tissue distribution of either barbiturate was similar. It is postulated that the higher tissue levels and reduced elimination rate of barbiturates in the presence of ethanol results from the reduction in urine excretion caused by the administration of the barbiturates with ethanol.

33. *Pharmacologic and Metabolic Studies on Ethanol-Amphetamine Combinations in the Mouse.* F. IVERSON, H. L. TRENHOLM, B. B. COLDWELL, and R. H. DOWNIE, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada. (J. F. Borzelleca.)

This investigation was initiated because of our continuing interest in the interaction of central nervous system-active drugs with ethyl alcohol. All experiments were conducted with male mice which were isolated 1 day prior to treatment. Ethanol (3 g/kg) was mixed with *d*-amphetamine sulfate and administered ip. Acute LD50 studies revealed a biphasic initial toxicity response with *d*-amphetamine at 2.5 and 24 hr, but not at 10 min. Ethanol eliminated the biphasic response at 2.5 hr but not at 24 hr. Ethanol had no effect on the blood, brain, and liver decay rates of tritiated *d*-amphetamine (20 mg/kg), nor did it significantly alter the concentrations of unchanged drug in these tissues 30 min after treatment with 20, 40, or 80 mg/kg *d*-amphetamine. Rectal temperature studies indicated that ethanol, in combination with 20 mg/kg *d*-amphetamine, produced a temperature profile falling between the hyperthermia produced by amphetamine, and the hypothermia produced by ethanol. In the final study, treatment with 20 mg/kg *d*-amphetamine-ethanol produced no significant changes in the blood ethanol levels found after administration of ethanol alone. These studies reveal that within the experimental conditions chosen, ethanol produces only subtle changes in amphetamine toxicity in the mouse.

34. *Toxicity of 1-Methylxanthine, Alone and in the Presence of Ethanol.* ROSEMARY L. ALSTOTT and ROBERT B. FORNEY, Indiana University Medical Center, Indianapolis, Indiana.

Studies of 1-methylxanthine, the major metabolite of caffeine in man, were made as part of a caffeine–ethanol study. The compound was administered ip alone and in combination with ethanol to young adult male white Cox mice in order to evaluate its toxicity. Both 24- and 48-hr LD50 values were obtained. It is believed that the 48-hr value more truly represents drug effects since the animals alive at this time appeared to be fully recovered at 72 hr. The LD50 for 1-methylxanthine was found to be 510 mg/kg and for ethanol, 6.655 g/kg. The toxicity of proportionally varied combinations of the two drugs was determined according to the mathematical method of Hewlett and Plackett and the graphical method of Gaddum as modified by Tarrant. These studies revealed a biphasic toxic interaction which became evident as the proportion of ethanol changed. Investigation into the mechanism of the delayed death observed after 1-methylxanthine treatment indicated that kidney damage may be the principal lethal event. Urea nitrogen concentrations in blood were greatly elevated, and a histologic examination of kidney tissue showed a large degree of pathologic alterations as well as the presence, within the lumen of the kidney tubules, of crystals which were morphologically similar to uric acid crystals.

35. *Toxicity of Caffeine, Ethanol, and Caffeine–Ethanol Combinations.* ROSEMARY L. ALSTOTT, and ROBERT B. FORNEY, Indiana University Medical Center, Indianapolis, Indiana.

In order to understand more fully the relationship that existed between caffeine and ethanol when administered concurrently, combined toxicity studies were done. The experimental animals were the weanling male Dutch rabbit and the young adult male Cox mouse. The individual acute toxicities of caffeine and ethanol were determined according to the method of Litchfield and Wilcoxon, and the toxicities of proportionally varied combinations of the two drugs were determined according to the mathematical method of Hewlett and Plackett and the

graphical method of Gaddum. The ip route of administration was used in all studies. The LD50 of caffeine in the rabbit was found to be 305 mg/kg, and in the mouse, 250 mg/kg. The deaths were accompanied in each case by intermittent clonic convulsions, and in most cases a tonic convulsion was the terminal event. The LD50 values for ethanol in the rabbit and mouse were found to be 3.200 g/kg and 6.655 g/kg, respectively. The deaths following ethanol administration occurred after progressive general depression. The deaths that followed the concurrent administration of proportionally varied doses of caffeine and ethanol more closely resembled those after ethanol alone, but required several hours longer to occur in both the rabbit and the mouse.

36. *Ethanol-Induced Adrenocortical Response and Related Metabolism in the Rabbit.* LAWRENCE W. MASTEN and HERBERT H. CORNISH, University of Michigan, Ann Arbor, Michigan.

Increasing interest in ethanol-induced metabolic derangements in the human population, specifically those of adrenocortical origin, has prompted this laboratory to search for a more versatile animal system for the study of these effects. Compared to mice or rats, rabbits readily afford the collection of a large number and/or amount of blood samples from a single animal and thus allow the monitoring of a complex sequence of metabolic events while maintaining an internal control. The present study clearly demonstrates the aforementioned advantages of this model. White Flemish rabbits (3–5 kg) were infused with several doses of 20% v/v ethanol in saline via the marginal ear vein. Controls received an equivalent amount of saline. According to a timed sequence, a number of pre- and postinfusion isometric blood samples were collected employing an in vacuo ear bleeding technique. No food or water was given during the test period, which lasted generally 1 or 2 days. Various metabolic indicators of adrenocortical response were determined, i.e., plasma corticosterone (p-B), plasma urea nitrogen (PUN), and plasma glucose (p-Glu), as well as those of direct ethanol metabolism, i.e., blood ethanol, blood acetaldehyde and a plasma lactate-pyruvate ratio (L:PR). The ethanol-induced adrenocortical response consists basically of 3 phases characterized by alternating p-B levels: an initial brief elevation followed by a long period of low levels, and finally a relatively prolonged elevation. During the first phase, plasma lactate (p-Lac) was elevated. In contrast, plasma pyruvate (p-Pyr), PUN, and p-Glu were unaffected during the initial p-B elevation. Beginning with the end of this phase while p-B was depressed, p-Lac, p-Pyr, L:PR, p-Glu and PUN were significantly elevated. In the final phase, PUN, p-Lac, p-Pyr, and the L:PR remained elevated or returned to normal levels. p-Glu, though, usually declined to normal or subnormal levels before a final elevation if a significant p-B concentration were present. It was not uncommon to have depressed levels separating elevations in each phase. The general response was found to be dependent on both the amount of ethanol given and the time of infusion, the former controlling the intensity and the latter the timing of the response. This work confirmed rat and human data in this area and extended the present knowledge to include both a second and third adrenocorticoid phase characterized by metabolic indicators of this response. (Supported in part by NIH Grant ES00106-04.)

37. *The Oral and Dermal Toxicity of Hexachlorophene in Rats.* T. B. GAINES and R. D. KIMBROUGH, Public Health Service, Department of Health, Education, and Welfare, Chamblee, Georgia.

The toxicity of hexachlorophene, a widely used fungicidal and bactericidal agent, was studied in Sherman strain rats. The acute oral LD50 for hexachlorophene in peanut oil solution was 56 mg/kg in female rats and 66 mg/kg in males. Adult female rats fed 500 ppm hexachlorophene in the diet for 97 days developed paralysis in the hindquarters; 1 of 10 rats died. Females fed 100 ppm exhibited no signs of poisoning. Examination of the central nervous system (CNS) by light and electron microscopy showed extensive vacuolization of the myelin sheaths resembling spongy degeneration of the white matter in all rats fed 500 ppm. The brains were slightly affected in 8 of 10 rats fed 100 ppm. Reproduction studies were conducted in male and female

rats fed 20 or 100 ppm hexachlorophene in the diet continuously from the time the F_0 generation was 4-wk-old through the F_{1a}, F_{1b}, and to weaning of the F_2 generation. No effect on reproduction was observed in rats fed 20 ppm, and the CNS was morphologically normal. In the rats fed 100 ppm the survival rate of the F_1 generations was slightly reduced, the effect being more pronounced, but not statistically significant, in the F_{1b} rats. No definite effect of hexachlorophene was seen in the F_2 generation. Injury to rat skin by stripping with cellophane tape did not appreciably enhance the effect of a single dermal dose of 600 mg/kg of hexachlorophene in 95% ethanol solution (highest dose tested). Two of 10 stripped-skin rats and 1 of 10 rats with intact skin died of hexachlorophene poisoning. A 3% commercial hexachlorophene preparation on the intact skin of female rats at a dose of 48 mg/kg/day for 30 days produced severe ulceration of the treated skin and extensive diffuse spongy degeneration of the white matter of the CNS; similar treatment with 1.5 and 0.75% hexachlorophene solutions (commercial preparation diluted with water) at doses levels of 24 or 12 mg/kg/day, respectively, caused less severe local reaction of the skin.

38. *Mechanism of Enteric Ulcer Formation in Rats and Dogs Treated with Nonsteroid Anti-Inflammatory Agents.* JINKS E. WALTER, and ROBERT M. DIENER, CIBA Pharmaceutical Company, Summit, New Jersey.

Intravenous doses of 10–25 mg/kg of indomethacin and certain nonsteroid anti-inflammatory agents produced multiple intestinal ulcers in rats and dogs at 4 and 12 hr post-treatment, respectively. Other routes of treatment, although capable of producing ulcers, required longer periods. The lesions were distributed throughout the mucosa of the small intestine but were frequently more severe in the duodenal region. Ligation of the common bile ducts of both rats and dogs prior to iv treatment with maximal nonlethal ulcerogenic doses of the test compounds successfully blocked ulcerogenesis. Bile collected over a period of 12 hr by catheterization of the bile ducts of surgically prepared dogs treated iv with the test compounds, induced ulcers when intubated into other dogs. In contrast, the catheterized, treated dogs remained free of ulcers. The mechanism of enteric ulcerogenesis in dogs and possibly rats is attributed to a hepatic metabolite(s) which enters the intestine through bile rather than to direct contact of the parent drug with the mucosa. Studies to characterize the metabolite(s) and to determine its anti-inflammatory activity are in progress.

39. *Effects of Barbiturates and Catecholamine Precursors on the Release of LH and Prolactin in the Proestrous Rat as Measured by Radioimmunoassay.* J. WEDIG, and V. L. GAY, The University of Michigan, Ann Arbor, Michigan. (H. H. Cornish.)

Barbiturates given just prior to the critical period (2–4 PM of proestrus) block ovulation, apparently by an inhibition of the central nervous system. Exogenously administered LH-releasing factor (LRF) has been shown to cause LH release and ovulation in this type of rat. We have used catecholamine precursors in an attempt to override the barbiturate blockade. Cyclic females were cannulated via the carotid artery (9 AM of proestrus), and blood samples, taken every 20 min throughout the afternoon, were analyzed for LH and prolactin. Pentobarbital (40 mg/kg, ip or 25 mg/kg, ia) caused serum prolactin levels to increase whereas sodium pentothal (20 mg/kg × 2, ia) did not. Both of these drugs blocked the LH peak and ovulation. Electrophoretically purified rat LH administered to pentothal-"blocked" rats resulted in ovulation in the absence of increased serum prolactin. L-3,4-Dihydroxyphenylalanine (L-DOPA; 25, 75 mg/kg, ia) given at 1:00 PM on the afternoon of proestrus induced an increase in serum LH in unanesthetized rats, the maximum effect occurring 40–70 min after the injection. Pentobarbital (40 mg/kg, ip) treatment of proestrous rats inhibited the response to L-DOPA either partially or totally. These data indicate that ovulation can occur in the absence of elevated serum prolactin and that barbiturates may prevent both the spontaneously and L-DOPA-induced LH release in the proestrous rat. The latter suggest that barbiturates block a

dopaminergic pathway involved in the process of LRF and/or LH release. (Supported by USPHS Grants ES-00106 and HD-05318.)

40. *Chronic Toxicity Studies with 2,2-Dichlorovinyl Dimethyl Phosphate (DDVP) in Dogs and Rats Including Observations on Rat Reproduction.* S. WITHERUP, W. J. JOLLEY, K. STEMMER, and E. A. PFITZER, College of Medicine, University of Cincinnati, Cincinnati, Ohio. (E. Homan.)

Diets prepared by adding DDVP to laboratory chow in amounts yielding nominal concentrations of 0.1, 1.0, 10.0, 100, and 500 ppm, were fed to rats and beagle dogs for a period of 2 yr and to male and female rats mated through three successive generations. Fresh diets were supplied each week because there was gradual loss of DDVP and a slow accumulation of dichloroacetaldehyde. There was inhibition of the cholinesterase activity in the plasma and erythrocytes of rats and dogs on the nominal 100 and 500 ppm feedings, in the brain of rats at the nominal 500 ppm level and of erythrocyte of dogs at the nominal 10 ppm level. The inhibition of cholinesterase activities had recovered in dogs but not in rats at the end of a 2-yr feeding period. Histopathologic examination of tissues from rats and dogs revealed no adverse effect attributable to DDVP. The content of DDVP (or its decomposition products) in the diet on which the procreating animals were fed had no effect on the number and size of the litters which they produced and did not reduce the probability of the survival of their offspring. No anomaly in anatomical structure was found in any of more than 6000 offspring. Growth and development of the offspring during the suckling period was not retarded by the DDVP fed to the dams.

41. *The Effect of Dietary Dichlorvos on Swine Reproduction and Viability of Their Offspring.* J. A. COLLINS, M. A. SCHOOLEY, and V. K. SINGH, Shell Development Company, Modesto, California; Shell Chemical Company, San Ramon, California; and Bio/Toxicological Research Laboratories, Inc., Spencerville, Ohio. (J. K. Kodama.)

The intent of this study was to provide chronic feeding experience and reproductive performance data in swine with the new ATGARD® V (dichlorvos) Swine Anthelmintic (1963–1966). Male and female swine were fed for up to 37 mo on diets containing polyvinylchloride resin formulations of dichlorvos at 0, 200, 250, 288, 400, and 500 ppm of the active ingredient. Progenitor gilts (primaparous females) were bred; after the weaning of their first litters (F_1), some were rebred to yield second, third, and fourth successive F_1 litters. Females randomly selected from the (first) F_1 generation were raised to sexual maturity, on dichlorvos-medicated rations and bred, producing the F_2 generation. F_2 pigs were then raised to market age on the same dichlorvos-medicated feeds. The dichlorvos in the diet of the procreating swine had no inhibitory effect on the numbers, viability, or growth rates of the offspring, nor were gross anatomic aberances observed in any of the piglets. No pathologic changes suggestive of neoplasia (gross or microscopic) were found in any of the young or adult animals examined at necropsy. A variable but dose-related depression of cholinesterase activity was observed in the whole blood. Activity of the enzyme in brain tissue was only slightly reduced with dichlorvos feeding, and there was never any clinical evidence of neurophysiologic impairment. These dietary levels of dichlorvos had no significant effects on the hemoglobin level, formed elements of the blood, blood glucose, components of the urine, hepatic or renal function, physical structure or Ca/P content of the femoral bone, or individual organ weights (except the liver, which was generally heavier in dichlorvos-fed animals).

42. *Teratology Studies with Dichlorvos in Rabbits.* E. E. VOGIN and S. CARSON, Food and Drug Research Laboratories, Inc., Maspeth, New York; and M. B. SLOMKA, Shell Chemical Company, San Ramon, California.

Teratogenic studies were carried out in rabbits given graded doses of polyvinyl chloride resin formulation of dichlorvos containing up to 62 mg/kg of the active ingredient. Positive

control groups received empty gelatin capsules, distilled water, the PVC resin, encapsulated lactose, or capsules containing dioctylphthalate. This broad group of control treatments was required to control the stress associated with the administration of drug and to determine the possible effects due to the inert ingredients comprising the total formulation of dichlorvos in polyvinyl chloride resin. Doses of 34 mg dichlorvos per kilogram of body weight or greater administered during the critical days of organogenesis were badly tolerated by the dam. Maternal toxicity seen in these animals was associated with the pharmacologic activity of dichlorvos on cholinesterase inhibition. Statistical comparisons revealed no significant differences at nontoxic dichlorvos doses on nidation, in utero survival, or neonatal survival. No evidence of teratogenic changes was observed on gross visceral examination of the fetuses obtained by cesarean section. Skeletal examination of alizarin red S-stained specimens indicated no abnormal skeletal signs of ossification or bone formation. Flexion or rotated limbs were not seen upon incubation of the newborn. The results indicated that at nontoxic doses of dichlorvos to the dam, 12 mg/kg or less, there was no demonstrated teratogenesis in the rabbit.

✓ 43. *Metabolic Fate of Ingested Dichlorvos in Swine.* A. C. PAGE, D. M. DEVRIES, R. YOUNG, and J. E. LOEFFLER, Shell Development Company, Modesto, California. (E. Homan.)

Young pigs were given ^{14}C-vinyl-labeled dichlorvos either as a single dose of slow release formulation in food or by a slow infusion into an isolated intestinal loop. Sows were administered daily doses of the slow release formulation for 4 wk. Results of the 4 hr infusion studies demonstrated intact dichlorvos, demethyl dichlorvos, dichloroacetaldehyde, dichloroethanol, and dichloroacetic acid in the intestinal lumen, but only dichloroethanol was observed in portal or peripheral blood. Even at 4 hr, degradation proceeds well beyond the chlorinated metabolites listed above. The single dose balance trials in young pigs demonstrated considerable radioactive CO_2 in expired air and retention of the radioactive carbon in tissues even 14 days after dosing. Multidose treatment of sows showed a similar pattern of $^{14}CO_2$ expiration and retention of ^{14}C in the tissues. Fractionation of liver and muscle tissue showed that the vinyl carbon entered glycine, serine and, at lower levels, glucose, cholesterol, fatty acids, and RNA. Because of this very broad distribution of ^{14}C in normal body constituents, similar experiments were performed with ^{36}Cl labeled dichlorvos. The ^{36}Cl retained in tissues was identified as chloride ion. Thus the degradation of dichlorvos proceeds rapidly through the chlorinated metabolites described in the literature to dechlorinated compounds which enter many normal metabolic pools. No chlorine containing product related metabolites could be found by either specific residue analytical methods or by radiochemical techniques.

✓ 44. *Metabolic Fate of Inhaled Dichlorvos in Pigs.* J. E. LOEFFLER, D. M. DEVRIES, R. YOUNG, and A. C. PAGE, Shell Development Company, Biological Sciences Research Center, Modesto, California. (J. F. Borzelleca.)

The degradation of dichlorvos in vitro using both blood and lung tissue slices was studied at dose levels related to the inhalation exposure from the NO-PEST ® insecticide. From ^{32}P labeled dichlorvos the metabolites found in vitro were demethyl dichlorvos and methyl esters of phosphoric acid. Short-term inhalation trials in anesthetized pigs did not show the presence of intact dichlorvos or demethyl dichlorvos in blood or lung tissues. Even in the 2–4 hr trials the degradation has proceeded to the stage where only methyl phosphates and phosphoric acid can be detected. Results of longer term ^{14}C trials will also be presented.

45. *The Metabolism of ^{14}C VAPONA® in Rats after Administration by Oral and Inhalation Routes.* D. H. HUTSON, D. BLAIR, E. C. HOADLEY, and B. A. PICKERING, Shell Research Limited, Sittingbourne, Kent, England. (J. F. Borzelleca.)

After oral administration of vinyl-1-^{14}C VAPONA ® to male rats, 42% of the radioactivity was recovered as metabolites during the first 24 hr. After 4 days, 39% was recovered as ^{14}C carbon dioxide, 13% was excreted in the urine, 3.4% in the feces, and the remainder was

found in the carcass. Radioactivity in the urine was divided between at least 9 metabolites, which were examined by chromatographic, isotope dilution, and chemical analyses. ^{14}C metabolites identified in 0–24 hr urine were dichloroethyl β-D-glucopyanosiduronic acid (5.4% of the administered radioactivity) dichlorovinyl methyl phosphate (2.2%), *N*-benzoyl glycine (1.7%), and urea (0.6%). The radioactivity associated with the liver (5% of that administered) 4 days after dosing was largely in the protein fraction and was identified as glycine-^{14}C and serine-^{14}C by isolation and by amino acid analyses. ^{14}C-labeled VAPONA was administered to male rats as a vapor for 1 hr, and the rats then housed for 4 days in metabolism cages. The rates and routes of excretion of radioactivity, and the retention of radioactivity in gut, liver, skin, and carcass were similar to that found after oral dosing. ^{14}C metabolites in the urine of these animals, and the radioactivity in the liver were very similar in their distribution to that found after oral dosing.

46. *Dichlorvos: Human Inhalation Studies.* C. G. HUNTER, Shell Research Limited, Sittingbourne, Kent, England.

Human inhalation exposures to the vapor of dichlorvos, an anticholinesterase insecticide, have been made to define dose-response relationships at concentrations of the order of the TLV and above. The clinical and laboratory observations before, during, and after partial and total body exposures remained unaffected, except for alterations in the activities of plasma acetylcholinesterases. These were reduced, and the degree of reduction was directly related to the intensity of the exposure, concentration of dichlorvos vapor inhaled and the time of exposure in minutes. For any defined exposure the amount of reduction or inhibition of the enzyme system may be estimated.

47. *An Evaluation of the Safety of NO-PEST® Strip Insecticide with Special Reference to Respiratory and Dietary Exposure of Occupants of Homes in Arizona.* J. S. LEARY, L. HIRSCH, E. M. LAVOR, E. FEICHTMEIR, D. SCHULTZ, B. KOOS, C. R. ROAN, C. FONTENOT, and C. H. HINE, Shell Development Company, Modesto, California; Shell Chemical Company, San Ramon, California; Associates in Laboratory Medicine, Tucson, Arizona; Hine Laboratories, Inc., San Francisco, California.

Investigations have been made as to the possible effects of near continuous exposure of man to the cholinesterase inhibitor dichlorvos (DDVP) under near maximal air concentrations and excessive concentrations as obtained with NO-PEST® insecticide strips. There were 3 separate study periods, 1 of which extended over a full year and involved 6 families. A second study of 6 mo duration included 16 families. In the final study of this series, 6 families were exposed to maximal to excessive rates of NO-PEST® insecticide strip use. Residue samples of food consumed during the 41 days of this latter exposure were analyzed. Blood cholinesterase values as well as other blood clinical values were obtained at regular intervals throughout the 3 studies. There were no effects on health related to the exposure to NO-PEST® strips at either maximum use level or at excessive use rates. There were no effects on RBC acetylcholinesterase and only slight depression of plasma pseudocholinesterase within the expected limits of populations with no known exposure to cholinesterase inhibitors.

48. *Exposure of Newborn Babies to VAPONA® Insecticide.* E. C. VIGLIANI, Institute of Occupational Health "Luigi Devoto", University of Milan, Milan, Italy. (J. Keller.)

Plasma and red cell cholinesterase levels were measured in 22 healthy babies at birth and at the end of a 5-day stay in a nursery where one VAPONA® insecticide strip was hung every 40 m^3 and where a time-weighted DDVP concentration of 0.05 mg/m^3 was measured. This exposure, which lasted for 18 hr per day for 5 days, caused no significant change in the red cells or plasma cholinesterase level. In another study, 22 healthy babies were kept 18 hr per day for the first 5 days of life in 2 poorly ventilated nurseries where one VAPONA strip was hung every

30 m^3. The time-weighted concentrations of DDVP in the two nurseries were 0.152 mg/m^3 and 0.159 mg/m^3, respectively. Blood cholinesterase levels were measured at birth and at the end of the fifth day. No adverse effect on health nor significant change in the red cell and plasma cholinesterase levels was observed in any of the 22 babies.

49. *The Metabolism and Binding to Cellular Macromolecules of Acetanilide under Chronic Conditions.* P. H. GRANTHAM, T. MATSUSHIMA, J. H. WEISBURGER, R. S. YAMAMOTO, and E. K. WEISBURGER, National Cancer Institute, Bethesda, Maryland.

Chronic treatment of rats with acetanilide inhibits the carcinogenicity of simultaneously administered *N*-2-fluorenylacetamide. In part, the effect may reside in competition at the level of processing enzymes or of molecular receptors. Thus, the fate and binding of isotope from acetanilide-ring-^{3}H alone to tissue elements after single ip doses of 200 mg/kg or 0.8% dietary intake for 4 wk was compared in male rats. After a single dose, 60% was in urine and 1% in feces. Blood and plasma contained 0.4 and 0.25 μmole/ml after 1 day. The liver had 74 nmole/g, and 4.3, 210, 3200, 66, and 81 pmole/mg were associated with DNA, microsomal and soluble RNA, and microsomal and soluble protein, respectively. After 4-wk dietary intake, 75–90% appeared in 24-hr urine and 3–4% in feces. Blood and plasma levels were 10- to 20-fold higher than after a single dose. The liver contained 15 times more isotope, and 10 to 20 times more was associated with DNA, RNA, and proteins. The quantities of some of the urinary metabolites, resolved on DEAE-cellulose columns and by thin-layer chromatography were different after the single dose as compared to chronic treatment. Thus, the metabolism of acetanilide and binding of metabolites to cellular and molecular receptors is changed by chronic treatment, compared to the generally studied single dose effects. Other drugs and chemicals may exhibit similar alterations in pattern from repeated administration.

50. *Effects of Chrysotile Asbestos on Trace Metals, Hydroxyproline, and Aryl Hydrocarbon Hydroxylase in the Hamster Lung.* J. T. MOUNTAIN, J. R. DIXON, D. B. LOWE, A. E. MOFFITT, JR., and D. H. GROTH, Bureau of Occupational Safety & Health, Cincinnati, Ohio.

In vitro studies showed that trace metals in amounts comparable to those found in chrysotile asbestos can be a factor in promoting carcinogenesis in lungs by inhibiting or activating aryl hydrocarbon hydroxylase. The present in vivo studies employed hamsters as subjects and chrysotile asbestos as the source of trace metals. Asbestos totaling 7 mg was given intratracheally in 3 doses over a 6-mo span; serial autopsies were performed over a period of 15 mo. Fibrosis was apparent early in the study. At final sacrifice comparisons with controls disclosed that (1) the treated animals exhibited increased hydroxyproline (25%) indicating substantially increased collagen; (2) aryl hydrocarbon hydroxylase was depressed 35%, and (3) Ni persisted to the extent of 15% of that introduced in the chrysotile while 30% of Cr was similarly accounted for. Cu decreased 25% as compared to controls. As Ni and Cr inhibit, and Cu activates the carcinogen-metabolizing enzyme, the net effect would favor benzpyrene carcinogenesis in addition to any other primary or secondary carcinogenetic activity due to Ni and Cr. The changes observed support the postulates derived from the in vitro studies which relate asbestos cancer to the metals associated with the asbestos.

51. *Experimental Neoplasia in ChR-CD Rats with the Oral Administration of 3,3′-Dichlorobenzidine, 4,4′-Methylenebis(2-chloroaniline), and 4,4′Methylenebis(2-methylaniline).* E. F. STULA, H. SHERMAN, J. A. ZAPP, JR., E. I. du Pont de Nemours and Company, Inc. Wilmington, Delaware.

It has been reported that 3,3′-dichlorobenzidine and 4,4′-methylenebis(2-methylaniline) were carcinogenic for the rat. 4,4′-Methylenebis(2-chloroaniline) is a rubber chemical whose

carcinogenicity had not been investigated. Because of the close resemblance in chemical structure it was decided to compare the carcinogenic potential of these three chemicals. Dietary ingestion of 1000 ppm of 3,3′-dichlorobenzidine for approximately one year resulted in the development of malignant mammary tumors in male and female rats. Skin tumors developed in both sexes with the ear sebaceous gland tumor occurring more frequently in male rats. The incidence of malignant lymphoma was increased in male rats. The ingestion of a diet containing 1000 ppm of 4,4′-methylenebis(2-chloroaniline) for about 18 mo resulted in lung tumors with some spreading in the pleural cavity. A low incidence of liver tumor was found. Ingestion of this compound in a low protein diet increased the incidence and malignancy of liver tumors in male rats and the malignancy of mammary tumors in female rats. Dietary ingestion of 1000 ppm of a polyamine-curing agent containing 42% 4,4′-methylenebis(2-chloroaniline) for about 18 mo resulted in tumors of the lung and liver in both sexes and skin tumors in males. Ingestion of a diet containing 200 ppm of 4,4′-methylenebis(2-methylaniline) for about 1 year or the gastric intubation of 50 mg/kg/day for about 180 days resulted in liver, lung, mammary, and skin tumors. A total of 650 test and control rats were used with the identification of 750 spontaneous and chemically induced tumors. Like 3,3′-dichlorobenzidine and 4,4′-methylenebis(2-methylaniline), 4,4′-methylenebis(2-chloroaniline) proved to be a carcinogen for rats when ingested. The type of tumors produced differed among the three chemicals.

52. *Carcinogenesis Studies in CAF_1 Mice of Reaction Products of β-Propiolactone (BPL) with Tissue and Blood Constituents.* M. J. BLEIBERG, D. S. BATES, and G. WOODARD, Woodard Research Corporation, Herndon, Virginia.

BPL is a carcinogenic alkylating agent. Thirteen groups of weanling CAF_1 mice (25 M and 25 F per group) each received 24 weekly sc injections (0.1 ml) of one of the following test materials and were observed an additional 12 mo or until death: normal saline, peanut oil, BPL in oil, reaction products of BPL with DNA, RNA, saline, plasma, lecithin, albumin cysteine, *Salmonella* organisms, adenine plus pyrimidines, adenovirus. Data included weekly body weights, time of appearance of tumors, general toxic signs. Selected organ weights were taken at necropsy and tumors were studied histopathologically. Differential leukocyte counts were taken at intervals. Also, 4 groups of newborn mice received one injection only, within 24 hr of birth, of peanut oil, BPL, and oil, urethane, or normal saline. None of the groups receiving BPL reaction products showed a significantly increased incidence of sc sarcoma. Female weanling mice after BPL in peanut oil showed increased mortality and incidence of subcutaneous sarcoma (19/25 vs oil controls, 3/25). Male weanling mice, after BPL and adenovirus (residual viable virus titrated in injected material) showed a greater incidence of adenoma of the lung (17/25 vs saline controls, 9/25). In newborn mice, BPL was not tumorigenic; however, urethane was. Other findings were within normal limits generally. (The research upon which this publication is based was performed pursuant to contract number PH-43-67-1397 with the Division of Biologic Standards, Public Health Service, Department of Health, Education, and Welfare.)

53. *Response of the Oral Mucosa, Cheek Pouch, and Facial Skin of Syrian Hamsters to Chronic Irritation, Polycyclic Hydrocarbons, and Chewing Tobacco.* F. HOMBURGER, Bio-Research Consultants, Cambridge, Massachusetts.

This study was designed to determine whether the oral mucosa of the Syrian hamster was susceptible to cancer induction by polycyclic hydrocarbons, chronic irritation or chewing tobacco. An apparatus was constructed permitting the placement into the gingivolingual fold for 30 min each day for as long as 1 yr of a bit containing cotton, cotton and benzo[*a*]pyrene (BAP), or dimethylbenzanthracene (DMBA), or chewing tobacco. The results of such studies showed that cancers followed the application only of chemical carcinogens and no carcinomas formed with tobacco or chronic irritation. BAP appeared to be a weak carcinogen for skin and mucosa of hamsters. Among 34 animals surviving 52 wk of BAP application to the oral mucosa, only 3 cancers were found, one each on the skin, on the cheek pouch and on the oral mucosa.

Of 16 carcinomas found in 14 animals surviving 30 wk of application of DMBA to the oral mucosa and killed 22 wk later, 11 were on the perioral and nuchal skin, 3 in the cheek pouch and only 2 in the oral mucosa. It would appear that skin is the most sensitive indicator for surface carcinogenesis in the Syrian hamster.

54. *Carcinogenesis Studies in Inbred Syrian Hamsters: Breast Cancer Induction by Methylcholanthrene Feeding.* F. HOMBURGER and S.-S. HSUEH, Bio-Research Institute, Cambridge, Massachusetts.

Induction of breast cancer by oral methylcholanthrene (MC) is subject to genetic control, much as in the case of sc cancer induction by polycyclic hydrocarbons in hamsters. Animals of 6 inbred strains of BIO ® hamsters and of one randombred strain were given a total dose of 250 mg of MC per hamster by stomach tube in 50 doses of 5 mg in 0.5 ml of corn oil, given over a 17-wk period. At least 15 females and 15 males of 7 strains survived to permit autopsy 9–23 wk after the last gavage. None of the males in any line had any breast tumor. All controls of each line receiving only corn oil survived free of tumors. Nine of 21 surviving MC-fed females in the randombred line had breast tumors, compared with ratios of breast tumors to survivors, ranging from 12/18 to 24/29 among the females of the inbred lines. This difference between the tumor incidence in randombred and inbred animals was significant at the 1% level of probability. The difference between the highest breast cancer incidence among the inbred lines (BIO 15.16 24/29, BIO 86.93 22/25) and the lowest incidences among the remaining inbred lines (12/18 in the BIO 4.22 line) was significant at the 5% probability level. Other neoplasms occurring in all lines at random were, in descending order of frequency, tumors of the forestomach, tumors of the ovaries, uterus, stomach, small intestine, large intestine, cecum, and lung. Rare tumors were seen in the skin, spleen, lymph nodes, subcutaneous tissue, liver, thymus, and kidney. Among MC-induced neoplasms in Syrian hamsters, only mammary gland tumors appear to be subject to genetic factors. (Supported by PHS NCI grant CA 10101 and a grant from the Fannie E. Rippel Foundation.)

55. *Toxicology of a New Pyrimidine Antimetabolite, 5-Azacytidine, in Mice, Hamsters, and Dogs.* P. E. PALM and C. J. KENSLER, Arthur D. Little, Inc., Cambridge, Massachusetts.

5-Azacytidine (NSC 102816), which shows strong antitumor activity against the Walker 256 im tumor in rats, in the spleen colony assay in mice, and in the L1210 culture assay, is of interest as a cancer chemotherapeutic agent. The LD50 value in mice administered a single-dose of the compound by the ip or po route was 115.9 and 572.2 mg/kg, respectively; and 2.48 and 4.35 mg/kg after 5 daily doses. In the hamster, iv injection of 15.6, 31.2, 62.5, 125.0, and 250.0 mg/kg produced a moderate to severe reduction in the rate of arteriole and venule blood flow with some hemostasis in the cheek pouch microcirculation 3–5 min after administration. Blood flow returned to normal within 1 hr after injection at all but the highest dosage. A dose of 13.3 mg/kg × 1 administered iv to a dog produced no immediate response, but by day 2 the animal appeared dehydrated and semiresponsive. Bradypnea with slight bilateral rales, hypotension, hypothermia, tremors, and markedly elevated BUN and SGPT values were also noted. The animal was sacrificed in a moribund condition ~25 hr after injection. Dosages of 6.65 and 3.32 mg/kg × 1 also produced elevated SGPT values and reversible leukopenia. The maximum tolerated repeated dose in dogs appeared to be ~0.28 mg/kg (5.5 mg/m^2), following either a 5-day or 5-day repeated-course regimen. Dose-related bone marrow depression ranging from slight-moderate was observed microscopically and detected clinically by a marked but reversible depression in circulating WBC and slight-moderate transient depression in RBC, Hb, Hct, and platelet values in dogs following repeated doses $\geq$0.55 mg/kg. Liver degeneration was suggested clinically by markedly high, but reversible SGPT values, with lesser elevations in alkaline phosphatase and SGOT, and was later confirmed by microscopic observations of focal liver necrosis and fatty metamorphosis in animals which received repeated dosages $\geq$1.1 mg/kg. These lesions were often more pronounced in dogs which received a second 5-day treatment. Death occurred in 1/4 and 2/2 dogs after doses of 1.1 mg/kg × 5(9)5 and 4.4 mg/kg

× 3, respectively. Local effects of the compound, as evidenced from tissues sampled at the site of injection, appeared to be slight. (Supported by Contract No. PH 43-65-61 with Chemotherapy, National Cancer Institute, National Institutes of Health.)

56. *Relationship in Mice between Inhibition of Salivation and Toxicity of Cigarette Smoke.* C. G. VAN DONGEN, Bio-Research Consultants, Cambridge, Massachusetts. (F. Homburger.)

A method has been developed for the quantitative determination of salivary performance in minimally restrained unanesthetized mice. This assay has previously been used to study physiological factors (sex and strain) affecting salivation, and pilot experiments revealed significant salivary inhibition when mice were exposed to whole smoke from various cigarette brands. The purpose of this study is to examine the relationship in mice between inhibition of salivation and acute toxicity of whole smoke from various cigarette brands. Male mice known to exhibit high salivation (C57BL/6J) were used. The salivary performance of the mice was determined 4 times, with 1 hr intervals between measurements. Measurements 1 and 2 served to establish the base salivation level of the mice during that day. Treatments were applied immediately prior to the third measurement whereas, the subsequent measurement was used to indicate aftereffects. Groups studied were untreated controls, machine-exposed air-treated animals, and mice inhaling various concentrations of smoke from various brands of cigarettes. Smoke caused dose-related salivary inhibition, the degree of which correlates well with the acute toxicity of the same smoke at higher concentrations. Salivary inhibition at low smoke concentrations may be predictive of acute toxicity of tobacco smokes.

57. *Maximum Tolerated Doses of Antitumor Agents: Correlation between Experimental Animals and Man.* ELTON R. HOMAN, Laboratory of Toxicology, National Cancer Institute, Bethesda, Maryland.

The predictive value of animal toxicity studies of cancer chemotherapeutic agents was examined by considering the quantitative relationships between maximum tolerated doses (MTD) established in preclinical studies using dogs and monkeys and in clinical investigations. The logarithms of mg/kg MTD of 37 compounds in the 3 species and the logs of the MTD ratios were found to be normally distributed. There is an approximate equivalence between human and dog mg/kg MTD (geometric mean MTD ratio = 0.94), but monkey mg/kg MTD are significantly greater than human MTD (geometric mean MTD ratio = 0.53, $p < 0.01$). The distribution of the logs of the MTD ratios of alkylating agents and purine and pyrimidine antimetabolites (Group I) was compared with the distribution of the whole sample. The variability of Group I data is considerably less as indicated by the coefficients of variation, possibly indicating a greater overall homogeneity of response to these drugs. For group I drugs as well as for the whole sample, the dog MTD is a more accurate predictor of the human MTD than is the monkey MTD when doses are compared on the basis of mg/kg. It has been suggested that for certain antitumor agents the use of body surface area provides better dosage equivalence than body weight (e.g., mg/m^2 rather than mg/kg). Data on the compounds included in the present study do not appear to support this hypothesis. The variability of the MTD ratios indicates that considerable care should be used in extrapolating from animal MTD.

58. *Toxicity of 1,3-Bis(2-chloroethyl)-1-nitrosourea in Newborn Mice.* J. L. GARGUS, L. E. DUDECK, G. F. CONGLETON, and R. W. VOELKER, JR., Hazleton Laboratories, Inc., P.O. Box 30, Falls Church, Virginia. (D. C. Jessup.)

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) was originally synthesized and screened for potential carcinostatic activity. Delayed toxicity, involving the hepatic, renal, pulmonary, and hematopoietic tissues, induced death in dogs and monkeys as long as 100 days after dosing. In these experiments, BCNU was administered to newborn Swiss ICR mice during the first 3 days following birth. Growth inhibition and retardation of hair growth were observed at doses

of 7, 12.5, 25, and 37 mg/kg. Mortality was high at 25 and 37 mg/kg while growth of the surviving animals was significantly reduced. At 7 mg/kg approximately 80% of the treated animals survived. The average body weight of these mice was reduced 25% at 6 mo of age. The lung adenoma incidence and mean in the mice which received 7 mg/kg BCNU was 40% and 0.55 compared to 16% and 0.20 in the controls. In a second group which received 3 injections, the incidence was 37% and the mean was 0.74. The delayed toxicity of BCNU included significant growth reduction when administered to newborn mice. At 6 mo the pulmonary adenoma incidence was also significantly increased.

59. *Potentiation of Effects of Chloroform Inhalation on Barbiturate Narcosis and Metabolism by Phenobarbital Pretreatment.* SURINDRA K. PURI, GEORGE FULLER, and HARBANS LAL, University of Rhode Island, Kingston, R.I.

In previous experiments an increase in the toxicity of CCl_4-inhalation was observed in animals pretreated with repeated injections of phenobarbital. The present study investigated the toxicity of chloroform in barbiturate-treated rats. Adult male rats were exposed to continuous inhalation of chloroform. At 24 hr after the termination of inhalation, hexobarbital narcosis was prolonged and the metabolism of hexobarbital by liver supernatant-fractions (9000 *g*) was inhibited. Pretreatment of rats with phenobarbital (40 mg/kg, ip, daily for 4 days, last injection 24 hr before chloroform inhalation) increased chloroform lethality. After chloroform inhalation, narcosis due to hexobarbital as well as to barbital was enhanced in the phenobarbital group. SKF 525A blocked the effect of phenobarbital pretreatment. These data indicate that pretreatment with repeated doses of phenobarbital which induced hepatic microsomal enzymes enhanced the toxicity of chloroform inhalation.

60. *An Increasing Dose Procedure for Cholinesterase Inhibition Studies in Dogs.* OTIS E. FANCHER, R. GRECO, D. LINDBERG, M. L. KEPLINGER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Much difficulty has been encountered in establishing minimal effect levels of cholinesterase inhibitors in dogs because of the instability of cholinesterase activity levels in untreated animals. In the present study a group of 8 animals, 4 males and 4 females, was maintained on a standard ration during a pretreatment period with weekly determinations of plasma and erythrocyte cholinesterase activity levels using the Autoanalyzer Method with a substrate of acetylthiocholine. After baseline levels or the patterns of variation for each animal had been established, daily treatment of 2 animals of each sex with a selected phosphate was initiated at a dose predicted to be well below an effect level based on results of a feeding study in rats. Two male and 2 female animals were continued as controls. The level of treatment was increased at 3-wk intervals until a clear effect was established for individual animals and for the groups. The existence of an effect was confirmed by a demonstration of recovery during a 3-wk period following cessation of treatment. This procedure is considered to offer advantages over the traditional 90-day study at arbitrarily chosen fixed dose levels.

61. *The Influence of Phenobarbital on Carbon Tetrachloride-Treated Hepatic Changes in the Rat and Dog.* T. M. FARBER, C. L. LITTERST, A. HEIDER, and E. J. VAN LOON, Food and Drug Administration, Washington, D.C.

In vitro microsomal drug metabolizing activity was determined before and after the po administration of 0.05 ml/kg of carbon tetrachloride (CCl_4) to normal and phenobarbital (PB)-treated beagle dogs. At 24 hr after the administration of the CCl_4, little or no reduction in microsomal drug-metabolizing activity was seen in the normal dogs. However, 24 hr after the administration of CCl_4 to the PB-treated dogs, a significant reduction was seen in aniline hydroxylation, aminopyrine and codeine demethylations and in the azoreduction and the nitroreduction of 1,2, dimethyl-4-(*p*-carboxyphenylazo)-5-hydroxybenzene and *p*-nitrobenzoic acid, respectively. A significant reduction in aniline hydroxylation activity was seen after 3 hr

in the PB-treated dogs. CCl_4, 1 ml/kg, was given po to normal and PB-treated rats and the increase in lipid peroxidation was followed by determination of the amount of diene conjugates present in the microsomal membrane at ½, 1, 2, 4, 8, and 24-hr intervals after the administration of CCl_4. Significant increases in lipid peroxidation were seen after the first and second hours in the PB-treated rats, which was over and above that seen with untreated rats given CCl_4. CCl_4, 1 ml/kg, was administered po to normal and PB-treated rats and the decrease in glucose-6-phosphatase activity was determined at ½, 1, 2, 4, 8, and 24 hr after the CCl_4 administration. PB alone produced a 20–30% decrease in activity over the entire period when compared to controls. CCl_4 produced a steady decline in activity in normal rats, reaching a minimum of 30% of control values in 8 hr. In the PB-treated rats, CCl_4 produced a 50% decrease in glucose-6-phosphatase activity after only 2 hr. This effect continued to increase until only 15% of normal activity remained after 24 hr.

62. *Relationship of Altered Hepatic Microsomal Enzymes to Potentiated Hepatotoxicity of Carbon Tetrachloride.* G. P. CARLSON, G. C. FULLER, K. A. SUAREZ, and A. R. JOHNSON, University of Rhode Island, Kingston, Rhode Island.

Previous investigations in this and other laboratories have shown that hepatotoxicity due to CCl_4 is potentiated by phenobarbital (PB). These studies suggest that the potentiation is related to changes in the microsomal enzymes responsible for drug metabolism. Male albino rats (250–350 g) were pretreated with PB (50 mg/kg, ip) or saline daily for 4 days. Another group received 3-methylcholanthrene (3-MC) in corn oil (40 mg/kg, ip) or vehicle alone for 2 days. In the PB experiments, the rats were exposed to CCl_4 vapor (~500 ppm) in a dynamic inhalation chamber 24 hr after the last dose. 3-MC or corn oil treated animals were exposed 48 hr after the last dose. After 3 hr of exposure to the CCl_4 vapor, SGPT was increased 4-fold and SGOT 3-fold in PB treated rats when compared to saline treated. In contrast, similar exposure to CCl_4 vapor elevated SGPT and SGOT of the 3-MC treated rats to less than one-half that of their corn oil controls.

Hepatic microsomal cytochrome P-450 of air exposed animals was elevated 5-fold in the PB pretreated groups and 2-fold in the 3-MC pretreated groups. Exposure to CCl_4 vapor for 3 hr reduced the P-450 content of the PB group to that of the saline treated, CCl_4 exposed group. In the 3-MC experiments, P-450 content in corn oil and 3-MC animals was reduced to one-half of respective control values by CCl_4. Hepatic microsomal NADPH-cytochrome *c* reductase was significantly elevated in the PB pretreated but not in the 3-MC groups. CCl_4 exposure for 3 hr had no effect on NADPH-cytochrome *c* reductase. Thus, potentiation of hepatotoxicity due to CCl_4 is associated with phenobarbital-mediated enzyme induction but not with 3-methylcholanthrene-mediated induction. This contrast may be related to the differential effects of the two inducing agents on NADPH–cytochrome *c* reductase.

63. *Effect of Microsomal Enzyme Inhibition on the Acute Pharmacologic Properties of Chlordiazepoxide.* M. E. GOLDBERG, K. SLEDGE, R. C. ROBICHAUD, B. DUBINSKY, and S. BLUM, Warner-Lambert Research Institute, Morris Plains, New Jersey.

Repeated doses of benzodiazepines result in tolerance to many depressant actions but not to disinhibitory ("antianxiety") effects, which appear to be enhanced. This difference suggests that alterations in metabolite formation or selective neuronal adaptation following chronic dosing may be implicated. The present study was undertaken to assess the acute properties of chlordiazepoxide (CDP) in animals treated with inhibitors of drug-metabolizing enzymes, in order to assess the relative importance of CDP metabolites. For this purpose, mice and rats were treated with SKF-525A or 4-(1-naphthylvinyl)pyridine (NVP) prior to CDP administration and studied using a variety of psychopharmacologic procedures. Microsomal enzyme inhibition prior to CDP produced no significant differences in motor depressant, antimetrazol, or inhibitory actions on discriminated or nondiscriminated avoidance as compared with animals given CDP alone. In chloralose-anesthetized cats, the interneuronal blocking effects of CDP appeared to be somewhat less after NVP pretreatment. In mice, inhibition of drug-metabolizing

enzymes potentiated the taming effects of CDP in electric-shock induced fighting (ED50 = 3.0 mg/kg *vs.* 8.0 mg/kg for CDP alone) and anticonvulsant activity following al 50 mA maxima electroshock seizure (ED50 = 7.5–9.0 mg/kg *vs.* 22.0 mg/kg for CDP alone). Furthermore, the antianxiety effect in a conflict situation in rats following 5.0 mg/kg of CDP was markedly potentiated after 5.0–10.0 mg/kg of NVP. These studies indicate that inhibition of CDP metabolism results in alterations of certain pharmacologic actions, which suggest that higher concentrations of CDP (or a metabolite) at certain neuronal sites do not uniformly alter expected activity.

64. *Hepatic Reticuloendothelial Stimulation and Microsomal Drug Metabolism.* D. W. BARNES and W. R. WOOLES, Medical College of Virginia, Richmond, Virginia, and Division of Medical Sciences, East Carolina University, Greenville, North Carolina.

In earlier findings, we have reported that hepatic reticuloendothelial stimulation results in a marked prolongation of barbiturate sleeping time and of the half-life of hexobarbital in mice. Present studies demonstrate that the extent of the depression of barbiturate metabolism is associated with the degree of enhancement of phagocytic activity, and this depression also correlates with the amount of hypertrophy of the liver, lung, and spleen when zymosan is used as the stimulation agent. In vitro studies employing liver 9000 *g* supernatant indicate a decreased rate of hexobarbital metabolism in stimulated mice. When RE-stimulated mice are treated with the microsomal inhibitor SKF-525A, the depression of the in vitro metabolism of hexobarbital is additive to that produced by zymosan stimulation alone. On the other hand, when phenobarbital is administered concurrently with zymosan pretreatment, the decrease in hexobarbital metabolism associated with the stimulated state is abated. These data and kinetic studies indicate that the zymosan-induced depression is a quantitative phenomenon, since there is no significant difference in the Michaelis constants of RE-stimulated and control mouse liver supernatants. These studies have been extended with the use of another microsomal substrate, antipyrine. Preliminary results indicate the demethylation of antipyrine is also depressed in RE-stimulated mice. The mechanism(s) for the depression of microsomal drug metabolism by RE-stimulants is yet to be established.

65. *Inhibition of Hepatic Microsomal Enzymes by N-Substituted Ethanolamines.* G. V. FOSTER, JR., R. HARTUNG, and H. H. CORNISH, School of Public Health, University of Michigan, Ann Arbor, Michigan.

In our laboratory, 2-aminoethanol (AE) and a number of its N-substituted derivatives have recently been found to significantly lower hepatic drug-metabolizing enzyme activity in rats. On an equimolar basis, aminoethanols may be ranked in order of increasing inhibitory action as follows: dimethyl-AE, butyl-AE, diethyl-AE, AE, diethanolamine (DEA), methyl-AE, and ethyl-AE. The present study was performed in order to characterize the dose- and time-response relationships for inhibition of liver microsomal hydroxylase and *N*-demethylase activities by aminoethanols. Diethanolamine was chosen in view of its widespread industrial and commercial use. Neutralized DEA was injected ip into adult male Sprague-Dawley rats at various doses (100–1000 mg/kg) for varying periods of time (1–14 days). After pretreatment with DEA, in vitro hydroxylation of acetanilide and *N*-demethylation of aminopyrine by rat liver 13,000 *g* supernatants were assayed and compared to untreated controls. DEA-induced inhibition of microsomal hydroxylation or *N*-demethylation activity was delayed in onset, occurring more than 24 hr after a single dose (1000 mg/kg). Dosing for 5 consecutive days at 250, 500, or 750 mg/kg/day caused a 35, 61, or 64% reduction in hydroxylase activity and a 55, 80, or 84% reduction in *N*-demethylase activity, respectively. Hexobarbital anesthesia was prolonged 55, 169, and 222% in similarly treated rats compared to controls. At 100 mg/kg/day for 14 consecutive days, there were 52 and 68% reductions in hydroxylase and *N*-demethylase activities, respectively, 24 hr after the last dose. Inhibition of both microsomal enzymes was long lasting. Both were 50% lower than controls 2 wk after the final dose. Although DEA inhibited microsomal enzyme activity in vivo, it did not inhibit hydroxylase or *N*-demethylase activity when

added to a normal in vitro system. These data suggest that DEA-induced inhibition of hepatic drug metabolism proceeds by indirect mechanisms. (Supported in part by N.I.H. Grant GM-15269 and TO1 EC 00037.)

66. *Synergistic Inhibition of Liver Protein and Nuclear RNA Synthesis Following Combined Oral Administration of Dimethylamine and Sodium Nitrite to Mice.* M. A. FRIEDMAN, G. MILLAR, M. SENGUPTA, and S. S. EPSTEIN, Children's Cancer Research Foundation, Inc., and Harvard Medical School, Boston, Massachusetts.

Nitrosamines are synthesized chemically from nitrite and secondary amines under acidic conditions. There is growing evidence that nitrosamines can be synthesized in vivo from simple precursors. Nitrosodimethylamine (DMN), a potent hepatocarcinogen, inhibits liver RNA and protein synthesis in mice and in other species. We report here inhibition of liver RNA and protein synthesis by combined oral administration to mice of large doses of $NaNO_2$ and dimethylamine-hydrochloride (DMA). Mice were dosed orally with 2000 mg/kg DMA and 150 mg/kg $NaNO_2$, either alone or in combination. Combined administration of DMA and $NaNO_2$ induced acute synergistic toxicity, as evidenced by 70% mortality, inhibition of liver RNA synthesis, as evidenced by a 60% decrease in uptake into nuclear RNA of a 45-min pulse of cytidine-^{3}H, and inhibition of liver protein synthesis, as evidenced by decreased uptake of a 30 min pulse of leucine-^{3}H. Single administration of DMA or $NaNO_2$ alone, produced no synergistic toxicity and no inhibition of RNA or protein synthesis. Additionally, combined administration of 150 mg/kg $NaNO_2$ and 1200 mg/kg methylbenzylamine-hydrochloride produced marked inhibition of leucine incorporation, in contrast with the null effect induced by either compound alone. (Supported by Grants C-6516 and FR-05526 from the National Institutes of Health.)

67. *Mycotoxicosis Induced in Mice by Cultures of Penicillium ochraceum.* WILLIAM W. CARLTON and JOHN F. TUITE, School of Veterinary Science and Medicine and School of Agriculture, Purdue University, Lafayette, Indiana.

In a continuing study of the mycotoxin-producing ability of penicillia, three isolates of *P. ochraceum* were tested for toxicity to mice. The isolates were grown on autoclaved rice at 24°C for 2 wk, treated with chloroform, dried at 100°F for 5 days, and ground for mixing with a purified diet at 50% concentration for all 3 isolates and at 33 and 25% concentration for the markedly toxic isolate. Male weanling mice were fed the diets ad libitum, weighed at weekly intervals, and necropsied when found dead or when terminated. One isolate was markedly toxic, causing high mortality and signs of toxicosis such as roughened hair coats, huddling, decreased activity, and humped backs. Mice became icteric, and multiple greenish foci of necrosis were observed in the liver at necropsy. The earliest microscopic change was an acute necrotizing cholangitis with periductal edema and inflammatory cell infiltration. Both intra- and extrahepatic ducts were involved. Later lesions consisted of the biliary changes and isoseminated foci of necrosis associated with affected bile ducts. The renal lesions varied from focal involvement of proximal tubules by hydropic degeneration and fatty change to extensive cortical necrosis with dystrophic calcification. The other tissues appeared normal. (Supported in part by Cooperative Agreement 12-14-100-9091(51), Market Quality Research Division, USDA and by NIH Grant No. ESCA 00463.)

68. *Acute Effects of Synthetic Aflatoxin M_1.* R. S. PONG and G. N. WOGAN, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Aflatoxin M_1 (M_1), the 4-hydroxylated metabolite of aflatoxin B_1 has been reported to be lethal to ducklings and carcinogenic to rainbow trout. No toxicity data on rats have been available. With the availability of synthetic racemic M_1, we have evaluated its acute effects in rats, and compared its potency with those of synthetic, racemic B_1, and natural B_1. Lethality was

estimated by ip injection of DMSO solutions of the individual toxins to 200 g male Fischer rats at doses of 0.4, 0.6, 0.8, 1.0, or 1.5 mg/kg. Natural aflatoxin B_1 killed 2/4 rats at 0.6–0.8 mg/kg and 6/6 at 1.0 and 1.5 mg/kg. In contrast, synthetic M_1 and B_1 killed 1/2 and 1/1 rats at 1.5 mg/kg and caused only transient weight loss at lower doses. Ability to inhibit liver nuclear RNA synthesis was also compared among the 3 compounds. Male rats weighing 100 g and fasted for 24 hr were intubated with natural B_1 at doses of 0.5 or 1.0 mg/kg, or synthetic M_1 or B_1 at 1.0 mg/kg. Twelve hours later, the rats were injected with 80 μCi cytidine-^{3}H and killed 45 min thereafter. Natural B_1 inhibited precursor incorporation into nuclear RNA to the extent of 90% at 1.0 mg/kg and 85% at 0.5 mg/kg. Synthetic M_1 caused an 80% inhibition and synthetic B_1 a 65% suppression at doses of 1.0 mg/kg. These changes were accompanied by proportionate declines in the nuclear RNA : DNA ratios. Fine structural changes in liver parenchymal cells were similar for all 3 compounds, and consisted of macrosegregation of nucleolar components, disorganization of RER, proliferation of SER, dissociation of ribosomes from RER, among other changes. These data suggest that only one isomer of the racemates of the synthetic compounds is active in rats.

69. *Aflatoxin B_1 Carcinogenesis and Partial Hepatectomy: Absence of an Interaction.* A. E. ROGERS, N. S. KULA, and P. M. NEWBERNE, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The influence of hyperplasia of liver cells on the response to toxins and carcinogens varies in experimental animals. Induction of hyperplasia by partial hepatectomy or by nutritional deficiency has been found to enhance or to have no effect on the induction of hepatocarcinoma by chemical carcinogens. In human populations, hepatic carcinoma and food contamination by the carcinogenic mold metabolite, aflatoxin B_1 (AFB_1) may coexist with viral hepatitis and malnutrition, both of which can induce hyperplasia of liver cells and have been suggested as carcinogens or cocarcinogens for the liver. We have examined the effect of hyperplasia, induced by partial hepatectomy, on AFB_1 carcinogenesis in rats. Young male rats, fed an adequate synthetic diet, were given carcinogenic doses of AFB_1 by gastric intubation either 3 mo before partial (2/3) hepatectomy or starting at intervals of 6 hr to 6 days after a 2/3 or a 1/3 surgical hepatectomy. Rats given AFB_1 3 mo before partial hepatectomy had normal regeneration of liver following hepatectomy, measured by thymidine-^{3}H labeling of liver cells evaluated in autoradiographs, mitotic counts, and liver weight. The development of preneoplastic changes and of carcinoma in the liver were not affected by hepatectomy. Rats given AFB_1 at intervals after partial hepatectomy developed hepatocarcinomas in the same incidence as rats treated after sham hepatectomy. Hyperplasia and other changes induced by partial hepatectomy performed before or after AFB_1 administration did not influence significantly the development of hepatocarcinomas in rats.

70. *The Response of the Hypothalamus to High Doses of Monosodium Glutamate in Mice and Monkeys.* R. ABRAHAM, W. DOUGHERTY, L. GOLBERG, and F. COULSTON, Albany Medical College, Albany, New York.

In an effort to arrive at a definitive assessment of the safety of monosodium L-glutamate (MSG) for human infants, we have examined the effects of MSG in mice (5–7 days old) and monkeys (*Macacca mulatta*, 4 days old). An aqueous solution of MSG (4% w/v) was given by stomach tube or sc, in doses of 4 g/kg to monkeys and mice, and 1 g/kg to mice. The hypothalamus was examined histologically in 100 control mice and 2 control monkeys, and was found to be normal in all respects. Cytochemical analysis of acid phosphatase activity revealed no evidence of abnormality in the number, character, or distribution of lysosomes. The nature and severity of hypothalamic lesions present in treated mice were dependent on the dose of MSG and the route of administration. With 1 g/kg, histologic changes were observed, involving 1–4 neuronal cells of the arcuate nuclei. MSG given po affected 16 of 61 mice, and by the sc route 11 of 26 mice had minor changes. With 4 g/kg, given po, a moderate lesion, involving

cells that were glial in origin, was seen in 27 of 95 mice. When the 4 g/kg MSG was given sc, the cells affected were neuronal and the arcuate nuclei were damaged in 36 of 60 mice treated. Striking structural changes were also seen in lysosomes, with formation of numerous membranous inclusions. Judged by all the criteria employed for assessing the changes brought about in mice, monkeys treated with 4 g/kg MSG (2 orally and 2 sc) revealed no effect in the arcuate nuclei, in the median eminence or elsewhere in the hypothalamus. The species difference in susceptibility to large doses of MSG, and individual variations among the treated mice, may be attributed to dissimilar degrees of myelination of the central nervous system, resulting in disparate permeability of the blood-brain barrier in neonatal animals. The rate of intestinal absorption of MSG is probably also a limiting factor in the production of hypothalamic damage in mice, treated by the oral route. (Supported by Research Grant 5PO1-ES00226-04 from the National Institute of Environmental Health Sciences, NIH, by the National Institutes of Health Training Grant ES 00103-04, and by Food and Drug Administration Contract No. FDA CPF 69-7.)

71. *A Comparison of Parenteral and Oral Administration of Monosodium Glutamate and Congeners to Neonatal Monkeys.* S. CARSON, G. COX, E. E. VOGIN, and B. L. OSER, Food and Drug Research Laboratories, Inc., Maspeth, New York.

Studies have been conducted in neonatal monkeys comparing the effects of single oral and parenteral doses of monosodium glutamate, monopotassium glutamate, sodium chloride, and sodium gluconate with respect to possible pathologic alterations in the eyes and central nervous system (CNS). In addition to microscopic examination of the eyes and hypothalamic region, including the median eminence and arcuate nuclei, blood concentrations of glutamic acid and glutamine were determined. No treatment-related changes were observed nor were any differences found among the test compounds administered by either the oral or parenteral route. The findings in the monkeys support earlier conclusions derived from studies in 3- and 12-day-old mice and rats and 3- and 45-day-old dogs. Monosodium glutamate did not cause any characteristic ocular or hypothalamic lesions of the CNS in any of the species examined.

72. *Effects of Prolonged Monosodium Glutamate and Other High-Salt Diets on Arterial Pressure and Learning Ability in Rats.* L. R. WEISS, J. F. REILLY, J. WILLIAMS, and S. KROP, Food and Drug Administration, Washington, D.C.

A study was undertaken to compare and estimate the toxicologic significance of certain high-salt diets in rats and to test pharmacologically for changes in learning ability, motor activity, blood pressure, and minimal convulsive thresholds. Diets containing monosodium glutamate (MSG) and monopotassium glutamate (MKG), each 10%, or sodium chloride (NaCl) and potassium chloride (KCl), each equivalent to the cation in the glutamate salt, were given to groups of male weanling rats for approximately 34 wk. Observations on growth rates and food intakes were made weekly. Pharmacologic measurements were made after 24 wk on these diets. After 12 and 24 wk, animals receiving MSG showed reductions in weight gain as a group significantly different from controls, while groups receiving the other substances had smaller changes in this respect. Food consumption was similar in all groups. After 24 wk, indirect arterial systolic pressures, as measured in unanesthesized rats by a tail-cuff (plethysmographic) technique, were significantly elevated in MSG rats, as were those in NaCl rats, though to a lesser extent; in contrast, MKG and KCl rats were normotensive. Motor activity was unchanged but amphetamine-induced stimulation of motor activity was reduced in MKG and KCl rats as compared with controls, MSG and NaCl rats. Tests of learning deficits in an avoidance-escape situation indicated that animals receiving MSG failed to develop avoidance lever-pressing behavior. This effect was also seen in some NaCl rats. In contrast, MKG, and especially KCl, animals showed a high rate of avoidance responding suggesting an enhancement of avoidance learning by potassium salts in rats; these results point to the possibility of pharmacologic interactions between drugs and high-salt diets.

73. *Household Toxicology: Appraising the Hazards and Communicating the Risks.* J. R. HICKMAN, G. S. WIBERG, and J. W. BLACK, Food and Drug Directorate, Department of National Health and Welfare, and Department of Consumer and Corporate Affairs, Ottawa, Canada.

Many serious accidents occur in and around the home because of improper storage and handling of household chemical products. The recently enacted Canada Hazardous Products Act has stimulated us to reconsider the question of adequate cautionary and precautionary labelling in an attempt to reduce the incidence and severity of such accidents. In a bilingual country (in which substantial minorities of immigrants also speak languages other than English and French), it was clear that pictorial representation of the hazards would be the most effective means of conveying cautionary information. Symbols were devised in which traditional symbols to denote toxicity, caustic and corrosive properties, flammability and explosiveness were combined with three basic shapes to indicate the degree of hazard. The shapes chosen were the octagon, diamond, and inverted triangle, the meaning of which is intuitively understood by many through analogy with highway signs. Such symbols will be required in a prominent position on the principal display panel of the label of products regulated under the Hazardous Products Act. At the time the symbols were developed, criteria were developed in order to decide upon the appropriate symbols that will be required on regulated products. Hazard ratings are based upon acute and chronic toxicity data obtained by the oral, percutaneous, and inhalation routes and information on skin and eye irritation properties. Flammability hazard is decided upon from consideration of flash point and lower explosion limits. There is already evidence that some manufacturers are reformulating their products to bring them into a lower category of hazard as a result of the new requirement to indicate the degree of hazard on the label.

74. *Toxicity Coding Scheme.* JOSEF V. MARHOLD, Research Institute for Organic Synthesis, Pardubice–Rybitvi, Czechoslovakia.

Toxicologic knowledge of substances and preparations forms the basis for a number of preventive and therapeutic measures. The results of research and experiences should, therefore, be easy and quickly available in the most useful, intelligible, and concise form possible. The proposed Toxicity Coding Scheme (TCS) condenses substantial toxicologic information in a short designation. Artificial code words of 6 letters have been used indicating the mode of action (and the recommendable first aid and basic treatment of intoxication summarized in an alphabetic key book). The position (number) of capital letters in the (first) code word denotes the toxicity range (lethal dose for an adult). Scales of toxicity and irritative effects ratings have been constructed, and the relations to results of animal experiments have been indicated. Possibilities have been provided for denoting the grades of confidence, side and late effects, etc. The TCS designation could form a part of the label of substances, preparations, and drugs and give a sufficient amount of toxicologic information without interfering with commercial interests by indicating the ingredients, using odious denominations or symbols and without serving as advice for any misuse. Care has been taken to avoid obvious hurdles of international use (e.g., by using some letters or preferring some language) and to provide for the possibility of evolution. However, the TCS, as well as every other project of this kind, could be really useful only if unfolded by a team, sponsored by influential organizations and broadly accepted.

75. *Electrocardiographic Telemetry with Arrhythmia Recording on Dogs Chronically Exposed to Air Pollutants.* WINSTON N. BLOCH, JR., WALDEN C. CROCKER, THOMAS K. WESSENDARP, and TRENT R. LEWIS, National Air Pollution Control Administration, 1055 Laidlaw Avenue, Cincinnati, Ohio. (J. F. Borzelleca.)

Electrocardiographic telemetry was performed on 93, 4½-year-old female beagle dogs that had been exposed to air pollutants daily for 4 years. The glued-on electrodes and vest-mounted

transmitter operated on nonrestrained dogs where 2 to 4 were simultaneously present in exposure chambers. An arrhythmia-monitoring system indentified widened beats and recorded them on a bar graph type recorder. Data were gathered during a diurnally identical 13.5–15-hr period for all dogs. The resulting bar graphs were analyzed by planimetric determination of area contained and division by the number of hours of the recording. This gave the area under the widened beat bar graph per hour, hereafter called the widened beat index. The mean widened beat index was similar for the 18 animals in clean air, the 11 animals each in raw auto exhaust (CO = 100 ppm), oxides of sulfur (100 $\mu g/m^3 H_2SO_4$ and 0.5 ppm SO_2), low nitric oxide (0.2 ppm) with high nitrogen dioxide (0.5 to 1 ppm); and in the 10 dogs exposed to raw auto exhaust with oxides of sulfur—the greatest deviation being by no more than 8.6% The 11 animals exposed to irradiated auto exhaust (CO = 100 ppm, O_3 = 0.3 ppm) with oxides of sulfur had a mean widened beat index 42.03% greater than that of the control animals. The 10 animals exposed to irradiated auto exhaust had a mean widened beat index 30.76% greater than that of controls. Four exposure atmospheres of this study comprised a 2 by 2 factorial based on the presence and absence of irradiated auto exhaust and oxides of sulfur. An analysis of variance was performed on pooled individual mean widened beat indices of dogs exposed to irradiated auto exhaust and their corresponding controls. A significance probability of 0.057 was obtained. This suggested that exposure to irradiated auto exhaust was causing an elevated widened beat index. These results indicate an association between chronic irradiated auto exhaust exposure and an increased incidence of widened electrocardiographic heart beats. The latter suggests cardiac dysfunction.

76. *Effects of Adrenalectomy and Phenobarbital Pretreatment on the Toxicity and Biochemical Effects of 1,1-Dichloroethylene.* LAWRENCE J. JENKINS, JR., JOSHUA TRABULUS, and SHELDON D. MURPHY, Harvard School of Public Health, Boston, Massachusetts.

1,1-Dichloroethylene (vinylidene Cl) is used as a monomer in the manufacture of thermoplastic polymers in industry and has been identified as a contaminant in the atmospheres of closed systems such as spacecraft and nuclear submarines. Information on its toxicity indicates that it is a central nervous system (CNS) depressant and hepatotoxin, but little is known concerning its biochemical effects. This investigation was undertaken to more clearly identify the biochemical response to vinylidene Cl. A time-course study following single po doses of 500 mg/kg to male rats showed that liver alkaline phosphatase (AP) activity was increased 11-fold and liver tyrosine transaminase (TT) activity 13-fold 20 hr after the administration of vinylidene Cl. Plasma alanine transaminase (AT) reached a maximum activity of 19 times controls 44 hr after vinylidene Cl and plasma AP was at maximum 5-fold increase at the same time. Liver glucose-6-phosphatase (G-6-P) activity reached maximum depression (52% of controls) at the 44 hr point. These effects resemble the biochemical effects of CCl_4, and since it is known that adrenalectomy alters the hepatotoxicity of carbon tetrachloride, we tested the effects of adrenalectomy on vinylidene Cl toxicity. Adrenalectomy 10–14 days prior to administration of vinylidene Cl reduced the 24-hr po LD50 from approximately 1550 mg/kg to 84 mg/kg. Garner and McLean reported that pretreatment with phenobarbital increased the susceptibility of rats to carbon tetrachloride poisoning. With vinylidene Cl, we found that pretreatment with phenobarbital (50 mg/kg ip daily for 5 days prior to administration) exerted a protective effect against its toxicity, when judged by the parameters of liver G-6-P depression, liver AP and TT stimulation, or plasma AT increase. Thus, although the toxicity of vinylidene Cl resembles that of CCl_4, it appears that these two materials are not subject to the same toxicologic interactions. (Supported in part by Training and Research Grants TO1 ES-00045 and RO1 EC-00216 USDHEW; L.J.J. supported by Outservice Training Program of U.S.N.)

77. *Effects of Halocarbon Compounds on Rat and Mouse Mitochondria Following Exposure in Vitro and in Vivo.* E. BACHMANN, and L. GOLBERG, Albany Medical College, Albany, New York.

The mitochondrial effects of halocarbon compounds, particularly fluorinated fire extinguishers and retardants, have been investigated as part of a study having as its object the

assessment of the safety of these agents for man. After inhalation exposure of weanling female Sprague-Dawley rats and male Swiss albino mice, mitochondrial and microsomal fractions were isolated from liver, kidney, lung, heart, and brain. Mitochondrial metabolic activities (electron transfer, oxidative phosphorylation, citric-acid cycle activities, and substrate-level phosphorylation) were compared with the corresponding activities of mitochondria isolated from untreated animals and of similar mitochondria exposed to the same halocarbons in vitro. Inhalation of bromotrifluoromethane and hexafluoroethane (5% by vol for 30 min in a closed system) had no effect on the metabolic activities of liver, brain, and lung mitochondria of rats or mice. Dibromotetrafluoroethane produced significant effects on mitochondrial activities after a single exposure of the animal to 4.3% by vol for 10 min or after repeated exposures (1 hr daily for up to 6 wk) to lower atmospheric concentrations (2.9% for rats and 1.3% for mice). Lung and brain mitochondria appear to be more susceptible to damage by inhaled halocarbons than heart, kidney, and liver mitochondria. Results obtained with the above-mentioned compounds, and with others of the same series, demonstrate a diversity of effects that may be correlated with changes induced in the intact animal. (Supported by NASA Contract NAS 9-9964, by Research Grant 5PO1-ES00226-04 from the National Institute of Environmental Health, NIH, USPHS, and by the National Institutes of Health Training Grant ES 00103-04.)

78. *Chronic Pulmonary Pathology in Rats after the Intratracheal Injection of 0.4, 4, and 40 μg of Beryllium as Beryllium Hydroxide.* DAVID H. GROTH and GEORGE R. MACKAY, Bureau of Occupational Safety and Health, Cincinnati, Ohio. (H. E. Stokinger.)

As part of an ongoing project to determine the variables that affect the pulmonary response to beryllium compounds in rats, 3-mo-old, female, Wistar rats were divided into 4 groups and injected intratracheally with distilled water, 0.4, 4, and 40 μg of Be as $Be(OH)_2$. They were sacrificed 8 mo later, and the tissues were examined grossly and microscopically. The injection of 40 μg of Be produced pulmonary interstitial fibrosis, lymphocytic and mast cell infiltrates, alveolar metaplasia, and adenocarcinoma. The 4-μg dose produced mast cell infiltrates and metaplasia, but no adenocarcinoma. This establishes a definite dose-response relationship between the amount of $Be(OH)_2$ injected and the production of lung tumors, and also represents the smallest amount of any inorganic compound that has been demonstrated to induce lung cancer in rats. Metaplasia is a consistent feature of low-level exposures and is probably a precursor to cancer.

79. *Ultrastructural Observations of the Pulmonary Epithelial Response to Beryllium Hydroxide in the Rat.* DAVID H. GROTH, GEORGE R. MACKAY, and MICHAEL L. MEAD, Bureau of Occupational Safety and Health, Cincinnati, Ohio. (H. E. Stokinger.)

A previous report described the ultrastructural character of the predominant cell types encountered in beryllium hydroxide-induced rat pulmonary epithelial tumors: an abundance of cuboidal cells containing cytoplasmic lamellar inclusions in the bronchioloalveolar cell tumor, ciliated columnar cells mixed with nonciliated columnar cells containing cytoplasmic lamellar inclusions in the adenocarcinoma, and flattened squamous cells containing elongated nuclei but few cytoplasmic organelles in the epidermoid carcinoma. The present report describes the ultrastructural morphology of preneoplastic pulmonary epithelium after a single low dose intratracheal injection with beryllium hydroxide in the rat. An evaluation of the cytologic ultrastructure is made, comparing the preneoplastic epithelium and the mature tumor types.

80. *Comparative Changes in Serum Enzyme Levels in Liver Necrosis Induced by Beryllium and Other Liver Toxins.* J. J. CLARY and L. A. BLAND, Bureau of Occupational Safety and Health, Cincinnati, Ohio. (H. E. Stokinger.)

This study was undertaken to compare changes in serum enzyme (ICD, SGOT, SGPT, and LDH) levels in beryllium-induced liver necrosis (midzonal) with liver necrosis caused by carbon

tetrachloride (centrolobular) or allyl alcohol (perilobular). Because of the recognized difference in enzyme concentration according to liver site, it was of interest to determine whether the serum enzyme response to Be differs from that of CCl_4 or allyl alcohol. Be (0.8 mg/kg of body weight), CCl_4 (2.5 mg/kg body weight) and allyl alcohol (24 mg/kg body weight) were injected in male albino rats which were serially sacrificed at 4, 8, 24, and 48 hr after injection. The first change in serum enzyme level was seen in the 4-hr CCl_4 group. ICD demonstrated the greatest elevation (9-fold), SGT and SGPT were about equally elevated (5-fold), and LDH showed only slight elevation (2-fold). Increased SGOT and SGPT (2- to 3-fold), a slight response of LDH (1- to 2-fold), and no change in ICD were first observed at 24 hr in the allyl alcohol group. No response was seen in the Be group until the 48-hr sacrifice when ICD, SGPT, and SGOT were all elevated to about the same degree (4- to 5-fold). The initial response of LDH at 48 hr was slight as observed in the previous groups. The level of ^{7}Be in the liver was appreciable in a very short time and had reached its maximum (35–40% total dose) by 24 hr after injection. The delay in the response to Be, as measured by the serum enzyme, may be due to the fact that Be is known to concentrate in the lysosome prior to the release of the lysosomal enzymes and cell death. The difference in both the serum enzyme pattern and the time of response observed demonstrates that the response to Be differs from that caused by CCl_4 or allyl alcohol.

81. *Subacute Oral Toxicity of Monochlorobenzene in Dogs and Rats.* W. K. KNAPP, JR., W. M. BUSEY, and WALTER KUNDZINS, Hazleton Laboratories, Falls Church, Virginia.

Little has been reported on the toxicity of monochlorobenzene (MCB), an important chemical widely used in industry. In undertaking the present study, consideration was given to the possibility of food contamination with MCB. Once a day po administration, via capsule, to dogs at doses of 27.25, 54.5, and 272.5 mg/kg/day, 5 days per wk over a 93-day period, led to a 4 out of 8 mortality at the high dose after 14–21 daily doses. Clinical studies prior to death revealed an increase in immature leukocytes, low blood sugar, elevated serum glutamic–pyruvic transaminase and alkaline phosphatase, and in some dogs, increases in total bilirubin and total cholesterol. At the high level, there was gross and/or microscopic pathology in liver, kidneys, gatrointestinal mucosa, and hematopoietic tissue of the dogs which died and, less extensively, in the dogs which were sacrificed after 65 or 66 daily doses. No consistent signs of MCB effect were evident in the dogs at the intermediate and low levels. Dietary MCB administration for 93 to 99 consecutive days to rats at doses of 12.5, 50.0, and 250.0 mg/kg/day retarded the growth of the male rats at the high level. Liver and kidney weights for some rats at the high and intermediate levels were significantly increased; however, histopathologic findings were not remarkable. The findings reported here indicate a higher sensitivity of the dog to MCB effect when compared to the rat.

82. *Pharmacologic Lexicography; or, My Definition Is Better Than Your Definition.* FREDERICK SPERLING, Howard University Medical School, Washington, D.C.

Pharmacologic and toxicologic terminology is often imprecise, inconstant, and vague. Furthermore, definitions and meanings differ in medical dictionaries and in various texts and references. There are almost as many definitions for commonly used terms as there are authors. Five of 9 standard sources differ in the definition of LD50. Ten of 11 differ in defining toxicity and toxicology, and 4 of 7 differ in defining tachyphylaxis. Four authors give 19 definitions involving variations and modifications of "antagonism." The problem has been compounded by the imposed definitions associated with inflexible library classifications in which pharmacology is classified with pharmacy, and toxicology is classed by itself. The widespread use of MeSH and MEDLARS has made standardization of terminology a critical issue, and FDA has found it necessary to develop COSTART. It is suggested that a standing committee of the National Research Council be formed to develop a standard lexicon and be responsible

for review, with additions, deletions, and revisions, as necessary, in a manner similar to the committee on zoological nomenclature and classification. The membership should include representatives from SOT, ASPET, AMA, IUPHAR, and interested governmental agencies. It should also include a librarian, preferably from the National Library of Medicine, and a lexicographer. Committee decisions should be published and the actions incorporated into MeSH and MEDLARS by the NLM.

83. *Effects of Starvation on the Mobilization and Redistribution of Pesticides, and on Hepatic Microsomal Enzyme Activity in Weanling, Adult, and Old Rats.* W. B. DEICHMANN, W. E. MACDONALD, and D. A. CUBIT, University of Miami School of Medicine, Miami, Florida.

The effects of starvation on mobilization and redistribution of pesticides in tissues and on hepatic microsomal enzyme activity were determined in weanling, young adult, and old rats. Groups of 12 male and 12 female Osborne-Mendel strain rats were fed for 4 wk the control diet or a diet supplemented with either DDT 50 ppm, aldrin 7.5 ppm or DDT 50 ppm plus aldrin 7.5 ppm. Six males and 6 females of each group were then sacrificed for the determination of total pesticide body burden and of pesticide concentrations in blood, abdominal fat, liver, kidneys, brain, testes, or uterus. Portions of each liver were analyzed for (a) enzymatic detoxication of EPN, (b) *O*-demethylation of *p*-nitroanisole, and (c) azo-reduction of methyl orange. The remaining 6 male and 6 female rates of each group were subjected to starvation (3 days for young rats and 6 days for adults) followed by analyses of the tissues for pesticides and hepatic enzyme activity as described above. Severe starvation was found to have a marked influence in modifying the concentrations of DDT, DDE, DDD, and dieldrin in the blood and body fat of Osborne-Mendel rats. The effects were not uniform and were influenced by sex, age, and whether the compounds (DDT, aldrin) were administered singly or in combination. As a result of starvation, hepatic microsomal enzyme activities increased significantly in adult male and female rats. In weanling males activities decreased, while in weanling females all activities increased. (Supported by USPHS Grant 2 PO1 ES00052-07).

84. *Increased Microsomal Enzyme Activity Due to Pyrethrum Administration.* A. C. SPRINGFIELD, G. P. CARLSON, and J. J. DEFEO, University of Rhode Island, Kingston, R.I. (G. C. Fuller.)

Liver enlargement in rats resulting from po administration of pyrethrum has been observed in various laboratories. This study was undertaken to investigate the nature of this enlargement as well as resulting changes in hepatic drug metabolism. Oral administration of pyrethrum at 200 mg/kg for 13 or 23 days resulted in decreases in hepatic DNA concentrations. While total lipid concentrations were increased significantly, the increases did not account for the enlargement. Protein concentrations of whole liver homogenates, 9000 *g* supernatants and the 105,000 *g* pellet were not different from control. No significant changes occurred in hepatic water concentrations. Accompanying histologic observations of the enlarged livers indicated cellular hypertrophy. Significant decreases in hexobarbital-induced hypnosis without concomitant changes in barbital-induced hypnosis suggested a pyrethrum-caused alteration in hepatic drug metabolism. The activities of hepatic microsomal enzymes responsible for *O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate (EPN) detoxification, *p*-nitroanisole (PNA) demethylation, and hexobarbital oxidation were increased to 150, 173, and 241% of control, respectively. No significant increases were noted in the *N*-demethylation of aminopyrine. Increases in liver weight, the detoxification of EPN and demethylation of PNA, were found to be dose related. Increased enzyme activities were observed at the lowest dose used of 85 mg/kg for 15 days. At a dose of 500 mg/kg liver weights and enzyme activities were increased continually during a 17-day period of treatment but returned to control levels within 7 days after cessation of treatment. These data show that pyrethrum causes significant liver enlargement with accompanying increases in microsomal drug metabolizing activity. They suggest a reevaluation of the use of pyrethrum as a noninducing insecticide in laboratory animal quarters.

85. *Induction of Hepatic Microsomal Hydroxylative Enzymes by Technical Piperonyl Butoxide and Some Related Compounds.* D. J. WAGSTAFF, and C. R. SHORT, University of Missouri, Columbia, Missouri. (J. C. Shupe.)

Although they are usually considered to be inhibitors, technical piperonyl butoxide (PB) and some of its analogs fed to female adolescent rats for 15 days increased activity of hepatic microsomal enzymes. Responses measured included hexobarbital sleep time, *o*-demethylation of *p*-nitroanisole, oxidative cleavage of EPN, microsomal cytochrome P-450, and concentration of microsomal protein. PB was fed at 10, 100, 1000, 10,000, and 100,000 ppm of the diet with marginally increased activity at 100 ppm, maximum activity at 10,000 ppm, and nonsurvival at 100,000 ppm. The PB analogs safrole, isosafrole, eugenol methyl ether, and isoeugenol methyl ether when fed at 5000 ppm, all produced marked induction. There was greater response for the methylenedioxyphenyl structures than for the dimethoxyphenyl compounds. One seemingly inconsistent observation was that safrole prevented reduction of hexobarbital sleep time; this was presumably due to an anesthetic effect of safrole itself counteracting the stimulatory effect of enhanced barbiturate metabolism. In 2 × 2 factorial experiments involving feed levels of 5000 ppm, additivity of inductive effects was observed for PB in combination with either phenobarbital or safrole. However, the combination of phenobarbital and safrole produced no greater response than either singly, indicating that these two compounds probably induce by different mechanisms. In general, these data support the theory that PB and related compounds are alternate substrates of hepatic microsomal enzymes.

86. *Effect of Chloroquin on Hepatic Microsomal Enzyme of the Squirrel Monkey.* M. CRANMER and A. PEOPLES, DHEW, Perrine Primate Research Branch, Box 490, Perrine, Florida.

Chloroquin, a widely used antimalarial drug, has been shown to inhibit bacterial DNA and RNA synthesis. Malarial infections have been shown to produce alterations in host hepatic P-450 content. The present work was done to examine the possible effect on the hepatic microsomal enzymes and P-450 content of squirrel monkeys treated with chloroquin. Four adult male squirrel monkeys were orally administered (2) 5 mg/kg per day and (2) 10 mg/kg per day of chloroquin phosphate for 30 days, and 4 similar animals served as controls. At the time of sacrifice several hepatic microsomal enzymes and P-450 were determined. Chloroquin was found to stimulate the *o*-demethylation of *p*-nitroanisole and the oxidative hydrolysis of EPN but not to increase hepatic P-450. A 30-day 10 mg/kg/day chloroquin pretreatment to 4 adult male squirrel monkeys blocked the stimulation of P-450 by a 3 day 5 mg/kg administration of *p,p'*-DDT and decreased the stimulation of hepatic microsomal enzymes by *p,p'*-DDT.

87. *Acute and Subchronic Administration of Acetylsalicyclic Acid on the Development of Tolerance to Pentobarbital in Male and Female Rats.* JASBIR M. SINGH. Xavier University of Louisiana, College of Pharmacy, New Orleans, Louisiana.

To test our hypothesis that acute or chronic administration of acetylsalicyclic acid (ASA) can affect the development of tolerance to pentobarbital in male and female rats, the following experiments were designed; (1) Pentobarbital (P), 30 mg/kg; (2) P + ASA 0.4 g/kg, was administered on the second day; P + ASA, 0.4 g/kg was administered on the first and second day. For acute experiments, the sleeping time was determined on the first and second day. For the subchronic study, ASA, 0.4%, containing 0.4% saccharin to mask the taste of ASA was added to the drinking water for 32 days and the sleeping time was determined on days 1, 2, 15, 16, 31, and 32. Percentage Tolerance Index (PTI) was calculated as follows: hypnotic effect or sleeping time of first injection hypnotic effect or sleeping time of second or subsequent injections; then multiply this ratio by 100. During the development of tolerance to pentobarbital, the hypnotic effect or sleeping time is decreased on subsequent administrations. This decreased hypnotic effect was not restored to normal values by the acute ip administration of ASA, 0.4 g/kg, whereas subchronic administration of ASA for 32 days significantly ($P > 0.05$) increased the development of tolerance to pentobarbital because at the end of 32 days the PTI was

significantly ($P > 0.05$) greater with ASA-treated animals than with pentobarbital-treated animals. From our experimental results it is proposed that subchronic administration of ASA for 32 days produces cross tolerance to pentobarbital in male and female rats. (In part supported by Reynold Foundation Grant.)

88. *Biochemical and Physiological Correlates of Altered Drug Sensitivity in Chronically isolated Animals.* IRWIN BAUMEL, Yale University, New Haven, Connecticut; and JOHN J. DEFEO and HARBANS LAL, University of Rhode Island, Kingston, R.I.

It is well established that the physical environment plays an important role in determining the biological responses of an organism to foreign chemicals. The present experiment demonstrates the significance of social environment in this respect. Mice deprived of social stimuli for various periods became aggressive and exhibited altered sensitivity to central nervous system (CNS) drugs. Biochemical studies showed enhanced hepatic drug metabolism and reduced threshold of brain neurons to chemically induced CNS depression. The behavioral changes due to social deprivation were not correlated with the alteration in chemical sensitivity. Aggression was not temporally related with reduced sensitivity to barbiturates. Female mice that failed to become aggressive still showed altered drug metabolism and altered drug sensitivity. Gonadectomy, but not adrenalectomy, prior to social deprivation prevented the effects of social environments. Ethionine administered prior to and during the social deprivation also blocked the environmental effects. Our data suggest that social aspects of environment can affect significant changes in the biochemical make up of an organism to alter its responsivity to chemical agents. (Supported by NIMH Grant 1 RO3 MH 17603-01 and USPHS Grant 1 TO1ES-00104.)

89. *The Reversibility of Increased Rat Liver Weights and Microsomal Processing Enzymes after Feeding High Levels of 2,2,4-Trimethyl-1,3-Pentanediol Diisobutyrate.* W. J. KRASAVAGE, K. S. TISCHER, and R. L. ROUDABUSH, Eastman Kodak Company, Rochester, New York.

Kodaflex® (TXIB) was fed to male and female albino rats at concentrations of 0.1 and 1.0% in the diet. In 3 experiments run concurrently, animals were fed the diets for (A) 52 days, (B) 99 days, or (C) 52 days then changed to a control diet for 47 days or vice versa. This design was adopted to determine if increased liver weights previously seen with this compound were reproducible or reversible if the compound was withdrawn. Body weights, feed consumption, general appearance and behavior of all animals were recorded. Routine hematologic examinations, alkaline phosphatase and SGOT determinations were performed. Tissues were collected for histopathology, and selected organs were weighed for organ weight comparisons. Liver tissue was prepared for assay of the microsomal glucose-6-phosphatase, *p*-nitroanisole demethylase, UDP-aminophenol and UDP-bilirubin glucuronyl transferase. The only consistent changes seen were an increase in relative liver weights and increases in *p*-nitroanisole demethylase and UDP-aminophenol and bilirubin glucuronyl transferase. These changes occurred in both sexes fed the high dose and only in those animals ingesting the compound at time of sacrifice. They were not seen in the animals fed the 1.0% diet for the first 52 days followed by the control diet for the final 47 days. It appears, therefore, that high doses of TXIB cause significant adaptive changes in the rat liver, and these changes are reversible if the animal is returned to a normal diet. Male rats given ip injections of 100 mg/kg TXIB for 7 days had elevated demethylase activity when compared to the controls. This change was not seen at 25 mg/kg, and neither dose level affected the bilirubin glucuronyl transferase activity.

90. *The Role of Intestinal β-Glucuronidase in the Absorption of Diethylstilbestrol Glucuronide in Immature Rats.* L. J. FISCHER, J. L. WEISSINGER, and T. H. KENT, University of Iowa College of Medicine, Iowa City, Iowa. (B. A. Becker.)

Following ip administration of diethylstilbestrol (DES) larger amounts of unconjugated drug were found in intestinal contents of 25-day-old rats than in 5-day-old animals. This suggested hydrolysis of biliary excreted conjugates was taking place in older animals but not in

newborns. Intestinal β-glucuronidase activity increased with age in conjunction with development of intestinal flora. Very little β-glucuronidase activity was found in the small intestine. Large intestine contents of 25-day-old animals exhibited high, adult levels of enzymatic activity in contrast to the low activity found in 5-day-old rats. DES was absorbed rapidly from closed loops of proximal and distal small intestine of 25-day-old rats with only 20–30% remaining 20 min after the dose. Absorption from the cecum was slower and 50% remained in the contents after 60 min. DES glucuronide was not hydrolyzed or absorbed in loops of proximal small intestine. Complete hydrolysis of the conjugate took place in the cecum of 25-day-old rats and 75% of the administered glucuronide could be recovered in the nonconjugated form at 60 min. The enterohepatic circulation of DES in rats does not appear to involve the rapid absorption of β-glucuronidase-liberated estrogen from the small intestine. Our studies show that DES glucuronide excreted in the bile is not absorbed as such, but is enzymatically hydrolyzed in the lower bowel where the unconjugated drug is slowly absorbed. (Supported by NIH Grants GM 12675 and AI 07587.)

91. *The Relationship of Cyclophosphamide Activation to its Perinatal Toxicity in Mice.* R. D. SHORT and J. E. GIBSON, Michigan State University, East Lansing, Michigan. (T. M. Brody.)

Cyclophosphamide adversely affects perinatal development of mice. The parent compound lacks alkylating activity; however, metabolites produced by the mixed function oxidase system of rodent liver are alkylating agents. The purpose of this study was to examine the relationship between cyclophosphamide activation (i.e., production of nitrobenzyl pyridine reactive metabolites) and its toxicity in perinatal mice. An in vitro system, containing the 9000 *g* supernatant of liver from female mice, was optimized with respect to pH, duration of incubation, and concentration of cofactors and cyclophosphamide. An apparent K_m of 9.2×10^{-4} M and a V_{max} of 1.7 nmoles alkylating activity/mg protein/min was obtained by means of a Lineweaver-Burke plot. This optimized system was used to examine the ability of the 9000 *g* supernatant of tissues from perinatal mice to activate cyclophosphamide. There was no significant difference ($P < 0.05$) in the ability of liver from mature females or males to form alkylating products (18 ± 1.6 *vs* 18 ± 3.2 nmoles alkylating activity/mg protein/10 min). The activity in whole fetuses was much less: 1.8 ± 1.0 3.0 ± 3.0 and 1.0 ± 1.0 nmoles alkylating activity/mg protein/10 min on gestational days 15, 17, and 19, respectively. The corresponding activity in placentae was 1.7 ± 0.2, 7.5 ± 1.9, 4.6 ± 1.9. Cyclophosphamide activation by neonatal liver on days 1, 3, 5, 7, 14, and 21 after birth, expressed as a percent of the activity determined simultaneously in mature females, was 28 ± 9, 14 ± 2, 15 ± 4, 9 ± 1, and 80 ± 13%, respectively. The differences between neonates and adults were significant until 3 wk after birth. Postnatal cyclophosphamide administration to 1-day-old mice at a dose of 40–50 mg/kg sc resulted in a significant decrease in average adult body weights to 70% of control and an increase in the 6 wk mortality rate from 0% to 22 ± 17%. It was concluded that cyclophosphamide toxicity occurs at a time when the affected organism has not developed its ability to activate the parent compound.

92. *Direct Effect of Paraoxon on Erythrocyte Metabolism as Measured by O_2 Uptake.* J. A. SANTOLUCITO and E. R. WHITCOMB, DHEW, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida. (W. F. Durham.)

In studying the effects of anticholinesterase compounds, the blood has been considered primarily as an index of exposure; however, there are reports in the literature which suggest that anticholinesterase compounds may have an effect on the erythrocyte itself. This study was undertaken to measure the metabolic O_2 uptake of rabbit erythrocytes and the rate of erythrocyte removal from general circulation after exposure to paraoxon. O_2 uptake was measured with a Gilson Differential Recording Respirometer. ^{51}Chromium-labeled erythrocytes were used to determine removal rate. The control rabbit erythrocyte utilizes O_2 at a rate of $1.5 \pm 0.2 \times 10^{-15}$ l/RBC/hr. Paraoxon blocked the O_2 uptake. Glutathion restored O_2 uptake following paraoxon inhibition. There was no apparent acceleration of removal rate of the paraoxon treated erythrocyte in the general circulation. These results suggested that paraoxon acted on —SH groups in blocking O_2 uptake.

93. *Effect of Solvents on Percutaneous Absorption of Organophosphate Pesticides in Rabbits.* DONNA F. FOSTER, T. E. SHELLENBERGER, and B. J. GOUGH, University of Southwestern Louisiana, Lafayette, and Gulf South Research Institute, New Iberia, Louisiana.

Phosdrin, DDVP, Azodrin, and Bidrin were applied as undiluted technical grade product or as solutions in xylene, dimethyl sulfoxide (DMSO), water, and acetone. The chemicals were applied to a 3.81 cm^2 area on the closely clipped mid-shoulder area of anesthetized rabbits. Absorption was calculated by comparing the rates of inhibition of blood cholinesterase after percutaneous application and iv infusion. Heparinized whole blood samples were obtained at periodic intervals from a cannula implanted in the external jugular vein for measurement of enzyme activity using acetylcholine bromide substrate in an electrometric constant pH titration assay. Technical grade Phosdrin and DDVP were more readily absorbed than were Azodrin and Bidrin. Water and acetone moderately enhanced absorption of Phosdrin and DDVP but had little effect on absorption of Azodrin or Bidrin. Xylene markedly enhanced the absorption of each of the 4 organophosphate compounds; blood cholinesterase was completely inhibited within 25 min after addition of the chemicals in xylene solution. DMSO enhanced absorption of Phosdrin and DDVP but appeared to have little, if any, effect on the absorption of Azodrin and Bidrin. (Supported in part by Public Health Service Research Grant ES-00297.)

94. *In Vivo Detoxication Potential and Selective Insecticidal Activity of Carbamate and Organophosphorus Compounds.* BRIAN R. SMITH and WILLIAM A. BRINDLEY, Department of Zoology, Utah State University, Logan, Utah. (R. P. Sharma.)

The increased use in agriculture of carbamate and organophosphorus insecticides has introduced significant difficulties due to the poisoning of pollinating insects (e.g., the alfalfa leaf cutter bee, *Megachile rotundata*, the alkali bee, *Nomia melanderi*, and the honey bee, *Apis mellifera*). These difficulties might be met by utilization of selective insecticides favoring the pollinator or by taking advantage of enzyme induction effects after exposing the insect to sublethal drug doses. Several commercial carbamate and organophosphorus insecticides and some experimental carbonyl-containing organophosphorus compounds were tested for their toxicity to the alfalfa leaf cutter bee and the alkali bee. The ability of the insects to detoxify the insecticides was estimated by synergistic ratios comparing the toxicity of the insecticide when topically administered alone or after application of piperonyl butoxide. The effects of chlorcyclizine and SKF-525A on the toxicity of parathion to the alkali bee were studied. The alkali and alfalfa leaf cutter bees were considerably less susceptible to the carbamate insecticides than the honey bee. This was also suggested by the much higher synergistic ratios for the wild bees indicating about a 10-fold advantage in carbaryl detoxication relative to the honey bee. The toxicity of the organophosphorus insecticides and the detoxication were similar in the wild bees to values for the honey bee. Chlorcyclizine increased parathion toxicity to the alkali bee whereas SKF-525A had variable effects. The studies indicated that some selective toxicity for wild bees with respect to honey bees can be achieved with carbonyl-containing compounds and that enzyme induction phenomena may offer, on occasion, some protection of the alkali bee against organophosphorus compounds.

95. *Rabbit Whole Blood Cholinesterase Inhibition and Reactivation after Intravenous Infusion of Azodrin.* B. J. GOUGH and T. E. SHELLENBERGER, Gulf South Research Institute, New Iberia, Louisiana.

The present study was initiated to determine the inhibition and reactivation of rabbit whole blood cholinesterase following iv infusion of Azodrin. Azodrin as saline solution was infused at various concentrations and times; whole blood cholinesterase activity was determined at periodic intervals during and after the infusion. Heparinized whole blood samples were obtained by means of a cannula implanted in the external jugular vein. Acetylcholine bromide was used as the substrate to determine enzyme activity in the electrometric constant pH titration assay. Azodrin produced time- and dose-related inhibition of whole blood cholinesterase

during infusion, followed by partial slow spontaneous recovery of inhibited enzyme during the immediate postinfusion period; the rapid spontaneous recovery noted with many organophosphate chemicals was not obtained. Short infusion periods at higher concentrations resulted in continued pronounced inhibition of cholinesterase for a period of 20–40 min after the infusion period. Injection of 2-PAM produced marked reactivation of inhibited enzyme within 30 min following compound infusion but had only slight effect if injected after 2–3 hr. The continued enzyme inhibition after infusion of Azodrin at higher concentration for a short period of time was probably due to an excess quantity of chemical in the blood system. This effect may be related to slow metabolism or elimination of the compound and/or to a rate-limiting reaction in the formation of dimethylphosphorylated enzyme in the inhibitory process. (Supported by USPHS Research Grant ES-00297.)

96. *The Effect of 2,4-Dichlorophenoxyacetic Acid (2,4-D) and Esters of 2,4-D on Rat Fetal and Neonatal Growth and Development.* B. A. SCHWETZ, G. L. SPARSCHU, and V. K. ROWE, Biochemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

The present study was performed to evaluate the effect of 2,4-D, the propylene glycol butyl ether (PGBE) ester of 2,4-D and the isooctyl (IO) ester of 2,4-D on fetal development and neonatal growth and survival when administered orally to pregnant rats during organogenesis. Pregnant Sprague-Dawley rats were treated with doses of 2,4-D up to a maximum tolerated dose (87.5 mg/kg/day) or equimolar doses (acid equivalent) of the PGBE ester (141.8 mg/kg/day) or the IO ester (131.9 mg/kg/day) on days 6 through 15 of gestation. In the first part of the experiment, fetuses were delivered by cesarean section 1 day before normal parturition, examined, measured, and prepared for examination for soft tissue and skeletal anomalies. Fetotoxic responses were seen at high dose levels, but teratogenic responses were not seen at any dose level. In a second part of the experiment in which litters delivered naturally, 2,4-D and its esters had little or no effect on fertility, gestation, viability, and lactation indices. There was no observable effect on neonatal growth and development.

97. *The Safety for Man of Small Daily Doses of an Organophosphate Acaricide (GS 13005).* J. H. WILLS, J. D. BRADLEY, J. C. RUSSELL, and F. COULSTON, Albany Medical College, Albany, New York.

Capsules containing *O,O*-dimethyl-[*S*-2-methoxy-1,3,4-thiadiazol-5-(4*H*)onyl-(4)methyl]-dithiophosphate (GS 13005) were given once daily for 6 wk to 2 groups of 4 men each in a dose of 0.04 mg/kg and to 2 other groups of 4 men each in a dose of 0.11 mg/kg. A fifth group of 4 men received placebo capsules of similar appearance. Neither dose of GS 13005 caused any significant change in the cholinesterase activities of red blood cells or plasma nor elicited any other sign nor any symptom referable to ingestion of GS 13005. Daily oral doses up to 0.11 mg/kg of GS 13005 appear to be safe for man during a period of 6 wk.

98. *Alterations in Liver Microsomal Metabolism of Parathion Caused by Chronic Administration of Dilantin, Chloroquine, or DDE.* J. E. DAVIS, M. F. CRANMER, and A. PEOPLES, DHEW, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

Monkeys and rats were fed dilantin, chloroquine, or DDE in order to assess the effects of these compounds on liver microsomal metabolism of parathion and the inductive mechanisms involved. Dilantin, chloroquine, or a combination of both drugs was fed to male squirrel monkeys for 1 mo and to female rats for 1 wk. DDE was added to the food of female rats for 18 mo. Liver microsomes were isolated and were incubated with parathion and a NADPH generating system. The parathion metabolites produced were analyzed by gas chromatography. Dilantin increased the cytochrome P-450 level and the metabolism of parathion in monkey liver microsomes, but had no significant effect on either of these parameters in rats. Chloroquine treatment of monkeys and rats did not affect the cytochrome P-450 level in, or parathion

metabolism by, the liver microsomes of either species. The feeding of dilantin and chloroquine affected neither the cytochrome P-450 level in, nor the parathion metabolism by, rat liver microsomes. DDE administration greatly increased the liver microsomal metabolism of parathion but had no effect on the cytochrome P-450 level.

99. *Fate of ^{14}C-Ring and ^{14}C-Ethyl Labeled Parathion in the Rhesus Monkey.* F. COPELAND, M. CRANMER, J. CARROLL, and W. OLLER, DHEW, Perrine Primate Research Branch, P.O. Box 490, Perrine, Florida.

There is a need for an experimental species which resembles man in the effects produced by exposure to parathion, a widely used insecticide. ^{14}C-Ring- and ^{14}C-ethyl-labeled parathion (0.3 mg/kg) was administered iv to 8 male rhesus monkeys (*Macaca mulatta*). Plasma and RBC cholinesterase depression, disappearance of parathion from the blood and metabolites in urine were monitored. Cholinesterase activity decreased sharply for the first 30 min with a maximum depression occurring after 6 hr. Comparison of radioisotopic and gas chromatographic determinations of urinary ^{14}C-ring label, ^{14}C-labeled diethylphosphate (DEP), and diethylthiophosphate (DETP) indicated that 75% of the administered ^{14}C-ring-labeled dose was recovered within 6 hr and 90% within 24 hr, as compared to 35% of the administered ^{14}C-ethyl labeled dose in 6 hr and 54% within 24 hr. The ratio of DEP:DETP changed with time but the total ^{14}C-labeled DEP + DETP to ^{14}C-ring label ratio remained constant.

100. *A Toxicologic Evaluation of 5-Ethoxy-3-trichloromethyl 1-1,2,4-Thiadiazole (Terrazole®).* JOSEPH F. BORZELLECA, PAUL S. LARSON, and EDWARD J. KUCHAR, Virginia Commonwealth University, Richmond, Virginia, and Olin Corp., New Haven, Connecticut.

The following studies were conducted on Terrazole®, a soil fungicide; acute oral toxicity in rats, rabbits, dogs; acute percutaneous toxicity in rabbits; 2-yr feeding to rats and dogs; and a 3-generation reproduction study in rats. The test material was 95.3% active ingredient. CD rats, albino rabbits, mongrel and beagle dogs were employed. The acute oral toxicity values (LD50 ± SD) were: rats, 1077 ± 78 mg/kg; rabbits, 779 ± 532 mg/kg; dogs, >5000 mg/kg (premedicated with morphine). The test material was added to the diet of rats at levels of 0, 10, 80, 640 ppm and to the diet of dogs at levels of 0, 10, 100, 1000 ppm. No consistent adverse dose-related effects were apparent in the 2 yr rat feeding study. The dog study revealed the following findings only in dogs that received the 1000 ppm diet; a lesser weight gain; increased SAP, SGOT, serum cholinesterase, BSP retention, liver weights; cholestatic hepatosis with secondary bile nephrosis. No adverse effects were apparent at 10 or 100 ppm. In the 3-generation reproduction study, no adverse effects were apparent on fertility, gestation, viability, lactation, number of stillborn, average number of pups born and weaned per litter. Weaning weights of offspring on the 640 ppm diet were notably depressed. The body weights of parents on the 640 ppm diet were also depressed. Adverse effects occurred only on the 640 ppm diet, and these consisted of lower weaning weights of offspring and depressed body weight gains of parent rats. (Supported by a grant from the Olin Corp.)

101. *Antagonism of Sodium Nitrite Intoxication.* JAMES L. WAY and MAUREEN H. SHEEHY, Colleges of Veterinary Medicine and Pharmacy, Washington State University, Pullman, Washington.

In an attempt to evaluate the action of oxygen and hyperbaric oxygen in antagonizing sodium nitrite intoxication, oxygen was administered in varying concentrations either alone or in combination with methylene blue. Fasted male Swiss-Webster mice weighing 18–20 g received sodium nitrite po. Subsequently, these mice were placed in a hyperbaric chamber and were treated with methylene blue. When methylene blue was employed, it was injected iv into the tail vein at a dose of 2 mg/kg or 20 mg/kg prior to the administration of oxygen. The mice were exposed to hyperbaric oxygen (2 ata) for 6 hr and the remaining 18 hr at 1 ata. The LD50 values

of sodium nitrite in mice pretreated with these various antagonists were determined and compared by means of potency ratios. The data were statistically analyzed by the method of Litchfield and Wilcoxon. These results indicate that the administration of oxygen alone at 1 ata protects against nitrite intoxication, and this effect was strikingly enhanced when hyperbaric oxygen was employed. Furthermore, when oxygen was employed in combination with methylene blue, an increased protection was observed, especially when a larger dose of methylene blue was employed. (Supported by NIH Grant GM 15871.)

102. *Some Aspects of the Reactions between Hydroxylamine and Hemoglobin Derivatives.* R. D. CRANSTON and R. P. SMITH, Dartmouth Medical School, Hanover, New Hampshire.

Recent evidence contradicts the older suggestion that nitrite and unsubstituted hydroxylamine are toxicologically equivalent forms in vivo. Hydroxylamine has effects on red cell metabolism which are not shared by nitrite. Hydroxylamine has a greater tendency to generate sulfhemoglobin (SulfHb)-like pigments and Heinz bodies (HB) in the red cells of mammalian species than do equal concentrations of nitrite. SulfHb and HB formation by hydroxylamine might be due to effects on red cell metabolism or to some fundamental difference between hydroxylamine and nitrite in the mechanisms of reaction with hemoglobin. Certain hemoglobins with abnormal amino acid substitutions appear to precipitate as HB because of a dissociation of the heme group from the globin moiety. When nitrite, hydroxylamine, and ferricyanide were tested for ability to produce specific heme loss, all 3 resulted in a significant loss of total hemoglobin relative to control preparations of oxyhemoglobin, but only ferricyanide produced a small but significant loss of heme groups. Ferricyanide and hydroxylamine convert deoxyhemoglobin to genuine methemoglobin, but there was no apparent reaction with nitrite. Hydroxylamine converts some oxyhemoglobin to methemoglobin, but SulfHb formation is extensive. Perhaps SulfHb and HB formation by hydroxylamine require molecular oxygen. Some evidence suggests that the heme oxygen is released in the hydroxylamine-oxyhemoglobin reaction, but that ferric heme groups which are formed catalyze the oxidative decomposition of hydroxylamine. It appears that methemoglobin formation by nitrite, by hydroxylamine and by ferricyanide each proceeds by a different mechanism. (Supported by USPHS Grants AP 00260, GM 01370, and 1-KS-GM-31, 784.)

103. *Systemic Absorption of Copper from Copper Sulfate Emetic.* WALTER J. DECKER, WILLIAM A. GOLDSMITH, ROY C. MILLS, and RALPH J. BANEZ, William Beaumont General Hospital, El Paso, Texas.

Copper sulfate ($CuSO_4$) has been advocated as a rapidly acting emetic for use in the immediate treatment of poisoning by ingestion. There is, however, a high potential for systemic absorption of toxic amounts of copper, especially when emesis does not occur. To test this possibility, a dose of $CuSO_4$ (2.5 mg in 0.25 ml H_2O, equivalent to the recommended human emetic dose of 250 mg) tagged with radioactive copper tracer ($^{64}CuSO_4$) was administered to rats, a species that cannot vomit. Rats were sacrificed 1, 3, 6, and 24 hr after copper administration and blood and other tissues were counted to determine copper content. Significant concentrations of administered copper were found in kidney and liver, as well as in plasma. These concentrations increased with time from 0.41, 0.35, and 0.04 μg/g at 1 hr to 2.7, 1.1, and 0.5 μg/g at 24 hr in the respective organs. These results militate against the use of copper sulfate as an emetic agent.

104. *Probability of Tumor Development in Control and Steroid-Treated Charles River Rats.* F. E. RENO, J. A. TRUTTER, L. D. SHOTT, Hazleton Laboratories, Inc., Falls Church, Virginia; and R. G. MCCONNELL, G. D. Searle and Company, Chicago, Illinois. (D. C. Jessup.)

The life-table technique was employed to determine the probability of tumor development in male and female rats which were treated with various contraceptive steroids or which served as

controls. The animals, numbering 40 per group, were approximately 8 wk of age at initiation, and observations were recorded for periods ranging up to 91 to 104 wk. After sacrifice, target organs and all tissue masses were subjected to histopathologic examination. Data on all histologically identified tumors were then analyzed by the life-table technique to determine the probability of development of any type tumor. These data were further classified into 4 categories: any mammary tumor, only mammary carcinomas, only benign tumors, and any malignant tumor. Data indicated that probability of tumor development ranged from 25 to 45% for control male rats and from 48 to 69% for control female rats. Generally, the probabilities determined for the steroid-treated animals were not significantly increased in female rats and were increased in male rats in only certain categories at higher dosage levels.

105. *Toxicity of Metolazone in Meat-Fed Beagle Dogs.* D. J. SULLIVAN and R. E. KANE, Pennwalt Corporation, Rochester, New York. (Z. Hadidian.)

Beagle dogs fed an all meat diet were medicated with metolazone for 14 wk (beginning after 9 wk on the diet). Clinical chemistry studies were done once each week throughout the experiment. Meat-fed dogs were the first animals to give any evidence of the effect of medication. Diuresis with associated hemoconcentration was seen in meat-fed dogs medicated with metolazone after the first week of medication. Diminished serum electrolyte values (Na, K, Cl) were most prominent in meat-fed dogs, although serum K and Cl values of medicated dogs fed Purina Canine Checkers also declined. Serum glucose levels of meat-fed male dogs rose during the first week of medication. The results of this experiment suggest that the effects of medication with metolazone are more severe in dogs receiving suboptimal diets.

106. *Toxicology and Fate of 2,2,4-Trimethyl-1,3-pentanediol Diisobutyrate in the Rat.* BERNARD D. ASTILL, C. J. TERHAAR, and D. W. FASSETT, Eastman Kodak Company, Rochester, New York.

Since Kodaflex® (TXIB) may be an indirect food additive from use in food packaging, a study of the toxicity and fate was done. Acute oral, ip, skin irritation, sensitization, and inhalation studies showed it to be practically nontoxic. Rats were fed for 103 days at concentrations of 0.1 and 1.0% in the diet. The only consistent finding was a slight but significant increase in the weight of the livers of rats fed 1.0% TXIB. Single oral 250 mg/kg doses of 3-^{14}C-labeled-TXIB were eliminated in the urine (64–88%) and feces (13–31%) almost completely within 8–10 days of dosing, 80% being eliminated within 3 days. $^{14}CO_2$ excretion was insignificant. At sacrifice after 14 days these doses, and one of 900 mg/kg, gave negligible increments of radioactivity in liver, brain, kidneys, lungs, and fat. After TXIB dosing, free 2,2,4-trimethylpentane-1,3-diol, 2,2,4-trimethyl-3-hydroxyvaleric acid, the glucuronides, and a monoisobutyrate of 2,2,4-trimethylpentane-1,3-diol, were found in the urine. Feces contained the same monoisobutyrate and unchanged TXIB. Large doses of TXIB are thus completely eliminated, with negligible retention by the organism. They appear to be handled by partial hydrolysis in the gut, and after incomplete absorption, by normal pathways such as hydrolysis, oxidation, and esterification.

107. *Toxicologic Studies with Polychlorinated Biphenyls.* M. L. KEPLINGER, OTIS E. FANCHER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Polychlorinated biphenyl compounds (PCB) have been reported to be in the environment, and the chronic toxicologic properties have not been reported. PCB compounds with 42, 54, or 60% chlorine (1242, 1254, and 1260) are being fed to rats and dogs, and rat and chicken reproduction studies are being conducted at 1, 10, and 100 ppm. After 18 mo in rats (interim sacrifice and histology at 3, 6, and 12 mo), compound 1242 produced no effects. Rats fed compounds 1254 or 1260 had increased liver weights at 100 ppm, but not at 1 or 10 ppm. No other effects have been observed. Dogs fed 1242 have shown no adverse effects, while those fed 100 ppm 1254 or 1260 have not gained weight as well as controls. In the rat reproduction study there was decreased survival of pups at 100 ppm of 1242 or 1254, and decreased mating indices with

1242. No adverse effects at any of the 3 levels of 1260 or at 1 or 10 ppm of the other two compounds have been observed. There was anorexia, loss of body weight, decreased thickness of egg shells, and poor hatchability of eggs from chickens fed 10 or 100 ppm of 1242, or 100 ppm of 1254. At 1, 10, and 100 ppm of 1260 and at other levels of 1242 and 1254 there were no effects.

108. *Cataracts in Parachlorophenylalanine-Treated Rats: Development, Clinical Appearance and Antagonistic Effect of Phenylalanine.* E. J. GRALLA and L. D. RUBIN, Yale University School of Medicine and University of Pennsylvania School of Veterinary Medicine.

Parachlorophenylalanine, a serotonin biosynthesis inhibitor, causes distinctive cataracts and depressed body weight gain when fed to weanling rats. Our studies explored the effect of dietary phenylalanine on this toxicity, while cataract development was followed biomicroscopically. Phenylalanine feeding partially prevented the ocular lesions without significantly affecting the toxic effect on growth patterns. The cataracts were subcapsular, and matured in characteristic patterns; they were permanent and apparently age-related since mature rats showed a marked resistance to eye changes. The results suggest that depletion of serotonin, or serotonin metabolites, may be an important factor in the pathogenesis of these cataracts.

109. *Studies on the Mechanism of the Cataractogenic Activity of 2,4-Dinitrophenol.* J. E. LEBEAU and P. J. GEHRING, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.

Using an in vitro lens culture system, 2,4-dinitrophenol (DNP) at a concentration of 2×10^{-5} M caused cataracts to develop in lenses obtained from rabbits 40 ± 5 days of age. Known metabolites of DNP (2 amino-4 nitrophenol, 2 nitro-4 aminophenol, and 2,4-diaminophenol) did not produce this effect even at a concentration of 1×10^{-3} M. The cataractogenic activity of DNP was found to have a positive temperature coefficient and was not influenced by the amount of oxygen in the system. The observation that control lenses maintain their clarity, even in the absence of oxygen, eliminates the uncoupling of oxidative phosphorylation as the mechanism for the cataractogenic activity. Having eliminated this possibility, the effect of DNP on ATPase activity was determined. Although ATPase activity was stimulated and oligomycin blocked the production of cataracts, a cause-effect relationship was not established due to the observation that the ATP content of lenses treated with DNP and ologomycin was lower than that of lenses treated with only DNP. DNP did not alter either the production of lactic acid or sorbitol by the lens. Although DNP caused a significant depression of the hexose monophosphate shunt activity of lenses, this depression was not alleviated when cataract induction was blocked with oligomycin.

110. *Storage, Metabolism, and Urinary Excretion of DDT and DDT Metabolites in Human Subjects.* D. P. MORGAN and C. C. ROAN, University of Arizona, Tucson, Arizona. (George Ware.)

Eight human subjects ingested 5–20 mg per day of either *pp'*-DDA, *pp'*-DDD, *pp'*-DDE, or technical DDT for periods of 21 days to 6 mo for the purpose of studying efficiencies of storage and rates of metabolic conversion and excretion. Serum and fat concentrations of DDT and/or metabolites, plus urinary excretion of DDA, were measured before, during, and after dosing. *pp'*-DDA was efficiently absorbed and excreted in the urine. *pp'*-DDD underwent adipose storage, but was nearly all excreted (mainly as urinary DDA) within a year of the end of dosing. Adipose storage of *pp'*-DDE accounted for most of the *pp'*-DDE ingested during dosing, after which release from storage was extremely slow. None was excreted as urinary DDA. The *pp'*-DDT technical DDT was more efficiently stored than the *op'*-DDT. There was only very slow conversion of *pp'*-DDT to *pp'*-DDE during and after DDT dosing. In the course of 1 yr after termination of DDT dosing, urinary *pp'*-DDA excretion accounted for 30–50% of estimated *pp'*-DDT storage loss. Comparative urinary DDA excretions during and immediately after DDT dosing suggest an important role of the intestine in DDA synthesis.

111. *Fate of ^{14}C-Labeled o,p′-DDT in the Rat.* M. CRANMER, W. OLLER, J. CARROLL, and F. COPELAND, DHEW, Perrine Primate Research Branch, Box 490, Perrine, Florida. (W. F. Durham.)

The intent of this work was to test the reported observation that *o,p′*-DDT is isomerically converted to *p,p′*-DDT by the rat. *o,p′*-DDT at 50 ppm in the feed was fed to 12 of 24 female Sprague-Dawley rats weighing 200–250 g for 12 wk. Three consecutive days prior to sacrifice, ^{14}C-labeled *o,p′*-DDT was administered to the 12 rats receiving *o,p′*-DDT in their diet and to 6 of the 12 controls. Fat samples were prepared for analysis by the Mills, Olney Gaither method, analyzed by gas–liquid chromatography and confirmed by thin-layer chromatography. Radioactivity of individual residues was determined by trapping from the gas-liquid chromatograph followed by liquid scintillation counting. The levels of *p,p′*-DDT or *p,p′*-DDE was not increased over the control levels in any of the 12 rats receiving 50 ppm *o,p′*-DDT for 12 wk. ^{14}C-labeled *o,p′*-DDT was converted to ^{14}C-labeled *o,p′*-DDD, but not to ^{14}C-labeled *p,p′*-DDT or ^{14}C-labeled *p,p′*-DDE.

112. *Metabolic Alterations in the Squirrel Monkey Induced by DDT Administration and Ascorbic Acid Deficiency.* R. CHADWICK, M. CRANMER, and A. PEOPLES, DHEW, Perrine Primate Research Branch, Box 490, Perrine, Florida. (W. F. Durham.)

In experimental animals, agents which induce drug metabolizing enzymes frequently stimulate the excretion of ascorbic acid via the glucuronic acid system. Ascorbic acid deficiency in the guinea pig, which cannot synthesize the vitamin, has been reported to inhibit hepatic mixed function oxidases. The effects of DDT stress and ascorbic acid deficiency on various mixed function oxidases, enzymes of the glucuronic acid system, and the metabolism of ^{14}C-γHCH and ^{36}Cl-DDT were studied in the squirrel monkey. Daily po injections of 5 mg DDT for a 2-wk period produced significant increases in liver weight, microsomal protein cytochrome P-450, and the in vitro activity of various mixed function oxidases. DDT pretreatment also significantly increased elimination and decreased storage of radioactivity from ^{14}C-γHCH but not from ^{36}Cl-DDT. Ascorbic acid deficiency significantly inhibited the ratio of glucuronic: glucaric acid excreted in 24 hr as well as the increase in the *O*-demethylation of *p*-nitroanisole resulting from the DDT pretreatment. Results from this study suggest that interactions between nutritional status and toxic stress may significantly affect some routes of detoxification in mammals.

113. *Paraquat Poisoning.* A. PASI and C. H. HINE, University of California, San Francisco, California.

Since 1966 20 suicidal and accidental poisoning cases, generally lethal, have been reported due to the herbicide Paraquat (1,1-dimethyl-4,4′-dipyridylium dichloride) among children and adults in Europe. Although Paraquat is used in the United States, no such reports appear in current American literature. We are reporting, therefore, a Paraquat poisoning in a 49-yr-old white man, who, though having taken a dose generally considered to be lethal (at least 10 ml of a 42% solution), failed to develop expected proliferative fibrosing alveolitis of the lungs. Presenting signs were superficial ulceration of the oropharynx but not of the esophagus or stomach, recurrent singultus, and continuing low-grade fever. He experienced immediate oral and pharyngeal burning, followed later by nausea and vomiting. The most significant pathologic finding was a transient necrosis of the renal tubules, with initial anuria, rise of the serum BUN and creatinine, transient glycosuria, and casts. Significant Paraquat levels were detected in both blood and urine. On the 12th post-ingestion day peritoneal dialysis was attempted, but was discontinued because the amount of Paraquat detected in dialyzate was insignificant. At no time were there more than minor transitory pulmonary changes, though pathologic change of this organ was anticipated. By the third week after ingestion all toxic symptoms had disappeared and biochemical values had returned to normal.

114. *Distribution and Metabolism of Paraquat in the Rat.* I. G. MOLNAR and W. J. HAYES, JR., Vanderbilt University, School of Medicine, Nashville, Tennessee.

The excretion and tissue concentration of paraquat were investigated in Sprague-Dawley rats following po and ip administration at rates of 160 and 30 mg/kg, respectively. Paraquat determinations were carried out both by the column chromatographic method of A. Calderbank and by a new, unpublished thin-layer chromatography method. An average of 85.2% of the total dose administered was recovered in the excreta during the first 3–4 days, regardless of the route of administration or of the age or sex of the animals. The rate of excretion and the amount of paraquat recovered in the excreta of pregnant rats, treated po at 160 mg/kg on the 9th day of pregnancy were the same as in nonpregnant animals given the same dose. Among the tissues analyzed (liver, kidneys, lungs), paraquat was detected only in the lungs, representing an average of 6.3% of the total dose given po or ip. Thus the average recovery of paraquat from the excreta and the lung accounted for 91.5% of the dose. The use of thin-layer chromatography led to the isolation of a metabolite from the lungs. This metabolite and paraquat extracted from lung tissue were subjected to further analysis by mass spectrometry and by nuclear magnetic resonance spectroscopy using paraquat as standard. The metabolite was found only in the lungs of paraquat-treated animals and in the total body extracts of young born to paraquat-treated mothers.

115. *Lethality and Pharmacokinetics of Paraquat in Rats.* R. E. MURRAY and J. E. GIBSON, Michigan State University, East Lansing, Michigan. (T. M. Brody.)

The acute oral toxicity of the herbicide paraquat (1,1′-dimethyl 4,4′-dipyridylium) was influenced by gastric content in human intoxications. The purpose of this study was to determine paraquat lethality and pharmacokinetics in normally fed and food-deprived rats. Three groups of 50 Sprague-Dawley rats weighing 150–175 g (1 group fed ad libitum; 1 group fasted 4 hr; and 1 group fasted 8 hr) were subdivided into groups of 10 and administered various doses of paraquat (cation) po. The LD50 of paraquat was calculated for each group by the method of Litchfield and Wilcoxon. Heart, kidney, lung, and liver tissue from 3–5 animals at each dose were analyzed for paraquat content at the time of death by a colorimetric method. Other nonfasted and 8-hr-fasted rats were divided into groups of 4–6 and administered 92 mg/kg paraquat (LD5, nonfasted). Blood was collected by cardiac puncture and centrifuged, and serum levels of paraquat were determined colorimetrically at 0.25, 0.5, 1, 2, and 4 hr after paraquat administration. Rate constants for absorption and elimination of paraquat were calculated. Paraquat deaths occurred between 3 and 7 days after administration. Seven-day LD50 values with 95% CL were 143 (123–166), 130 (106–159), and 126 (102–156) mg/kg, respectively, in rats fasted 0, 4, and 8 hr. Tissue levels of paraquat at death were dose related. At the dose of 101 mg/kg, tissue levels of paraquat (± SE) were 1.7 ± 0.6, 2.6 ± 0.7, 1.7 ± 0.6, and 1.4 ± 0.2 μg/g in heart, kidney, lung, and liver, respectively. Paraquat was poorly absorbed as peak serum levels in rats administered 92 mg/kg po were 1.4 ± 0.4 and 1.7 ± 0.4 μg/ml in animals fasted 0 and 8 hr. The corresponding rate constants for paraquat absorption and elimination were: $k_a = 0.3$ and 1.8 hr^{-1}; $k_e = 0.9$ and 0.9 hr^{-1}. The $t_{1/2}$ for the initial phase of paraquat disappearance from serum was 48 min in fasted and nonfasted animals. It was concluded that in the rat orally administered paraquat is poorly absorbed at a low rate but has a rapid initial rate of elimination from serum. Paraquat tissue levels at death (3–7 days) indicated tenacious tissue binding. Food deprivation did not influence the above parameters in rats.

116. *Neurologic and Behavioral Effects of Dietary Dieldrin in Juvenile Mallard Ducks.* R. P. SHARMA, D. S. WINN, and J. L. SHUPE. Utah State University, Logan, Utah.

Studies were undertaken to determine the behavioral effects of long-term exposure to sublethal amounts of dieldrin in ducks and the associated changes in brain biogenic amines. Ducks were maintained on 0, 4, 10, or 30 ppm dieldrin in feed. Ducklings hatched from these birds were placed on the same feed as the adults. Group observation periods of 2 hr were conducted

with randomly selected drakes (70–100 days old) from each treatment. At the conclusion of the investigation on group behavior, paired contests were initiated with randomly selected pairs of different treatment and equal age groups. Brain biogenic amines, i.e., norepinephrine, dopamine, and serotonin, were measured in the brains of randomly selected birds at 70–76 days of age. The 3 amines were quantitated in brain tissues by the method of Ansell and Beeson. Encounters between individuals of different treatments resulted in obvious trends of control birds dominating those on the various pesticide levels. An appreciable decrease in all the brain amines measured was noted in the birds maintained at highest levels of dieldrin. Results indicated a possible relationship of dietary levels of dieldrin to alterations in behavior which in turn was related to the biochemical alterations in brain. (Supported in part by USPHS Grant No. MH 18589-01 and contract No. 14-16-0008-1020 between the U.S. Fish and Wildlife Service and Utah State University.)

117. *Inhibition of Diglyceridase and Cholinesterase Activity in Rats by Organophosphorus and Carbamate Esters.* R. LAUWERYS and J. P. BUCHET, Louvain University, Brussels, Belgium. (S. D. Murphy.)

The present investigation was conducted to compare the inhibition of intestine and heart diolein hydrolase (diglyceridase) with the inhibition of brain, heart, intestine, and serum cholinesterase produced in rats by several organophosphate esters and carbamates. In vitro, paraoxon, DDVP, haloxon (di-(2-chloroethyl)3-chloro-4-methylcoumarin-7-yl phosphate), PE 304 (di-(2-chloroethyl)4-nitrophenyl phosphate), trichlorphon (dimethyl 2,2,2 trichloro-1-hydroxyethyl phosphonate) and coroxon (diethyl 3-chloro-4-methylcoumarin-7-yl phosphate) inhibit intestine and heart diglyceridase, and at a given inhibitor concentration the loss of enzyme activity follows first-order kinetics. Two carbamates (phenyl *N*-α-methylbenzylcarbamate and phenyl *N*-3-chlorobenzylcarbamate) inhibit heart and intestine diglyceridase in vitro, but the reaction is nonprogressive and competitive. Three methylcarbamates (Zectran, Carbaryl, Baygon) have no effect on these enzymes at a final concentration of 150 μM. The organophosphate esters inhibit brain acetylcholinesterase in vitro at a lower concentration than that required for diolein lipase. The ratios of the respective I50 are between 10 and 150. The inhibition of cholinesterase and diglyceridase activity produced 3 hr after ip administration of 3 indirect organophosphorus inhibitors was as follows: 20 mg/kg of Dursban produced 0 and 44.2% inhibition of heart and intestine diglyceridase and 49.7, 61.8, 48.9, and 67.7% inhibitions of brain, heart, intestine, and serum cholinesterase, respectively. Trichloronate (20 mg/kg) produced 24.3 and 38.5% inhibition of heart and intestine diglyceridase and 39, 56, 36.3, and 50.2% inhibition of brain, heart, intestine, and serum cholinesterase. Triamiphos (10 mg/kg) inhibited heart and intestine diglyceridase by 72.6 and 52% compared to 26.2, 84, 94.6, and 91.2% inhibition of brain, heart, intestine, and serum cholinesterase, respectively. This work indicates a need to further investigate the possible effect of some organophosphorus esters on fat metabolism. (Supported by Grant 1198 from the Fonds de la Recherche Scientifique Médicale, Belgium.)

118. *Age-Dependent Sensitivity of the Dog Testis to Drug-Induced Atrophy.* H. REINERT, D. A. RUTTY, and R. M. QUINTON, Pfizer Ltd., Sandwich, Kent, U.K.

We have recently experienced an increased incidence of testicular atrophy during 1 and 3 mo toxicity trials in dogs. Since testicular atrophy was produced by drugs which differed widely from the chemical and pharmacologic point of view, we felt that mechanisms other than drug-related target organ toxicity may contribute to this effect. In previous trials we used dogs which were above 12 mo of age, and drug-induced testicular atrophy was not observed. During last year, for supply reasons, we used dogs of 6 mo, an age which is generally recommended for long-term toxicity trials primarily because the effects of drugs on the growth curve can be measured. In our trials those drugs which induced testicular atrophy did so in a dose-related fashion, but there was also a dose-related increase in the incidence of gastrointestinal side effects

such as anorexia, vomiting, and diarrhea. This observation drew our attention to dietary deficiency as a cause of the frequently observed testicular atrophy in young dogs. We studied the effect of dietary restriction in young dogs and obtained evidence which supported partly our hypothesis that testicular atrophy can be due to malabsorption of essential nutrients and does not have to be per se a direct drug-related effect, *but* we also found that the germinal epithelium of young dogs is extremely sensitive to drug-induced atrophy. Our experience reemphasizes the need to monitor carefully gastrointestinal side effects during toxicity trials and to supplement the diet if such side effects occur; we question whether it is valid to use immature animals for long-term toxicity trials because of an increased rejection rate of therapeutically novel and valuable compounds on account of an effect observed only in young dogs.

119. *Spontaneous Neoplastic and Nonneoplastic Diseases Commonly Encountered in Aging Sherman Rats.* S. OHTAKE, A. L. KNEZEVICH, B. SPARANO, and R. MORASKI, Lederle Laboratories, Division of American Cyanamid Company, Pearl River, New York.

It is of considerable importance to recognize the incidence of neoplasms in the control animals utilized for evaluation of carcinogenic effects of various therapeutic agents. There is a marked difference with respect to the incidence and the type of neoplasms in various strains of rats. The literature reviewed contains no systematic investigation concerned with spontaneously occurring neoplasms in aging Sherman rats. The purpose of the report is to present the incidence and the morphologic types of naturally occurring neoplasms and briefly describe nonneoplastic diseases in approximately 540 male and female Sherman rats from the Lederle Wyckoff colony. Of the rats examined, approximately 45% showed neoplasms. The most common sites were pituitary gland, mammary glands, adrenal glands, and lymphoid tissue. Chronic murine pneumonia, senile nephrosis, and cystic lesions in the adrenals and lymph nodes were among the most common nonneoplastic diseases encountered.

120. *Apparent Hydronephrosis as a Stage of Renal Papillary Growth in Fetal Rats.* R. M. HOAR and D. C. WOO, Biological Research Division, Hoffmann-La Roche Inc., Nutley, New Jersey. (S. E. Sader.)

It has been reported that rat kidneys underwent marked changes in size, weight, and histologic structure during late gestation. In this investigation, we have studied the relationship between growth of the renal papilla and renal mass in fetuses from day 19 of gestation until day 1 after birth in normal and methyl salicylate-treated rats. Female rats in the treated group were given 0.1 ml methyl salicylate ip on days 10 and 11; control animals received no treatment. The fetuses, recovered either at cesarean section or after birth, were fixed in Bouin solution. Fetal kidneys were weighed, sectioned transversely through the hilus, and examined under a dissecting microscope to determine the growth of the renal papilla. The length of the papilla was graded from 0 (no papilla) through +, ++, +++, and ++++ (overgrowth). It was found that although there was a steady growth of the renal papilla, it was accompanied by an exponential increase in renal mass (weight) which could result in an apparent hydronephrosis, i.e., large pelvis, small papilla, and normal ureter, on gestation days 20 and 21. By day 22 or lactation day 1, however, the hydronephrotic appearance disappeared. Utilizing the above observations, an attempt has been made to differentiate between retarded renal growth and hydronephrosis in methyl salicylate-treated fetuses.

121. *Normal Hematology and Blood Chemistry Values for Standard Laboratory Animals.* R. E. VERMETTE and D. C. JESSUP, Hazleton Laboratories, Inc., Falls Church, Virginia.

Normal hematologic and blood chemical values are presented for male and female albino rats, young purebred beagle dogs, and young rhesus monkeys fed basal diets. The purpose of this investigation was to obtain, for use in toxicologic research, baseline data of the more commonly analyzed blood parameters. Hematologic parameters consisting of hematocrit, hemoglobin, erythrocyte and total white blood cell counts; blood chemical determinations including

blood glucose, alkaline phosphatase, serum glutamic-pyruvic transaminase, serum glutamic-oxaloacetic transaminase, bilirubin, blood urea nitrogen, and electrolytes were analyzed from large populations of laboratory animals (over 1500 dogs, 1500 monkeys, and 2000 rats). Mean, standard deviation, and normal range values were computed for each investigated parameter and reported by sex and species.

122. *Applicability of CUSUM Techniques for Evaluation of Biological Data from Chronic Studies.* N. M. FIELDS, J. R. DUFFETT, A. KRUMM, and Y. ALARIE. Hazleton Laboratories, Inc., Falls Church, Virginia, and Graduate School of Public Health, Pittsburgh, Pennsylvania.

The cumulative sum (CUSUM) technique was extended for comparison between a control group and treated groups for time-paired and non-time-paired data obtained from pulmonary function tests performed on cynomolgus monkeys exposed to various pulmonary irritants for 12–18 mo. The same data were analyzed by standard regression techniques. A comparison between these analysis techniques revealed the usefulness of the CUSUM technique, which was applied to monitor changes as the experiments were progressing (real-time analysis) as well as when the experiments were completed (postmortem analysis). The advantages of the CUSUM technique over the standard regression analysis are as follows: simplicity in computation, rapid detection of changes occurring as experiments are in progress, exact determination of the time at which a change occurs in the treated group, and meaningful graphical presentation of the results. The CUSUM technique can be best applied in cases where sudden changes occur rather than when steady or cyclical trends occur, and a minimum of 6–8 data points are required.

123. *Toxicity Testing of Biomaterials and Ingredients by a Special Tissue Culture Technique.* E. O. DILLINGHAM, W. H. LAWRENCE, and J. AUTIAN, University of Tennessee Medical Units, Memphis, Tennessee.

In order to reduce time and expense of routine screening, a method was developed for the direct visual examination of mature monolayers of mouse fibroblast cells which had been exposed 24 hr to serial dilutions of toxicants or extracts. Eagle medium, saline, or distilled water were used to prepare test solutions or extracts. Materials were extracted at 50°C for 24 hr or at 121°C for 1 hr in an autoclave, using 5 g of material per 20 ml of saline or distilled water. Visual estimation of the effect of the toxicant or extract was recorded as percent survival compared to untreated monolayers. ID50 (inhibitory dose, 50%) and ID0 values of phthalate esters, obtained by the above technique, and similar values obtained by protein assay of growing monolayers after 72 hr exposure to toxicant agreed with respect to both rank and magnitude except for the ID50 of diethyl phthalate. The ID50 values for phthalate esters were directly related to water solubility, whereas the LD50 values in mice were inversely related to solubility. A direct relationship was found between the product of the intrinsic toxicity (slope of the dose-response curve by protein assay in tissue culture) and the maximum effective concentration (limited by solubility) and LD50 values in mice. The relative toxicities of aqueous extracts of polyurethanes are presented.

124. *Rotarod Performance and Sciatic Nerve β-Glucuronidase Activity as Indices of Acrylamide Neurotoxicity in Rats.* MICHAEL L. KAPLAN and SHELDON D. MURPHY, Harvard School of Public Health, Boston, Massachusetts.

Acrylamide has produced occupational poisonings characterized by weakness of the limbs, tremors, and reversible ataxia. In laboratory animals a typical dying-back type of peripheral neuropathy has been reported in which the distal parts of the longest nerve fibers show Wallerian-like degeneration. In this investigation, we sought to determine the efficiency of the rotarod technique as an objective means of evaluating neurological deficit in acrylamide-treated rats. Initially, the ability of rats to remain for 2 min on a screen-covered, 1 in diameter rod, rotating at 24 rpm, was taken as an index of normal neurologic function. A cumulative dose of

350 mg/kg of acrylamide given ip at the rate of 50 mg/kg/day produced loss of normal function in 50% of the animals (ED50). A major inadequacy encountered with the use of this technique for chronic studies was that the rats learned to jump off the rod, precluding recovery rate measurements. A modified rotarod was constructed which contained an electrode floor programmed to administer continuous shocks. Male rats were trained to remain on the rod. When acrylamide was given ip at the rate of 50 mg/kg/day the cumulative ED50 was estimated at 260 mg/kg, and the recovery time after receiving a cumulative dose of 350 mg/kg was 10 days. Determinations of sciatic nerve β-glucuronidase activity were conducted at various time intervals during the onset, duration, and recovery from acrylamide poisoning. Acrylamide was administered ip to male rats at the rate of 50 mg/kg/day for 8 days. Increased levels of β-glucuronidase were observed the day following the last dose, and this rise continued, reaching a peak of 340% of control values 30 days after the first dose. At this time, the rats had recovered clinically and displayed no evidence of neurologic deficit. The changes in neural β-glucuronidase following acrylamide poisoning were of a lesser magnitude but identical in time profile to those reported for Wallerian degeneration. (Supported by Training and Research Grants TO1 ES-00045 and RO1 EC-00216 from USDHEW.)

125. *The Absorption of Different Chemical Compounds of Mercury from the Gastrointestinal Tract.* T. W. CLARKSON, University of Rochester, Rochester, New York.

Mercury is present in food in different chemical forms including complexes of inorganic and methylmercury cations. Information on the relative rates of absorption would be helpful in assessing the potential hazard to man. Mice, CBA strain, body weight 18–20 g, were allowed to eat Purina chow containing the mercury compound labeled with the ^{203} Hg isotope. Levels of mercury varied from 0.05 to 5.0 ppm of dry food. Exposure to radioactive food was continued for periods of up to 3 wk before the animals were returned to normal food. Radioactivity was measured in the whole animal, in the excreta, in the food, and in various fractions of the food. In the case of methylmercury compound the data were subjected to compartmental analysis. More than 90% of the methylmercury compounds were absorbed into the blood stream. Less than 2% of the radioactivity was absorbed from food to which mercuric chloride had been added. Fractionation of the food by centrifugation and by precipitation with trichloroacetic acid indicated that 90% or more of the mercury was bound to nondiffusible components. The results indicated that it is important to identify the chemical form of mercury in food if we are to assess the hazard to human health arising from the ingestion of contaminated food. (This work was supported under AEC contract No. W. 7401-Eng-49 and by USPHS Grant GM 105190-04.)

126. *Methyl Mercury-^{203}Hg Excretion by Lactation in Guinea Pigs.* H. LOCKSLEY TRENHOLM, CHARLES J. PAUL, HAROLD BAER, and FRANK IVERSON, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada. (W. J. Hayes, Jr.)

It is well documented in the literature that methyl mercury is excreted primarily in the feces and urine. Since methyl mercury has a high affinity for protein sulfhydryl groups, a study was carried out to determine the amount of methyl mercury excreted in the milk of lactating guinea pigs. Radiotracer techniques were used to compare the levels of ^{203}Hg in the milk and whole blood of lactating animals. When the methyl mercury-^{203}Hg was administered ip as a single injection (maximum dose: 1 mg Hg/kg) under the following conditions: 2 days prior to parturition, at parturition, and 3 days after parturition, the levels of methyl mercury in the milk were only 5–10% of the blood concentrations. When methyl mercury was administered 12 days prior to parturition, the mercury levels in the milk approached those observed in the blood. In contrast, the blood levels of methyl mercury in the young from mothers dosed 2 days prior to parturition were comparable to the methyl mercury blood levels in the mothers. The data suggested that the methyl mercury is not readily transferred to the milk. On the other hand, placental transport of the methyl mercury was very rapid.

127. *Effects of Monomethylhydrazine on Blood-Cerebrospinal Fluid Glucose Flux.* BENNETT A. SHAYWITZ, WILLIAM T. GORMLEY, KENNETH C. BACK, and M. E. GEORGE, 6570 Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio.

It has been shown by Fortney and Clark that the administration of monomethylhydrazine (MMH) to fasted, anesthetized dogs results in profound hypoglycemia and convulsions after a 2-hr period. This suggests that the convulsions following MMH may be due to the hypoglycemia which results in deprivation of substrate glucose to the brain. Cerebrospinal fluid (CSF) is a better representative of brain extracellular fluid than is blood, and if indeed convulsions result from hypoglycemia, CSF glucose should be similarly decreased. We have investigated plasma and CSF glucose in fasted pentobarbital-anesthetized rhesus monkeys given MMH 25 mg/kg iv. CSF was collected by continuous drainage from a needle placed in the cisterna magna. This afforded a measure of formation of CSF as well as glucose concentration. Glucose was determined by the glucose oxidase method. Fasting blood glucose and CSF glucose in mg/100 ml averaged 48.8 ± 2.5 (SE) and 45.1 ± 2.9 (SE), respectively. The CSF:blood ratio of 0.923 was similar to that reported for dogs but differed from the ratio of 0.6 for humans. Formation of CSF prior to MMH was 0.003 ml/min. Following MMH, both blood and CSF glucose increased to 90.6 ± 8.0 and 63.8 ± 5.5 mg/100 ml, respectively, and similarly the CSF:blood ratio decreased to 0.704. Formation of CSF continued at 0.03 ml/min. Convulsions occurred in half the monkeys 120 min after MMH, a period when both blood and CSF glucose were elevated. It is concluded that, in pentobarbital anesthetized monkeys, MMH does not cause hypoglycemia and the convulsions are not related to either decreased blood or CSF glucose.

128. *Some Effects of $CBrF_3$ Inhalation on Myocardial Glycolysis.* RICHARD A. RHODEN and KARL L. GABRIEL, Drexel University, Philadelphia, Pennsylvania.

Bromotrifluoromethane ($CBrF_3$) would have considerable potential as a closed environment fire-extinguishing agent, except for membership in that large group of hydrocarbons capable of precipitating irregular heart action when inhaled by persons with elevated levels of blood epinephrine. These experiments were an effort to determine whether myocardial glycolysis was impaired in male Wistar rats breathing 79/21 $CBrF_3/O_2$ and challenged with *l*-epinephrine in what would ordinarily constitute subtoxic dosages. Central nervous system effects were the major observations in rats exposed to both $CBrF_3$ and epinephrine; 17 of 20 rats so exposed suffered respiratory arrest within 40 min, and several exhibited erratic convulsive behavior prior to the respiratory difficulties. All control rats survived, and myocardial levels of glycolytic intermediates were compared in animals breathing air on $CBrF_3/O_2$, with and without epinephrine challenge, using "crossover plots." With air–epinephrine and $CBrF_3/O_2$–epinephrine there were crossovers at phosphohexose isomerase (deactivation), phosphofructokinase (activation), and aldolase (deactivation). $CBrF_3/O_2$ alone showed a single deactivation crossover at the aldolase step, and the general picture was that of an FDP "bottleneck" (accumulation), possibly due to anoxia but certainly exacerbated by the $CBrF_3$–epinephrine combination. (Supported by a grant from the Environmental Control Administration, CPEHS, USPHS, EC 44453.)

129. *Continuous Exposures of Human Subjects to SO_2.* F. W. WEIR, C. ROSS, P. BROMBERG, and J. H. SCHULTE, Department of Preventive Medicine, Ohio State University, Columbus, Ohio. (C. H. Hine.)

This report represents the first phase of a continuing investigation designed to quantify effects of continuous exposures to low levels of SO_2 with and without selected contaminants in healthy subjects and ambulatory patients with chronic obstructive pulmonary disease. In this phase of the investigation 4 groups of 3 healthy young males were continuously exposed for 120-hr periods to 0, 3, 6, or 8 ppm SO_2. Exposures were conducted in a 30 m^3 dynamic flow chamber. Temperature was controlled at $22 \pm 1°C$; water vapor concentration was controlled at 300 ± 25 mg/l. Daily pulmonary function measurements included airway resistance at varying lung volumes, functional residual capacity, dynamic lung compliance, and total lung

volume. Subjects were exercised on a treadmill at the end of each exposure to a progressive work load sufficient to produce a pulse of 160 bpm. Electrocardiogram, pulse, respiratory frequency, minute ventilation, CO_2 production, and O_2 uptake were measured at rest and during exercise. In addition arterial blood gases (P_{CO_2} and P_{O_2}) were measured at the end of the control and 8 ppm exposure periods. Dose related changes were observed in subjective complaints, clinical evaluations, blood gases, airway resistance, and dynamic lung compliance. Results of this study will provide baseline information for future experiments of this series using SO_2 and particulates on healthy subjects, and SO_2 alone and with particulates on subjects with chronic obstructive pulmonary disease. (Supported by Research Contract No. CAWC 8-15 from the American Petroleum Institute.)

130. *The Hyperbaric Toxicity of Sulfur Dioxide.* T. A. HILL, L. KURLANSIK, and J. SIEGEL, National Naval Medical Center, Bethesda, Maryland.

Although much work has been done on the toxicity of "inert" or noble gases under hyperbaric conditions, there is little information on the in vivo effects of toxic gases under pressure. Previous studies with CO, an asphyxiant, have indicated that there is no difference in toxicity at 1 and 8 atm absolute (ata). In the present study, male Sprague-Dawley derived rats were exposed for 4 hr to SO_2, at 1 and 8 ata to evaluate the pressure-related response to an irritant gas. Oxygen partial pressure was automatically controlled at 160 ± 10 mm Hg, and chamber temperature was regulated to provide a nonstressful thermal environment. Carbon dioxide and humidity were controlled by a constant purge of the chamber with the exposure mixture. There was no significant difference between the 4 hr LC50 expressed as mg/m^3 at 1 and 8 ata. These data support the hypothesis that within the range of pressures studied a constant mass concentration of toxic contaminant elicits a response independent of the total environmental pressure.

131. *Protection against Toxicity of Oxygen under High Pressure by Pargyline as Related to Alteration in Brain GABA Metabolism.* ROBERT SCHATZ and HARBANS LAL, University of Rhode Island, Kingston, R.I.

Wide use of oxygen under high pressure (OHP) in medicine prompted a search for drugs to provide protection from OHP toxicity. Pargyline, a monoamine oxidase inhibitor, provided considerable protection against convulsions, pulmonary edema, pulmonary hemorrhage, and postexposure mortality. There was no correlation between the effects of pargyline on brain amines and protection from OHP convulsions. Pargyline was effective in the absence of amine synthesis. Moreover, administration of 3,4-dihydroxyphenylalanine or 5-hydroxytryptophan, precursors of important brain amines, did not provide significant protection. Reduction in brain levels of γ-aminobutyric acid (GABA) resulting from OHP is well documented. Pargyline increased markedly the brain GABA levels in normal as well as OPH exposed mice. Pargyline-induced elevation of brain GABA was temporally related to protection from OHP convulsions; therefore, it is suggested that pargyline-induced protection from OHP toxicity is not mediated through elevation in catecholamines or indolealkylamines but rather through prevention of OHP-induced fall in brain GABA, an amino acid with known neuroinhibitory actions. (Supported by PHS Grant No. 1 TO1 ES 00104-03 through Institute of Environmental Biology.)

132. *Formation and Persistence of S-Sulfonate in Plasma Following Intravenous Administration of Sulfite.* A. F. GUNNISON and E. D. PALMES, New York University Medical Center, New York, New York.

It has been assumed that inhaled sulfur dioxide is rapidly oxidized to sulfate after absorption and is, therefore, of little systemic toxicologic importance in mammals. Recent work by Gunnison and Benton at Pennsylvania State University revealed that *S*-sulfonate could be demonstrated in rabbit plasma many hours after inhalation exposure to sulfur dioxide. The reaction involved is apparently sulfitolysis of disulfide bonds to yield the *S*-sulfonate which is remarkably persistent in plasma. Studies at this laboratory have been aimed at confirmation and extension of these results by use of iv injection of sulfite rather than inhalation of sulfur dioxide.

The injection procedure permitted accurate specification of time of dosage and made it possible to estimate in plasma both the rates of conversion of sulfite to *S*-sulfonate and of disappearance of *S*-sulfonate. The production and relative stability of the *S*-sulfonate have been confirmed and there are indications that free sulfite, demonstrated immediately post injection, is rapidly removed from plasma.

133. *Toxicologic and Teratologic Studies with ORF 3858 (2-Methyl-3-ethyl-4-phenyl-4-cyclohexene Carboxylic Acid).* L. E. VAN PETTEN and T. O. KING, Ortho Research Foundation, Raritan, New Jersey, and Bio/dynamics, Inc., East Millstone, New Jersey.

Acute toxicity studies with ORF 3858, a nonsteroidal antifertility compound, showed the oral LD50 in mice and rats to be 680 ± 70 mg/kg and 2350 ± 290 mg/kg, respectively. Intraperitoneal toxicity in mice was 150 ± 17 mg/kg. A dose of 20 mg/kg iv in dogs produced convulsions and death within 16 min after dosing. Rats were fed ORF 3858 at dietary levels of 25, 10, or 2 μg/kg/day for 14 wk and 75, 25, or 5 μg/kg/day for 1 and 2 yr. Decreased body weights and food consumption, decreased erythrocytic values, increase in BUN and SGPT, increase in relative weight of liver, pituitary gland, adrenal glands, and uterus were present in the high dose animals when compared to the controls. Histologic evaluation revealed toxic heptatic injury, kidney pigmentation, uterine and mammary gland hyperplasia, aspermatogenesis, atrophy of the male gonads and accessory sex organs and an increased incidence of mammary gland neoplasia in high dose male animals. Female monkeys receiving daily po doses up to 10 mg/kg of ORF 3858 for 1 yr showed hyperemia of the sex skin, fewer instances of vaginal bleeding, a decline in mean hemoglobin concentration, decrease in terminal serum, alkaline phosphatase, depressed relative thyroid gland, and ovarian weights. Histologically the test animals revealed ovaries of a follicular pattern, uteri in varying states of endometrial hyperplasia, vaginal epithelium in a state of cornification, and mammary gland proliferation. Teratologic studies in rats and rabbits showed a dose-related decrease in litter size. No visceral or skeletal abnormalities were observed in either test. Perinatal and postnatal studies in rats revealed a decrease in neonatal birth weight and postnatal growth. The F_1 generation female rats showed significant infertility.

134. *Comparative Toxicity of o-Phenylphenol and an o-Phenolphenol-Containing Disinfectant.* FREDERICK W. OEHME, Kansas State University, Manhattan, Kansas.

The comparative toxicity and biotransformation of phenol was described in an earlier report. The current investigation compares the toxicity and excretion of a phenol derivative, *o*-phenylphenol (OPP). OPP was administered po to dogs and cats as the chemically pure compound and as a component of a commercial spray disinfectant. Tracer quantities of ^{14}C-OPP were added to all doses. Groups of 6 dogs and 6 cats were each given 1 or 3 g OPP/kg body weight. Similar groups received 3 or 9 ml of the disinfectant concentrate (containing 0.125% OPP)/kg body weight. Toxicity, plasma levels, urinary excretion, and tissue residues of OPP were determined. Dogs required approximately 3 times the OPP dosage as cats to develop similar toxicity. This species difference was also reflected in OPP plasma disappearance and urinary excretion. Similar toxicity and species variations occurred following the administration of the disinfectant, but vomiting was an additional common result. Since the amount of OPP in the doses of disinfectant was much less than that given in the chemically pure form, an apparent additive effect occurs due to the other ingredients. Under practical circumstances it is unlikely that dogs or cats would consume and retain sufficient disinfectant to be adversely affected.

135. *The Effect of Restricted Food Intake on the Cardiotoxicity of Isoproterenol in Rats.* T. BALAZS, E. ARENA, and C. N. BARRON, Smith Kline & French Laboratories, Philadelphia.

Isoproterenol hydrochloride at acutely toxic sc doses in rats produced acute cardiac failure or myocardial necrosis. Obese rats are particularly sensitive. We examined the effect of obesity,

loss of weight, and age on sensitivity to these effects. The sc median lethal doses were determined for 3 groups of male Charles River (CD) rats. One group (50 rats), 7 mo old, had been fed ad libitum. Another group (54 rats), 7 mo old, had been fed ad libitum except for the last mo when intake of food was restricted. A third group (24 rats), 4 mo old, had been fed Purina Laboratory Chow ad libitum. The terminal body weights of the latter 2 groups were comparable. The LD50 for these groups were 0.35, >900 and 0.66 mg/kg, respectively. The severity of cardiac lesions following sc injections of 0.075 mg/kg isoproterenol HCl given on 2 consecutive days was evaluated in 3 groups similar to those above. The rats fed ad libitum had a strikingly greater incidence and severity of cardiac lesions than those on restricted feeding. In another test with 3- to 4-mo-old rats, marked cardiac necrosis occurred with sc doses of 0.5 mg/kg of isoproterenol HCl on 2 consecutive days in those fed ad libitum but not in those on restricted feeding for 8 days. The resistance acquired by restricted intake of food persisted for a week upon return to ad libitum feeding, even though the weight lost was regained. The chronotropic effect of isoproterenol HCl on isolated, perfused hearts from rats on either ad libitum feeding or restricted intake of food was the same. Indirect evidence suggests that pharmacokinetic change may not be responsible for the decreased cardiotoxicity of isoproterenol in rats kept on restricted diet. The development of a myocardial metabolic change by dietary restriction which increases the resistance to the ischemic effect of this catecholamine is postulated.

136. *Acute Oral Toxicity of Cannabinoids in Various Species.* G. R. THOMPSON, ULRICH H. SCHAEPPI, and HARRIS ROSENKRANTZ, Mason Research Institute, Worcester, Massachusetts; and MONIQUE C. BRAUDE, NIMH, Chevy Chase, Maryland.

For preclinical toxicologic evaluation, Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), and *Cannabis* extract were administered po to rats, dogs, and monkeys as solutions in either absolute ethanol, sesame oil, or sesame oil with 2.5–9.0% ethanol. All three compounds were significantly more potent to male than to female Fisher rats. However, within the dosage range of 225–3600 mg/kg, Δ^9-THC and Δ^8-THC produced the same lethality while both isomers were approximately twice as potent as the *Cannabis* extract. Death due to these compounds invariably occurred between 36 and 72 hr after treatment, regardless of compound, dose level, or sex. Toxicity was characterized by severe hypothermia, decreased respiratory rate, rapid weight loss, inactivity, wide stance, ataxia, muscle tremors, and prostration. Rat mortality apparently resulted from severe hypothermia and other central effects. In dogs and monkeys, single oral doses of Δ^9-THC and Δ^8-THC as high as 525 mg/kg in the dogs and 1050 mg/kg in the monkeys were nonlethal. Predominant toxicity in dogs included drowsiness, ataxia, prostration, anesthesia, tremors, moderate hypothermia, salivation, emesis, and anorexia. Toxic signs in monkeys included hyperreactivity to stimuli, lethargy, drowsiness, characteristic huddled posture, slow movements, abnormal eating procedures and sedation. With equal doses (in mg/kg) of the three compounds, the extent and severity of clinical signs followed the pattern Δ^9-THC > Δ^8-THC > *Cannabis* extract.

137. *Maternal Distribution and Placental Transfer of ^{14}C-Δ^9-Tetrahydrocannabinol in Pregnant Mice.* R. D. HARBISON, School of Medicine, Tulane University, New Orleans, Louisiana. (K. H. Jacobson.)

Maternal distribution and placental transfer of Δ^9-tetrahydrocannabinol (THC) has not been extensively investigated. The purpose of this study was to investigate these parameters. ^{14}C-Labeled THC was used in this study to measure distribution and placental transfer. Timed pregnant Swiss-Webster mice were administered THC, 100 mg/kg and 10 μCi/kg, ip, on gestational day 14. Maternal and fetal tissue were removed and solubilized at various periods of time following injection. ^{14}C was measured by liquid scintillation spectroscopy and disintegrations per minute converted to equivalent amount of THC injected (THC equivalents). THC absorption was rapid, and peak plasma levels, 15.8 $\pm$ 1.7 μg equivalents of THC/0.5 ml of plasma, were found 1 hr after injection. Disappearance of THC from plasma was rapid, and at 4 hr approximately 1/3 of the peak level remained, 6.3 $\pm$ 1.2 μg equivalents of THC/0.5 ml. A sig-

nificant amount of THC, 2.5 ± 0.3 μg equivalents of THC/0.5 ml, was also measured at 16 hr. THC was rapidly taken up by liver tissue and peak levels of 23.9 ± 0.3 μg equivalents of THC/100 mg of tissue was measured at 30 min after injection and 8.2 ± 1.6 and 4.8 ± 1.1 μg equivalents of THC/100 mg of tissue at 4 and 16 hr, respectively. Highest levels of THC were measured in fat tissue at 30 min after injection, 128.9 ± 20.2 μg equivalents of THC/100 mg of tissue. At 4 and 16 hr, respectively, 52.7 ± 25.0 and 32.8 ± 13.6 μg equivalents of THC/100 mg of fat were measured. Rapid transfer of THC to brain tissue was demonstrated. Peak levels of 2.2 ± 0.1 μg equivalents of THC/100 mg of tissue were measured 30 min after injection. At 4 hr after injection of THC, 0.63 ± 0.05 μg equivalents of THC/100 mg of brain tissue remained, and only 0.22 ± 0.07 μg equivalents of THC/100 mg of tissue was measured at 16 hr. Fetal tissue levels of THC were highest at 1 hr after injection, 1.0 ± 0.1 μg equivalents of THC/100 mg of tissue. Disappearance of THC from fetal tissue was rapid and 0.32 ± 0.02 and 0.11 ± 0.02 μg equivalents of THC/100 mg of tissue remained at 4 and 16 hr, respectively. In conclusion, significant amounts of THC were measured in maternal liver, brain, muscle, fat, kidney, and placenta. In addition, THC was measured in fetal tissue. These data indicate that THC is distributed to most tissues of the maternal animal and is transferred across the placenta to the fetus. (Supported by USPHS 5-S01-FR-05377-09.)

138. *Circulating Levels of Nicotine in the Blood of Human Smokers.* A. N. WORDEN, I. E. BURROWS, and B. F. J. PAGE, Huntingdon Research Center, Huntingdon, England.

Recent interest has been shown in correlating the physiological effects of nicotine with the circulating levels found in the blood of smokers. A method has been developed specifically to measure the levels of nicotine found in the blood of human smokers. This has been applied to the measurement of nicotine in blood samples taken by venipuncture while a subject has been smoking a cigarette. The levels have been studied by taking blood samples at different intervals of time, before, during, and after the smoking of a cigarette. Nicotine levels have also been studied in relation to various parameters, e.g., diet, type of cigarette smoked, frequency of smoking. Preliminary studies have shown that with smokers a true zero level of nicotine is not found in the blood, but rather some resting level. The actual resting level is rather variable and has been further investigated, particularly with reference to the metabolic implications. The method has also been used to measure blood levels of nicotine found in experimental animals. The animals, mainly primates, have received smoking doses of nicotine either iv, or by actually smoking a cigarette. The blood levels have been examined in relation to acetylcholine output and EEG, etc.

139. *Subacute Toxicity of Water-Suspended Δ^9-Tetrahydrocannabinol in Rats.* R. N. PHILLIPS, D. J. BROWN, R. C. MARTZ, J. D. HUBBARD, and R. B. FORNEY. Indiana University School of Medicine, Indianapolis, Indiana.

Thailand marihuana plant stock was extracted by the method of Turk. Purity of the Δ^9-tetrahydrocannabinol (THC) obtained was 99% as determined by nuclear magnetic resonance, mass spectroscopy, and gas–liquid chromatography (GLC). Water suspensions of THC were prepared as follows: 1.0 g of THC was dissolved in 25 ml ethanol. To this solution was added 200 ml distilled water with vigorous shaking. The suspension was steam distilled to remove the ethanol. Purity of the THC in water suspension remained at 99% as determined by GLC using two different columns. Five groups of male Holtzman rats, 10 rats per group, were given daily ip injections of the water-suspended THC for 30 days. Group I received 30 mg/kg THC, group II received 15 mg/kg, group III received 7.5 mg/kg, group IV received 3.75 mg/kg, and group V received distilled water. Animals receiving the highest concentration of THC exhibited the following signs approximately 10 min post injection: hypersensitivity to touch or sound, lacrimation, depression, ataxia, and diarrhea. At the end of the 30-day test period, the body weight of the highest dose group was significantly less than that of the control group. The same was true for liver and lung weights. Histologic sections were made of the following tissues: liver, lung, heart, testis, kidney, brain, spleen, muscle, and nerve.

140. *Metabolism of γ-Dimethylaminopropyldiphenylphosphine by Rat Liver Homogenates.* R. A. WILEY, The University of Kansas, Lawrence, Kansas; H. A. SASAME and J. R. GILLETTE, National Heart and Lung Institute, Bethesda, Maryland. (M. D. Faiman.)

A number of phosphine analogs of nitrogenous drugs have been recently prepared in these laboratories; some displayed marked pharmacologic activity together with evidence of chronic toxicity. In order to determine the metabolic transformations carried out on γ-dimethylaminopropyldiphenylphosphine (I) in the rat, a series of studies was performed using various subcellular fractions of rat liver homogenates. Rate of disappearance of (I) was followed by ultraviolet spectrophotometry. The disappearance rate of (I) in the presence of whole liver homogenate was found to be 0.6 μmole/g of liver/min, and similar rates were found for 600 *g* and 9000 *g* supernatant fractions. In the presence of the microsomal fraction, a disappearance rate of 1.1 μmoles/g of liver/min was found. When microsomes were recombined with the 100,000 *g* supernatant, the disappearance rate was identical with the lower value observed for the 9000 *g* supernatant preparation. Rate of disappearance of (I) was shown to be unaffected by catalase and to be markedly inhibited when the reaction tubes were gassed with CO. After large-scale incubations, two metabolites were isolated by thin-layer chromatography and identified by mass spectrometry. These were I P-oxide and I N,P-dioxide. No evidence of *N*-demethylation, as measured by careful assays for formaldehyde production, was found.

141. *Preclinical Toxicity Studies with Tilorone Hydrochloride, an Oral Interferon Inducer.* MICHAEL W. ROHOVSKY, JAMES W. NEWBERNE, and JOHN P. GIBSON, The Wm. S. Merrell Co., Cincinnati, Ohio.

Tilorone hydrochloride, 2,7-bis-[2-(diethylamino)ethoxy]-fluoren-9-one dihydrochloride, has broad spectrum antiviral activity in vivo against both RNA and DNA viruses following oral administration to mice. The compound stimulates the production of an antiviral protein with properties characteristic of interferon. In acute studies, the oral LD50 for 24 hr was 959 for mice and 852 mg/kg for rats. In subchronic studies, oral doses up to 180 mg/kg/day in rats and mice produced a dose-related depression of body weight gain and food consumption. In dogs and monkeys oral administration of ≥20 mg/kg/day produced clinical signs of anorexia, emesis, ptosis, salivation, ataxia, and tremors. Oral doses of 3, 10, or 30 mg/kg/day in pregnant rabbits during days 9 through 16 of gestation produced no teratologic effects. Tilorone HCl produced interesting phenomena in the hematopoietic and reticuloendothelial systems of dogs, monkeys, rats, and mice. A single oral dose (range 2–100 mg/kg) resulted in the appearance of vacuoles and/or granules in the cytoplasm of leukocytes in peripheral blood. Basophilic spherical structures accumulated concomitantly in the cytoplasm of cells of the reticuloendothelial system; namely, Kupffer cells of the liver and fixed macrophages of the spleen and lymph nodes. Regression patterns for these phenomena were generally similar; however, they varied according to species.

142. *Morphological Skin Reactions to 2-Chloroethanol.* J. V. BRUCKNER and W. L. GUESS, College of Pharmacy, The University of Texas at Austin, Austin, Texas.

The presence of residual 2-chloroethanol has been demonstrated in ethylene oxide sterilized plastic materials. Previous studies from this laboratory have shown 2-chloroethanol to be acutely toxic to various rabbit tissues. The present study was undertaken to more accurately assess the nature of the damage to dermal and epidermal structures. Intracutaneous injections of several concentrations each of ethanol and 2-chloroethanol were carried out utilizing the shaved back of albino rabbits. Tissue samples for light and electron microscopic examination were taken at time intervals chosen to maximize the acute toxic response. Appraisal of the toxic response of each compound with the light microscope, revealed a classical inflammatory reaction, the magnitude of which varied directly with the administered concentration. In zones of higher concentration, coagulative necrosis of collagen, muscle, and dermal cellular structures was present. The peripheral areas, like the lower concentration injection sites, showed plentiful

polymorphonuclear leukocytes and edema, but no visible changes in fibroblasts or other cellular structures. However, scrutiny with the electron microscope revealed a number of degenerative subcellular changes, including unusually shaped nuclei and nucleoli, vacuolization, intracellular inclusion bodies, plasmalemmal alteration, swelling of the endoplasmic reticulum, and appearance of necrotic foci and intracellular fibrils. Both ethanol and 2-chloroethanol exhibited similar degradative manifestations, although the latter was 2–4 times as toxic. It is well established that both alcohols are active as protein denaturants and lipid solvents. Our data suggest that ethanol and 2-chloroethanol exert toxic effects through such a mechanism, with the chlorine atom potentiating the lipophilicity and protein denaturing properties of the ethanol moiety.

143. *Dermal Phototoxic Reaction from an Insecticide: Clinical and Laboratory Studies.* W. C. FELSENSTEIN, T. GAINES, and D. C. STAIFF, Food and Drug Administration, Department of Health, Education, and Welfare, Wenatchee, Washington, and Atlanta, Georgia.

Since its introduction into North Central Washington orchards in 1965 application of the cyclic carbonate pesticide Morestan has frequently produced a peculiar skin reaction. Recognition of the influence of weather, involvement of exposed skin surfaces only, and sunburn-like character of the reaction, all suggested a photoactive mechanism. Contact phototoxicity appears confirmed by clinical studies which indicate requirement of presence of the compound on the skin in presence of sunlight, prompt reactivity without the "incubation period" seen in photoallergies, positive photopatch tests, failure of skin protected from contact with Morestan to become sun-sensitive, and protection of susceptible spraymen by application of a sun-screen lotion which forms no mechanical skin barrier. Appropriate analyses revealed no evidence for systemic absorption of Morestan or secondary skin photosensitization via abnormal porphyrin production in exposed individuals. Limited animal studies have produced positive skin reactions in rats. In the laboratory, photoactivity has been demonstrated in vitro with an interesting biological test. Prompt photodecomposition of the compound occurs with unknown reaction product(s) which are under study elsewhere. Evidence was found that under special conditions Morestan can also produce simple irritations and true allergic contact dermatitis.

144. *The Effects of Acrolein Inhalation on the Tracheal Mucosa of the Chicken.* E. P. DENINE, Arthur D. Little, Inc., Cambridge, Massachusetts; S. L. ROBBINS, Boston University School of Medicine, Boston, Massachusetts, and C. J. KENSLER, Arthur D. Little, Inc., Cambridge, Massachusetts.

Acrolein has been found to inhibit mucus transport activity both with isolated tracheal preparations and in vivo in the chicken. In order to better define the action, in vivo experiments were conducted to determine the histologic effects of repeated acrolein inhalation at concentrations of 200 ppm and 50 ppm. Experiments were conducted in chickens exposed at these concentrations for 5 min daily via an endotracheal cannula. The effects of this regimen were characterized morphometrically and descriptively in histologic studies of tissue sections of trachea harvested from animals after 1, 3, 6, 13, 20, and 27 days of exposure to acrolein. Comparison of these data with those derived from sham-treated control animals indicates that repeated inhalation of acrolein produces: (1) decreases in the trachea complement of ciliated and goblet cells and mucous glands, and (2) a predominantly lymphocytic inflammatory lesion in the tracheal mucosa. The severity of these changes is related to the number of exposures and the concentration of acrolein.

145. *The Effects of the Chronic Inhalation Exposure of Primates to Nitrogen Dioxide.* V. ALARIE, W. M. BUSEY, H. N. MACFARLAND, H. E. SWANN, JR., and A. A. KRUMM, Hazleton Laboratories, Inc., Falls Church, Virginia. (L. W. Hazleton.)

Two groups of 9 cynomolgus monkeys were exposed to the nominal levels of 0.5 and 7.5 ppm nitrogen dioxide, and a control group was exposed to filtered room air. The exposures were

conducted 24 hr/day, 7 days/wk for 104 wk. Measurements of body weight, mechanical properties of the respiratory system, such as dynamic compliance, pulmonary flow resistance, work of breathing, the distribution of ventilation, diffusing capacity of the lung, arterial blood gases, and hematologic and serum biochemical determinations, were conducted periodically throughout the study. The most striking effects were seen in the distribution of ventilation and in arterial blood gases. A significant deterioration in the distribution of ventilation and alterations in blood oxygen tension were observed in the group exposed to 7.5 ppm nitrogen dioxide. Histopathologic effects resulting from exposure to nitrogen dioxide were also present. (Supported by the Coordinating Research Council.)

146. *The Effects of Chronic Inhalation of Sulfuric Acid Mist on Primates.* W. M. BUSEY, V. ALARIE, J. W. CLAYTON, JR., H. N. MACFARLAND, H. E. SWANN, JR., and A. A. KRUMM, Hazleton Laboratories, Inc., Falls Church, Virginia.

Four groups of 9 cynomolgus monkeys were exposed to 0.38 and 2.43 mg/m^3, 1–5 μ particle size, and 0.48 and 4.79 mg/m^3, <1 μ particle size, of sulfuric acid mist, a control group was exposed to filtered room air. The exposures were conducted 24 hr/day 7 days/wk for 78 wk. Measurements of body weight and growth revealed no deleterious effects of sulfuric acid mist. A significant increase in respiratory rate occurred in the groups exposed to 0.38, 2.43, and 4.79 mg/m^3 sulfuric acid mist. Very slight changes occurred in the diffusing capacity of the lung during the exposure period; however, all groups were comparable at the end of the exposure. A significant deterioration in the distribution of ventilation was observed in the groups exposed to 2.43, 0.48, and 4.79 mg/m^3 sulfuric acid mist. Alterations in blood oxygen tension occurred in the groups exposed to 2.43 and 4.79 mg/m^3 sulfuric acid mist. Hematologic and clinical biochemical measurements were not affected by the exposure. Histopathologic evaluation of the trachea, liver, heart, and kidneys revealed that these tissues were similar in both control and sulfuric acid mist-exposed animals. The 78 wk of exposure to sulfuric acid mist resulted in distinct histopathologic alterations in the respiratory bronchioles of the lung in the groups exposed to 0.38, 2.43, and 4.79 mg/m^3. These microscopic changes were characterized by focal epithelial hyperplasia and focal thickening of the bronchiolar wall. (Supported by the Air Pollution Research Program of the Electric Research Council.)

147. *The Role of Tolerance in Pulmonary Defense Mechanisms.* D. L. COFFIN, D. E. GARDNER, and E. J. BLOMMER. Biological Research Branch, DHEW, Environmental Health Service, Cincinnati, Ohio.

A phenomenon is well known wherein one exposure to ozone (and certain other gases) protects against a subsequent lethal dose. While there is no doubt that such "tolerance" prevents death by acute pulmonary edema, evidence exists which indicates that continued exposure to such a gas causes chronic injury to the lung. Questions which may be posed are: (1) Does continuous or repeated exposure overcome tolerance? (2) Is the more subtle cytological injury mediated via a system which is incapable of exhibiting tolerance? Answers to these questions are of fundamental importance from the standpoint of ozone toxicity, for it is of value to know whether more than one mode of injury exists. From the standpoint of air quality criteria it is important to know whether repeated exposures to ozone might or might not protect man against the noxious effects of this gas. Experiments reported here suggest that tolerance development does not ablate the enhancement of pulmonary infections attributable to ozone. Furthermore, tolerance did not prevent injury to various cellular components of pulmonary defense, i.e., depression of macrophage numbers and lysosomal enzyme activity. Interestingly, while tolerance successfully blocked edema formation, a previous dose of ozone appeared to actually augment the chemotactic response as evidence by heightened influx of polymorphonuclear leukocytes. Data suggest that while tolerance development successfully blocked the edemagenic effect of ozone other more subtle toxic reactions were not affected.

148. *The Role of the Trigeminal Nerve in Sensory Irritation Produced by Airborne Chemical Irritants.* C. E. ULRICH, M. P. HADDOCK, and V. ALARIE, Hazleton Laboratories, Inc., and University of Pittsburgh, Pittsburgh, Pennsylvania (J. W. Clayton, Jr.)

Reflex inhibition of respiration often results from exposure to airborne irritants. This reflex is important as a protective mechanism for the lower respiratory system. A study was conducted to define the role of the trigeminal nerve in sensory irritation. Three different techniques were used: (1) electrical stimulation of trigeminal nerve, (2) bilateral transection of the trigeminal nerves, and (3) microelectrode recording from trigeminal fibers during stimulation by a chemical irritant. The following relationships were demonstrated: electrical stimulation of the trigeminal nerves resulted in a decrease in respiratory rate which was related to the strength of the stimulus. Bilateral transection of the trigeminal nerves blocked the decrease in respiratory rate produced by exposure to chemical irritants, and afferent nerve impulses were shown to result from these irritants. These data support the premise that the trigeminal nerve operates reflexly to modulate respiration.

149. *The Comparative Toxicity of Natural and Synthetic Nordihydroguaretic Acid (NDGA) in Rats.* S. E. SADEK and R. BANZIGER, Hoffmann-La Roche Inc., Nutley, New Jersey.

NDGA was reported to cause cystic reticuloendotheliosis of paracecal lymph nodes and vacuolation of the kidney tubular epithelium. It was believed that the above effects could have been induced by the impurities in natural NDGA material. We undertook a short-term study to ascertain whether synthetic NDGA would produce such effects. Charles River CD rats of either sex were divided into 3 groups. One group was fed natural NDGA at 2% in the diet, the second group received synthetic NDGA at the same level, and the third group received only basal diet. Body weight gains and food consumption were slightly less in treated animals as compared to controls. Serum alkaline phosphatase levels were moderately elevated in rats receiving NDGA. Other clinical chemical determinations and urinalyses were within normal limits. The cystic enlargement of the lymph nodes as reported by Grice *et al.* was not seen in this study. Histopathologic changes were primarily in the kidneys and of focal distribution. Some convoluted tubules showed evidence of cloudy swelling and narrowing of the lumen, others showed desquamation of the epithelium. The most severely affected tubules were dilated, the lining epithelium was denuded, and the lumen contained eosinophilic casts. Most of these dilated tubules were surrounded by a zone of peritubular reactive proliferation of interstitial cells intermingled with plasma cells; macrophages containing a brownish pigment and sometimes polymorphonuclear leukocytes were scattered in the reactive zone. These changes were seen in both the synthetic and natural NDGA-fed rats.

150. *Effect of Mode of Oral Administration on Toxicity Produced by EL 273 [α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol] in the Rat.* D. G. HOFFMAN, H. M. WORTH, J. L. EMMERSON, and R. C. ANDERSON. Eli Lilly and Company, Greenfield, Indiana.

EL 273 was more toxic to male rats (100–120 g) when administered by gavage (2–500 mg/kg/day) than when administered in the diet (20–5000 ppm). Dietary concentrations of EL 273 as high as 5000 ppm produced no deaths, but 4/4 and 3/4 rats receiving gavage doses of 500 and 250 mg/kg, respectively, died during a 14-day study. The difference in toxicity could not be explained by a difference in EL 273 intake since, on the basis of actual food consumption, the rats given diets containing 2500 and 5000 ppm of EL 273 were receiving daily doses comparable to the respective gavage doses of 250 and 500 mg/kg. Food intake and weight gain were not affected by dietary concentrations less than 1250 ppm or gavage doses less than 125 mg/kg. At the higher doses, EL 273 administered by gavage produced a greater and more prolonged effect on weight gain and food intake than when given in the diet. EL 273 produced a dose-related increase in the rate of hepatic microsomal drug metabolism. With po doses less than 1250 ppm (diet) or 125 mg/kg (gavage), the mode of administration had no apparent effect on the extent of microsomal induction or the no-effect level. The threshold for enzyme stimulation

was approximately 80 ppm. These experiments indicate that the mode of administration is an important variable in subacute oral toxicity studies in rats.

151. *Toxicity of Formose Sugars.* A. FURST and H. B. CHERMSIDE, University of San Francisco, and J. SHAPIRA, NASA-Ames Research Center, Moffett Field, California.

Formose sugars, a possible synthetic source of carbohydrates, arise from the catalytically induced self-polymerization of formaldehyde in the presence of calcium hydroxide. These comprise an extremely complex mixture of both sterioisomers of trioses, tetroses, pentoses, hexoses, higher monosaccharides, the corresponding polyols of these sugars and minor amounts of partly oxidized materials. For toxicity evaluations, several formulations of formose sugars were administered to groups comprised of Sprague-Dawley rats of both sexes. Animals developed diarrhea when fed the crude mixture at a level of 5% in the diet; when purified or partially fractionated the formose mixture was less toxic. Formaldehyde did not cause the toxicity. Rats could also tolerate the materials better if the formose sugars were dissolved in the drinking water and administered ad libitum. At 20% in the water, the animals did not drink. An operationally defined index of "food utility" was developed with the aid of a computer algorithm to better understand the weight changes observed in the animals. This showed a dose-relationship with toxicity. The symptoms were reversible with cessation of treatment unless systemic damage occurred after prolonged dehydration. Histologic changes at high doses were marked reduction of splenic extra medullary hematopoesis, mild acute catarrhal enteritis, and acute thymic involution. (This study was aided by NGR-050-29-001 from the NASA-Ames Research Center.)

152. *Relation of Pesticides to Abortion in Dairy Cattle.* WILLIAM E. RIBELIN and A. W. MACKLIN, University of Wisconsin, Madison, Wisconsin.

Abortion is a common event among dairy cattle, but the cause is infrequently determined. As part of a survey to determine causes of abortion in Wisconsin dairy cattle one of the parameters examined was the role of pesticides. Of 32 aborted fetuses, 18 contained traces of 3 pesticides: Dieldrin, DDT, or heptachlor epoxide. When compared with residues in non-aborted fetuses these levels were found to be insignificant. No carbamates or organic phosphates were detected. Feeding of Ciodrin, Diazinon, Methoxychlor, Sevin, or Vapona to pregnant cattle failed to produce abortion. Investigation of 6 farms having milk withheld from commerce because of pesticide contamination failed to reveal any abortions. It is concluded that pesticides do not contribute significantly to the incidence of abortion on Wisconsin dairy farms. (Supported by U.S.D.A.—University of Wisconsin Hatch Act Funds, Project 6016.)

153. *Comparison between Ibogaine and Serotonin Metabolism in Rats.* H. I. DHAHIR, A. B. RICHARDS, N. C. JAIN, and R. B. FORNEY, Indiana University School of Medicine, 1100 West Michigan Street, Indianapolis, Indiana.

Ibogaine, an alkaloid from the West African shrub *Tebernanthe iboga*, is a psychotropic agent which is considered as a serotonin analog. Ibogaine hydrochloride and serotonin creatinine phosphate were given to white male Sprague-Dawley rats in doses of 10, 20, 30, or 40 mg/kg. Urine was collected and analyzed for freely excreted ibogaine using a spectrophotometric method. Identification of ibogaine and its metabolite was made by thin-layer chromatography using benzene:ethanol (4:1) as the developing system. Ibogaine gave an R_f value of 0.6 whereas its major metabolite gave an R_f value of 0.35. This metabolite gave a positive test for 5-hydroxyindoleacetic acid (5-HIAA), and the amount excreted depended on the dose of ibogaine administered. No other metabolite was detected by this method. Urine was also analyzed for 5-HIAA using a colorimetric method. Of the administered dose of ibogaine, 4–5% was found to be excreted unchanged in the urine. Amounts of 5-HIAA excreted after administration of ibogaine and/or serotonin were identical. About 12–14% of the administered dose was recovered in the urine of rats as 5-HIAA in both cases. It appears from this study that ibogaine and serotonin are degraded so that similar end products result.

154. *Tissue Distribution of Ibogaine Hydrochloride in Rats.* H. I. DHAHIR, A. B. RICHARDS, N. C. JAIN, and R. B. FORNEY. Indiana University School of Medicine, 1100 West Michigan Street, Indianapolis, Indiana.

Ibogaine, 1 of the 12 alkaloids obtained from the West African shrub *Tebernanthe iboga*, is classified as a psychotropic drug which has been reported to be used by addicts. Ibogaine hydrochloride was given to white male Sprague-Dawley rats in a dose of 50 mg/kg ip. The rats were decapitated after 1-, 4-, 8-, and 12-hr periods. Blood was collected in heparinized test tubes and the liver, kidneys, and brain were carefully removed and weighed. Tissues were then homogenized, the homogenate made alkaline and extracted with petroleum ether. Solvent extract was then reextracted with weak acid and analyzed by UV spectrophotometry. About 42% of the dose was recovered in the whole rat after 1 hr of administration of the drug. The highest concentration of ibogaine was found in the liver at this time. After 8 hr only 12% was recovered in the whole rat and none could be detected after 12 hr. At 8 hr post injection, although the whole rat contained about 12% of the dose, no significant amount of ibogaine was detectable in liver, kidneys, brain, or blood.

155. *Behavioral Analysis of the Effects of Trifluoroperazine and Chloral Hydrate in Patients under Treatment with Psychopharmacologic Agents.* HARBANS LAL, YANI KARKALAS, NELSON SMITH, and MOHINDER SINGH, Rhode Island Medical Center, Howard, R.I.

Behavioral analysis of drug toxicity in animals has reached a high degree of sophistication; however, technology to employ behavioral analyses in humans to evaluate therapeutic or toxic effects of drugs has not been developed. Because of differences between man and animal with respect to variables which affect measurable drug responses, we investigated drug actions in humans using automatically recorded behavioral measures. Patients who were being treated with drugs for illness were selected. Schedules of drug administration were rearranged to enable us to analyze the data reliably. This report describes effects of trifluoperazine (0.1–2.4 mg/kg) and chloral hydrate (5–20 mg/kg) on button-pushing for pennies under fixed ratio (FR 100) and fixed interval (FI 1 min) schedules in chronic schizophrenics. Chloral hydrate depressed responding under both schedules. Trifluoroperazine was without an effect on FI responding but depressed FR responding at higher doses. The effects were not cumulative from day to day. Responding under placebo did not differ from those under no-drug.

156. *Effect of Fluoride on Urine Flow Rate and Renal Sodium Concentration Gradients in the Rat.* GARY M. WHITFORD and DONALD R. TAVES, University of Rochester, Rochester, New York.

While it has been known for some time that large doses of inorganic fluoride (F) iv will cause polyuria, the serum concentrations of F associated with this effect and the mechanism of this effect have not been investigated. Determining the serum F concentrations necessary to produce polyuria has recently become important in order to evaluate the hypothesis that metabolism of methoxyflurane to F causes the polyuria seen in some patients after anesthesia. In order to maintain nearly constant serum F concentrations, anesthetized rats were given continuous infusions of graded doses of F in isotonic solutions infused at 20 μl/min. A diuresis was established in all groups receiving F. Plasma concentrations of 50, 270, and 600 μM were associated with increased urine flow rates of 1.8, 3.0, and 2.5, respectively, relative to saline-infused controls (<3 μM F). Marked reductions of the renal Na concentration gradients in the F-infused groups were observed. The inner medulla : cortex Na concentration ratios for the F groups were 0.61, 0.55, and 0.47 relative to the control values. These findings are consistent with the above hypothesis and published observations of patients who received methoxyflurane. (Supported in part by USPHS Toxicology Training Grant 1-TO1-GM-01781-01 and USPHS Research Center Grant 1-P11-GM-15190.)

157. *The Effects of Mestranol and Several Progestins on Chromosomes and Fertility in the Rat.* C. W. EDWARDS, F. J. CALHOUN, and S. GREEN, Food and Drug Administration, Washington, D.C. (John T. Litchfield.)

Mestranol and progestins used in several oral contraceptives were tested to determine their effects on bone marrow chromosomes and fertility in rats. Holtzman female rats were treated for 5 days po with one of the following compounds: mestranol (0.2, 0.02 mg/kg); norethynodrel (0.2 mg/kg); norethindrone (4 mg/kg); ethynodiol diacetate (0.2 mg/kg) or medroxyprogesterone acetate (10 mg/kg). Corn oil or ethanemethane sulfonate (EMS), a known mutagen, was administered to control rats. On day 6, animals from each group were sacrificed, and the bone marrow harvested for chromosomal examination. Others were caged with males on a 1:1 basis. The stage of the estrous cycle was monitored daily, and the day of mating (day 0 of gestation) was recorded. At autopsy (day 14) the numbers of corpora lutea, implants, and resorptions were noted. Of the compounds tested only EMS increased the frequency of damage to bone marrow chromosomes as compared to corn oil treated controls. Only mestranol at 0.2 mg/kg prolonged the return to fertility and no increase in preimplantation or postimplantation loss was noted in any group. Studies of the effects of 1 mo treatments on these parameters are in progress.

158. *A Comparison of the Carcinogenicity and Mutagenicity of Some Epoxy Compounds,* C. H. HINE, J. H. NEMENZO, and G. H. EISENLORD, University of California, San Francisco, California, and The Hine Laboratories, San Francisco, California.

Eight epoxy compounds were evaluated for carcinogenicity and mutagenic potency. All compounds contain either 2 epoxy groups or an epoxy group with an additional active group on the terminal carbon. Carcinogenicity was evaluated by repeated skin application to mice for at least 6 mo. Mutagenicity was determined by 1 of 2 methods: the short-term dominant lethal test in which treated male mice are mated during the second and third postinjection weeks, and the chromosome analysis of lymphocytes cultured from rats administered the compound sc. Teratogenic activity was also evaluated for 3 compounds. Four of the test compounds, all diepoxides, were found to be carcinogenic. The mutagenic index never exceeded 10 with any compound. Mitotic indices were not depressed, and chromosomal changes were noted with only the lowest molecular weight diepoxy compound. No gross teratogenic changes were noted, but significant differences between the test and control groups were demonstrated by mensuration of specific anatomical parts.

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