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Abstracts of Papers for the Eleventh Annual Meeting of the Society of Toxicology, Williamsburg, Virginia March 5–9, 1972

1. *Reversibility of Effects Caused by Hexachlorophene in the Rat.* G. L. KENNEDY, JR., I. A. DRESSLER, W. C. RICHTER, M. L. KEPLINGER, AND J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Ill.

Hexachlorophene has been shown to produce symptoms of central nervous system disorders following po administration to albino rats. A correlation between doses of hexachlorophene, blood levels of the chemical, and lesions in the brain was obtained in subacute feeding studies. Rats were given daily doses of 40 mg hexachlorophene per kg for 6 wk. Groups of animals were sacrificed 0, 7, 28, 56 and 84 days following the last exposure to the chemical, and histological evaluation of the brain and spinal cord was conducted. Vacuolization was observed in the white tracts. The severity of the lesions decreased as the time following the last exposure increased. No evidence of demyelination was observed in the treated animals and the pathological changes observed were reversible.

2. *The Electroencephalogram and Visual Evoked Potential of the Squirrel Monkey Fed Hexachlorophene.* J. A. SANTOLUCITO, Environmental Protection Agency, Perrine, Florida.

Young adult, female Squirrel monkeys were administered po hexachlorophene in oil daily for 28 days. Two monkeys were placed on each of the following treatments: oil only, and 1, 5 or 15 mg/kg body wt. Following the treatment period one animal of each group was sacrificed. The four remaining animals were held an additional 28 days without further treatment before sacrificing. Before, and at intervals throughout the experiment, EEG recordings were obtained; just prior to sacrifices an averaged visual evoked potential to light flash was taken. Electrode placements used were right and left occipito-parietal (RO and LO) and midfrontal (MF). On-line computer processing of the brain biopotentials was performed using a Digital Equipment Corp. LAB-8. Microscopic examination of brain and spinal cord was performed by Dr. Kimbrough, Chamblee Toxicology Laboratory, Perrine Primate Laboratory. At the end of 28 days of treatment the primary component of the visual potential was attenuated at all three dose levels. This effect was still seen in those animals removed from treatment for 28 days. The EEG underwent a decrease in mean frequency throughout the treatment and posttreatment periods in all animals, suggesting a possible influence of repeated anesthesia. However, the decrease in hexachlorophene monkeys was approximately twice that of controls. No differences were seen in degree of bilateral correspondence of spontaneous electrical activity attributable to hexachlorophene. Histology of the CNS did not reveal lesions seen previously in rats given high doses of hexachlorophene. In view of the limited data, we regard these findings as tentative. Nevertheless, they appear suggestive enough to warrant further studies on the feasibility of using EEG as an early indicator of the effects of hexachlorophene.

3. *Determinations of Hexachlorophene in Human and Experimental Animal Tissues.* A. G. ULSAMER, P. D. YODER, and F. N. MARZULLI, Food and Drug Administration, Washington, D.C.

An average hexachlorophene (HCP) whole blood level of 1.21 μg/ml has been associated with the threshold of brain damage in adult rats at the end of a 97-day dietary feeding study (5 mg HCP/kg/day). No overt signs were noted at this blood level. In our work newborn rats dosed by gavage from day 2 (10 mg/kg/day; 10–20 doses) showed signs of CNS poisoning and extensive cerebral white matter degeneration. Their average HCP level in whole blood 20 hr

after the last dose was 1.9 (0.8–3.7) μg/ml. Their HCP concentrations in liver (μg/g) were similar to blood, while their HCP concentrations in brain and kidney were about half these values. Protein, RNA, DNA, glycogen, ATP, phospholipids, sterols, and triglyceride were determined in the brains and livers of these rats with no differences in concentrations between controls and experimentals. Only liver phospholipids showed an effect in the incorporation of radioactive precursors with an increased uptake of ^{32}P-labeled H_3PO_4 in HCP-treated animals. We also analyzed HCP by glc in the blood of humans exposed to various uses of HCP. A randomly selected group had HCP levels of 0.00–0.23 μg/ml in whole blood; medical personnel using a 3% HCP surgical scrub on the hands had levels of 0.02–0.11 μg/ml; dentists using a 1% HCP hand cleanser had levels of 0.00–0.05 μg/ml; use of a 0.75% HCP soap for up to 16 yr gave values of 0.03–0.18 μg/ml with no correlation with length of use. Experimental whole body washing for 3 wk with a 3% HCP cleanser increased the HCP in whole blood (from less than 0.01 μg/ml) to 0.10–0.38 μg/ml. Random human sc fat samples contained 0.00–0.08 μg/g. At present, the suitability of the rat as a model system for extrapolation to man is not known.

4. *A Possible Mechanism of Action of Hexachlorophene Intoxication.* L. E. BLOCKUS, D. H. M. CHAN, J. W. GOODE, M. L. KEPLINGER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois.

Accidental ingestion of soaps containing hexachlorophene (G-11) by dogs has caused death. A systematic study of the events of such intoxication was undertaken. Administration of shaved soap suspensions produced emesis, so retained dosage was questionable. Cannulation of the jejunum and introduction of a suspension eliminated this variable. Anesthetized dogs were given a lethal dose (300 mg/kg) of G-11. Continuous monitoring over 6 hr revealed an elevation of body temperature (2–5°C), increased respiratory rate (4–6 times control) and a decrease of CO_2 in expired air from 5.0–6.0% to 1.5–2.0%. Death ensued as the body temperature reached 43.0°C. Surgical removal of a ligated 50-cm segment of jejunum containing unabsorbed G-11 reversed the hyperthermia and hyperventilation, and prevented death. The striking similarity between these events and those seen with 2,4-dinitrophenol suggested an investigation of tissue O_2 uptake. Mice were given 100, 200, and 400 mg/kg of G-11 po. After 1, 2, 3, and 4 hr mice were sacrificed, and the rate of O_2 uptake was measured with a Clark oxygen electrode in liver and brain. The slopes of the rates of O_2 uptake were calculated and analyzed. G-11 markedly increased the rate of O_2 uptake in liver and brain. These findings suggest that G-11, like 2,4-dinitrophenol, may uncouple oxidative phosphorylation.

5. *Persistent Effects of Monomethylhydrazine Administered at a Continuous Very Low Dose Rate or in Single Doses.* F. N. DOST, D. E. JOHNSON, and C. H. WANG, Oregon State University, Corvallis, Oregon.

Monomethylhydrazine (MMH) interferes with oxidation of glucose-^{14}C and of methylamine-^{14}C to $^{14}CO_2$ by intact animals. Continuous low-level exposure to MMH has a much greater effect than equimolar single doses. Glucose-^{14}C was continuously infused iv or intragastrically at 150 mg/hr/rat for 11 hr. Methylamine-^{14}C was infused iv or given as a single ip dose. Respiratory $^{14}CO_2$ was measured with flow ion chambers coupled to vibrating reed electrometers. MMH at 0.4 mmol/kg (LD50 = 0.5 mmol/kg for this strain rat) frequently caused severe depression of $^{14}CO_2$ production from glucose-^{14}C and a somewhat lower CO_2 output and RQ. When MMH was infused iv at 0.035 mmol/kg/hr for 14 hr beginning 4 hr before glucose infusion, the glucose catabolism was reduced from the outset; this depression persisted as much as 1 mo. Single doses of 0.01 mmol MMH/kg almost totally inhibited oxidation of methylamine-^{14}C, and inhibition was still detectable 3 wk later. Oxidation of putrescine-^{14}C was inhibited as strongly but returned to normal in 24 hr. MMH infused continuously at 0.002 μmol/kg/hr for 60 hr (total 0.12 μmol/kg) decreased oxidation of methylamine by 30–40%. Similar interference with methylamine oxidation was observed in squirrel monkeys at somewhat higher MMH doses. Increased MMH effect at low intake rates is related to the dose-dependent metabolism of MMH previously reported.

6. *The Chronic Inhalation Toxicity of Monomethylhydrazine.* J. D. MACEWEN, C. C. HAUN, and A. A. THOMAS, SysteMed Corp., Dayton, Ohio; Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio.

Chronic exposures of four animal species to monomethylhydrazine were conducted to evaluate the degree of safety in the current industrial threshold limit value (TLV) which was established by analogy to hydrazine and UDMH chronic toxicity. Exposures conducted 6 hr daily for a 6-mo period at MMH concentrations from 0.2 to 5.0 ppm showed dose related growth depression in rats and hemolytic effects in dogs and monkeys. A dose dependent change in the myeloid/erythroid elements of bone marrow was also observed. One continuous experimental exposure of animals was performed, further confirming the dose dependency of MMH induced pathological effects. There appears to be no threshold effect level for this highly reactive chemical.

7. *Effect of Methylhydrazine (MMH) on Cerebrospinal Fluid Catecholamine Efflux in the Rhesus Monkey.* BENNETT A. SHAYWITZ, WILLIAM T. GORMLEY, and KENNETH C. BACK, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Recent evidence suggests that methylhydrazine (MMH) may exert prominent effects on brain catecholamine metabolism, but the mechanistic nature of this effect is unclear. As a first step in our investigations of mechanisms, we have utilized the technique of ventriculocisternal perfusion to investigate the effect of MMH on the rate at which catecholamine added to the cerebrospinal fluid will leave the ventricular system to enter brain or blood. The efflux, representing the amount of artificial cerebrospinal fluid cleared of labeled catecholamine each minute, is analogous to renal clearance and was determined by perfusing the ventricular system with synthetic cerebrospinal fluid that contained either ^{14}C-dopa or ^{14}C-dopamine. After a steady state efflux was established, MMH was added to the perfusion solution and a new steady state efflux determined. Each animal served as his own control. The average clearance of ^{14}C-dopa in ml/min $\pm$ mean SE increased from 0.0478 $\pm$ 0.0124, before, to 0.0693 $\pm$ 0.0174 after MMH, an increase of 145%. Similarly the clearance of ^{14}C-dopamine increased from 0.0273 $\pm$ 0.0031, before, to 0.0483 $\pm$ 0.0077 after MMH, an increase of 171%. Formation of cerebrospinal fluid (0.036 ml/min) and clearance of inulin did not change, showing that bulk absorption and formation of cerebrospinal fluid at the choroid plexus were not affected by MMH. The percentage of both ^{14}C-radioisotopes excreted in the urine (33%) and the amount remaining in the brain (15%) were not increased by MMH. Results suggest that the increased efflux of ^{14}C-dopa and ^{14}C-dopamine found after MMH may be due to either an increased permeability of the blood–brain or blood–cerebrospinal fluid barriers by effects of MMH on catecholamine metabolism or both.

8. *In vivo Effects on Ornithine Decarboxylase Activity in Hyrdazine-Treated Rats.* V. S. HUBBARD and W. L. BANKS, JR., Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) catalyzes the conversion of ornithine to putrescine, which is believed to be the rate limiting step in the biosynthesis of polyamines. Rapid growth systems, such as in the developing chick embryo, regenerating liver, or certain tumor tissues show a correlation between ornithine decarboxylase (ODC) activity and RNA synthesis. Since hydrazine treatment and partial hepatectomy produce similar hepatic biochemical changes which include increased protein and RNA levels, the effect of hydrazine on ODC activity was studied. Adult rats were injected with neutralized hydrazine (40 mg/kg, ip) and fasted for various times. ODC activity was assayed in a 20,000 *g* supernatant from rat liver by trapping and counting released $^{14}CO_2$ from ornithine-1-^{14}C. In comparison to the controls, ODC activity was enhanced within 2 hr following treatment with hydrazine. The enzyme activity was maximal 4 hr after administration of the compound and returned to near control levels by 12 hr. The supernatants prepared from hydrazine-treated rats were found to have increased endogenous ornithine levels which would magnify the difference in ODC activity between the controls and hydrazine-treated animals when corrected for the ornithine

pool size. Although the changes in ODC activity occur sooner, they are comparable in magnitude to those observed in regenerating liver. Aromatic amino acid decarboxylase activity, using phenylalanine-1-^{14}C as substrate, was decreased compared to controls at the time of maximal ODC activity. Since both enzymes require pyridoxal phosphate as a cofactor, a direct effect of hydrazine on the pyridoxal phosphate cannot account for the changes observed in both enzyme activities. The similarities of the biochemical responses following either partial hepatectomy or hydrazine treatment could suggest that hydrazine might produce a "chemical" partial hepatectomy. (Supported in part by the A. D. Williams Fluid Fund for Research at the Medical College of Virginia.)

9. *Drug Related Mesovarial Leiomyomas in Rats.* L. W. NELSON and J. H. WEIKEL, JR., Mead Johnson Research Center, Evansville, Indiana.

Benign smooth muscle neoplasms were observed in the mesovarial tissue of rats given either of two β-adrenergic stimulant compounds for 18 mo. With soterenol hydrochloride given at dose levels of 4.6, 10.0, or 21.5 mg/kg/day po, 3 of 30 low dose, 6 of 30 middle dose and 10 of 30 high dose rats had benign mesovarial leiomyomas. In the study of mesuprine hydrochloride given at dose levels of 40, 100, or 250 mg/kg/day po, similar benign neoplasms were found in 2 of 30 low dose, 7 of 30 middle dose and 7 of 30 high dose rats. Smooth muscle neoplasms were not observed in any of the male rats nor in any of the female control rats in either study. The spermatic cords including the cremaster muscles were given close gross examination. With mesuprine, nonprogressive epithelial changes of acanthosis, hyperkeratosis, and parakeratosis developed in the esophagi of some middle and most high dose male and female rats. No other distinct or consistent compound related gross or microscopic changes were observed in the tissues from the rats in either study.

10. *Thin-Layer Chromatography of Carcinogenic Substances.* SOUHEIL LAHAM, J-P. FARANT, and M. POTVIN, Department of National Health and Welfare, Ottawa, Canada.

During metabolic studies on a potent bladder carcinogen, 4,4′-dinitrobiphenyl, 22 derivatives of biphenyl (viz., amino-, nitro-, acetamido-, and hydroxy-derivatives as well as *O*- and *N*-sulfates) were synthesized, and a thin-layer chromatographic procedure developed. The compounds were chromatographed on both SiO_2 and Al_2O_3 plates (250 μm) and developed in five different solvents. After drying and examination in day and ultraviolet light, they were sprayed with one of the following reagents: Ehrlich, Gibbs, Folin–Ciocalteu, beta-resorcylaldehyde and potassium ferrocyanide. The color tests and R_f observed in the procedure were sufficient to separate and identify very small amounts (μg quantity) of each of these biphenyl derivatives.

11. *Direct-Acting Alkylating Carcinogens. Chloro Ethers and Related Compounds.* B. L. VAN DUUREN, C. KATZ, and B. M. GOLDSCHMIDT, New York University Medical Center, New York, New York.

Direct-acting alkylating carcinogens such as epoxides, β-lactones, sultones and α-halo ethers have been found to be active in several species of laboratory animals. Since they do not have to be metabolized to proximal carcinogens, they may also be carcinogenic for man. Some of these compounds have found wide use in the chemical industry because of their versatile chemical reactivity. This report is concerned with the carcinogenicity assays of a series of compounds belonging to these chemical categories. The compounds were tested in mice by sc injection. In other experiments some of the compounds were also tested by skin application in mice and sc injection in rats. Twenty-one compounds were tested by sc injection in female ICR/Ha mice together with a number of control groups; these included vehicle controls, no-treatment controls and a positive control, β-propiolactone; there were 50 mice per group. The doses used were selected on the basis of short-term toxicity experiments and were the highest doses possible that did not result in early deaths, severe irritant, or cytotoxic effects. The animals were injected sc in the left flank, once a week, for 26 wk and were then observed for the duration of the experiment. One year after the beginning of testing, the following compounds resulted

in significant numbers of sarcomas at the injection site: glycol sulfate, diethyl-β,γ-epoxypropyl phosphate, epichlorhydrin, dimethylcarbamoyl chloride, and 2,3-dichloro-*p*-dioxane. Several of these compounds are used in chemical and related industries. In addition, these findings provide new insight with regard to chemical structure–carcinogenicity relationships.

Supported by USPHS Contract PH 71-2020 from the National Cancer Institute and grant ES 00260 from the National Institute of Environmental Health Sciences.)

12. *Vitamin A, Liver and Colon Carcinoma in Rats Fed Low Levels of Aflatoxin.* PAUL M. NEWBERNE and ADRIANNE R. ROGERS, Massachusetts Institute of Technology, Cambridge, Massachusetts.

It has been demonstrated that the toxicity and carcinogenicity of aflatoxin can be influenced by some nutritional factors, but vitamin A has not been investigated. Since vitamin A has been associated with decreased incidence of respiratory cancer in hamsters, the effects of this vitamin on tumor induction in rats have been examined. Rats fed continuously 0.1 ppm aflatoxin B_I (AFB_1) in the diet over a 24-mo period and subjected to one of three vitamin A levels developed liver tumors in the following incidence: (1) control diet, 24/50; (2) excess vitamin A, 19/50; and (3) low vitamin A, 11/50. Significantly, only those in the low vitamin A group developed colon carcinomas with an incidence of 6/50. Results suggest that low levels of AFB_1 and vitamin A may be associated with a decreased incidence of liver tumors and production of metabolite(s) specific for colon epithelium. (Supported by a grant from the William S. Merrell Company.)

13. *Vitamin A and Respiratory Carcinogenesis in Hamsters.* ADRIANNE E. ROGERS and PAUL M. NEWBERNE, Massachusetts Institute of Technology, Cambridge, Massachusetts.

An adequate intake of vitamin A is required to maintain normal epithelium in the respiratory tract, and high doses of vitamin A have been associated with a decrease in benz(*a*)pyrene-induced respiratory cancer in hamsters. We have examined the effect of adequate or increased levels of vitamin A given as retinyl palmitate (RP) or as retinoic acid (RA) in hamsters fed a synthetic diet and given either (1) no additional treatment; or (2) BP and hematite; or (3) hematite only. Treatment with high levels of RP alone, 5000 IU by gavage 2–5 times per wk, resulted in fatty livers with decreased acid phosphatase. Increased RA had much less effect on the liver and neither treatment had a consistent effect on morphology or histochemistry of the respiratory mucosa. Treatment with BP and hematite induced a high mortality; 6 mo following BP treatment, squamous carcinomas or polyps of larynx or trachea were as follows: (1) adequate RP, 18%; (2) high RP, 10%; and (3) high RA, one of two survivors with tumor. Results suggest beneficial effect of high RP but not RA in decreasing BP-induced respiratory carcinomas in hamsters. (Supported by NIH grant NO. NCI-69-2083.)

14. *Carcinogenicity of Simple Aromatic Amine Derivatives in Mice and Rats.* F. HOMBURGER, G. H. FRIEDELL, E. K. WEISBURGER, and J. H. WEISBURGER, Bio-Research Consultants, Inc., Cambridge, Massachusetts; St. Vincent Hospital, Worcester, Massachusetts; National Cancer Institute, Bethesda, Maryland.

In order to study their carcinogenicity, the following aromatic amine derivatives were fed in lifetime studies to CD male rats and to CD mice of both sexes obtained from Charles River Laboratories: *o*-toluidine-HCl, 4-chloro-*o*-toluidine-HCl, 2,4,5-trimethylaniline, 2,5-dimethoxy-4′-aminostilbene, at two to four dose levels of which the highest approximated the experimentally determined maximal tolerated dose. Controls received powdered Purina chow. All of these compounds produced significant tumor incidences in either rats (*o*-toluidine-HCl, 2,5-dimethoxy-4′-aminostilbene) or mice (2,4,5-trimethylaniline, 4-chloro-*o*-toluidine-HCl). The following neoplasms were observed: with 4-chloro-*o*-toluidine, 40-hemangiosarcomas of various organs in 82 mice; with 2,4,5-trimethylaniline, 17-hepatomas and 12 lung adenomas in 37 mice; with *o*-toluidine-HCl, 35 sc fibrosarcomas among 50 rats; with 2,5-dimethoxy-4′-aminostilbene, 34 earduct tumors among 50 rats. Controls showed none of these types of

tumors. This report on the first four compounds is part of a study including eighteen related simple aromatic amine derivatives. It is concluded that even monocyclic aromatic amines may be carcinogenic.

15. *Antioxidants and Carcinogenesis: Butylated Hydroxytoluene, but not Diphenyl-p-phenylenediamine, Inhibits Cancer Induction by N-2-Fluorenylacetamide and by N-Hydroxy-N-2-Fluroenylacetamide in Rats.* B. M. ULLAND, J. H. WEISBURGER, R. S. YAMAMOTO, and E. K. WEISBURGER, Bionetics Research Laboratories, Inc., Kensington, Maryland, National Cancer Institute, Bethesda, Maryland.

In continuation of our studies on inhibition of cancer induction with the carcinogens *N*-2-fluorenylacetamide (FAA) and the *N*-hydroxy derivative (NOHFAA) by acetanilide and related compounds, we now report on the effect of the antioxidants butylated hydroxytoluene (BHT) and diphenyl-*p*-phenylenediamine (DPPD). Groups of rats were fed 223 ppm FAA or 239 ppm NOHFAA (1 mmol) carcinogen and a 30 mole excess, namely 7850 ppm DPPD or 6600 ppm BHT, in Wayne Chow for 24 wk (male Charles River strain rats) or 32 wk (female rats). Male and female rats continued on control diet for another 12 wk. With FAA alone 70% of males had hepatoma, 20% of females mammary adenocarcinomas. With NOHFAA, 60% of male rats had hepatoma, and 70% of females had mammary adenocarcinoma. The simultaneous administration of DPPD failed to alter induction of cancer in male or female rats. However, BHT reduced the incidence of hepatoma in males to 20% when the carcinogen was FAA. With NOHFAA, BHT lowered the liver tumor incidence to 15% and the mammary cancer incidence in females to 35%. Similar data on the inhibition by BHT were obtained utilizing different levels of carcinogens in Fischer strain rats. Equimolar levels of sulfate failed to increase liver tumor incidence in animals given NOHFAA and BHT. Also, BHT failed to depress the excretion of free urinary inorganic sulfate. Liver and esophageal tumor induction with 51 ppm diethylnitrosamine in drinking water for 24 wk was not affected by BHT or DPPD. Thus, BHT, but not DPPD, inhibits cancer induction by FAA and NOHFAA by as yet unknown mechanisms.

16. *A Rapid Screen for the Assessment of Potential Carcinogenic Activity of Compounds Using the Sebaceous Gland Suppression Test with Particular Reference to Tobacco Condensates and Polycyclic Hydrocarbons.* L. E. MAWDESLEY-THOMAS, D. H. BARRY, and P. HEALEY, Huntingdon Research Centre, England.

The sebaceous gland suppression test for carcinogenic activity of polycyclic hydrocarbons utilizing histochemical demonstration of nonspecific esterase activity and quantitation by image analyzing computer was first described by the authors in 1970. The original test was devised for short term testing of carcinogenic potential of tobacco condensates and involved painting the backs of mice for 3 days with various doses of test compound. Statistical evaluation of results has shown that there is an optimum regime for painting and evaluation of sections if erroneous results are to be avoided. Application of test substances to the skin of the mouse must be controlled more accurately than in the case of long term tests. If this optimum regime is strictly adhered to, results of short and long term tests show correlation for many polycyclic hydrocarbons and tobacco condensates.

17. *The Kinetics of Formation of Nitrososarcosine from Sodium Nitrite and Amines.* MARVIN A. FRIEDMAN, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia. (J. F. Borzelleca.)

Nitrosamines may constitute a major class of chemicals responsible for human cancer in industrialized society. Possibly of greater significance than exposure to preformed nitrosamines is their synthesis in vivo from simple presursors: nitrite and secondary amines. Among the most commonly occurring secondary amines is sarcosine (*N*-methylglycine); nitrososarcosine, the corresponding nitrosamine, is an esophageal carcinogen in the rat. We report here in vitro kinetic constants for nitrosation of sarcosine. Unlike previously studied amines, the nitrosation

of sarcosine is linear with respect to hydrogen ion concentration over a pH range of 2.0–4.5. With the sarcosine concentration constant at 0.05 mM, the first order reaction constant at pH 2.0 for reaction of sodium nitrite with sarcosine was 2.52×16^6/hr × mM. Similarly with the sodium nitrite concentration constant at 0.05 mM and buffered with 10 mM HCl, the reaction constant for nitrosation with respect to sarcosine concentration was 3.24×10^6/hr × mM. Since there have been reports that the nitrosation of dimethylamine was second order with respect to nitrite rather than first order (as was the case with sarcosine), the order of dimethylamine nitrosation was determined. This also was first order. It is our conclusion, therefore, that the hazard from nitrosamine formation is linearly related to environmental exposure to nitrosamine precursors rather than second order as has previously been assumed.

18. *Carrageenan: an Ulcerogenic Agent?* K.-F. BENITZ, R. ABRAHAM, L. GOLBERG, and F. COULSTON, Albany Medical College, Albany, New York.

Earlier reports of cecal and colonic ulceration induced by carrageenan in guinea pigs and other species of laboratory animals attempted to establish a rather questionable analogy with ulcerative colitis in man. We have compared a native form of carrageenan (HMR), derived from *Chondrus crispus*, with a degraded form (C16) obtained from *Eucheuma spinosum*, both administered in drinking water to male and female rhesus monkeys (*Macacca mulatta*) for 7–14 wk. With a 1% solution of HMR (about 1.3 g/kg/day) all animals gained weight and remained in good condition; fecal occult blood occurred sporadically, as in controls. At autopsy only minor changes were found in the lower intestinal tract and were later confirmed microscopically. Monkeys drinking 0.5 or 1.0% C16 solution gained weight, but those on 2% C16 (corresponding to approximately 2.9 g/kg/day) did not. All animals on C16 lost blood frequently from the intestinal tract and developed a slight degree of anemia. Pathological changes seen in the colon ranged from shallow mucosal erosions to ulceration associated with cellular infiltration, granulation tissue in the lamina propria and formation of multiple crypt abscesses. Preliminary results from similar experiments in guinea pigs and rats confirmed the distinction between the biological effects of the two types of carrageenan. The conclusion drawn is that HMR is not ulcerogenic under the conditions used. (Supported by Research Grant 5P01-ES-226-05 from the national Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant 5T01-ES00103-05.)

19. *The Kupffer-cell Response to Degraded Carrageenan.* R. ABRAHAM and L. GOLBERG, Albany Medical College, Albany, New York.

Degraded carrageenan (C16) derived from *Eucheuma spinosum* is utilized in the therapy of peptic ulcer. A comparison of this product with a food additive, undegraded carrageenan (HMR) derived from *Chondrus crispus*, was carried out in rats, guinea pigs, and rhesus monkeys (*Macacca mulatta*). Both carrageenans were administered in drinking water (5, 2, 1, or 0.5% C16 or 1% HMR) for 10–14 wk. Histologically, the livers of animals ingesting C16 had enlarged and vacuolated reticuloendothelial cells engorged with material displaying the properties of a sulfated polysaccharide. Cytochemical demonstration of acid phosphatase and β-glucuronidase in vacuoles containing sulfated polysaccharide was confirmed by electron microscopy. The Kupffer cells displayed fibrillar material located in membrane-bound vesicles that also contained acid phosphatase reaction product. Such Kupffer cells exhibited altered functional characteristics, manifested by a failure to take up horseradish peroxidase into lysosomes. In rats and guinea pigs given C16, comparable Kupffer-cell effects were associated with hepatocellular damage, which was not seen in monkeys. Six months after administration of C16 was discontinued, fibrillar material was still present in Kupffer cell lyososomes. None of the changes described were seen in livers of animals drinking 1% HMR solution. The present study in three species thus suggests that degraded carrageenan is taken up, stored and retained for a considerable time in lysosomes of reticuloendothelial cells. The long-term consequences of such thesaurosis cannot be predicted. (Supported by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant 5T01-ES00103-05.)

20. *Fixation and Preparation of Ocular Tissues for Toxicological Evaluation.* D. M. ALBERT and E. J. GRALLA, Yale University School of Medicine, New Haven, Connecticut.

We have encountered an increasing interest in the histopathologic examination of eyes in toxicology studies. Successful sections require special handling and differences in technique from general histology. The eye should be enucleated at the time the animal is sacrificed, as autolysis of the retina rapidly ensues. Enucleation, particularly in larger animals, is aided by the use of muscle hook, conjunctival forceps, and special scissors. Zenker-formal solution in our hands is the best general fixative. When this is not available or impractical to use, buffered 10% formaldehyde is our next choice. It is not necessary to inject fixatives into the eye. The eye should not be opened until fixation is complete. At gross examination the eye should be properly oriented by the extra ocular muscles and a peripheral cap (calotte) should be cut; careful gross examination should be carried out to determine sites of pathology. Routinely, a second calotte is then removed and the portion containing the pupil and optic nerve embedded in Bioloid-type paraffin and cut at 6 μm. A method of systematic examination and recording is suggested. Possible pitfalls include the use of metallic containers, an insufficient quantity of fixative, and too low water bath temperature for the sections. Successful electron microscopy may be done from either wet tissue or paraffin embedded tissue.

21. *Ocular Lesions Induced in Rats by Feeding Cultures of Penicillium viridicatum.* WILLIAM W. CARLTON, MALCOLM MCCRACKEN, and JOHN TUITE, School of Veterinary Science and Medicine and School of Agriculture, Purdue University, Lafayette, Indiana.

Previous studies have established the renal and hepatic toxicity of cultures of *P. viridicatum* for the rat, mouse, guinea pig, and swine. Recently ocular lesions were observed in most rats fed a rice culture of the fungus. The isolate of the fungus was grown on autoclaved rice at 23°C for 2 wk. The cultures were treated with chloroform, dried at 40°C for 5 days and ground for mixing with a purified diet at concentrations of 50 and 25%. Male rats, weighing approximately 300 g were fed the diets ad libitum. The ocular lesions began after 7–10 days of feeding as a slight cloudiness of the cornea, and increased in severity until the cornea was opaque yellow and vascularized. Microscopically, the initial lesion was edema of the corneal stroma which was thickened and pale staining. These changes were followed by cellularity of the stroma and proliferation and hydropic degeneration of the corneal epithelium. In more severely affected eyes the lesions were those of suppurative keratitis, corneal vascularization and fibroplasia, hypopyon and anterior synecia formation. (Supported in part by Cooperative Agreement 12-14-100-9091 (51), Market Quality Research Division, USDA and NIH Grant No. ESCA 004163).

22. *Quantitative Histochemistry of the Effect of Cosmetics on Mouse Skin.* DAVID H. BARRY and LIONEL E. MAWDESLEY-THOMAS, Huntingdon Research Centre, Huntingdon, England.

The normal laboratory method of evaluating primary irritation involves the comparative study of treated skin areas in animals. Although this type of animal experimentation is of proven use for screening high irritant compounds, weak responses may be impossible to detect by visual assessment. The method frequently involves the use of exaggerated conditions such as high concentration of material and skin occlusion. This is not the normal method of applying cosmetic compounds; although marked responses may be obtained, it is inadequate to demonstrate the subtle changes induced by cosmetics. Accordingly, a more delicate system of testing is required. The present study involves the assessment of metabolic changes in mouse skin resulting from topical applications of various cosmetic compounds and the evaluation of these changes using quantitative histochemical techniques. The cosmetic compounds investigated include shampoos, handcreams, sunscreens, deodorants or antiperspirants, and color-containing cosmetics. Several histochemically demonstrated hydrolytic enzyme systems were studied in the skins from treated and control animals, and the reaction products quantitated using scanning microdensitometry. The results obtained for the various enzyme systems on each compound tested were incorporated into enzyme profiles. These profiles for different compounds were then compared; any deviations in the response pattern were immediately

apparent. The preliminary study indicates the possibility of using this type of methodology to differentiate the effects of the various cosmetic compounds investigated on the activity of hydrolytic enzymes of the skin.

23. *Sources and Classification of Toxicological Data: A Study of Carbaryl (Sevin®).* K. E. WESTON, National Library of Medicine, Bethesda, Maryland.

The Toxicology Information Program at the National Library of Medicine was initiated in 1967 as a result of recommendations of the President's Science Advisory committee. The National Academy of Science–National Research Council advisory body for this program, the Toxicology Information Program Committee, has directed its major efforts toward the computerization of validated toxicology information so that it may become available to interested scientists. Beginning efforts were directed toward the toxicity of pesticides. In order to aid this effort a manual file to be used as a module for the gathering of pertinent information was constructed. It was designed with the research toxicologist's needs in mind, but was adaptable to the use of anyone who might need general concepts or detailed fragments. It was also designed so that its organizational plan and condensed abstracts could be used as a guide for a vocabulary of computer systems input when they are available. The pesticide, carbaryl (1-naphthyl-*N*-methyl carbamate, Sevin) was used as an example. Many aspects of the time, effort, cost, library (and other facilities) services, sources, human skills, and subject matter organization were discussed and illustrated. Suggestions were made for economy of time and the use of more efficient methods of obtaining and recording pertinent information.

24. *A Computer System for the Collection and Analysis of Toxicology–Pathology Data.* ROBERT T. DREW, WERNER W. LOHMANN, and KAREN WOLFORD, Merck Institute for Therapeutic Research, West Point, Pennsylvania. (H. M. Peck.)

In 1968 the Department of Safety Assessment (formerly Toxicology–Pathology Department) initiated a formal study, involving departmental toxicologists and computer systems personnel, to develop and implement a computerized scientific information collection and retrieval system. The system as it exists today stores all hematologic and biochemistry parameter values (utilizing an in-lab Linc-8 and IBM 360 computer) and animal body weights, and prints out this data in a variable tabular form for review by departmental scientists. In addition to this table preparation, statistical analysis, as requested by the responsible toxicologist, is also performed by the computer. All data collected on a toxicology study that has been input to the computer can be available to the toxicologist for review in a structured format in 12–24 hr. Included in this system is the capability to provide the scientific staff with normal value data (either individual values or summarized) on more than 10,000 animals. Also, all tables printed out in this system are of such format that they can be submitted to appropriate government regulatory agencies without editing or retyping.

25. *Detecting Shifts in Laboratory Profiles During Drug Studies in Man.* R. J. KUZMA, C. R. BUNCHER, and J. W. NEWBERNE, Merrell-National Laboratories, Cincinnati, Ohio.

A computer-assisted procedure devised to characterize and screen laboratory profiles during investigational drug studies in man is presented. The procedure utilizes those values obtained during "no treatment" conditions to establish baseline or local "norms" for the group under study. All subsequent values are expressed as percentiles of the appropriate "norm". Various indices are generated to aid the reviewer in discerning abnormalities in the data. Benefits are realized in the convenient and comprehensible review of large bodies of laboratory data oriented toward detecting aberrant laboratory values as defined by the user, and small but consistent shifts in laboratory test values. These reviews are amenable to statistical comparisons. Another important advantage is the additional time afforded for evaluation and interpretation by qualified laboratory reviewers. In one routine evaluation of ten batteries of tests for 150 patients, a small, consistent but clinically insignificant elevation in SGOT and alkaline phosphatase was revealed that probably would not have been detected by other methods.

26. *The Mission and Program of the National Center for Toxicological Research.* M. F. CRANMER, Environmental Protection Agency, Perrine, Florida. (W. F. Durham.)

The National Center for Toxicological Research was established by an interagency agreement between the DHEW and the Environmental Protection Agency on April 1, 1971. The Center will be administered by the Commissioner of the Food and Drug Administration as a national resource, and participation by other government agencies, universities, research institutes and industry will be encouraged. The primary mission of the National Center for Toxicological Research shall be to conduct research programs to study the biological effects of potentially toxic chemical substances found in man's environment with the following emphases: (1) determinination of the adverse health effects resulting from long term, low level exposure to chemical toxicants; (2) determination of the basic biological processes involving chemical toxicants; (3) development of improved methodologies and test protocols for evaluation of the safety of chemical toxicants; (4) development of data that will facilitate the extrapolation of toxicological data from laboratory animals to man. The secondary mission of the National Center for Toxicological Research shall be to conduct such additional research programs as may make appropriate use of the facilities and expertise of the NCTR and contribute to its overall scientific capability without interfering or detracting from the primary mission.

27. *The Toxicology Information Program.* H. M. KISSMAN, National Library of Medicine, Bethesda, Maryland.

The Toxicology Information Program (TIP) at the National Library of Medicine has been in operation for 4 yr. The basic mission of this Program is to implement recommendations made in 1966 by a Presidential Science Advisory Committee panel on "The Handling of Toxicological Information." The objectives of the Program include: (1) creation of computerized, toxicological information and data banks from the scientific literature, from the files of other government agencies, and from such other data files as can be obtained; and (2) the repackaging and distribution of this information in various formats to the scientific community. To have an instrument for the performance of information services in toxicology for the scientific community, TIP has organized a Toxicology Information Response Center (TIRC) at the Oak Ridge National Laboratory. This information analysis center performs in-depth literature searches, analyzes and evaluates information and data, and prepares reviews and state-of-the-art reports. Because of the availability of professional expertise and extensive computer and library facilities, TIRC is also involved substantially in building computerized toxicology data bases for the Toxicology Information Program. Dissemination of the information and data collected and processed by TIP takes three basic routes through: (1) services provided by the TIRC; (2) a variety of printed products such as the *Directory of Information Resources in the United States–General Toxicology*; *a Toxicology Vocabulary*; secondary journals such as the *Toxicity Bibliography* and *Health Effects of Environmental Pollutants*; and (3) an on-line, interactive toxicology information and data system to which users will have access via remote terminals on a pay-as-you-use basis. At the present time TIP is building data files and services in the area of the toxicology of pesticides and other environmental toxicants, and in the field of drug toxicology, with particular emphasis on interactions.

28. *Evaluation of Normal and Organophosphate and Carbamate Insecticide Inhibited Blood Cholinesterase Activity Utilizing Dimethylbutylacetate as a Substrate.* M. F. CRANMER and A. J. PEOPLES, Environmental Protection Agency, Perrine, Florida. (W. F. Durham.)

The availability of an extremely sensitive and precise gas chromatographic method for the analysis of human blood cholinesterase led to the attempt to apply this method to common laboratory animals where analysis of limited blood samples is important. Of particular importance was the comparison of plasma activities, since the plasma of different species vary greatly in esterase profiles. In addition to the need to compare activities obtained with dimethylbutylacetate to those expected with standard methods utilizing acetylcholine was the requirement to compare activities resulting after the administration of an anticholinesterase pesticide. Normal and inhibited blood cholinesterase values are presented for weanling and mature rats, mice, and

rabbits and for mature Rhesus and squirrel monkeys. The inhibited samples were analyzed 30 min after the animals were given a po dose of parathion and carbaryl equivalent to $\frac{1}{4}$ the LD50. The results demonstrate DMBA to be acceptable as a cholinesterase substrate both for the determination of normal and inhibited activities in all species except the mouse, where high levels of nonspecific plasma esterases complicate interpretation.

29. *Cholinesterase Inhibition Studies in Man with Ethion.* R. J. PALAZZOLO, O. E. FANCHER, J. F. MCCARTHY, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois; Niagara Chemical Division, FMC Corporation, Middleport, New York.

The minimum effect dose (MED) for cholinesterase inhibition was determined for Ethion (ethyl methylene phosphorodithioate) in human volunteers using an increasing dose procedure. Following a pretreatment period for determination of baseline plasma and erythrocyte cholinesterase activity, the subjects were treated on a tid po schedule at a dose level (mg/kg) corresponding to a fraction of the maximum no-effect level previously determined in rats and dogs. The dose level was increased incrementally at 3-wk intervals until an effect on plasma and/or erythrocyte cholinesterase activity was observed. Although each subject served as his own control, simultaneous placebo controls were also employed. A minimum effect level of 0.10 mg/kg was determined for Ethion.

30. *Effect of Acute and Subacute Inhibition of Cholinesterase on the Response of the Rat Uterus to Cholinergic Drugs.* JOSEPH J. MCPHILLIPS and DENNIS J. FOLEY, West Virginia University, Morgantown, West Virginia.

The rat uterus, in contrast to the ileum, does not exhibit diminished sensitivity to carbachol following subacute administration of disulfoton. This may be the result of sparse cholinergic innervation of the rat uterus and a low cholinesterase content. Inhibition of the enzyme, therefore, may not be accompanied by measurable changes in the sensitivity of this organ to drugs. In the present study the cholinesterase content of the uterus was compared to that of the ileum. In addition the effects of acute and subacute inhibition of cholinesterase on the sensitivity to cholinergic drugs was also measured. The uterus hydrolyzes acetylcholine (ACh) at a rate of 25.6 μmol/g of protein/hr, whereas the ileum hydrolyzes ACh at a rate of 172.0 μmol/g of protein/hr. Methacholine is hydrolyzed at a rate of 1.8 μmol/g of protein/hr by the uterus and at a rate of 9.0 μmol/g of protein/hr by the ileum. A dose of 1.0 mg/kg of disulfoton/day for 8 days caused a 63% reduction in the hydrolysis of ACh by the ileum. This was accompanied by subsensitivity to carbachol and an increased sensitivity to ACh. The ED50 of ACh decreased from 0.64 to 0.32 μmol/l. In the uterus, there was a 58% reduction in ACh hydrolysis. The sensitivity of the uterus to both ACh and carbachol was unchanged. A single dose of disulfoton, 1.5 mg/kg, produced 65% inhibition of ACh hydrolysis in the ileum. The sensitivity to ACh was increased. The same dose of disulfoton produced a 67% decrease in ACh hydrolysis in the uterus, but there was no change in the sensitivity of the uterus to ACh. Inhibition of the cholinesterase of the uterus, therefore, does not affect the response of the tissue to cholinergic drugs. (Supported by a grant from the National Institute of Environmental Health Sciences.)

31. *Relationship Between Cholinesterase Activity and Sensitivity to Cholinergic Drugs in the Rat Ileum.* DENNIS J. FOLEY and JOSEPH J. MCPHILLIPS, West Virginia University, Morgantown, West Virginia.

Repeated administration of disulfoton, an organophosphate cholinesterase inhibitor, produces a decrease in the sensitivity of the rat ileum to carbachol. In the present study experiments were designed to determine the onset and duration of reduced sensitivity to carbachol in female rats. At the same time the hydrolysis of acetylcholine (ACh) and methacholine (MeCH) was measured in segments of ileum from the same rats. Twenty-four hours after the first dose of disulfoton, there was no change in the sensitivity of the ileum to carbachol. Hydrolysis of ACh was reduced 36% and MeCh hydrolysis was reduced 40%. After 2 days of treatment there was a slight decrease in the sensitivity of the ileum to carbachol. The ED50 of carbachol increased from 0.32 to 0.50 μmol/l ($P < 0.05$). The hydrolysis of ACh was reduced 76% and the hydrolysis

of MeCh 45%. After 4 days of treatment the ED50 of carbachol increased from 0.29 to 0.78 μmol/l, and after 8 days the ED50 had increased from 0.26 to 0.96 μmol/l. However, the maximum change in sensitivity occurred at 4 days since there was no difference between the changes in sensitivity at 4 and 8 days. After 4 days of treatment hydrolysis of ACh was inhibited 66% and MeCh hydrolysis 59%. Reduced sensitivity to carbachol was still present 5 days after subacute treatment with disulfoton was stopped. Hydrolysis of ACh and MeCh, however, was essentially normal 2 days after treatment was stopped. Changes in the sensitivity of the ileum, therefore, do not seem to be directly related to the degree of cholinesterase inhibition. (Supported by a grant from the National Institute of Environmental Health Science.)

32. *Lethality, Antiesterase and Hemicholinium-like Actions of Bis-quaternary Ammonium Compounds Containing the 4-(Tetramethylene-2-One) phenacyl Chain.* TED A. LOOMIS, HAROLD Z. SOMMER, and JANE A. COLLINS, University of Washington School of Medicine, Seattle, Washington.

Several analogues of a series of heterocyclic hemicholinium-3 compounds have been shown to have various degrees of anticholinesterase and hemicholinium-3 types of pharmacologic action. The possibility of having both of these actions in the same molecule prompted one of us to synthesize a series of bis-quaternary compounds containing the 4-(tetramethylene-2-one) phenacyl chain. This report consists of results obtained on the ortho pyridinio (1); the meta pyridinio (2); and the 2-hydroxyethyldimethyl ammonio moieties (3). In vitro tests showed that compounds 1 and 2 were potent antiesterase agents but compound 3 was inactive as an antiesterase agent. The iv LD50 values (mice) for compounds 1, 2, and 3, respectively, were 0.64, 0.25, and 0.09 mg/kg. The ip LD50 values (mice) for the respective compounds were 5.2, 0.27 and 0.06 mg/kg. Pretreatment of mice with SKF 525A did not alter the ip LD50 of compound 1. Pretreatment with pentobarbital significantly protected the mice from the lethal effects of all of the compounds. Compounds 1 and 2 showed characteristic anticholinesterase action on the intact and isolated nerve muscle preparations of the rat and delayed hemicholinium-like action on the same preparations. Compound 3 showed only hemicholinium-like action on the nerve–muscle preparations.

33. *Some Actions of a New Organophosphorus Compound and One of Its Major Metabolites at the Neuromuscular Junction.* B. A. HEMSWORTH and J. M. CHOLAKIS, University of Rochester School of Medicine, Rochester, New York. (H. C. Hodge.)

The organophosphorus insecticide, *O,O*-dimethyl-*S*-[(2-methoxy-1,3,4-thiadiazol-5(4*H*)-on-4-yl)-methyl]dithiophosphate (GS 13005), inhibits both acetylcholinesterase and cholinesterase in vivo. Indirectly elicited contractions of the cat tibialis muscle are initially potentiated by iv injections (30 mg/kg) of the anticholinesterase. High frequencies of nerve stimulation eventually block the muscle contractions, and it has been suggested that in addition to its anticholinesterase action the insecticide may have an effect on the motor nerve terminal. In vitro, GS 13005 caused an inhibition of acetylcholine (ACh) synthesis by an action on the enzyme choline acetyltransferase (ChAc), and the anticholinesterase was found to inhibit the uptake of choline into nerve ending particles (synaptosomes). The major degradation of GS 13005 in plants is via the oxygenated metabolite GS 13007. This metabolite is a more effective anticholinesterase in vitro than GS 13005 but has no effect on ACh synthesis or choline uptake. It is suggested that in vivo GS 13005 may affect ACh synthesis, thus modifying the amount of transmitter released from the motor nerve terminal and affecting neuromuscular transmission.

34. *Comparison of the Metabolism of Parathion with the Amounts of High Spin and Low Spin Cytochrome P-450 in Various Sub-fractions of Rabbit and Rat Liver Microsomes.* B. J. NORMAN and R. A. NEAL, Vanderbilt University, Nashville, Tennessee.

The metabolism of parathion to paraoxon and to diethylphosphorothioic acid plus *p*-nitrophenol are apparently catalyzed by two different mixed function oxidase enzyme systems. One evidence for this is induction of the mixed function oxidase enzymes of rat liver by 3,4-benzopyrene stimulates the metabolism of parathion to paraoxon to a greater extent than to

diethylphosphorothionate plus *p*-nitrophenol. It has also been shown that induction of the mixed function oxidase enzymes with 3,4-benzopyrene and other polycyclic hydrocarbons selectively induces the amount of high spin cytochrome P-450 in hepatic microsomes. We therefore undertook studies in which we compared the amount of high spin and low spin P-450 in various samples of microsomes and subfractions of microsomes with the ability of these microsomal fractions and subfractions to metabolize parathion to paraoxon and to diethylphosphorothioic acid. The purpose of these studies was to determine if high spin P-450 was selectively catalyzing paraoxon formation. The results of these experiments indicate there is no direct relationship between the amount of high spin P-450 in a particular sample of microsomes and the ability of these microsomes to metabolize parathion to paraoxon. (Supported by Training and Research Grants ES-00075, ES-00267, and ES-00112.)

35. *Enhancement of Aniline Metabolism by Paraoxon.* JAMES T. STEVENS, ROBERT E. STITZEL, and JOSEPH J. MCPHILLIPS, West Virginia University, Morgantown, West Virginia.

During a series of experiments, we observed that paraoxon, in contrast to most other anticholinesterase agents, enhanced the in vitro metabolism of aniline. This study examines that observation in more detail. Paraoxon, when added to microsomal preparations at concentrations greater than 2×10^{-5} M, enhanced the *para* hydroxylation of aniline. This effect was observed in hepatic microsomal preparations of the mouse, rat, rabbit, and dog. Enhancement of aniline metabolism could also be produced by pretreating mice with paraoxon. Mice were given 1/2 LD50 of paraoxon po and sacrificed 1 hr later. The in vitro metabolism of aniline was found to be increased 20–30% in livers which were taken from these mice. Although the addition of paraoxon to microsomal preparations enhanced aniline metabolism, the side chain oxidation of hexobarbital and the *N*-demethylation of ethylmorphine were inhibited. *p*-Nitrophenol, a major metabolite of paraoxon, was also found to have some enhancing properties. (Supported by U.S.P.H.S. grant ES 0036, GM 16433, K4-GM 12522 and GRS-5-S01-FR-05433.)

36. *Effect of Carbon Monoxide on Response to Parathion and Paraoxon.* C. BAEZA, A. M. GOLDBERG, and R. J. RUBIN, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland. (D. Blake.)

Inhalation of carbon monoxide (CO) has been shown to prolong the in vivo response to drugs by inhibiting the hepatic enzyme system responsible for their inactivation. Since this same CO-sensitive enzyme system is responsible for the metabolism of parathion to its toxicologically active form, paraoxon, it became of interest to study the effects of CO in the in vivo response to parathion. Parathion (10 mg/kg ip) produced a 50% reduction in red blood cell cholinesterase after 1 hr in rats maintained in room air. Prior equilibration of rats for 90 min in various levels of CO resulted in a concentration-related inhibition of the anticholinesterase effect, with complete reversal occurring at 1000 ppm CO. At 42 mg/kg ip, parathion resulted in a mean time to death of 58.6 min in animals maintained in room air. Exposure to CO resulted in a concentration-related prolongation in the time to death, such that at 1500 ppm CO the mean time to death was 129.3 min. It was also observed that a lowered inspired O_2 concentration produced effects qualitatively like those seen with CO. In rats maintained in room air, paraoxon at 1.06 mg/kg ip produced neither deaths nor signs commonly associated with cholinesterase inhibition. At 1000 ppm CO, this same dose of paraoxon resulted in specific signs in 80% of the animals and death in 40%. Paraoxon, at 1.42 mg/kg ip, resulted in signs of intoxication in all rats maintained in room air, but no deaths were observed. In the presence of 2500 ppm CO, 70% of the animals given this dose of paraoxon died. This level of CO alone was not lethal to control animals. As with parathion, lowering the inspired O_2 concentration produced effects on the response to paraoxon similar to those seen with CO. These data indicate that CO or lowered inspired O_2 cause a decreased and/or delayed response to parathion. These results are consistent with a decreased rate of metabolic activation of parathion. The total response, however, is complicated by the demonstrated concomitant enhancement of paraoxon toxicity.

37. *Ultraviolet Determination of Mesitylenic Acid in Urine.* SOUHEIL LAHAM and ELMERA O. MATUTINA, Department of National Health and Welfare, Ottawa, Canada.

In the course of studies on comparative metabolism of 1,3,5-trimethylbenzene (mesitylene), it was found necessary to determine one of the metabolites, 3,5-dimethylbenzoic acid (mesitylenic acid), which is present in the urine of humans and various animal species exposed to this chemical. This metabolite is first removed from acidified urine by steam distillation followed by several ether extractions of the distillate. The extract is then chromatographed on thin-layer plates (SiO_2, 250 μm) and, after development in a suitable solvent, is viewed under ultraviolet light. The spot of mesitylenic acid is marked, eluted with EtOH, and finally read against a blank in a spectrophotometer (290 nm). This method has been applied successfully to human urine and is specific for 3,5-dimethylbenzoic acid. In the conditions used for the extraction and identification of this material, there were no interferences from other aromatic acids usually found in human and animal urine.

38. *Genesis of a Drug Screening Laboratory.* CHARLES R. ANGEL and DOUGLAS J. BEACH, Walter Reed Army Institute of Research, Washington, D.C.

President Nixon's Drug Abuse Counter Offensive stimulated the genesis of a laboratory totally dedicated to screening for drugs. Screening systems were surveyed and two technologies selected, the free radical assay technique and thin layer chromatography. Since any screening system requires confirmation, gas–liquid chromatography was chosen. The composition of the laboratory, its capacity and the personnel required to operate it will be discussed. Establishing a full operation in a field situation poses a myriad of operational problems that are not normally faced. A description of the methodologies used, their sensitivities and the interfering substances encountered will be presented.

39. *A Rapid Procedure for Analyzing Large Numbers of Urine Specimens for the Presence of Drugs.* LEO R. GOLDBAUM and ABEL M. DOMINGUEZ, Armed Forces Institute of Pathology, Washington, D.C.

The reported widespread use of narcotics and dangerous drugs has precipitated an urgent demand for the testing of large numbers of urine specimens for drugs of abuse. A rapid, simple, and reliable procedure has been developed to establish either the absence or the presence of drugs of abuse in urine in a concentration of 1 μg/ml and for morphine at a level of 0.1 μg/ml. Urine (5 ml) is adjusted to pH 9.0 $\pm$ 0.2 by adding 1 ml of a heated solution of sodium borate (20%); then a single direct extraction is made with 15 ml of 5% isobutanol in chloroform. The solvent containing the basic, acid, and neutral drugs is extracted with acid to remove basic drugs, including morphine, with alkali to remove acid drugs; the remaining solvent is evaporated to dryness for the detection of neutral drugs. The presence of morphine is established by the use of a fluorometric procedure. The identification of the other isolated drugs is made by gas–liquid chromatography. A number of columns with different liquid phases are used to detect the presence of drugs as well as their retention times. This analytical procedure is presented in relation to its application as a model for the large-scale screening of urine samples for drugs.

40. *The Effect of Feeding an Elemental Chemical Diet on Mature Rats.* A. H. CAMPBELL, W. R. SEWELL, M. CHUDKOWSKI, J. E. WILLSON, G. H. LORD, and K. MOHAMMED, Johnson & Johnson Research Foundation, New Brunswick, New Jersey.

An 8-wk feeding study was conducted in mature Sprague-Dawley rats to determine the effect of feeding a liquid elemental chemical diet as the sole source of nutrition. Periodic studies of urine and blood were normal except for a slight depression of urine specific gravity, blood urea nitrogen, and hemoglobin–hematocrit levels in some of the animals. Prolonged prothrombin times and bleeding observed in some animals were ascribed to a questionable deficiency of vitamin K. Histopathologic examinations of the tissues showed no abnormalities except for the indication in some animals that fatty metamorphic changes had occurred in the liver. Apparently these changes resulted from a liptropic factor deficiency that was reversed by the addition of choline to the diet. In all other respects the diet appeared to be well tolerated.

41. *A Comparative Study of Inhalation of Lead Particulates in Animals and Man.* T. B. GRIFFIN, F. COULSTON, L. GOLBERG, J. BRADLEY, and J. C. RUSSELL, Albany Medical College, Albany, New York.

Albino rats and rhesus monkeys (*Macacca mulatta*) were exposed to atmospheric lead particulates (21.5 μg Pb/m^3) 22 hr daily for 1 yr. Blood lead levels in controls remained at about 5 μg Pb/100 ml but rose in exposed rats to 25–30 μg/100 ml by 3 mo and in exposed monkeys to 15–17 μg/100 ml by 4 mo, with slight increases in urinary and fecal excretion of lead. These blood levels remained unchanged at the end of 1 yr of exposure, by which time erythrocyte levels of δ-aminolevulinic acid dehydrase (ALAD) activity in rats had fallen to 30% of control values. It was not possible to assess the effects of lead on the enzyme in monkey erythrocytes due to the intrinsically lower levels of activity. Urinary levels of δ-aminolevulinic acid (ALA), porphobilinogen (PGB) or coproporphyrin III (CP) were not measurably increased in the exposed animals of either species. Tissue levels of lead were markedly increased in bone, kidney, liver, and lung. Fourteen male human volunteers were similarly exposed on a continuous basis to 10.9 μg Pb/m^3; eight of these remained in the exposure chamber for about 4 mo. Six other volunteers served as controls living under comparable conditions except for exposure to atmospheric lead. Blood levels of lead, initially 18–20 μg/100 ml, rose to an apparently stable level averaging 36 μg/100 ml, with a slight increase in urinary lead. Erythrocyte ALAD fell to just under 50% of the preexposure value, without measurable changes in urinary ALA, PBG, or CP. Concentrations of blood lead and levels of ALAD activity returned to preexposure values within 4 mo after termination of exposure. (Supported by Environmental Protection Agency Contract No. CPA 70-85, by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 5T01-ES00103-05.)

42. *Toxic Effects of Triphenyllead Acetate in Laboratory Animals and Humans.* G. EISENLORD, G. J. GOBLE, and J. F. COLE. Hine Laboratories, Inc., San Francisco, California; International Lead Zinc Research Organization, Inc., New York, New York.

Triphenyllead acetate (TPLA) has been shown to be an effective molluscicide; concentrations as low as 10 ppm are adequate in an aquatic environment. In addition, it has a relatively high LD50 in mammals due to its low absorption in the gut. This study was undertaken to explore further the toxic and irritating properties of TPLA in laboratory animals and humans. The current investigation includes a 180-day rat feeding study, a 90-day rat inhalation study, a 45-day rabbit dermal application study, a guinea pig skin sensitization test, and a human repeated skin-patch test. In the rat-feeding study, the effects of three dose levels of TPLA incorporated into the diets are documented: rat growth and food intake for each sex are measured, and comparisons made of blood counts, blood chemistries, histopathology, lead depots in tissues, and food:stool lead ratios. Comparable studies are made on rats exposed to TPLA aerosols. Dermal and systemic effects in the rabbit are reported along with blood and urine changes. Data on skin sensitization in guinea pigs and human skin effects are presented. (Sponsored by the I.L.Z.R.O., Inc. of New York.)

43. *Distribution Study of Arsenic, Mercury and Lead in Human Fatal Poisonings.* S. K. NIYOGI, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania.

The distribution of heavy metals in various tissues is of obvious toxicological interest. Determination of metal from biological materials would also be a significant value in assessing the toxic level. In the present study, fourteen fatal cases including adults and children were investigated. Arsenic as well as mercury was estimated by Reinsch test combined with the Gutzeit technique. Lead was quantitated by the Dithizone method. For confirming arsenic or mercury in a positive test, these metals can be sublimed, arsenic giving octahedral crystals, mercury giving silvery globules. Comparing the toxic levels, these findings indicate that death occurred to two adults by arsenic, one adult by mercury, and five children by lead poisoning. The rest, who had slightly toxic or normal levels, may have died by other causes. The results will be presented for further discussion.

44. *The Effect of Folinic Acid on Diphenylhydantoin-Induced Teratogenicity in Mice.* J. L. SCHARDEIN, D. L. HENTZ, J. A. PETRERE, J. E. FITZGERALD, and S. M. KURTZ, Parke, Davis and Company, Ann Arbor, Michigan.

Diphenylhydantoin (Dilantin, DPH) has been shown to be teratogenic in rodents, inducing primarily cleft palate. One of the mechanisms proposed for the teratogenic activity is its alleged antagonism to folic acid. To test this hypothesis, inseminated female Spartan-source mice were treated with teratogenic doses of DPH with and without concurrent treatment with folinic acid, the biologically active form of folic acid. Treatment with 50 mg/kg DPH ip on days 11–13 resulted in cleft palate in 24.8% of the resultant offspring. When 100 mg/kg of folinic acid was concurrently injected each day, the incidence of cleft palate was increased to 42.4%, while reduction of the daily folinic acid to 20 mg/kg reduced the incidence to 11.6%. Further experiments are in progress to define more completely the apparent potentiating and protective effects of folinic acid on DPH-induced cleft palate in mice.

45. *Excretion of Three Antimalarial Drugs, WR-38,839, WR-33,063, and WR-122,455, in Rhesus Monkeys with Chronic Biliary Cannulae.* CARL C. SMITH, GERALDINE F. WOLFE, and STEELE F. MATTINGLY, University of Cincinnati College of Medicine, Ohio.

Biliary excretion of three recently developed antimalarial agents, WR-38,839 (4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3,4-dichlorobenzyloxy)-1,3,5-triazine, labeled with ^{14}C), WR-33,063 (6-bromo-α-di-*N*-heptylaminomethyl-9-phenanthrenemethanol hydrochloride, labeled with ^{3}H), and WR-122,455 (α-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol, labeled with ^{3}H), has been followed in rhesus monkeys with chronic biliary cannulae. The volume of bile is measured and, except for 20% set aside for analytical studies, the bile is returned to the animal via a separate indwelling cannula. In the case of WR-38,839, biliary excretion of ^{14}C following a single po 10 mg/kg dose was highest 4–6 hr after treatment, accounted for 8.5–30% of the dose over a 2–4-day period and was essentially complete at this time. For WR-33,063, highest excretion of the label following a single po 30 mg/kg dose occurred during day 1. Excretion of ^{3}H proceeded slowly but was essentially complete by the end of the second week and accounted for about 10% of the dose administered. WR-122,455 was excreted much more slowly with peak excretion of 0.4–1.1% of the dose/hr occurring at 36–120 hr after a single po 5 mg/kg dose. Biliary excretion continued for 15–20 days and accounted for a total of 27–100% of the dose administered. There is evidence that all three compounds undergo enterohepatic circulation to varying degrees. (Supported in part by Army Contract DADA17-67-C-7065.)

46. *Observations on the Metabolism of Saccharin.* J. L. BYARD, Albany Medical College, Albany, New York. (F. Coulston.)

Following po doses of 40 mg/kg of ^{14}C-sodium saccharin uniformly labeled in the aromatic ring, urine was collected for 24 hr in mice, golden hamsters, guinea pigs, and dogs; and for 96 hr in rats and monkeys (*Macacca mulatta*). Most of the ^{14}C was recovered in the 0–24 hr urines, which were filtered, streaked on thin-layer silica gel plates and developed with isopropanol:water:dimethylformamide (92:6:2, v/v/v) to separate saccharin from its two hydrolysis products, *o*-sulfamoylbenzoic acid and *o*-sulfobenzoic acid. No significant radioactive peak, other than saccharin, was observed. Radioactivity from the urinary saccharin peak recrystallized as pure saccharin. Chromatography of a methanol extract of rat feces, collected for 48 hr after dosing, also revealed no metabolite of saccharin. During a 6-hr collection, less than 0.3% of a po dose was excreted in rat bile, while 11% passed into the bladder; the ^{14}C in the bile had the same R_f as saccharin. Induction of mixed function oxidase activity in rat liver with phenobarbital did not influence saccharin metabolism. Daily po doses of 20, 100, or 500 mg/kg sodium saccharin, given to monkeys for over 2 yr had no effect on the metabolism of a single po dose of labeled saccharin. Hence in a number of species, under varying conditions, and to the limit of the analytical methods employed, there was no evidence that saccharin was metabolized. An evaluation of the method used by workers who have reported the formation of saccharin metabolites indicates that artifactual peaks may be produced which are not true

metabolites. (Supported by FDA Contract 69-7, by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 5T01-ES00103-05.)

47. *Metabolism of Caffeine-^3H in the Rat.* K. L. KHANNA, H. SUBA RAO, and H. H. CORNISH, University of Michigan, Ann Arbor, Michigan, National Heart and Lung Institute, NIH, Bethesda, Maryland.

Caffeine, a xanthine alkaloid, is being consumed daily by the general public in large quantities in beverages such as coffee, tea, cocoa, and soft drinks, as well as in a number of prescription and nonprescription drugs. The methodology for the unequivocal identification of caffeine and fifteen possible metabolites (seven mono-, di-, and tri-*N*-demethylated products, uric acid, and seven mono-, di-, and tri-*N*-methyluric acids) based on TLC, UV, and mass spectrometry has been developed. Sprague-Dawley rats, 250–300 g, were utilized in these studies. Upon ip administration of caffeine-^{3}H to the rat, 64–67% of the radioactivity was recovered in the urine over a period of 24 hr. The chloroform–methanol (9:1) extract of the urine accounted for about 35% of the administered radioactivity. Water soluble metabolites constitute approximately 30% of the injected caffeine-^{3}H. With the aid of preparative TLC, 8.8% of unchanged caffeine and the following metabolites were isolated from chloroform:methanol extract: theophylline (1.2%), theobromine (5.1%), paraxanthine (8.8%) and trace amounts of 1,3,7-trimethyluric acid and 3-methyluric acid. An additional major unidentified metabolite accounts for approximately 11% of the administered caffeine-^{3}H. This unidentified metabolite appears to be a xanthine derivative. The metabolites present in the water soluble fraction are presently under investigation. (Supported in part by PHS Grant GM15269.)

48. *The Metabolism and Urinary Excretion of o-Phenylphenol in Dogs and Cats.* FREDERICK W. OEHME and THOMAS H. F. SMITH, Kansas State University, Manhattan, Kansas; Lehn and Fink Products Co., Montvale, New Jersey.

Biotransformation and excretion of *o*-phenylphenol (OPP) was studied in mature dogs and cats using ^{14}C-OPP. Early metabolite investigations indicated that unchanged OPP, conjugation products, and phenol were excreted in urine. To establish the specific source of the OPP-derived phenol, phenol-ring-UL-^{14}C-OPP and phenyl-ring-UL-^{14}C-OPP were synthesized and administered po with the chemically pure compound or with a commercial spray disinfectant containing OPP. Groups of dogs and cats were each given 1 or 3 g OPP/kg body wt, or 3 or 9 ml of the disinfectant concentrate (containing 0.125 % OPP)/kg body wt. Toxicity, plasma levels, urinary excretion, urine metabolites, and tissue residues were determined. Obvious species differences in toxic effect, OPP plasma disappearance, urinary excretion, and metabolites were noted. Dogs were capable of successfully biotransforming and excreting three times the OPP cats were. Plasma OPP concentrations were consistently higher in dogs and resulted in less severe toxic signs than in cats. This suggested greater receptor tolerance for OPP in dogs. Urinary metabolites were unchanged OPP, glucuronide and sulfate conjugates, and phenol derived from cleavage of the phenyl–phenol bond followed by ring hydroxylation. The phenol metabolites result from both OPP-ring moieties.

49. *The Excretion Rates of Chlormadinone Acetate, Mestranol, Norethynodrel, and Norethindrone in Female Rats.* G. K. HANASONO and L. J. FISCHER, University of Iowa, College of Medicine, Iowa City, Iowa. (B. A. Becker.)

Steroidal po contraceptives are known to undergo protracted urinary excretion in several species including man. As part of a study to examine the role of the enterohepatic circulation in the bodily retention of these drugs and their metabolites in various species, comparisons of the biliary, urinary and fecal excretions of chlormadinone-1-^{3}H-acetate (I), mestranol-9,11-^{3}H (II), norethynodrel-6,7-^{3}H (III), and norethindrone-9,11-^{3}H (IV) were made in female rats. Following iv doses of 0.161 μmol/kg, extensive biliary elimination of total radioactivity (cumulative percentage of dose) occurred over an 8-hr period: 56.0% I, 68.9% II, 71.0% III, and 80.1% IV, with most of the biliary excretion complete within the first 2–3 hr. The values for the biliary excretion of III and IV are much higher than those reported for rabbits or humans.

Characterization of biliary metabolites in the 0–2 hr-pooled bile samples as percent $CHCl_3$-extractable radioactivity prior to ketodase hydrolysis was 19.8% for I and less than 7% for II–IV. Ketodase hydrolysis produced an additional 26.3% I, 47.9% II, 21.3% III and 60.4% IV. The percentages of biliary metabolites of III and IV present as glucuronide conjugates were less than those reported in other species. Both the cumulative percentage of the dose excreted in urine over a 7-day period following ip doses to intact animals, and the percent $CHCl_3$-extractable radioactivity in 24-hr urine samples after ketodase hydrolysis were lower for II–IV than values reported for rabbits and humans in other studies. In contrast to the extent of urinary excretion, the cumulative percentage of fecal excretion over 7 days amounted to 90.0% I, 95.1% II, 77.9% III and 81.8% IV, showing that this was by far the major route of excretion in the female rat. (Supported by NIH Grant GM-12,675.)

50. *The Effect of Altered Hepatic Function on the Plasma Disappearance and Biliary Excretion of Diethylstilbestrol.* CURTIS D. KLAASSEN, University of Kansas Medical Center, Kansas City, Kansas.

The present investigation was prompted by the observation that the toxicity of diethylstilbestrol (DES) was 140 times greater in bile duct ligated (BDL) rats than in control rats when dimethylsulfoxide was the vehicle (24-hr LD50 = 104 vs. 0.75 mg/kg). The DES was also more toxic (74 times) in the BDL rats when ethyl alcohol was the solvent (34 vs. 0.48 mg/kg) and was five times more toxic in the BDL rats when administered in propylene glycol (525 vs. 100 mg/kg). Since it appeared that altered hepatic function markedly alters the acute toxicity of DES, the plasma disappearance and biliary excretion of DES (0.118 mg/kg, iv) were measured in control rats and rats with altered hepatic function (produced by surgical removal of 2/3 of the liver, ip administration of 1 ml/kg CCl_4 or bile duct ligation). All three procedures of altering hepatic function markedly decreased the plasma disappearance of diethylstilbestrol. The total (free plus conjugated) concentration of diethylstilbestrol normally found in the plasma was 0.45 μg/ml 2 hr after administration. It was 2.04 μg/ml for the 2/3 hepatectomized rats, 3.98 μg/ml in the CCl_4-treated rats, and 34 μg/ml in the BDL rats. The plasma nonconjugated DES concentration in the plasma at 2 hr after administration was also greater in rats with impaired hepatic function; 0.20 μg/ml in control rats, 0.62 μg/ml in the 2/3 hepatectomized rats, 1.09 μg/ml in CCl_4-treated rats, and 0.53 μg/ml in BDL rats. The biliary excretory rate of DES was 2.1 μg/min/kg in the control rats during the first 15 min after administration and 1.2 μg/min/kg in the rats that had a 2/3 hepatectomy or CCl_4. Therefore, impaired hepatic function increased the plasma level and toxicity of DES as it decreased the conjugation and biliary excretion of DES. (Supported by USPHS Grant AM 14513.)

51. *The Metabolism of Quazodine in the Rat, Dog, and Man.* M. J. BARTEK, J. A. LABUDDE, and J. H. WEIKEL, JR., Mead Johnson Research Center, Evansville, Indiana.

The absorption, metabolism and excretion of ^{14}C-labeled quazodine, a new bronchodilator, were studied in the rat, dog, and man, while distribution of the drug was measured only in rats. After po administration, quazodine was rapidly absorbed in both man and dogs, a peak plasma level observed at 0.5 hr in man and at 1 hr in dogs. The drug did not localize in cerebrospinal fluid of dogs. Radioactivity was found in all tissues of rats 1 hr after po dosage, and no evidence for extreme drug localization or prolonged retention was found in any tissue including brain. In rats, 71.9% of the dose was recovered in urine and 14.2% in feces during the first 3 days after dosing. The 72-hr recoveries in dog urine and feces were 61.4 and 25.8%, whereas in humans these values were 84.1 and 1.1%, respectively. After iv doses, the rapid quazodine disappearance from both human and dog plasma followed a biexponential curve. Mean half-times describing the α- and β-slopes were 42 min and 2.6 hr in man, and 47 min and 3.3 hr in dogs. The major pathway for metabolism of quazodine in man, and to a lesser extent in the dog and rat, was demethylation at the 7-position of the quinazoline ring-system followed by conjugation with glucuronic acid or sulfate. The glucuronide conjugate accounted for 78.0% of the radioactivity in human urine, 45.1% in dog and 27.4% in rat. The amount of radioactivity present as the sulfate conjugate was 3.1, 15.3, and 10.5% in human, dog, and rat urine, respectively.

52. *Effect of Salicylate on the Biological Half-Life of Bishydroxycoumarin in the Rat.* B. H. THOMAS, B. B. COLDWELL, W. ZEITZ, and G. SOLOMONRAJ, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada. (J. F. Borzellaca.)

The interaction of other drugs with anticoagulants such as bishydroxycoumarin (BHC) is an area of concern due to the necessity of controlling precisely the degree of anticoagulation produced. Results from our laboratory previously reported showed that acetylsalicylic acid and sodium salicylate antagonize the hypoprothrombinemic action of bishydroxycoumarin in rats. Using ^{14}C-BHC (15 mg/kg, ip) we have found that both acetylsalicylic acid and sodium salicylate (100 and 88.9 mg/kg, respectively) when given po substantially reduce the blood level of radioactivity. The half-life of elimination ($T_{1/2}$) was reduced from 7.7 hr in the controls to 2.9 hr in the sodium salicylate-treated rats. In contrast the liver concentration of radioactivity was considerably higher in the salicylate-treated rats. In vitro plasma–protein binding studies showed that salicylate displaces BHC which is normally over 99% bound. It is postulated that the elimination of BHC is more rapid in the presence of salicylate due to displacement from plasma–protein binding sites.

53. *Toxic Effects of Methylmercury in the Rat.* I. C. MUNRO, S. M. CHARBONNEAU, R. F. WILLES, A. MOODIE, E. NERA, V. MONTPETIT, T. GOODMAN, D. STOLTZ, and H. C. GRICE, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Studies were undertaken to examine the toxicological effects of acute and subacute administration of methylmercury in the rat. Groups of 8–10 male Wistar rats were given methylmercuric chloride (10, 5, 1, or 0 mg Hg^+/kg/day) by po dosing or 0.5, 0.05, or 0 mg Hg^+/kg/day in the diet. Clinical signs of methylmercury intoxication occurred following 7, 14, 65, and 120–140 days of treatment in the 10, 5, 1, and 0.5 mg/kg groups, respectively. After 280 days of dosing no clinical signs of toxicity were noted in the 0.05 mg/kg group. Reduced body weight gain and food consumption was noted in all animals showing signs of toxicity. Renal tubular changes, similar to inorganic mercury intoxication, and accompanied by renal hypertrophy and elevated BUN levels, were observed in the 10, 5, 1, and 0.5 mg/kg groups. Pathological changes occurred in the nervous system of all treated rats and consisted of Wallerian degeneration of sensory nerves, swelling with loss of myelin in the posterior white column of the spinal cord, secondary loss of nerve cells in the dorsal root ganglia, and atrophy of the cerebellar vermis due to loss of granular cells and some Purkinje cells. Bradycardia and electrocardiographic changes consisting of *P* wave inversion and increased *R* wave amplitude were noted in the 10 and 5 mg/kg groups. Left ventricular hypertrophy with bulging of the septum into the right ventricle was observed in the myocardium. Animals given 10, 5, or 1 mg/kg showed a dose-related reduction in the size of the SA and AV nodes with accompanying degnerative changes and replacement fibrosis.

54. *Toxic Effects of Methylmercury in the Cat.* S. M. CHARBONNEAU, I. C. MUNRO, A. MOODIE, R. F. WILLES, E. NERA, V. MONTPETIT, D. STOLTZ, H. L. TRENHOLM, T. GOODMAN, and H. C. GRICE, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Methylmercury was administered po to two groups of 4–6 cats either as methyl mercuric chloride (in corn oil as a vehicle) or as methylmercury contaminated fish. In both treated groups the daily dose was 0.25 mg Hg^+/kg. A control group received only corn oil. Weight change, daily food consumption, hematology, blood electrolytes, ECG, urinalysis, neurological examinations consisting of tests of motor, sensory, auditory, and visual function, and blood mercury levels were carried out at regular intervals throughout the study. Clinical signs of methylmercury intoxication consisting of ataxia, intention tremor, impaired righting reflex, and convulsions developed between 76–100 days in both treated groups, at which time the total dose received was between 19–25 mg Hg^+/kg. Tissue mercury levels were correlated with clinical signs and pathological findings. Lesions were found in the cerebellar vermis and

adjacent parts of the cerebellar hemispheres, deep cerebellar nuclei, cerebral cortex, and brain stem. Essentially the changes consisted of loss of nerve cells with replacement by reactive and fibrillary gliosis.

55. *Acute Toxicity of Methylmercury, Its Tissue Distribution and Elimination in Guinea Pigs.* R. H. DOWNIE, F. IVERSON, C. J. PAUL, and H. L. TRENHOLM, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Methylmercury is recognized as a hazardous environmental pollutant. Documented cases of poisoning in Japan and elsewhere have prompted extensive research into the toxicological properties of this compound. In our laboratory preliminary experiments suggested that the guinea pig might be sensitive to methylmercury, and a study was designed to determine its acute toxicity, tissue distribution and tissue elimination rates in female guinea pigs. Acute toxicity studies showed that the guinea pig was quite susceptible to methylmercury intoxication. LD50 values were 5.5 (4.4–6.3) mg Hg/kg ip and 16.5 (5.5–23.5) mg Hg/kg po. Tissue distribution and pharmacodynamic studies after a single po dose of radiolabeled methylmercuric chloride at 1 and 10 mg Hg/kg revealed that most of the 17 tissues sampled showed a rapid absorption of mercury, while cerebrum, cerebellum, and muscle exhibited a delayed uptake of the alkyl mercurial. In CNS tissue the concentrations of mercury decreased in the order cerebrum > cerebellum > spinal cord. During the 49-day sampling period, kidney and liver consistently contained the highest levels of mercury and plasma the lowest. In the majority of tissues, concentrations of mercury were directly proportional to the dose administered. Most of the tissues displayed a biphasic decay profile with a half-life of 2–3 days for the initial rapid phase of decline. This initial phase was followed by a slower tissue excretion rate where the mean half lives for mercury were 15 ± 0.9 days and 15 ± 0.8 days for the low and high dose, respectively.

56. *Subchronic Administration of Methylmercury to Guinea Pigs: The Tissue Distribution and Elimination of Mercury.* F. IVERSON, H. L. TRENHOLM, R. H. DOWNIE, and C. J. PAUL, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Previous studies in this laboratory on the tissue distribution of an acute po dose of methylmercury in the guinea pig showed that brain, kidney, and liver tissue contained high concentrations of mercury. These studies have now been extended to show the distribution pattern after daily dosing with subtoxic levels of methylmercury. Female guinea pigs were dosed po every day for 10 wk with 0.4, 4, 40, and 400 μg/kg radiolabeled methyl mercury. Samples of kidney, liver, whole blood, plasma, muscle, and six brain sections (caudate nucleus, frontal and occipital lobes, calcarine cortex, hypothalamus, and cerebellum) were taken at 2-wk intervals, and the mercury concentration determined by liquid scintillation counting. After 8 wk mercury levels began to approach steady state values. In CNS tissue the caudate consistently had the highest and cerebellum the lowest mercury concentration. Generally, the mercury levels at 8 wk increased in the order plasma, whole blood, CNS tissue, muscle, liver and kidney with a 200–600-fold difference in the plasma:kidney concentrations, depending on the dose level. While the mercury dose administered varied 1000-fold, all tissues except plasma showed at least a 2000-fold increase in mercury concentration from the low to high dose levels. This indicated that a greater percentage of the mercury dose was eliminated at the lower dose levels. Tissue elimination rates and the excretion rates of mercury are now being studied.

57. *Consummatory Indices of Chronic Methylmercury Ingestion.* DANIEL R. SNYDER and J. JAY BRAUN, Yale University, New Haven, Connecticut. (E. J. Gralla.)

Rats have evolved a particularly sensitive ability to learn a long-lasting avoidance of a distinctive taste or odor that has been associated with visceral discomfort. This phenomenon might be utilized to study the early effects and course of heavy metal poisoning. Since the clinical signs of alkylmercury poisoning follow the degeneration of neural tissue, specific behavioral changes sensitive to alterations in brain function during the onset of toxicosis might serve as

an indicator of impending disease before irreversible damage has occurred. The present experiment examined the dose relationships between behavioral and physiological indices of chronic methylmercury ingestion in rats who were presented a daily choice of water or a highly preferred sucrose solution adulterated with scaled concentrations of methylmercuric chloride. Records of sucrose consumption, water consumption, and body weight were kept for 41 days, and the rats were observed for signs of metabolic or neurologic disturbances associated with mercury intoxication. When the rats were allowed to regulate behaviorally methylmercury intake, they exhibited markedly different manifestations of toxicosis depending on dose level: a concentration of 25 g CH_3Hg/l in a 5% sucrose solution was initially drunk in large volume, but was uniformly rejected before any outward signs of neurological or physiological damage appeared, i.e., a behavioral sign of poisoning prior to any clinical signs. A lower dose of 5 g CH_3Hg/l, on the other hand, was vigorously consumed well beyond the onset of toxic deterioration which included progressive weight loss, loss of motor coordination, ataxia, postural abnormalities, and renal failure. Neurohistological examinations were performed to correlate the sites and extent of CNS damage with amounts of mercury consumed. The dissociation between high and low dose rejection is curious because other poisons such as arsenious oxide and dicoumarin, both used for rodent extermination, are known to produce "bait-shyness" in significant portions of a rat population even when chronically ingested at low doses. The particular properties of methylmercury which prevent animals from developing such a negative association at low doses remain to be determined.

58. *A Study of the Effects of NTA on the Toxicity of Mercury Compounds.* P. L. WRIGHT, M. L. KEPLINGER, I. D. HILL, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois; Monsanto Company, St. Louis, Missouri.

Experiments were conducted to determine effects of sodium nitrilotriacetate (NTA) upon the teratogenicity of methylmercuric hydroxide. Pregnant rats (170) were dosed po with 1.5, 3.0, 4.5, 6.0, or 8.0 mg Hg/kg body wt from days 6 to 19 of gestation. The mercury was administered in an aqueous solution containing 4.5 mol NTA/mol Hg or an equivalent amount of sodium from sodium sulfate. Maternal mortality was lowest (27 vs. 43%) among those given the combination with NTA. Mercury doses of 1.5 or 3.0 mg/kg did not reduce fetal survival. The numbers of viable fetuses per 100 implantation sites for females given 4.5 or 6 mg/kg with sodium sulfate were 88 and 4. Comparable values for the NTA combinations were 62 and 26. All of the fetuses from dams given 4.5 or 6 mg Hg/kg with sodium sulfate were grossly abnormal, while only 30 and 21% were abnormal from dams given the same mercury doses with NTA. The most prevalent abnormality was fetal edema. The incidence of skeletal abnormalities, including angulated ribs, was not influenced by the material with which mercury was combined (518 fetuses examined). Internal abnormalities (351 fetuses examined) included hydrocephalus, cryptorchidism, enlarged or shrunken atria, renal ectopia, and large or small urinary bladder. There were no differences in incidences between the NTA and sodium sulfate treated groups.

59. *Effect of NTA Chelation on the Subacute Oral Toxicity in Rats of Cadmium and Methylmercury.* KEITH H. JACOBSON, Tulane University School of Medicine, New Orleans, Louisiana.

Male and female rats were intubated for 30 consecutive days with aqueous solutions of cadmium ($CdCl_2$) or methylmercury (CH_3HgOH) and of nitrilotriacetic acid (NTA) chelates of Cd or CH_3Hg. Chelate solutions had 5 mol NTA/mol Cd or CH_3Hg. Daily dose levels were 0, 0.5, 2.5, and 12.5 mg Cd/kg, or 0, 0.3, 1.5, and 7.5 mg CH_3Hg/kg. CH_3Hg at the highest dose (7.5 mg/kg) was very toxic, and all rats given CH_3Hg or CH_3Hg-NTA at this dose died or were killed because of imminent death by day 14. Based on clinical signs, organ weight ratios and pathologic changes, no significant differences in toxicity were found between the metal and its chelate at the same dose of metal. Based on body weight changes, each metal was more toxic than its chelate. Livers, kidneys, urine, feces, blood, and (for Cd) testes, and (for Hg) brains were analyzed for metal content. Deposition of Hg was not significantly different for CH_3Hg and CH_3Hg-NTA. A few differences in deposition of Cd in animals dosed with Cd or Cd-NTA were found, but no pattern was apparent.

60. *A Comparison of the Metabolism of Nitrilotriacetate in Dog and Man.* JOHN A. BUDNY and JOHN D. ARNOLD, Procter and Gamble Company, Cincinnati, Ohio; University of Missouri at Kansas City, Missouri. (W. R. Michael.)

Nitrilotriacetate (NTA) is a potential replacement for polyphosphates in synthetic detergents. There is the possibility, even though small, of animal and human ingestion. Therefore, the absorption, biotransformation and excretion of NTA-^{14}C was measured in both dog and man. In addition, tissue distribution was determined in dogs 72 hr after administration. After po administration of 20 mg/kg to fasted purebred female beagle dogs, NTA-^{14}C was rapidly (blood peak ~ 1 hr) and extensively (~90%) absorbed. Excretion was also rapid since 75% of the administered dose appeared in the urine within 4 hr. Tissue distribution of NTA-^{14}C 72 hr after dosing was greatest in bone (2–3 μg/g) and kidney (0.4 μg/g). When 10 mg NTA-^{14}C was given in a gelatin capsule to eight healthy human male volunteers, 5–20% of the dose was absorbed. Within 24 hr, approximately 85% of the absorbed dose was excreted via the urine. Using reverse isotope dilution and cellulose thin layer chromatography, no biotransformation could be detected in dog or man. It is concluded from these comparative studies on NTA that the main metabolic difference between dog and man is absorption.

61. *Calcium and Zinc Metabolism in the Presence of Na_3NTA.* W. R. MICHAEL and J. M. WAKIM, Procter and Gamble Company, Cincinnati, Ohio.

In a preliminary experiment, trisodium nitrilotriacetate (Na_3NTA), a metal complexing agent at physiological pH, was fed to rats at dietary levels up to 2%; the most significant effects on mineral metabolism were apparent increases in Zn and Ca absorption. Increased Zn absorption was confirmed by physiological and chemical means by feeding to rats for 91 days diets containing 10, 18, and 25 ppm Zn and 0, 0.03, 0.15, and 0.5% Na_3NTA. Na_3NTA enhanced growth of animals receiving the diet containing 10 ppm Zn; it reduced RBC count, hematocrit volume, and hemoglobin values toward the normal range in rats receiving diets containing 10, 18, and 25 ppm Zn, and caused an increase in Zn concentration in the skeleton of all groups. These results show that Na_3NTA increased Zn absorption and that the metal ion is available for use in normal biological processes. In this 91-day study, neither physiological nor chemical evidence indicative of enhanced Ca absorption could be found. Na_3NTA is known to be deposited in the skeleton, yet no changes in tibia weight, total ash or percent ash were found. There was also no change in serum alkaline phosphatase in animals fed Na_3NTA (2% of diet) for 30 days, a finding which suggests no gross change in metabolic activity of the skeleton. Although Na_3NTA forms strong complexes with Ca, it does not deplete body stores of the metal. It is concluded that Na_3NTA enhances both Zn and Ca absorption, and the absorbed metal ions are available for use by the body in normal biological processes. These effects should be of no toxicologic or physiologic significance to man at the maximum anticipated daily consumption of NTA in drinking water at a level of much less than 1 mg/day.

62. *A Study of the Mercury Content of Fish From Various Sources.* MARK M. LUCKENS, University of Kentucky, Lexington, Kentucky.

This paper presents the results of an analytical study of the mercury content in fish from various sources during the period 1969–71. The fish were obtained from various sources including: (a) farm ponds receiving drainage from fields on which crops had been grown from seeds treated with mercurial seed dressings; (b) the Great Lakes; (c) various ocean fishing grounds; and (d) rivers receiving drainage from farming areas on which crops had been raised using seeds treated with mercurials or had received wastes containing inorganic mercury. The receiving waters and the bottom sediments of the receiving waters were analyzed whenever possible. The total mercury content of the fish tissues examined ranged from zero to 2 ppm. It was noted that the mercury content usually related to the feeding habits of the fish. Additionally, it was found that the mercury content was not uniform throughout the entire filet but was related to the type of musculature (above or below the mid-line) from which the sample for analysis had been taken. (Supported by Grant No. F.D. 00245, E.P.A.)

63. *Genetic Nonhomogeneity of Microsomal Metabolism Within a Single Strain of Young Rats.* I. GUT and B. BALINOVA, Institute of Industrial Hygiene and Occupational Diseases, Prague, Czechoslavakia. (B. A. Becker.)

Male and female Wistar rats were tested for the duration of hexobarbital sleeping time (HST). Animals with short and long HST were bred, and HST in the F_1 generation followed the pattern of the parents. F_1 generation animals with shortest and longest HST were bred to obtain the F_2 generation. An F_3 generation was likewise obtained from F_2. Throughout the experiment offspring followed the HST pattern of the parents. HST (min) for short vs. long HST group by generation was: F_0, females 42 vs. 93, males 33 vs. 46; F_1, females 54 vs. 86, males 18 vs. 30; F_2, females 94 vs. 144, males 18 vs. 35 (Significance—$p < 0.01$). In vitro hexobarbital (HB) hepatic (9,000 *g* fraction) biotransformation did not show any significant differences in females. In males short vs. long HST group (μg HB/g liver) was: F_2, 1564 vs. 804; F_3, 1444 vs. 731. In vitro aminopyrine biotransformation also showed little difference in females, but substantial differences in males (short vs. long HST, males): F_2, 33 vs. 13; F_3, 74 vs. 40 μg/g liver. In vitro benzene biotransformation showed less differences in females (animals with short HST vs. long HST, μg/g liver): F_1, females 37 vs. 21, males 33 vs. 23; F_2, females 40 vs. 39, males 57 vs. 43; F_3, females, 38 vs. 39, males 36 vs. 24. These experiments indicate a genetic nonhomogeneity of hepatic microsomal metabolism within a single strain of rats. By use of HST as a criterion for selective inbreeding, the apparently "single" strain of rats can be separated into two substrains with more or less effective metabolic capability.

64. *Microsomal Activity in Endrin Susceptible and Resistant Pine Mice.* R. W. HARTGROVE, V. J. PETRELLA, and R. E. WEBB, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. (J. F. Borzellaca.)

Studies were conducted to characterize hepatic microsomal oxidase activity in endrin susceptible and resistant pine mice. Endrin resistant mice showed a greater capacity to metabolize ^{3}H-benzpyrene (^{3}HBP) as evidenced by kinetic properties developed from initial velocity studies for ^{3}HBP disappearance in the microsomal fraction. The ^{3}HBP-hydroxylase activity of first generation resistant offspring indicated that the higher hydroxylase activity of resistant mice is heritable. Further, the kinetic properties of BP-hydroxylase were determined for 2-, 4-, 6-, and 8-wk-old first generation offspring of the two strains. These studies showed that the rate of development of the microsomal BP-hydroxylase activity was faster in the resistant strain and that both strains showed adult level kinetic properties at 8 wk of age. A comparison between strains of ethylmorphine (EM) metabolism has indicated that endrin resistance is also correlated with EM *N*-demethylase activity in the two strains. No correlation was found with aniline hydroxylation. This indicates that endrin may be a type I substrate similar to EM. Preliminary oxygen uptake studies indicate that the resistant strain utilizes more oxygen than the susceptible, which further implicates differences in the mixed function oxidases as a factor in the strain differences noted with regard to the ability to handle endrin in the environment.

65. *Effect of the Butyl Alcohols on Liver Microsomal Enzymes.* DAVID H. BECHTEL and HERBERT H. CORNISH, School of Public Health, The University of Michigan, Ann Arbor, Michigan.

The butyl alcohols as a group are of considerable importance as industrial solvents and as intermediates in the synthesis of other compounds. There is a scarcity of literature defining the systemic effects of these alcohols. This paper presents our findings on the effects of the butanols on microsomal acetanalide hydroxylase and aminopyrine demethylase. Male Sprague-Dawley rats were dosed either po or ip injection with aqueous solutions of the alcohols. The rats were sacrificed about 18 hr after the last dose, and measurement of the enzyme activities was carried out on 9000 *g* liver supernatants. Normal, secondary, iso-, and tertiary butyl alcohols all increased microsomal enzyme activity approximately three-fold. PO administration appears to be more effective than the ip route in eliciting this response. At relatively high doses (1 g/kg) maximum induction of about three-fold was seen within 24 hr after a single po dose. Liver weights in treated animals were not significantly elevated. The ability of the butanols to increase

microsomal enzyme activity is of considerable importance in view of the potential interactions with other chemicals normally metabolized by this enzyme system. (Supported in part by PHS Grants GM15269 and 1-R01-ES00138.)

66. *Microsomal Enzyme Systems in Rabbit Lung and Liver During the First Month of Life.* J. R. FOUTS and T. R. DEVEREUX, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, North Carolina.

Rabbit lung microsomes metabolize a variety of chemicals and drugs at rates which are easily measured and often similar to rates using liver microsomes. Age-related changes in liver microsomal enzymes have been studied in detail, but similar studies in lung have not been made to our knowledge. We studied the development of cytochromes, cytochrome reductases, and certain drug metabolisms in liver and lung microsomes at four ages: 3–5 days, 2 wk, 1 mo, and 4–6 mo (adult). No general pattern emerged, although all components were lowest at 3–5 days in both tissues. Microsomal cytochromes P450 and b_5 reach adult levels in liver at or before 1 mo of age, while lung cytochromes continue to increase after 1 mo of age. In both tissues, microsomal cytochrome b_5 content is the same as cytochrome P450 content until 1 mo of age; then P450 content becomes greater. Liver cytochromes are approximately five times lung cytochromes (per mg microsomal protein) at all ages. Liver microsomal NADPH-cytochrome *c* and P450 reductases reach a maximum at around 1 mo of age. Lung microsomal NADPH cytochrome *c* reductase is similar in activity to the liver enzyme but may mature slower. NADPH cytochrome *c* in both tissues is stimulated about twofold by increased ionic strength (0.17 M KCl). Benzpyrene hydroxylase reaches a maximum in liver microsomes at about 1 mo of age, but appears to mature more evenly and much slower in lung microsomes. Activity in liver (per mg microsomal protein) is five to seven times greater than lung at all times. Benzphetamine metabolism per mg microsomal protein is the same in liver and lung at all ages, except in the adult, where lung activity is greater than liver. Results suggest the possibility that age-related changes in microsomal enzymes occur in both lung and liver, but individual components do not behave the same in both tissues.

67. *Effects of 1,3-Bis(2-chloroethyl)-1-nitrosourea on Hepatic Drug Metabolizing Systems in the Rat.* IRENE LU and R. E. LARSON, Oregon State University, Corvallis, Oregon.

Studies on the hepatotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) have revealed that a prolonged effect upon drug metabolism is exerted by single doses of this compound. The side-chain oxidation of pentobarbital by hepatic microsomes was significantly depressed by 7 days, and became progressively more impaired through 21 days following BCNU administration. The present investigation was an attempt to elucidate the mechanism(s) underlying this toxic effect. All experiments employed the 9000 *g* fraction or isolated microsomes obtained from livers of rats on day 13 after administration of BCNU (30 mg/kg ip). In vitro studies on the side-chain oxidation of pentobarbital and hexobarbital, *N*-demethylation of ethylmorphine, nitroreduction of *p*-nitrobenzoic acid, and *p*-hydroxylation of aniline, indicated BCNU inhibited all systems considered. Total microsomal protein concentration was only slightly depressed but cytochrome P450 levels were markedly reduced. When BCNU-treated animals were challenged with optimal enzyme inducing doses of phenobarbital the enzyme systems were variably affected. Total microsomal protein was increased in these animals; however, phenobarbital pretreatment had little effect with regard to the induction of cytochrome P450. Thus, it appeared that the major effect of BCNU on the hepatic drug metabolizing systems was upon cytochrome P450.

68. *Effect of Four Antiviral Agents on the Hepatic Microsomal Mixed Functional Oxidase Enzymes.* A. E. MUNSON, W. REGELSON, and J. A. MUNSON, Medical College of Virginia, Richmond, Virginia. (J. Borzelleca.)

Pyran copolymer (PCP), polyacetyl carboxylic acid (COAM), poly rI:rC, and endotoxin (*Salmonella typhosa* Difco 0901) all inhibit the hepatic microsomal mixed functional oxidase enzymes responsible for metabolizing Type I and Type II substrates. A 9000 *g* liver supernatant

fraction was prepared from male NYLAR-A mice 24 hr after an iv injection of the antiviral agent. PCP and COAM were administered in a dose of 25 mg/kg, while poly rI:rC was given in a dose of 2 mg/kg. Endotoxin was administered in a dose of 10 mg/kg. Aminopyrine and aniline were used as representatives of type I and type II substrates. The metabolism was monitored by measuring the formation of formaldehyde in the case of aminopyrine and *p*-aminophenol for aniline. In the case of aminopyrine metabolism, PCP causes a 10-fold increase in the K_m, while COAM, poly rI:rC and endotoxin increased the K_m 6.4-, 7.2-, and 1.6-fold, respectively. The V_{max} was the same for PCP and poly rI:rC but was decreased 14.3 and 27% for COAM and endotoxin. When a constant amount of aniline was used (5 μmoles) the metabolism was inhibited 55% for PCP, 51% for poly rI:rC, 48% for endotoxin, and 27% for COAM. Drugs which stimulate these enzymes such as phenobarbital and chlorcyclizine reverse the inhibitory action of the antiviral drugs. Reversal of this inhibitory action does not alter their antiviral activity. Since PCP and poly rI:rC are in clinical trial for their antitumor activity, attention should be paid to their ability to alter the action of other drugs.

69. *Investigation of Multiple Mechanisms for Potentiation of Malaoxon (MX) by Triorthotolyl Phosphate.* S. D. COHEN, J. E. CALLAGHAN, and S. D. MURPHY, Harvard School of Public Health, Boston, Massachusetts.

Potentiation of malathion (MAL) toxicity by low doses (<20 mg/kg) of triorthotolyl phosphate (TOTP) has been shown to be closely associated with TOTP-inhibition of hydrolysis of MAL by liver carboxylesterase (CE). However, MAL potentiation was observed to continue to increase with increasing doses of TOTP beyond those which produced maximum inhibition of liver CE. This suggested that an additional mechanism might be involved in TOTP potentiation of MAL toxicity. To determine if the continued increase in degree of potentiation of MAL resulted from TOTP inhibition of the detoxification of MX (the active cholinesterase-inhibiting metabolite of MAL), dose-response relationships between TOTP potentiation of MX toxicity and inhibition of tissue CE, cholinesterase (CHE) and liver inactivation of MX by binding were investigated. Groups of five male mice were injected ip with TOTP and challenged 18 hr later with selected doses of MX. They were sacrificed 5 min after challenge, and inhibition of brain CHE measured. Pretreatment with 10, 17, 50, or 125 mg/kg of TOTP resulted in 1.8-, 3.6-, 12.3-, and 31.4-fold potentiation of MX toxicity, respectively. Other groups of identically pretreated mice were sacrificed without challenge, and liver and plasma CE, liver, lung, plasma, and brain CHE, and liver inactivation of MX by binding were measured. Liver and plasma CE and plasma and lung CHE were maximally inhibited by 50 mg/kg of TOTP. In contrast, liver CHE inhibition increased from 90 to 98%, and inhibition of liver MX binding increased from 89 to 99% (both increases significant; $p < 0.05$) when the pretreatment dose of TOTP was increased from 50 to 125 mg/kg. TOTP pretreatment did not inhibit brain CHE nor alter the sensitivity of mouse brain CHE to inhibition by MX in vitro. These data suggest that the increase in degree of MX and/or MAL potentiation, observed with doses of TOTP greater than those that cause maximal inhibition of CE, may result from an additional increase in inhibition of MX binding and that liver CHE may contribute to binding of MX. (Supported by RG ES-00084 and ES-00002 NIEHS, U.S. Dept. H.E.W.)

70. *The Identification of Five Unreported Lindane Metabolites Recovered from Rat Urine.* R. W. CHADWICK and J. J. FREAL, Environmental Protection Agency, Perrine, Florida.

Though lindane has been widely applied as an insecticide since the Second World War, the metabolic fate of this compound in mammals is still unresolved. Recent data from this lab and others indicate that the metabolism of lindane in mammals is considerably more complex than was previously realized. Through an improved analytical method based on gas liquid chromatography and Coulson electrolytic conductivity detection, it has been possible to characterize five previously unreported metabolites from the urine of rats fed diets containing 400 ppm lindane. The lindane metabolites were identified as 3,4-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,5-tetrachlorophenol, 2,3,4,6-tetrachlorophenol and 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol. While the 3,4-dichlorophenol is a minor metabolite, the others are excreted

in greater quantities than either of the previously identified metabolites, 2,3,5- or 2,4,5-trichlorophenol. The excretion of the tetrachlorophenols and 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol require a revision in the currently accepted theory regarding the metabolism of lindane by mammals.

71. *The Fate of Thiodipropionic Acid, Its Didodecyl Ester and Its Polyester with Cyclohexanedimethanol and Stearyl Alcohol in Rats.* R. C. REYNOLDS, D. W. FASSETT, and B. D. ASTILL, Eastman Kodak Company, Rochester, New York.

Thiodipropionic acid and its esters are preservatives and stabilizers in food and food packaging. The po fate in rats, hitherto unknown, of thiodipropionic acid (TDPA), didodecylthiodipropionate (DDTDPA) and of a polyester of thiodipropionic acid with cyclohexane-1,2-dimethanol terminated with stearyl alcohol (poly TDPS-2000) was elucidated in evaluating poly TDPS-2000 as a polymer stabilizer. Single doses of TDPA-1-^{14}C were rapidly eliminated, 94% of a 3 mg/kg dose being recovered in 4 days in urine (90%), feces (0.5%), and as $^{14}CO_2$ (3%). About 0.3% remained in the carcass at sacrifice, while radioactivity in tissues and organs was <2× background. A 627 mg/kg dose of TDPA-1-^{14}C was handled identically. Urinary radioactivity at the higher dose was due almost entirely to unchanged TDPA, while the lower dose apparently gave an acid labile conjugate of TDPA. Single po doses of DDTDPA-1-^{14}C (100 and 200 mg/kg) were rapidly eliminated, mostly in the urine (85–88%), with less in feces (1.8–3.5%), and as $^{14}CO_2$ (3–4%, all in 4 days). A 1-day dietary feeding of 166 mg/kg gave similar results. Some tissue and organ levels of radioactivity at sacrifice were 3–4× background, higher than for TDPA-1-^{14}C. Fat levels after dietary DDTDPA-1-^{14}C were 5 ppm 34 days after dosing. Urinary radioactivity was found to be mostly unchanged TDPA or an acid-labile conjugate. A 5 hr feeding in the diet of 304 mg/kg of ^{14}C-labeled poly TDPS-2000, prepared from TDPA-1-^{14}C, was almost entirely eliminated in 4 days in urine (95%), feces (about 0.7%), and as $^{14}CO_2$ (about 6%). At sacrifice 4 days later tissue and organ radioactivity was 4–6× background, falling to 1–2× background 30 days later. In the same period fat levels fell from about 0.2 to 0.02 ppm. Almost 2/3 of the urine radioactivity was due to TDPA-1-^{14}C or a conjugate. TDPA behaves after po intake as a typical dicarboxylic acid in being rapidly absorbed and eliminated in the urine largely unchanged. Simple and polyesters appear to be readily hydrolyzed in the organism to the parent acid, which is then eliminated similarly to that given po.

72. *Synthesis and Biologic Activity of Para-Hydroxylaminopropiophenone.* F. G. DEFEO, T. J. FITZGERALD, and J. DOULL, University of Kansas, Kansas City, Kansas.

Para-hydroxylaminopropiophenone (PHAPP) has been shown to be responsible for the methemoglobinemia (MHb) producing effect of *para*-aminopropiophenone (PAPP). PAPP is also an effective radioprotective agent, and it has been suggested that this effect is related to its MHb-producing activity. It was of interest, therefore, to synthesize PHAPP and to compare its radioprotective activity with that of PAPP. PHAPP was prepared from PAPP in a two-step synthesis employing a fluoroboric acid adaptation of the diazonium salt replacement reaction followed by a partial reduction to the hydroxylamine. The overall yield for both reactions was 25%, and the PHAPP was identified by elemental and IR analysis. Adult male Sutter mice (23–27 g) were used to compare the acute ip toxicity of PHAPP and PAPP. The LD50 (10 days) for PHAPP was 134 ± 10 mg/kg and that of PAPP was 277 ± 26 mg/kg. Methemoglobin determinations were also carried out. When added to shed mouse blood, PAPP did not produce MHb, whereas PHAPP converted the hemoglobin to MHb at an equimolar ratio with respect to iron. Both compounds produced MHb when given ip to mice. To compare the radioprotective activity of PHAPP and PAPP, groups of 16 male Sutter mice were given PHAPP (30 or 60 mg/kg ip) or PAPP (30 or 60 mg/kg ip) and 15 min later were exposed to 750 r of whole-body X-irradiation (250 kvP, 15 ma, target-skin distance 50 cm, dose rate 45 r/min). Control groups of mice were given the vehicle (propylene glycol) and irradiated with the treated mice. Mortality was observed daily for 30 days. None of the irradiated control mice (48) survived, whereas over half of the animals treated with PHAPP (17/32) or PAPP (19/32) were alive at 30 days after the

X-ray exposure. There was, however, no significant difference in the number of survivors in the two dose groups of either compound; it does not seem likely that PHAPP is directly responsible for the radioprotective effect of PAPP in mice. (Supported by PHS GM-15956.)

73. *Observations Consequent upon a Long-term Study of Diphenylamine in Mice.* W. FORD, R. ABRAHAM, W. ROCKWOOD, and L. GOLBERG, Albany Medical College, Albany, New York.

A total of 1200 Charles River CD-1 albino mice of both sexes were fed diets containing 0, 0.005, 0.01, and 0.025% diphenylamine (DPA) for periods up to 92 wk. No effect of DPA exposure was noted on growth, clinical condition, survival, pattern of spontaneous disease, principal hematologic parameters (including methemoglobinemia), iron contents of liver and spleen, nature and incidence of histopathologic changes (in particular the times of appearance, nature and incidence of tumors). There was a striking increase in the proportion of erythrocytes containing Heinz bodies (HB); at 92 wk females on 0.025% DPA had levels of 30.3 ± 15.8 and males 62.7 ± 21.2%. Withdrawal of DPA from the diet for 5 wk brought about rapid decreases in HB levels, but not quite to the mean value of 0.01% in controls.

Further study of this phenomenon was undertaken in groups of mice fed on the same levels of DPA as before and on additional groups given diets containing 0.0005, 0.001, and 0.1% DPA for periods up to 12 wk. At the four highest dietary levels, HB were first observed on days 7, 9, and 14 of feeding. In mice on the three highest levels of DPA, 70–80% of red cells contained HB by day 30, 20% contained HB in mice on 0.005% DPA, and the two lowest groups remained normal throughout the test. Levels of methemoglobin, osmotic fragility and other hematologic measurements were unchanged in all groups. Significant depression of the combined activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in red cells first appeared on day 3 in females and on day 9 in males of the three highest dose groups, while glutathione reductase was first increased on day 11. After day 15, these enzyme activities were normal in all groups. Hence, a dietary level of 0.001% DPA is established as having no adverse effect under these conditions. (Supported by USDA Contract 12-14-100-9543, by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 5T01-ES00103-05.)

74. *Oxalate Excretion Following Oral Administration of Glycolate and Glyoxylate.* E. W. MCCHESNEY, LEON GOLBERG, and E. S. HARRIS, Albany Medical College, Albany, New York.

Earlier studies involving po administration of ethylene glycol (EG) to monkeys (*Macaca mulatta*) revealed that urinary oxalic acid accounted for only 0.3% of a dose of 1 ml EG/kg. In order to assess further the role of oxalate in EG metabolism, pairs of monkeys were given po doses of 500 mg/kg ^{14}C-labeled glycolate or glyoxylate. Fecal excretion of radioactivity over 96 hr amounted to 1–3%, while urinary ^{14}C accounted for about 50% of the dose given. Glycolic acid was excreted in urine as follows: 39% in unchanged form, 1.2% as glyoxylate, 0.3% as hippurate (by isotope dilution) and 0.8% as oxalate (intradermally). In view of the unexpectedly large output of unchanged glyoxylate, administration to the same two monkeys was repeated at 60 mg/kg glyoxylate. The excretory pattern was now: glyoxylate, 1.3%; hippurate 0.4% (intradermally); and oxalate, 14%. Urinary output of oxalate thus assumes increasing importance at moderate doses of precursors, but this study confirms the view that it is a minor product of EG metabolism in monkeys. (Supported by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, by the National Institutes of Health Training Grant 5T01-ES00103-05, and by joint NASA and Air Force sponsorship under Air Force Contract no. F29600-68-C-0031.)

75. *Modification of Acrylamide Neuropathy in Rats by Various Factors.* MICHAEL L. KAPLAN and SHELDON D. MURPHY, Harvard School of Public Health, Boston, Massachusetts.

We have previously demonstrated that the ability of rats to maintain balance on a rotating rod (electrorod) can be used to measure the peripheral neuropathy produced by acrylamide. In this investigation, the electrorod was used to determine if selected factors would modify the

course of onset and recovery from acrylamide neuropathy. Deficiencies in pyridoxine or thiamine were studied because they are known to be essential for normal peripheral nerve function. Treatment with hydrocortisone or bilateral adrenalectomy were selected since it has been reported that corticoids enhanced recovery from triorthocresyl phosphate neuropathy. Pretreatments with hepatic microsomal enzyme inducers (DDT and phenobarbital) were tested to determine if these would alter the susceptibility of rats to acrylamide. Acrylamide was administered daily (40 or 50 mg/kg, ip) until all rats in a group had failed to maintain balance on the electrorod. Neither the vitamin deficiencies nor daily hydrocortisone injections altered the cumulative neurotoxic action of acrylamide. The total cumulative doses of acrylamide required for neurotoxicity were 300 and 400 mg/kg for adrenalectomized and sham-operated rats, respectively. When acrylamide administration was begun 5 days following a single ip dose of 200 mg/kg DDT, the onset of acrylamide neurotoxicity was delayed appreciably. A similar protective effect was observed when acrylamide administration was started on day 6 of phenobarbital pretreatment (50 mg/kg/day, ip), and the administration of both compounds was continued daily. The total cumulative doses of acrylamide required for failure on the electrorod for DDT and phenobarbital pretreated rats were 520 and 600 mg/kg, respectively, compared to 360 mg/kg for the controls. Although the pretreated rats were more resistant to the onset of acrylamide neurotoxicity, once the injury had been produced they recovered more slowly. The ability of DDT and phenobarbital to induce the metabolism of many foreign organic compounds suggests a possible basis for their protective action against acrylamide neuropathy. (Supported by Training and Research Grants T01 ES-00045 and R01 OH-315 from USDHEW.)

76. *Studies on the Acute Toxicity of Monochloroacetic Acid in Rats.* FORREST D. HAYES, PERRY J. GEHRING, and JAMES E. GIBSON, Michigan State University, East Lansing, Michigan.

The acute lethality of monochloroacetic acid (MCA) was determined in male Sprague-Dawley rats and similarly compared to its close structural analog monofluoroacetic acid (MFA). The purpose of the study was to assess similarities or differences in modes of toxic action. Additionally the effect of MCA and MFA on the in vitro oxidation of ^{14}C-acetate (sodium) by whole homogenates of rat liver was determined to compare biochemical lesions of MCA and MFA poisoning. Aqueous solutions of MCA or MFA were administered sc to groups of 5–10 rats, and the number dead in each group determined 24 hr later. Other rats in groups of 10–20 were administered LD90 doses of MCA or MFA and the time of death (LT) noted. LD50 and LT50 values and the 95% confidence limits were calculated. In vitro ^{14}C-acetate oxidation in the presence and absence of MCA or MFA was determined in homogenates (2:1 in 0.1 M Tris) of rat liver. Acetate concentrations were 2.8, 7.0, 28 and 70×10^{-7} M, and MCA or MFA concentrations were 10^{-6} M. Homogenates with substrate (S) and MCA or MFA (total volume of incubate: 2.5 ml) were incubated for various times at 37°C with 95% O_2 in a Dubnoff incubator. $^{14}CO_2$ evolution was measured by absorption in hyamine hydroxide and liquid scintillation counting. Velocities (v) were plotted against $v/[S]$ (Hofstee plot) to determine reaction kinetics. The LD50 for MCA and MFA was 5.0 (4.2–5.9) and 108 (88–133) mg/kg, respectively, and the relative potency ratio 21.6 (95% confidence limits, 16.6–25.1). LT50 values at LD90 doses for MCA and MFA, respectively, were 130 (112–151) and 310 (292–360) min. The slopes were parallel. The reaction parameters for the oxidation of ^{14}C-acetate were V_{max}: 30.5×10^2 dpm $^{14}CO_2 \cdot min^{-1}$, and K_m, 2.4×10^{-6} M. MCA and MFA inhibited ^{14}C-acetate oxidation in vitro: MCA $k_i = 9.1 \times 10^{-7}$ M and MFA $k_i = 11.0 \times 10^{-7}$ M. MFA showed noncompetitive inhibition but MCA uncompetitive (coupling) inhibition. It was concluded that MCA and MFA, although close structural analogs, produce acute toxicity in the rat by dissimilar mechanisms of action.

77. *Acute Toxicity of Sodium Pentafluorostannite in Rats and Mice.* D. L. CONINE, J. C. MUHLER, and R. B. FORNEY, Indiana University School of Medicine, Indianapolis, Indiana.

Sodium pentafluorostannite is a compound possessing anticariogenic activity when administered to rats either in the diet or prenatally. Solutions of sodium pentafluorostannite dissolved in distilled water were administered by iv, ip, and po routes to male albino Cox/Swiss mice and to male and female albino Harlan/Wistar rats. The test animals were observed for 14 days,

and LD50 values determined thereafter. Very similar LD50 values were determined for male and female rats. They were 12.0 and 11.6 mg/kg, iv, 72.0 and 65.5 mg/kg, ip, and 221.0 and 227.00 mg/kg, po, respectively. Sodium pentafluorostannite in mice was less toxic iv (LD50 value being 19.0 mg/kg), about equally toxic, ip, and less toxic po (LD50 value of 595 mg/kg) than in the rats. The major toxic symptoms observed in both species and in both sexes were ataxia, general depression, and fore and hind leg weakness advancing to flaccid paralysis prior to death. In addition, rats dosed po developed diarrhea.

78. *Toxicology of Santicizer 334F.* O. E. FANCHER, G. L. KENNEDY, JR., J. B. PLANK, D. C. LINDBERG, W. H. HUNT, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois; Monsanto Company, St. Louis, Missouri.

Two year po toxicity studies with Santicizer 334F (a 1,3-butylene glycol–adipic acid polyester terminated by a mixture of myristic, palmitic, and stearic acids) were conducted in rats and dogs at dietary levels of 1000, 5000, and 10,000 ppm. At these levels no effects were observed in either species upon weight gain, food consumption, mortality or clinical or pathological parameters. In a three-generation reproduction study in albino rats at the same dietary levels, no effects were observed upon either parental animals or progeny except for slight decreases in weight gains for parental males at the highest dose level and for parental females at levels of 5000 and 10,000 ppm, and decreased liver weights for parental females at the two higher dose levels. The latter effect appeared to be a consequence of decreased body weights rather than being related to a specific toxic action of Santicizer 334F. These data indicate a large margin of safety for this material, which is used as a plasticizer for polyvinyl chloride formulations which come into contact with foodstuffs.

79. *Single and Repeated Dose Toxicity of Thalicarpine (NSC 68075) Administered Intravenously in Monkeys: A Comparison with Dogs.* P. E. PALM, M. S. NICK, and E. P. ARNOLD, Arthur D. Little, Inc., Cambridge, Massachusetts.

Thalicarpine, a dimeric isoquinoline alkaloid of interest as a cancer chemotherapeutic agent, has been studied for toxicological effects in the monkey when administered as: a single iv dose by (1) rapid injection or (2) 2-hr infusion; and repeated-dose schedules of (3) daily doses $\times 14$, (4) daily doses $\times 5(9)5$, and (5) Q4d $\times$ 8 wk. A comparison was made of the toxic effects noted with those reported earlier in dogs to aid in determining the dose regimen of choice for clinical trials. Rapid injection of 41.6 mg/kg $\times$ 1 was lethal to male and female monkeys causing mydriasis, convulsions, hypotension, apnea, and death by cardiac arrest within 23 min post-injection. The maximum nonlethal dose administered by the above five regimens was: (1) ~31.3 mg/kg (455 mg/m^2) in males and somewhat less in females compared to more than 41.6 mg/kg (800 mg/m^2) in dogs; (2) $\geqq$62.6 mg/kg (890 mg/m^2) compared to $\geqq$62.6 mg/kg (1249 mg/m^2) in dogs; (3) 5.2 mg/kg (72 mg/m^2); (4) 20.8 mg/kg (276 mg/m^2) and 10.4 mg/kg (138 mg/m^2) in the male and female, respectively, compared to $\geqq$20.8 mg/kg (427 mg/m^2) in dogs; and (5) 31.3 mg/kg (415 mg/m^2) in the male and 20.8 mg/kg (242 mg/m^2) in the female. Data in both species indicate infusion for ~2 hr to be the regimen of choice for clinical trials. On a mg/m^2 basis, monkeys are about two times as sensitive to the lethal effects of the drug as dogs, and when dosed by regimens (1), (4), and (5), the female monkey is somewhat more sensitive to thalicarpine toxicity than the male (a finding also observed in dogs). The most reliable early indicators of toxicity in both species were elevated SGPT/OT levels and reduced WBC counts. Histopathology noted in monkeys at the higher dose levels included: congestion of the lung, kidneys and liver, with some myocardial involvement, hypoplastic bone marrow, and liver degeneration characterized by reduced glycogen, parenchymal cloudy swelling and fatty metamorphosis. (Supported by Contract No. PH-43-65-61 with Chemotherapy, National Cancer Institute, National Institutes of Health.)

80. *Factors Affecting Hemolysis Induced by Ellipticine, an Antineoplastic Agent.* M. A. INNIS and I. P. LEE, National Cancer Institute, Bethesda, Maryland.

Ellipticine (5,11-dimethyl-6*H*-pyrido-[4,3-*b*]-carbazole) (NSC-71795), is an antineoplastic agent which is active against L1210 lymphocytic leukemia in mice. Preclinical toxicologic

studies demonstrated extreme hemolysis in dogs and monkeys following iv administration of even low doses of the hydrochloride salt of ellipticine. This finding prompted us to investigate the mechanism of hemolysis and to study various factors that might ameliorate this effect. Human red blood cells (RBC) were used throughout the study. Initial experiments determined that RBC were completely hemolyzed at a drug concentration of 10^{-3} M, while 10^{-4} M ellipticine stabilized the RBC against hemolysis induced by 150 mOsmol NaCl. Surface tension measurements demonstrated that the critical micellar concentration of ellipticine corresponded to the drug concentration at which extensive hemolysis occurred. Cellular uptake of ellipticine was linear with concentration and was not temperature dependent. It appears that ellipticine-induced hemolysis is due to its surface activity and lipophilic properties. It was determined that citrate, Na_2EDTA, oxytetracycline, and ICRF-159 (NSC-129,943) each protected against ellipticine induced hemolysis, suggesting that calcium is involved in the hemolytic process and perhaps in the cellular uptake of the drug. In fact, when ellipticine was formulated with citrate buffer and administered to Rhesus monkeys iv, no hemolysis occurred.

81. *Halocarbon-Epinephrine-Induced Cardiac Arrhythmia Potential of Some Common Industrial Solvents.* CHARLES F. REINHARDT, LINDA S. MULLIN, and MARY E. MAXFIELD, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

The inhalation of certain unsubstituted and halogenated hydrocarbons can make the mammalian heart abnormally reactive to epinephrine resulting in cardiac arrhythmias. These arrhythmias are usually ventricular in origin and may result in sudden death. This phenomenon is generally referred to as cardiac sensitization. There have been a small number of unexplained sudden deaths associated with common industrial solvents which could possibly be attributed to this phenomenon. Generally, the fatalities have been accompanied by either emotional or physical stress. In these experiments, therefore, the cardiac sensitization potential of some of the common industrial solvents was studied. These included methylene chloride, methyl chloroform, perchloroethylene, trichloroethylene, and trichlorotrifluoroethane. Beagle dogs were given a control injection of epinephrine, 0.008 mg/kg iv, prior to exposure to the test compound. The control period was followed by inhalation of the compound for 5 min, and then a challenge injection of epinephrine (same dose as above) was given. The effect upon the cardiac rhythm was monitored by an electrocardiogram which was continuously recorded throughout each experiment. The significant arrhythmias were considered to be either the appearance, after the challenge injection, of multiple consecutive ventricular beats or ventricular fibrillation. Methyl chloroform, trichloroethylene, and trichlorotrifluorethane caused cardiac sensitization at concentrations of 0.5–1.0% by volume, while methylene chloride and perchloroethylene did not produce cardiac sensitization at concentrations up to 2.0 and 1.0%, respectively. The onset of central nervous system effects precluded testing the latter two compounds at higher concentrations. It is concluded on the basis of this study that some of the common industrial solvents are capable of sensitizing the mammalian heart to epinephrine at moderately elevated concentrations and, therefore, this property should be taken into account when assessing potential toxic hazards associated with their use.

82. *Sensitization of the Heart to Catecholamine-Induced Arrhythmia by Haloalkanes.* J. H. WILLS, P. BRADLEY, H. KAO, H. GRACE, W. HULL, T. B. GRIFFIN, F. COULSTON, and E. S. HARRIS, Albany Medical College, Albany, New York; NASA Manned Spacecraft Center, Houston, Texas.

Anesthetized guinea pigs, cats, and dogs have been made to breathe through tracheal cannulae gas mixtures containing up to 20% of fluorotrichloromethane (I), trifluorobromomethane (II), tetrafluorodibromoethane (III), hexafluoroethane (IV), and octafluorocyclobutane (V). All increased the arrhythmogenic effect of iv infusion of epinephrine. The most potent compound was I, which was effective in 40–50% of the concentrations of the other compounds. The next most effective compound was III, which was about 1.2 times as potent as II, IV, and V. Further studies with I have shown that, although anoxia intensifies the sensitizing effect on the myocardium, addition of O_2 to the inspired gas does not remove completely the sensitizing

action. Vagotomy also reduced, but did not abolish, the sensitizing action of I to infusion of epinephrine. (Supported in part by NASA Contract No. NAS9-9964 and by NIH Grant No. 5T01 ES 00103-04.)

83. *Cardiac Function in Mice Following Exposure to Haloalkane Propellants Alone and in Combination with Bronchodilators.* JOHN L. EGLE, JR., JAMES W. PUTNEY, JR., and JOSEPH F. BORZELLECA, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia.

Recent studies have suggested that haloalkane gases, used as aerosol propellants, produce cardiotoxic effects which could account for sudden deaths associated with inhalation of aerosols. In the present study, the effect of inhalation of haloalkanes on cardiac responses to asphyxia has been observed in anesthetized mice. In these experiments animals were exposed to haloalkanes alone, an aerosol containing haloalkanes and one of two bronchodilators (isoproterenol or salbutamol) or nitrogen. The mice were then subjected to asphyxia for 2 min. During asphyxia the heart rate and number of instances of A–V block were recorded at 6-sec intervals. Exposure to haloalkanes alone or with a bronchodilator did not shorten the onset time or increase the intensity of the bradycardia induced by asphyxia. The development of cardiac slowing was slightly more rapid in animals exposed to nitrogen than in control mice. The frequency of A–V block was no higher in mice exposed to propellant or a propellant–bronchodilator mixture than in controls. There was a greater number of A–V events in animals inhaling nitrogen before asphyxia. The results of this study do not indicate that inhalation of propellant gases sensitizes the heart to asphyxia-induced arrhythmias in mice. (Supported in part by a research grant from the Schering Corp.)

84. *Alveolar Instability Following Administration of Fluorocarbon.* Y. ALARIE, M. A. CHOBY, and W. E. POEL, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

The halogenated hydrocarbon, 1,1,2,2-tetrachloro-1,2-difluoroethane (TCDF), was injected via the jugular vein in anesthetized rats (180–250 g). The total dose varied between 0.5 and 0.8 ml. Immediately following injection, the animals exhibited severe respiratory distress and died within 1 min. Upon opening the thorax the lungs were found to be completely atelectatic. The lungs were removed from the thoracic cavity and pressure–volume measurements (PVM) were made with air or with saline solution as the inflating media. PVM were compared with those of normal control rats in which pulmonary atelectasis was obtained by oxygen absorption following nitrogen washout of the lung. The differences in PVM with air between control and TCDF-treated rats were striking. They indicated instability of the alveolar spaces. With saline as the inflating media, pulmonary porosity was observed in TCDF treated animals. Histological examination indicated atelectasis of the alveolar spaces with overdistention of some conducting airways in TCDF-treated animals. A proteinaceous transudate present in the alveolar spaces formed a membranous hyalin film. A similar film lined some of the bronchioles. Because of TCDF immiscibility with water, low surface tension, and ability to solubilize lipids, TCDF is apparently capable of removing the surfactant normally present on the alveolar surface. As a consequence of the change in surface elastance, the alveoli are rendered mechanically unstable, and atelectasis apparently follows. Transudation occurs because of the effects of TCDF on cell membrane permeability as well as a change in surface tension in the alveoli. (Supported by PHS Grant No. 2A10AH00626-06 and Special Fellowship No. 1F03-ES46198-01, NIEHS.)

85. *The Hypotensive Response in Dogs Exposed to Bromotrifluoromethane.* ETHARD W. VAN STEE and KENNETH C. BACK, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

Mean arterial blood pressure has been observed to decrease reversibly in dogs exposed by inhalation to concentrations of bromotrifluoromethane greater than 10% in oxygen. Peripheral vascular resistance was determined in five dogs before, during, and after 50-min continuous exposures to 70% bromotrifluoromethane in O_2 and was found to decrease significantly during the exposure. In order to determine the mechanism of the resistance change, experiments were

performed in which arterial blood from a donor dog was pumped at a constant flow rate through the hindlimb of a recipient dog. The hindlimb was in vascular isolation from the recipient while the normal innervation remained intact. Exposure of the donor dog to 70% bromotrifluoromethane resulted in no change in the perfused hindlimb vascular resistance, whereas exposure of the recipient resulted in a fall in vascular resistance, indicating that the decrease in peripheral vascular resistance accompanying exposure to bromotrifluoromethane is a function of changes in vasomotor tone rather than a peripheral or direct vascular smooth muscle effect. The resistance change in the perfused hindlimb was blocked by administration of adrenergic blocking agents to the perfused limb or ganglionic blocking agents to the recipient dog.

86. *Inhalation of Aerosols by Multi-Species in a Head Exposure Chamber with Simultaneous Cardiopulmonary Measurements.* H. E. SWANN, JR., S. CARSON, J. SCHEIMBERG, and O. P. MCSHANE, Food and Drug Research Laboratories, Inc., Maspeth, New York.

This system was designed as a screening procedure for inhalation of aerosols by more than one species with simultaneous cardiopulmonary measurements while the animals were exposed under conditions simulating human exposures. The chamber can accommodate at one time one representative animal of each of six different species: monkey, dog, cat, rabbit, guinea pig, and rat or mouse. The number of cardiopulmonary parameters measured is limited only by the number of recording channels available. The exposure volume of the chamber is approximately 125 liters. Aerosol enters through a cone-dome shaped top. Air flow through the chamber can be regulated from 1–100 l/min. The animals are enclosed in body plethysmograph with heads protruding into the dynamic chamber and at a uniform level. Tidal volume, respiratory rate depth and configuration, as well as blood pressure and electrocardiography, on two species have been measured simultaneously during exposure. Stumptailed monkeys and guinea pigs have been exposed to monofluorotrichloromethane (Freon 11) and dichlorodifluoromethane (Freon 12). The aerosols have been used in a 50–50 combination or individually with concentrations of 400,000, 40,000, and 4000 ppm. Duration of exposure was 10 min. Chamber concentrations were determined by gas chromatography at minute intervals during the exposure. Samples were taken just above animal heads. The results indicated changes in tidal volume, respiratory rate depth and configuration; blood pressure decreased, and electrocardiograms showed various arrhythmias. Marked changes occurred at 400,000 ppm, and animals died when exposed to Freon 11 + 12 or Freon 11 alone. The animals survived the 40,000 ppm of Freon 12 and Freon 11, and the changes were less intensive with Freon 12. At 4000 ppm the animals responded, but the response to Freon 12 was far less marked than with Freon 11. This exposure system has utility for screening aerosols.

87. *Production of a Bronchial Asthma-Like Syndrome in Guinea Pigs by Inhalation of Sensitizing and Challenging Antigen for Testing of Therapeutic Drugs.* S. CARSON, H. E. SWANN, JR., J. SCHEIMBERG, O. P. MCSHANE, H. C. DANG, and D. SERLIN, Food and Drug Research Laboratories, Inc., Maspeth, New York.

This study was designed to develop an animal model with a bronchial asthma-like syndrome produced by inhalation of the antigen using repeated challenges with respiratory flow resistance measurements to indicate the response. Forced inhalation or systemic infusion of a foreign protein with challenges produces a bronchoconstrictive anaphylactic-type reaction; the most dramatic form of the response is shock reaction with death. The asthmatic syndrome is a mild anaphylactoid reaction to the antigen challenge. If the guinea pig survives the anaphylactic shock reaction, he may develop an asthma-like syndrome with subsequent inhalation challenges. Guinea pigs of mixed sex weighing 300–350 g inhaled egg albumin (purified five times) dissolved in distilled H_2O. The inhalation dose of 8 mg/ml was given with DeVilbliss Nebulizer calibrated to deliver 2 ml to the guinea pig face mask during a 3-min exposure. The guinea pigs were maintained in body plethysmograph, during exposure, and respiratory flow resistance was measured before and after challenge. Respiratory rate, tidal, and minute volumes were also recorded. The animals were divided into six subgroups of six animals. One subgroup was challenged on days 5, 10, 15, 20, 25, and 30. The second group on days 10, 15, etc. The third group

on days 15, 20, etc. The results indicate an anaphylactic shock reaction occurring at day 15, or the first exposure day; thereafter, for groups exposed on days 20, 25, and 30. Some animals died on the first day of reaction. Those that survived showed an increase in resistance. With repeated challenge, they continued to show an increase in resistance. This increase was of the same magnitude following each repeated challenge. This model with a bronchial asthma-like syndrome may be used to evaluate anti-asthmatic drugs.

88. *Evaluating the Toxicology of Household Aerosols.* G. S. WIBERG, Department of National Health and Welfare, Ottawa, Canada.

The use of household aerosol products has expanded rapidly in the last few years, and recent advances in aerosol technology will assure that their popularity and sales will continue to increase. Many of these products, particularly the non-food, drug, and cosmetic items, have been developed and marketed by manufacturers who hitherto have not had to consider toxicity hazards apart from those associated with accidental ingestions and eye and skin exposures. It is known, however, that the inspiration of many of these products in the form of fine droplets or volatilized material can occur coincident with the generation of aerosol sprays in the home. The evaluation of the risks involved from this adventitious exposure will require the development of new techniques and criteria of assessment. Conventional inhalation toxicology has been concerned primarily with prolonged repetitive 8-hr exposures for establishing TLV values, or with 1–4 hr exposures at high concentrations for estimating LC50 values. Household aerosol exposures differ markedly from either of the above situations and it will be necessary to employ different experimental designs. These will include selection of appropriate exposure chambers, consideration of the meaning of "dose" in terms of concentration and time ($C \times T$) for complex mixtures, comparison of static and dynamic exposures, choice of experimental animals, biochemistry and pathology of lung tissues including lung weights and trace metal shifts, evaluation of pulmonary sequestration and toxication of entrapped particles, ciliary clearance and its inhibition, in addition to the more conventional response criteria usually employed in inhalation studies.

89. *Investigations of a Lung Edema Toxin from Sweet Potatoes* (Ipomoea batatas). BENJAMIN J. WILSON and MICHAEL R. BOYD, Vanderbilt University, Nashville, Tennessee.

The loss of 50 head of cattle in Georgia due to consumption of moldy sweet potatoes led to investigation of the toxin(s) involved. Respiratory distress with lung edema were the principal disease signs. These were reproduded in mice by feeding ether extracts of the damaged tubers. Although ipomeamarone, a hepatotoxin discovered several years ago by Japanese investigators was present in the toxic sweet potatoes, no liver damage was observed in the cattle. Also, pure ipomeamarone did not cause lung edema in mice. A search for the lung edema factor(s) led to isolation of two isomers with a molecular weight of 168. One of these, 1-(3-furyl)-4-hydroxy-1-pentanone (trivial name 4-ipomeanol), is a potent lung toxin. It has been synthesized using diethyl 3,4-furandicarboxylate as starting material. The racemic compound has an iv LD50 in mice of 20.9 ± 1.3 mg/kg, an ip LD50 of 35.5 ± 6.1 mg/kg and an intragastric LD50 of 37.6 ± 5.0 mg/kg. Studies are underway on its mechanism of action in susceptible animals. The lung edema toxin and several other β-furan metabolites are frequently noted in sweet potatoes offered for sale in food markets. Methods of toxin bioproduction have been developed using common fungus pathogens of the plants as inocula for sweet potato slices.

90. *The Effect of Ascorbic Acid Deficiency and Protein Quality on Stimulation of Hepatic Microsomal Enzymes in Guinea Pigs.* R. CHADWICK, A. PEOPLES, and M. CRANMER, Environmental Protection Agency, Perrine, Florida. (W. F. Durham.)

It has been established that a number of nutritional and physiological variables affect hepatic microsomal enzyme induction in mammals. Standardization of such variables will permit results between labs to have a basis for comparison. Protein quality and ascorbic acid deficiency were found to affect the storage and excretion of ^{14}C-lindane together with the stimulation of various hepatic enzyme systems in the guinea pig. Low quality dietary protein enhanced urinary but not

fecal excretion of radioactivity. While radioactivity stored in the liver and fat was not significantly altered, that stored in the kidney was reduced by protein inadequacy. Ascorbic acid deficiency, on the other hand, inhibited urinary excretion of radioactivity and permitted a high level to be stored in the kidney. The dietary variables produce both qualitative and quantitative alterations in the metabolism of ^{14}C-lindane. The effect of dietary variables on pesticide induction of various in vitro enzyme systems was not clear cut. Thus while enhancing the *o*-demethylation of *p*-nitroanisole, protein inadequacy impairs the oxidative hydrolysis of EPN, the glucuronidation of *p*-nitrophenol, and exerts no definite influence over the azo reduction of methyl orange or the microsomal protein and cytochrome P450 content of the liver. While soy protein stimulates the excretion of glucaric acid, gelatin appears to inhibit it. Similarly ascorbic acid deficiency promotes glucuronyl transferase activity while depressing the *o*-demethylation of *p*-nitroanisole. These data support a possible role of dietary "stress" in the stimulation of some hepatic microsomal enzyme systems in the guinea pig.

91. *Interaction of Inhaled 1,1-Dichloroethylene and Microsomal Enzyme Inducers.* G. P. CARLSON, G. C. FULLER, and B. I. GONET, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island.

Rats pretreated with the enzyme-inducing agent phenobarbital (PB) did not demonstrate increased hepatotoxicity due to po administration of 1,1-dichloroethylene but were actually protected against it. Because of the contrasting effects of PB and 3-methylcholanthrene (3-MC) induction on the hepatotoxicity due to CCl_4, the effects of these inducing agents on the toxicity due to inhalation of 1,1-dichloroethylene were compared. Unexpected lethality was found in animals pretreated with PB (50 mg/kg, ip for 4 days) or 3-MC (40 mg/kg in corn oil, ip 48 and 72 hr prior to exposure) when exposed to 32,500 ppm for 1 hr. 0/4 Controls, 4/4 of the PB-pretreated, and 4/4 of the 3-MC-pretreated animals died during exposure. When the concentration was dropped to 20,000 ppm, 0/4 controls, 4/4 3-MC pretreated, and 3/4 PB pretreated rats died. Lethality was not found in the controls until the concentration was raised to 41,500 ppm. Under these conditions, controls lived 90 ± 11.8 min and rats pretreated with SKF 525A (50 mg/kg, ip 30 min before exposure) lived only 48 ± 10.3 min. When controls, 3-MC pretreated, and PB pretreated rats were exposed to 1440 ppm of 1,1-dichloroethylene for 1 hr, SGOT and SGPT values obtained 24 hr after exposure did not indicate any potentiation of the hepatic injury due to the 1,1-dichloroethylene by pretreatment with the enzyme-inducing agents. It is apparent from these studies that the potentiated lethality associated with PB or 3-MC pretreatment and subsequent exposure to 1,1-dichloroethylene is not associated with a potentiation of hepatotoxic effects. (Supported by NIH Grant No. ES-00596.)

92. *Interactions of Cyclodiene Insecticides with Components of the Hepatic Microsomal Mixed Function Oxidase System from Male and Female Rats.* FRANK E. GREENE, M. S. Hershey Medical Center, The Pennsylvania State University, Hershey, Pennsylvania.

The characteristics of aldrin (A) and heptachlor (H) epoxidation indicate that this biotransformation is catalyzed by the mixed function oxidase enzyme (MFO) system: the reaction is microsomal, requires a source of NADPH and O_2, and is inhibited by carbon monoxide. To further characterize the metabolism of these compounds, we have examined the effects of the substrates, A and H, as well as their respective epoxide metabolites dieldrin (D) and heptachlor epoxide (HE), on components and kinetic properties of the hepatic MFO system. All compounds tested bound avidly to cytochrome P450, ($K_s \approx 10^{-2}$ mM) producing a typical Type I difference spectra (absorption maxima at 390 nm and minima at 420 nm). The affinity in decreasing order was H > D > HE > A. The ability to stimulate NADPH oxidase paralleled the affinity of the compounds for cytochrome P450. The rate of reduction of cytochrome P450 by NADPH was increased from 150 to 200% above control values when either A, D, H, or HE was added to the reaction cuvette containing microsomes from male rats. However, there was no correlation with binding affinity since HE and A stimulated the rate of reduction more than did H and D. A sex difference was seen: in microsomes from female rats, affinity for cytochrome P450 and stimulation of NADPH oxidase was less, and the rate of reduction of cytochrome P450 could not be stimulated. These results indicate that cyclodiene insecticides and

their epoxide metabolites produce similar effects on components of the MFO system, which are not related to the ability of the compounds to act as substrates, since neither D nor HE is metabolized to an appreciable extent by this system in rats. The ability of epoxides to inhibit the metabolism of their parent compounds in vitro may be explained by their common affinity for cytochrome P450. An additional effect via stimulation of NADPH oxidase may be important in vivo since the availability of NADPH within the cell may be rate limiting in the metabolism of foreign compounds. (Supported by Grant 9-R01 ES00638-02.)

93. *Arsenic Trioxide: Stimulation of Liver Enzyme Detoxication Activity.* D. J. WAGSTAFF, University of Missouri, Columbia, Missouri. (J. L. Shupe).

Arsenicals usually inhibit enzymatic processes. This inhibition is the basis for their toxicity. However, I have observed that detoxication activity of liver microsomal enzymes is enhanced by dietary As_2O_3. The activities of EPN detoxication and *O*-demethylation of *p*-nitroanisole were increased to 227 and 280% of control, respectively, by feeding adolescent female Holtzman rats a diet containing 1000 ppm As_2O_3 for 15 days. Hexobarbital sleep time was decreased by feeding a diet containing as little as 100 ppm arsenical. The enzyme stimulation effect of As_2O_3 was additive with that of other chemical and physical factors. There was a summation of effects of feeding 500 or 1000 ppm As_2O_3 in the same diet with 500 ppm phenobarbital. Also the effect of As_2O_3 was additive with the enzyme-stimulatory effect of exposure to cold for 15 days. Rats which survived 1 wk of eating toxic levels of As_2O_3 tended to recover during the second week. Also, severity of arsenic intoxication was decreased by phenobarbital in the diet.

94. *Vinylidene Chloride Hepatotoxicity and Dissociation from a Lipoperoxidative Mechanism.* RUDOLPH J. JAEGER, JOSHUA TRABULUS, and SHELDON D. MURPHY. Harvard School of Public Health, Boston, Massachusetts.

Vinylidene chloride (VC), similar to carbon tetrachloride (CCl_4), depresses hepatic glucose-6-phosphatase (G6Pase) and increases serum alanine-α-ketoglutarate transaminase (AKT) activities in rats. The present investigation was conducted to determine the effect of VC on pentobarbital sleeping time (PST), hepatic and serum triglycerides (HTG and STG), and on two indices of hepatic lipoperoxidation, malonyldialdahyde (MDA) production, and diene conjugation (DC) in rats. VC (100–800 mg/kg) caused slight to moderate increases in HTG 24 hr after a single po dose. STG were significantly elevated by 400 mg/kg. PST was increased by doses greater than 100 mg/kg. Maximal depression of G6Pase occurred at 16 hr after 400 mg/kg, while AKT elevation was maximal at 8 hr. The greatest increase in PST occurred at 16 hr; however, even at 2–4 hr after VC, when G6Pase and AKT were not greatly affected, PST was significantly prolonged. To determine if the mechanism of VC hepatotoxicity was similar to that proposed by Recknagel and others for CCl_4, measurements of DC and MDA production were made. VC (5 μl in vitro) did not increase MDA production by 7.5% liver homogenates, while the same concentration of CCl_4 did. In vivo treatment of rats with CCl_4 (2.5 ml/kg) increased MDA production by liver homogenates in vitro, while VC (1 ml/kg) significantly decreased it. Similarly, DC was increased at 1 and 4 hr after CCl_4 but VC reduced the in vivo level of conjugated dienes. Treatment of rats with VC (1 ml/kg, 20 hr) or CCl_4 (2.5 ml/kg, 1 hr) resulted in equal decreases in G6Pase activity. The two treatments, when given together, resulted in G6Pase depression and DC elevation that was not greatly different from the combination of the effects of each chemical alone. These studies suggest that VC and CCl_4 share some common hepatotoxic effects but may act by a dissimilar mechanism. (Supported by Research Grants OH-315 and ES-00002 from USDHEW.)

95. *Enzyme Deficiencies in the Rabbit: Correlation with Pharmacological and Toxicological Effects.* D. J. ECOBICHON, Dalhousie University, Halifax, Nova Scotia, Canada.

Atropinesterase in rabbits was the first fully documented observation of a heritable modification of a pharmacological response. This enzyme, found in varying frequency in the sera and tissues of some but not all rabbits, readily degrades tropinaceous esters. Other drugs, cocaine, procaine, and benzoylcholine, have been reported to be hydrolyzed by a serum esterase in some

rabbits. The question arose regarding the pharmacogenetics involved and whether these agents were hydrolyzed by one enzyme, a group of closely-related esterases, or by distinctly different proteins. Techniques including electrophoresis, titrimetric, and spectrophotometric analysis of substrate specificity, and inhibition sensitivity were employed to characterize the plasma enzyme in adult male and female albino New Zealand rabbits. Atropinesterase was found to be a non-specific carboxylesterase occurring with a 50% frequency in either sex. Its occurrence was readily detected by the hydrolysis of *alpha*-naphthyl acetate (α-NA). The frequency distribution of atropine, procaine and benzoylcholine hydrolysis paralleled that of α-NA. Electrophoretically, three distinct phenotypes could be observed, the low rate of hydrolysis of the three drugs and α-NA occurring with only one phenotype. The rabbit population investigated could be divided into three groups on the basis of rate of substrate hydrolysis. The importance of this enzyme in toxicological studies was assessed by a study of atropine-induced mydriasis and procaine convulsion time in animals known to possess "low" and "high" plasma carboxylesterase activity.

96. *Cadmium Potentiated Hexobarbital Sleep Time in Male and Female Albino Rats and Mice.* W. M. HADLEY and T. S. MIYA. School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, Indiana.

The almost universal occurrence of cadmium in our environment, its cumulative nature and its toxicity to a wide variety of organs and metabolic pathways has recently stimulated renewed interest in cadmium toxicity. Liver damage is one of the toxicities reported after acute exposure to cadmium in both man and animals. The effect of cadmium on the liver suggested that cadmium might affect hexobarbital sleep times (HST) since the duration of HST is almost entirely determined by hexobarbital metabolism by the liver. Cadmium acetate (2 mg/kg, ip) was injected into male and female albino rats and mice. Controls received sodium chloride 0.9% (rats 0.5 ml/kg, ip; mice 5 ml/kg, ip). After 2 days in mice and 3 days in rats, HST (100 mg/kg, ip) were determined; HST were potentiated in all cadmium-treated animals (male mice, 28.3%; female mice, 44.8%; male rats, 131.0%; female rats, 28.7%). The potentiation of sleep time may be associated with decreased metabolism, although other factors may be involved, e.g. increased sensitivity of the brain to hexobarbital. The sleep times of male rats were potentiated to a much greater extent than were the sleep times of female rats. The greater potentiation of sleep times in the male rats might be attributed to hormonal differences. Further experiments are being carried out to clarify the mechanism of the potentiated sleep times and the sex difference. (Supported by Training Grant ES00071.)

97. *Effects of 2,6-Dichloro-4-nitroaniline and its Metabolites on Rat Liver Mitochondria.* M. A. GALLO, E. BACHMANN, and L. GOLBERG, Albany Medical College, Albany, New York.

The metabolites formed from 2,6-dichloro-4-nitroaniline (DCNA, Botran) in rodents have been reported to be 3,5-dichloro-*p*-aminophenol (DCAP) and 2,6-dichloro-*p*-phenylenediamine (DCPD), an observation confirmed in our laboratory. Studies carried out in vitro on liver mitochondria from untreated rats indicated that addition of DCNA or DCAP in solution in ethoxyethanol–ethanol (12:88, v/v) uncoupled oxidative phosphorylation and inhibited mitochondrial electron transport at concentrations of 5×10^{-5} M and 2×10^{-4} M, respectively. The uncoupling concentration of 2,4-dinitrophenol is 1×10^{-5} M. Concentrations of DCPD 10 times greater than that of DCNA did not uncouple oxidative phosphorylation, and addition of the solvent alone was without effect on the mitochondria. PO administration of 1000 mg/kg DCNA daily for 4 days to adult female rats did not influence oxidative phosphorylation of isolated mitochondria but did result in an increase in succinate-stimulated respiration. When rats were sacrificed 24 hr after 1–4 daily administrations of 1000 mg DCNA/kg, parallel increases were observed in relative liver weight, biphenyl hydroxylation and succinate-stimulated respiration. Biphenyl hydroxylation was stimulated most powerfully by DCPD and least so by DCHA. (Supported by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, and by National Institutes of Health Training Grant 5T01-ES00103-05.)

98. *A Strategy for the Seventies: The Role of the Toxicologist in Environmental Maintenance and Repair.* MARK M. LUCKENS, University of Kentucky, Lexington, Kentucky.

This decade calls for a new strategy to meet the demands of environmental degradation. While many groups have come forward with plans and solutions to meet this challenge, relatively little has been heard from the toxicologist. The toxicologist, by definition, is recognized as the evaluator of the toxicity of the products of science and technology and of the safety of the many formulations and effluvia of modern industry. He is both a generalist in this multidisciplinary science and a specialist in a particular area of his profession. The intentional as well as unintentional dispersal of bioactive compounds into the environment over the years has created problems whose dimensions are causing great concern. Because of his background and expertise, the toxicologist may well hold the key to meeting the increasing problems associated with the earth's capacity to adequately handle the contaminants and pollutants that are continually being dispersed within its life support systems. It is the purpose of this presentation to consider some of the more pressing environmental problems, their pollutional capacity, their interactions, and their effect on the planet's ecosystems as well as their direct and indirect effect on man. This paper will present the toxicologists' qualifications to develop scientific and design data to provide for the mitigation and possible elimination of these problems. Examples will be given of several of such problems and will trace the role of the toxicologist in the delineation of these problems.

99. *The Frequency of Occurrence of Teratological Anomalies in Control New Zealand White Strain Rabbits.* B. N. BANERJEE and R. S. DURLOO, Woodard Research Corporation, Herndon, Virginia.

In order to evaluate the occurrence of teratological anomalies in a normal population, an experiment was conducted in New Zealand White Strain rabbits. A total of 1081 rabbit fetuses was studied. The dams received a daily ration of 150 g of Dietrich and Gambrill Guinea Pig and Rabbit Chow and water ad libitum. On day 30 of gestation, each female was sacrificed, and the fetuses were delivered by Caesarean section. Viable offspring from each litter were placed in an incubator to observe 24-hr survival; survivors were sacrificed at the end of this time. All pups, whether sacrificed or previously dead, were examined grossly and skinned. They were then examined for visceral anomalies. After examination, the viscera were removed and discarded, or, if indicated, preserved in neutral formalin. The skinned, eviscerated fetuses were examined for skeletal anomalies by the KOH–Alizarin Red S technique. The most common skeletal anomalies observed were incomplete ossification of frontals (17.21%), incomplete ossification of parietals (17.76%), incomplete ossification of supraoccipitals (2.59%), and abnormal curvature of hyoid wings (2.50%). No pronounced visceral anomalies were observed except for pale kidneys (2.30%).

100. *Sensitivity of Blastocyst Implantation to Toxic Agents.* R. ABRAHAM, J. C. FULFS, L. GOLBERG, and F. COULSTON, Albany Medical College, Albany New York.

Successful nidation requires survival and normal development of the blastocyst, as well as the appropriate sequence of changes at the implantation site in the uterine wall. Invasion by the trophoblast is preceded by striking alterations in the uterine lysosomes, with formation of autophagic vacuoles and residual bodies. Administration of a copolymer of mixed phenylmethylcyclosiloxanes (PMxMMy, 100 mg/kg by stomach tube) to rabbits on days 4 and 5 of pregnancy prevents implantation. Blastocysts recovered on day 6 were found to contain large lysosomal aggregates, while the lysosomes in the uterine mucosa on days 6–11 had failed to undergo their normal development and to bring about autolysis of the uterine tissues. A primary cytotoxic action of PMxMMy on the blastocyst is postulated, but whether there is a concomitant direct effect on uterine lysosomes or whether the abnormality is secondary to blastocyst degeneration remains to be determined. The action of copper on uterine lysosomes has also been studied in an effort to clarify one possible mechanism by which intrauterine devices bring about their effects. (Supported by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant 5T01-ES00103-05.)

101. *Methylmercury Toxicity in Pregnant and Nonpregnant Rats.* W. L. MARCUS and B. A. BECKER, University of Iowa, College of Medicine, Iowa City, Iowa.

Methylmercury lethality was determined in pregnant and nonpregnant female Sprague-Dawley rats. Rats were injected ip with CH_3HgCl dissolved in 10% alcohol on gestational days 8, 9, 10, 13, 14, or 15. Pregnant animals were caesarian-sectioned on gestational day 22 or allowed to litter on day 23; nonpregnant survivors were sacrificed 14 days after injection. The LD50 for pregnant rats was 11.2 mg/kg (10.5–12.0, 95% confidence limits). The LD50 for nonpregnant rats was 10.8 (10.3–11.4) mg/kg. Morbid animals had hemorrhagic lungs, congested right ventricles, atria, and engorged right vena cavae. Gross and histologic examinations of heart, lungs, liver, and kidneys, indicated possible right-sided heart failure. Methylmercuric chloride, 1 mg/kg/hr, was infused iv, and venous and arterial blood pressures were measured. Venous blood pressure increased between 20–30 mm Hg with little or no increase in systolic arterial blood pressure. The LD50 values and slopes of the lethality regression lines for CH_3HgCl-induced lethality in pregnant and nonpregnant rats did not differ significantly. Accordingly, CH_3HgCl can be assumed to have caused death in both groups by a similar mechanism which involves water loss and increased pulmonary resistance. (Supported by NIH grant GM-12,675.)

102. *The Teratogenic Effects of Cadmium in the Rat.* NEIL CHERNOFF, Environmental Protection Agency, Perrine, Florida. (W. F. Durham.)

The present study was initiated to elucidate the teratogenic effects of cadmium on the rat fetus. SC injections of 4–12 mg/kg $CdCl_2$ were administered to CD strain rats on 4 consecutive days between days 13 and 19 of gestation. Dose responses were established for fetal death, fetal weight, and teratogenic effects. When cadmium was administered on days 14–17 of gestation, fetal death rates rose from 4% at the 4 mg/kg dose to 52% at the 12 mg/kg dose. Fetal weights decreased from 4 g at the 4 mg/kg dose to 3 g at the 12 mg/kg dose. The rate of anomalies rose from 4% at the 4 mg/kg dose to 70% at the 12 mg/kg dose. Anomalies produced included micrognathia, cleft palate, club foot, and small lungs. Micrognathia and small lungs were the most common. Data from the administration of 8 mg/kg $CdCl_2$ on varying days of gestation indicated that fetuses were more sensitive when treatment was begun on days 14 or 15 than on days 13 or 16. Cadmium retards the growth of the fetal lung. Fetuses of animals injected with 8 mg/kg $CdCl_2$ on days 13–16 or 14–17 had lung/body weight ratios which were significantly reduced to 2.5% from an average of 3.4% in controls. The data indicate that this was a specific retardation and not merely a reflection of differential organ growth rates and overall fetal retardation.

103. *Effects of Cadmium Chloride on Mouse Spermatogenesis Studies by Velocity Sedimentation Cell Separation and Serial Mating.* I. P. LEE and R. L. DIXON, National Cancer Institute, Bethesda, Maryland.

It is well known that cadmium damages testes of experimental animals, although the mechanism of action has not been completely elucidated. The use of the velocity sedimentation cell separation technique to study drug effects on spermatogenesis had been reported previously. Therefore, we have studied the in vitro and in vivo effects of cadmium chloride on the incorporation of tritium-labeled thymidine, uridine, and L-leucine by spermatogonia, early elongated spermatids, and late elongated spermatids, respectively. In addition, the effect of cadmium chloride treatment on the fertility of male animals has been assessed using the serial mating technique. CDF_1 male mice were treated with a single dose of cadmium chloride (1 mg/kg). For biochemical studies two mice were sacrificed 24 hr after treatment, and two additional mice were sacrificed every 7 days for the next 35 days. Seminiferous tubules were isolated and incubated with either tritium-labeled thymidine, uridine, or L-leucine for 30 min at 32°C. At the end of the incubation, seminiferous tubules were washed with solutions containing cold substrates and processed for spermatogenic cell separation by the velocity sedimentation separation technique. Radioactivity present in the acid-insoluble cellular fraction was determined by liquid scintillation counting. At the dose used, cadmium chloride resulted in a 50% reduction

of the incorporation of thymidine by spermatogonia, while not significantly affecting the incorporation of uridine or L-leucine. Similar results were obtained when cadmium chloride was incubated directly with the seminiferous tubules in vitro. Serial mating studies demonstrated cadmium chloride induced infertility.

104. *Alteration of Salicylate-induced Embryotoxicity by Phenobarbital and SKF 525A Pretreatments of Sprague-Dawley Rats.* J. L. MINOR and B. A. BECKER, University of Iowa College of Medicine, Iowa City, Iowa.

The embryotoxicity of single ip doses of sodium salicylate to Sprague-Dawley rats on gestational day 10 following treatments of phenobarbital (60 mg/kg, ip, 60, 36, and 12 hr), SKF 525A (40 mg/kg, ip, 25, 13, and 1 hr) prior to sodium salicylate and control injections with sodium chloride (90 mg/kg, ip, as above) was determined. Implant viability (number of live pups at delivery/number of implants) was affected by the pretreatments. In dams treated with 374 mg/kg of sodium salicylate, SKF 525A pretreatment reduced the implant viability from $90 \pm 4\%$ to $46 \pm 19\%$, while phenobarbital increased the implant viability at 570 mg/kg from $50 \pm 24\%$ to $85 \pm 15\%$. Fetal body weight and maternal weight gain were similarly affected by the pretreatments. No clear trend on the teratogenicity was established. Both phenobarbital and SKF 525A increased anomalies due to sodium salicylate (374 mg/kg), but phenobarbital appeared to decrease anomalies resulting from 500 or 570 mg/kg of sodium salicylate. Additional pretreatment effects on dams included depression of food intake, water intake, and urine volume for SKF 525A, while phenobarbital increased both water intake and urine volume. The alteration in sodium salicylate embryotoxicity caused by phenobarbital and SKF 525A may be due to either an altered metabolism or distribution, storage, and excretion. The relative importance of metabolism compared to the other factors remains to be elucidated. (Supported byNIH Grant Gm-12,675 and 5T1-GM-141.)

105. *Effect of Prenatal Treatment with Some Antineoplastic Agents on Gonadal Function in Mice.* R. L. DIXON and J. A. MCLACHLAN, National Cancer Institute, Bethesda, Maryland.

Anticancer drugs are continually being used to a greater extent in nonneoplastic diseases where the possibility of unknown pregnancy is increased. The purpose of these experiments was to assess the effects of prenatal exposure to selected anticancer agents on gonadal development and function of the offspring. Procarbazine (NSC-77213) is an effective antineoplastic methylhydrazine derivative which induces sterility in adult male laboratory animals and man. Cyclophosphamide (NSC-26271), a unique alkylating agent which requires in vivo activation, has been reported to result in infertility in mature female mice and patients. Large groups of pregnant mice were treated once with a nonteratogenic dose of either antineoplastic agent on days 10, 12, or 17 of gestation. Anatomical and functional alterations were evaluated for each treatment period. One week postpartum, representative male and female neonates were sacrificed and the ovaries, testes, adrenals, and pituitaries examined histologically for alterations. The remaining newborn were allowed to attain puberty and were tested for reproductive capacity. Animals exposed to anticancer drugs during gestation were mated with untreated control animals. Fertility, litter sizes, forced breeding performance, birth intervals, fertility span, and lordosis quotients were determined for the treated female offspring. The fertility profiles of the males were obtained by serial mating techniques. Although degeneration of germ cells was apparent in both the ovary and testes, the breeding studies indicated that the functional reproductive capacity of the female offspring from treated pregnant mice was more greatly reduced than that of the corresponding male offspring.

106. *Embryo–Fetal Toxicity and Teratogenic Effects of a Group of Methacrylate Esters in Rats.* A. R. SINGH, W. H. LAWRENCE, and J. AUTIAN, College of Dentistry and College of Pharmacy, University of Tennessee Medical Units, Memphis, Tennessee.

Polymeric materials made from acrylates and methacrylates find a host of applications today, including use as biomaterials ranging from dentures to contact lenses; they have recently been investigated for use as "implantable teeth". It is conceivable that these esters may reach

the body via inhalation by workers or from residual unpolymerized ester in a final material. The interest of the Laboratories, in developing toxicity data on biomaterials and their component parts, and in studying structure–activity relationships within a homologous series, investigated some of these monomers for their embryo–fetal toxicity and teratogenic activity. Methyl, ethyl, butyl, isobutyl, and isodecyl methacrylates, as well as glacial acrylic acid, were administered to female Sprague-Dawley rats on days 5, 10, and 15 of gestation. Each compound was employed at 1/10, 1/5, and 1/3 of the acute ip LD50 dose at each time interval. Untreated animals served as controls, while distilled water and normal saline were used at 10 ml/kg and cottonseed oil at 5 and 10 ml/kg. Each rat was sacrificed with ether on day 20 of gestation. The uterine horns and ovaries were surgically exposed to permit counting and recording of the number of corpora lutea, resorption sites, and viable and dead fetuses. The number of corpora lutea observed ranged from 51 to 62 per group of five rats, with no apparent distribution according to treatment. Embryo–fetal toxicity (mortality) varied from 0–44.2%. Fetal malformations ranged from 0–16.7%, both for gross and skeletal abnormalities. Fetuses from all methacrylate ester- and acrylic acid-treated groups were smaller ($P \leq 0.01$) than the untreated controls. Hemangiomas of the neck, shoulder, hind quarter and legs, and twisted hind legs were the most common gross abnormalities, while elongated and fused sternebrae and frontal ribs, and one or two missing ribs were the most common deformities found in the stained skeletal specimens.

107. *Studies on the Prenatal Toxicity and Teratology of N,N-Diethylbenzene Sulfonamide in Rats.* THOMAS M. LELAND, GERALDINE F. MENDELSON, MARSHALL STEINBERG, and MAURICE WEEKS, U.S. Army Environmental Hygiene Agency, Edgewood Arsenal, Aberdeen Proving Ground, Maryland.

N,N-diethylbenzene sulfonamide has been proposed for human use as a topical mosquito repellent. Comparative observations were made on pregnant albino rats receiving *N,N*-diethylbenzene sulfonamide by either ip injection or intragastric intubation. The pregnant dams received the chemical on days 5, 6, 7, 8, 9, 10, or 13 of gestation. The dose was 100, 200, 300, 400, or 500 mg/kg. Lower fetal weights, fetal resorptions or fetal malformations were produced at all dose levels. The fetal malformations observed included missing tail, umbilical hernia, lateral rotation of hind limbs, ectromelia, amelia, and exencephalia. The highest incidence of fetal anomalies occurred when the dams were exposed to *N,N*-diethylbenzene sulfonamide on day 9 of gestation.

108. *Light Microscopy and Ultrastructure of Liver of Rats Fed Polychlorinated Biphenyls.* RENATE D. KIMBROUGH, RALPH E. LINDER, and THOMAS B. GAINES, Toxicology Branch, EPA, Chamblee, Georgia.

Polychlorinated biphenyls (PCB) are widely distributed in the environment. Two PCB were fed to groups of 10 male and 10 female weanling Sherman strain rats in their diet at levels of 0, 20, 100, and 500 ppm Aroclor 1254 and 0, 20, 100, 500, and 1000 ppm Aroclor 1260 for 8 mo. In a preliminary study, 5 of 10 male rats and 8 of 10 female rats fed 1000 ppm Aroclor 1254 for 3 mo died. In the 8-mo study one female and two males fed 500 ppm Aroclor 1254 died. Eight females fed 1000 ppm, two females fed 500 ppm and one female fed 100 ppm of Aroclor 1260 died. All other rats survived. The livers of all rats exposed to the Aroclors weighed more than those of the controls. This difference was significant for all exposed male rats ($p < 0.025$) and for the females fed 500 ppm of either compound. Light microscopic examination of the liver of the exposed groups revealed enlarged hepatocytes with occasional inclusions in the cytoplasm. The cytoplasm of the liver cells was often either foamy or coarsely vacuolated and contained a great deal of lipid. A brown pigment was present in Kupffer cells of a number of livers. Adenofibrosis, particularly at the higher dietary levels, was also observed. Ultrastructural changes included increase in smooth endoplasmic reticulum, concentrically arranged membranes in the cytoplasm which surrounded lipid vacuoles, atypical mitochondria, "moth-eaten" appearance of the cytoplasm, and intracytoplasmic vacuoles with strands of electron-dense material. The area of adenofibrosis showed collagen, fibroblasts, and epithelial cells

with granular cytoplasm. The epithelial cells formed rosettes which surrounded cellular debris. Their free surfaces were lined by microvilli. Some of the epithelial cells contained a great deal of mucus and resembled goblet cells.

109. *Reproductive Effects of Polychlorinated Biphenyls in White Leghorn Chickens.* D. H. JENKINS, M. L. KEPLINGER, O. E. FANCHER, E. P. WHEELER, and J. C. CALANDRA, Industrial BIO-TEST Laboratories, Inc., Northbrook, Illinois; Monsanto Company, St. Louis, Missouri.

Several reports have indicated that polychlorinated biphenyls (PCB) were found in birds. The PCB investigated were Aroclor 1242 (biphenyl chlorinated to 42% by weight), Aroclor 1254 (biphenyl chlorinated to 54%) and Aroclor 1260 (biphenyl chlorinated to 60%). White Leghorn chickens were fed 1242 at levels of 1, 2, 4, 8, 10, and 100 ppm or 1254 or 1260 at 1, 10, or 100 ppm in the diet. Effects on the chickens as well as on egg production, hatchability of the eggs, and physical characteristics of the eggs were determined. Body weights of birds fed 1242 at 10 or 100 ppm or 1254 at 100 ppm were low. They exhibited anorexia, and some died from malnutrition. Most of the birds did not show abnormal gross changes; however, some of those fed 1254 at 100 ppm showed changes in the heart, kidneys, and overies. Egg production in hens fed 1242 at 100 ppm or 1254 at 100 ppm was low. There was reduced hatchability of eggs from hens fed 1242 at 8 or 10 ppm. None of the eggs hatched from hens fed 1242 or 1254 at 100 ppm. There were no significant differences in specific gravity of eggs. The egg shells from hens fed 1242 at 10 or 100 ppm or 1254 at 100 ppm were not as thick as shells of eggs from hens fed control diet. Therefore, ingestion of 1242 at 1, 2, or 4 ppm, 1254 at 1 or 10 ppm or 1260 at 1, 10 or 100 ppm caused no apparent effects on reproduction in the chicken.

110. *Toxicology of Brominated Biphenyls. I. Oral Toxicity and Embryotoxicity.* J. G. AFTOSMIS, R. CULIK, K. P. LEE, H. SHERMAN, and R. S. WARITZ, E. I. du Pont de Nemours and Company, Newark, Delaware.

Brominated biphenyls have shown promise as flame retardants for synthetic fibers and molded plastic parts. Octabromobiphenyl (BB-8) has low acute po toxicity (2 g/kg) in male rats and Japanese quail (>12.5 g/kg). Single po doses did not cause liver enlargement in either species. Four groups of young adult male rats were fed diets containing 1, 10, 100, or 1000 ppm (w/w) BB-8 for 28 days. Rats were sacrificed from each group after 2 and 4 wk feeding and after 2, 6, and 18 wk recovery. Ten ppm was an effect level as judged by bromine accumulation in fat, liver, and muscle. Bromine levels in body fat did not decrease during the recovery period. Bromine analysis was by neutron activation. One hundred ppm was an effect level as judged by liver weight to body weight ratios and histopathologic examination of tissues. Three groups of primigravid rats were fed diets containing 100, 1000, or 10,000 ppm (w/w) BB-8 from day 6 through 15 of pregnancy. The pups were delivered by Caesarian section on day 20. Anasarca was observed in one fetus at each of the two highest levels. Gastroschisis was observed in another fetus at each of these levels. No other gross effects were observed in either the mothers or the pups. Dose-related levels of bromine were found in the fetuses.

111. *Toxicology of Brominated Biphenyls. II. Skin. Eye, and Inhalation Toxicity and an Acute Test Method for Evaluating Hepatotoxicity and Accumulation in Body Fat.* J. G. AFTOSMIS, O. L. DASHIELL, F. D. GRIFFITH, C. S. HORNBERGER, M. M. MCDONNELL, H. SHERMAN, F. O. TAYFUN, and R. S. WARITZ, E. I. du Pont de Nemours and Company, Newark, Delaware.

Octabromobiphenyl (BB-8) has low acute skin absorption toxicity in the rabbit, the approximate lethal dose (ALD) being >10 g/kg. One gram per kilogram applied daily for 10 days caused significant ($p < 0.01$) liver enlargement, but 0.1 g/kg did not. It did not cause primary irritation or sensitization in the guinea pig and was mildly irritating to the rabbit eye, but did not cause corneal irritation. Hexabromobiphenyl (BB-6) had a skin absorption ALD of 5 g/kg and caused significant ($p < 0.01$) liver enlargement at single doses of 1 g/kg and higher. Atmospheric levels

of >3.1 μg/l (as BB-8) of pyrolysis products from BB-8 heated at 290°C caused liver enlargement in rats after a 4-hr exposure. Ten 4-hr exposures to 3.1 μg/l did not cause liver enlargement, a characteristic toxic effect of many chlorinated and brominated biphenyls, which usually occurs only after repeated administration of the material. An acute predictive test is desirable for preliminary screening. It was found that a single ip injection of ~1 g/kg of various brominated and chlorinated biphenyls suspended in corn oil caused liver enlargement in rats in 7 days. The enlargement was significant at the 99% confidence limit (CL) after treatment with BB-6 or chlorinated biphenyl (68% Cl); enlargement was also significant at the 95% CL after treatment with BB-8 and nonabromobiphenyl. Decabromobiphenyl, polybrominated terphenyl and polychlorinated terphenyl (60% Cl) did not cause significant enlargement in this test. Body fat from rats treated with any of the brominated materials had excessive bromine levels when sacrificed 7 days after treatment.

112. *Metabolism of 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) in Rats.* W. N. PIPER, J. Q. ROSE, and P. J. GEHRING, The Dow Chemical Company, Midland, Michigan.

A definitive study of the pharmacokinetics of 2,4,5-T in animals has not been reported. The purpose of this investigation was to determine the rate of clearance of 2,4,5-T from the plasma and the rate of excretion of 2,4,5-T from rats administered single po doses of 2,4,5-T-^{14}C. Groups of three male and three female rats were given 5, 100, or 200 mg/kg of 2,4,5-T-^{14}C. Blood collected by orbital sinus puncture for up to 7 days was centrifuged and the plasma was analyzed for ^{14}C activity. Urine, feces, and expired air were collected every 24 hr and analyzed for ^{14}C activity. The half-lives ($t_{1/2}$) for clearance of ^{14}C from the plasma and the volume of distribution increased as the dose increased. The $t_{1/2}$ for the disappearance of ^{14}C activity from the body also increased as the dose increased. The results indicate that rate of clearance of 2,4,5-T from plasma and the body of rats decreases and that volume of distribution increases with increasing doses.

113. *Teratogenic Effects of 2,4,5-T in Mice.* E. ROSS HART and MARION G. VALERIO, Bionetics Research Laboratories, Inc., Falls Church, Virginia.

Two commercial samples of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) stated to be >99% pure were used. Solutions in propylene glycol or dimethyl sulfoxide were prepared daily and administered by sc injection at a dosage of 10 or 100 mg/kg to pregnant female CD-1 mice. Comparable volumes of the solvents were used as controls. Administration began on day 6 of pregnancy and continued through day 15. On day 18, the mice were killed, and uterine contents carefully examined grossly. Subsequent examination was by the Wilson technique for soft tissue abnormalities and after clearing and staining with Alizarin Red S for skeletal abnormalities. At the larger dose, one sample produced a marginally significant increase in incidence of cleft palate as compared with solvent controls. Other abnormalities were observed only in very small numbers and so scattered as to preclude any causal relationship to the treatment.

114. *The Metabolism and Distribution of 2,4,5-Trichlorophenoxyacetic Acid in Female Rats.* S. C. FANG, M. L. MONTGOMERY, and V. H. FREED, Oregon State University, Corvallis, Oregon.

^{14}C-labeled 2,4,5-trichlorophenoxyacetic acid (2,4,5-T-1-^{14}C) was fed po to adult rats at a dose of 0.04–10 mg per rat, and expired air, urine, feces, and internal organs, and tissues were analyzed for radioactivity. No ^{14}C was found in the expired air during a 7-day period following dosing. During the first 24 hr, 75 ± 7% of the radioactivity was excreted in the urine and 8.2 ± 4.6% in the feces. The average recoveries after 7 days were 85% in the urine and 11% in the feces. Between 90 and 95% of the radioactivity in the first and second day urine samples was unchanged 2,4,5-T. There was no significant difference in the rate of elimination between rats receiving 0.04, 1, or 10 mg of 2,4,5-T. Radioactivity was found in all organs and tissues examined. The maximum concentration in all tissues was generally reached 6–12 hr after dosing and then started to decline rapidly. The highest concentration of 2,4,5-T was found in the kidneys; the next highest was in the blood, lung, heart and liver, respectively. The average

biological half-life of 2,4,5-T in the blood and vital organs was 3.5 hr. Very little radioactivity remained in the organs after 3 days. The excretion and tissue distribution of 2,4,5-T after multiple doses in pregnant rats were also investigated. (Supported by USPHS Research Grants ES 00141 and ES 00210.)

115. *Studies on Rat Reproduction and Guinea Pig Teratology of Carbaryl Fed in the Diet* vs. *Stomach Intubation.* C. S. WEIL, M. D. WOODSIDE, J. B. BERNARD, N. I. CONDRA, and C. P. CARPENTER, Carnegie-Mellon University, Pittsburgh, Pennsylvania.

These studies were undertaken to ascertain if differences in effect of carbaryl on reproduction and teratology were related to the two modes of po administration: gastric intubation or dietary feeding. Following repeated intubation, reduced reproduction of rats had been reported at low dose levels and teratology was reported in guinea pigs at an extremely high level. Randomized groups of rats were, therefore, concurrently presented daily doses of carbaryl by gastric intubation or by dietary inclusion. Each parental generation was bred three times during a year of dosing, and the F_{1a} generation bred to produce the F_2. The F_2 rats, in turn, were bred (a) to produce F_{3a} rats which were killed for micropathological examination; (b) to produce F_{3b} fetuses for teratological study; and (c) for a dominant-lethal test. Currently, the primary differences seen include signs of cholinesterase-inhibition and deaths in the highest intubation level of 100 mg/kg/day, as contrasted to no symptoms or mortality at 200 mg/kg. No consistent reproductive effects have been seen in any group of rats, as contrasted to previous reports by the Russians of reduced reproduction at 5 mg/kg. Guinea pigs were similarly dosed by gastric intubation or by inclusion of carbaryl in their diets on selected days of their gestation period during organogenesis. While some skeletal and visceral abnormalities previously had been reported at the 300 mg/kg LD50 level following intubation, no gross abnormalities were found in this concurrent study from dose levels as high as 200 mg/kg by gastric intubation or from 300 mg/kg in the diet. These concurrent studies demonstrate for carbaryl (a) the differences in results seen between gastric intubation and dietary administration and likewise (b) the dose-related incidence of teratology, if and when it is found.

116. *Use of the Fathead Minnow (Pimephales promelas Rafinesque) in Estimating the Toxicity of Chemicals to Fish.* C. J. TERHAAR, W. S. EWELL, and S. P. DZIUBA, Eastman Kodak Company, Rochester, New York.

There is an increasing need to characterize the toxicity of materials being discharged into receiving bodies of water. Over the past 5 yr we have attempted to measure the toxicity of many chemicals to fish. Several species have been used including various species of trout, goldfish, catfish, bluegills, and fathead minnows. The fathead minnow appears to be the species of choice in our laboratory because it is a cosmopolitan species, is readily available from commercial sources, is used in other laboratories, is susceptible to many toxins and is relatively resistant to temperature changes. Data will be presented on several compounds relating chemical structure and photodegradation to toxicity to fish. Methods for the analysis of these data will also be discussed. A static and a dynamic system of exposure, with and without simulated sunlight (Xenon arc), will be described.

117. *Toxicity of Phthalate Esters in Tissue Culture: Effects on Growth, Amino Acid Uptake, and Protein Turnover.* E. O. DILLINGHAM, CHENG-HSIEN WU, and J. AUTIAN, University of Tennessee Medical Units, Memphis, Tennessee.

Mouse fibroblast cells, strain L-929, were grown in monolayer cultures and examined for inhibition of growth (protein assay), rate of ^{14}C-amino acid uptake per cell in replicating and nonreplicating cultures, and for protein turnover (by following relative dilution of label in labeled cell populations grown in unlabeled medium) with respect to concentration of dimethyl and diethyl phthalate. Growth inhibition was observed at 0.002 M concentration with both compounds, but the inhibition of growth with respect to concentration was greater with dimethyl phthalate. The rate of protein synthesis (amino acid incorporation) showed a peak at 24 hr in the control cultures which was suppressed with increasing concentration with both

compounds. In the controls, the rate of protein synthesis decreased to the level at zero time by 48 hr and showed a six-fold increase over the zero time rate by 96 hr. At concentrations of 0.004 M diethyl phthalate or less, the protein synthesis rate approached that of the controls at 144 hr. At concentrations above 0.004 M, the rate remained suppressed or decreased. Non-replicating cells showed relatively uniform protein synthesis rate through 72 hr with a slight increase at 96 hr. Increasing rates of protein turnover were observed with respect to concentration; an approximately four-fold increase occurred with respect to the controls at a toxic concentration (0.01 M) which completely inhibited growth. Concentrations of dimethyl phthalate effecting significant inhibition by 48 hr show little inhibition at 24 hr, suggesting a specific inhibition of cell division followed by increased protein turnover rates. This increased turnover was also observed with diethyl phthalate and was correlated with growth inhibition. Membrane instability in the presence of elevated protein turnover may be an important factor in phthalate ester toxicity.

118. *Pulmonary Function Studies of Men Exposed for 120 Hours to Sulfur Dioxide.* F. W. WEIR, D. H. STEVENS, and P. A. BROMBERG, The Ohio State University, Columbus, Ohio.

This report represents a second set of experiments in a continuing investigation designed to quantify effects of exposures of healthy subjects to low levels of SO_2. Results of earlier experiments indicated the presence of increased small airway resistance after exposure to 3 or 6 ppm SO_2 as indicated by development of frequency dependence of dynamic compliance. During the present study, four groups of three healthy males were continuously exposed in randomized sequence for 120-hr periods to 0, 0.3, 1, and 3 ppm SO_2. Exposures were conducted in a 30 m^3 dynamic flow environmental chamber. Temperature was maintained at $22 \pm 1°C$; relative humidity at $50 \pm 5\%$. Daily pulmonary function measurements included airway conductance at several lung volumes, functional residual capacity, frequency dependence of dynamic lung compliance, total lung volume, single breath, and continuous nitrogen washouts, timed vital capacity and maximal expiratory flow–volume curves. On the last day of each exposure, pulmonary gas exchange was evaluated before and after a series of maximal inspirations. Measurements were made of minute ventilation, expired gas O_2 and CO_2 concentrations; alveolar–arterial O_2 tension gradients were calculated. No dose-related changes were observed in subjective complaints, clinical evaluation, or most pulmonary function measurements. Significant but minimal, reversible decreases were noted in airway conductance and in compliance at high frequencies at the 3 ppm level. These results provide baseline information for future experiments using SO_2 and particulates on healthy subjects and on subjects with chronic obstructive pulmonary disease. (Supported by Research Contract No. CAWC 8-15 from the American Petroleum Institute.)

119. *Attempt to Demonstrate S-Sulfonate in Human Plasma.* A. R. GUNNISON and E. D. PALMES, New York University Medical Center, New York, New York.

Endogenous *S*-sulfonate compounds have been demonstrated in the plasma of unexposed rabbits and the levels have been increased manyfold in this species by exposure to sulfite by several routes including po in drinking water, iv injection, and inhalation as sulfur dioxide. Endogenous *S*-sulfonates have not been found by us in normal human plasma. In preparation for analysis of samples of plasma of exposed persons to be supplied by Weir and coworkers in the Department of Preventive Medicine at Ohio State University, we demonstrated the in vitro formation of *S*-sulfonates in human plasma and found that this species is quite stable during storage at low temperature. At O.S.U. four groups of three young males each were to be continuously exposed for 120-hr periods to each of the following concentrations of sulfur dioxide. 0, 0.3, 1, and 3 ppm; this would require a total of 16 exposure weeks. Plasma samples were to be taken immediately following the exposure periods and shipped to us for analysis for *S*-sulfonate formed in vivo. One half of the exposures including all four concentrations have been completed, and samples taken following exposure have been analyzed. The results to date have shown neither exogenous *S*-sulfonate as a result of sulfur dioxide exposure nor endogenous *S*-sulfonate in control plasma at levels detectable by the colorimetric method used.

120. *Long-Term Exposure of Primates and Guinea Pigs to Mixtures of Sulfur Dioxide and Fly Ash.* R. J. KANTZ, II, W. M. BUSEY, Y. ALARIE, C. E. ULRICH, and A. A. KRUMM, TRW/Hazleton Laboratories, Inc., Vienna, Virginia. (J. W. Clayton, Jr.)

Four groups of nine cynomolgus monkeys, both male and female, and four groups of fifty male and fifty female Hartley strain guinea pigs were exposed by inhalation to mixtures of sulfur dioxide (SO_2) and fly ash (FA). Contaminant concentrations for these exposures were 0.1, 1.0, and 5.0 ppm SO_2 and 0.5 mg/m^3 FA. The duration of exposure was 22 hr/day, 7 days/wk for 52 wk for the guinea pigs and 78 wk for the monkeys. Measurements of body weight, pulmonary diffusion, total airway resistance, and biochemical determinations were made during pre-exposure and periodically throughout the study for both species. In addition, the distribution of ventilation and arterial blood gas measurements were performed on the primates. At the termination of the exposure, all surviving animals were sacrificed and necropsied. Tissues from the respiratory system and the major organs were examined microscopically. Measurements of body weight, growth, survival, and pulmonary function revealed no deleterious effects from the exposures. Hematological and serum biochemical determinations were not affected by the exposures. Similarly, no adverse effects attributable to SO_2 or FA were inferred from the organ weights or organ/body weight ratios of the major organs. (Supported by the Air Pollution Research Program of the Electric Research Council.)

121. *An Indirect Action of Ozone on the Pulmonary Macrophage.* DONALD E. GARDNER, Environmental Protection Agency, Research Triangle Park, North Carolina. (D. L. Coffin.)

Alveolar macrophages (AM) may be washed out of the lungs and maintained for short periods for study of their stability, viability, and phagocytic function. A model system was developed for such studies. It was found that when AM were separated from the acellular portion of the lavaged fluid and maintained at 37°C in physiological saline, they were very unstable. However, stability of AM was markedly improved when they were maintained in lavaged fluid from the lung. It was, therefore, hypothesized that a protective factor was present in the lung fluid. Using this model system, it was noted that the exposure of animals to ozone had an adverse effect on AM stability in vitro. A dose-response relationship was established over the range of concentrations of 0.1–10.0 ppm for 2.5 hr. A similar loss of cell stability was observed when normal AM were suspended in normal acellular lavaged fluid which had been previously exposed to 30 min of ozone in vitro. The data indicates that the deleterious effects of ozone on lung cells may be due to an indirect action mediated by the presence of a protective factor in lung fluid. Alveolar macrophages from ozone exposed animals could be partially protected if they were transferred to normal lavage fluid. The in vitro phagocytic capabilities of the normal AM in ozone-treated lavaged fluid were not adversely affected. It is believed that this is the first evidence for an indirect action of ozone on alveolar macrophages mediated by the presence of a protective factor in lung fluid.

122. *Altered Central Nervous System Function Resulting from Ozone Exposure.* C. E. ULRICH and M. F. SOBECKI, TRW/Hazleton Laboratories Inc., Vienna, Virginia. (J. W. Clayton, Jr.)

A methodology was developed for evaluating central nervous system (CNS) effects applicable to inhalation toxicology studies. The basis of the method is the concept of overtaxing the CNS with closely spaced pairs of light flashes and evaluating the resulting evoked responses. Averaging techniques were used to extract the evoked responses from background electrical activity. Latencies of the evoked responses were the measured variable. The effect of ozone at 1.5 ppm for 4–6 hr on rats with chronically implanted electrodes was evaluated with this methodology. Electrodes were sterotaxically implanted in the red nucleus, mid-brain reticular formation, ventral–medial nucleus of the thalamus, posterior nucleus of the hypothalamus, and the visual cortex. The results indicate that O_3 produces an increase (18–26%) in the evoked response latencies and that the effect is similar for all areas of the brain investigated. Overtaxing the CNS was shown to increase the sensitivity of the method for detecting CNS changes during these exposures.

123. *Chronic Oral Toxicity of Cannabinoids in Rats.* GEORGE R. THOMPSON and MONIQUE C. BRAUDE, Mason Research Institute, Worcester, Massachusetts; NIMH, Rockville, Maryland. (M. M. Mason.)

The preclinical toxicities of Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-THC and a crude marihuana extract (CME) were evaluated in Fischer rats. The compounds were dissolved in sesame oil and administered po for a maximum of 119 consecutive days at doses of 50, 250, 400, or 500 mg/kg/day for Δ^9- and Δ^8-THC, or 150, 750, 1200, or 1500 mg/kg/day for CME. These high dose levels were 30–300 times the usual po dose in man and were chosen specifically to induce toxicity in rats and not to simulate human doses. Cumulative toxicity was indicated by significantly increased mortalities in groups treated with the two largest doses of each compound when compared to mortality levels for these doses in acute toxicity studies. For example, daily treatments with the single dose LD1 and LD4 for Δ^9-THC produced 23 and 70% mortalities in male rats treated in the present study. In addition, a higher incidence of mortalities occurred in females than in males, but essentially all deaths in both acute and chronic studies with all three compounds occurred during the interval 36–72 hr postinitial treatment. Durations of hypothermia and hypopnea coincided with the interval for mortalities, and the severities of temperature and respiration decrements were dose related. Growth rates in all treated groups were significantly decreased throughout the treatment period despite an increased consumption of food and water after approximately day 50, and abdominal fat stores were depleted at extended periods. The severities of decreased growth rates were also dose-related, and the smallest dose of each compound decreased the growth rate by approximately 30%. Organ weight changes indicated atrophy of uterus, prostate, and spleen, and hypertrophy of adrenal and liver, but pathology in these organs was limited to splenic hypocellularity and occasional hyperemia and necrosis of the adrenal. Hematological parameters were generally normal. Moderately increased SGOT and markedly decreased coagulation times were the primary blood chemical changes. Toxicity induced by doses from 30–300 times the human po dose was similar for the three compounds and suggested a prominent effect on endocrinological systems. (Supported by NIMH Contracts HSM-42-70-95 and HSM-42-71-79.)

124. *Marihuana Related Pathology in Rats.* MARCUS M. MASON and GEORGE R. THOMPSON, Mason Research Institute, Worcester, Massachusetts.

The toxicity of large doses of marihuana in animals is critical for predicting possible effects in man. Single po doses of Δ^9-tetrahydrocannabinol, Δ^8-THC, and crude marihuana extracts (CME) in rats are relatively nontoxic (average LD50 for Δ^9-THC is 1130 mg/kg; Δ^8-THC is 1420 mg/kg and CME is 2340 mg/kg). Two synthetic cannabinols (Δ^9-THC and Δ^8-THC) and a crude marihuana extract (CME) were given to Fischer rats by gavage daily for 5, 28, 91, and 119 days at doses ranging from 50 mg/kg to 1500 mg/kg (30–300× the usual po dose in man). At doses above 400 mg/kg deaths occurred by day 3 due to a fall in blood pressure and central depression with little or no gross or cytological changes. At doses of the cannabinols below 400 mg/kg, severe depression was observed after the first three treatments, and then tolerance developed. Outstanding changes included retarded weight gains, increasing hyperactivity, and aggressiveness manifested in chewing of the feet and tails of cagemates. Light microscope histopathology seen in rats treated with any of the three compounds was similar as to organs affected. Splenic changes were seen as decreased size and development of the germinal centers and/or decreased numbers of lymphocytes in the red pulp. Bone marrow changes showed an increased fat deposition with a corresponding decrease of all cellular elements, a decreased number of megakaryocytes, many of which showed hyperchromatic or condensed nuclei. Testes of many rats showed delayed maturation in the spermatogonia with plugs of desquamated cells. These changes were mild and neither progressive nor cumulative. The adrenal changes were slight with an increase of soluble substances in the zona fasciculata of the cortex that disappeared during the process of making the slides. Hyperemia at the corticomedullary border and in the medulla was quite common. Necrotic changes seen in the adrenal only occurred in rats given high doses of Δ^8-THC. These lesions indicated that focal necrosis had taken place in the cortex followed by mineralization. Gross atrophic and hypoplastic cytological changes in

the uterus and prostate were not accompanied by obvious changes in the pituitary. Although most histopathological lesions were mild at these high doses, the involvement of the nervous system and the endocrine complex on a molecular level is strongly indicated. (Supported by NIMH Contracts HSM-42-70-95 and HSM-42-71-79).

125. *The Effects of Tobacco Smoke on Mucociliary Activity.* E. E. VOGIN, C. RUBENSTEIN, J. SCHEIMBERG, and S. CARSON. Food and Drug Research Laboratories Inc., Maspeth, New York.

Studies have been carried out evaluating exposure of cigarette smoke on the mucociliary activity of cats. Definite changes were shown in the flow and mucus viscosity of the mucociliary apparatus, and correlations were found relating these two, indicating that mucus flow appeared to be the dependent variable and mucus viscosity the independent variable. Additional studies have shown that the surface tension of the mucoid sheath in rats could be altered subsequent to insult to cigarette smoke; the same phenomenon was not true in cats. Further, while the resistivity was increased in rats following daily exposure to cigarette smoke for 75 days, the converse was true in cats. Additional analytical studies have indicated that while the direction of change of mucus flow and resistivity was the same in cats, subsequent to cigarette exposure no correlation existed between these two responses; each can therefore be treated as an independent variable with regard to the other. In a biophysical approach undertaken to evaluate the electronic aspect of the mucociliary system, resistivity curves show that the tissue may act as a semiconductor diode at low frequencies. A model has been made of the tracheal mucosa at low frequencies, and also an all-frequency model has been postulated which is substantiated by theories proposed by other investigators.

126. *Immunological Factors in the Etiology of Pulmonary Berylliosis.* ANDREW L. REEVES, ROBERT H. SWANBORG, and NEIL D. KRIVANEK, Wayne State University, School of Medicine, Detroit, Michigan.

Guinea pigs respond to intradermal injections of $BeSO_4$ with development of delayed hypersensitivity, and a study was conducted to clarify the correlation of this factor to pulmonary berylliosis. A colony of guinea pigs was divided into three equal groups. In one group, delayed hypersensitivity to $BeSO_4$ was produced as stated. The second group received low levels of $BeSO_4$ in the drinking water for the purpose of producing a state of immunosuppression. The third group of guinea pigs remained untreated. All groups were then subjected to the inhalation of $BeSO_4$ aerosol at selected atmospheric concentrations, and the histopathologic and hematologic response was determined at periodic necropsies. The typical response in the lungs was pneumonitis with occasional granulomas, fibrotic nodules, and alveolar epithelial metaplasia. The severity of these lesions was correlated to the extent of exposure, but in each exposure group the intradermally treated animals showed an alleviated tissue reaction. Lung weight/body weight ratios showed significant inverse correlation to the size of the delayed cutaneous hypersensitivity reaction in individual animals. These results suggest that an association exists between the immune status of the host and its vulnerability to beryllium inhalation. (Supported by U.S.P.H.S. Grant No. ES-00353-04.)

127. *Fluoride Intoxication: Correlation of the Fate of ^{18}Na with Fluoride Effects upon Glucose Catabolism in the Rat.* R. M. KNAUS, F. N. DOST, and C. H. WANG, Oregon State University, Corvallis, Oregon.

Fluoride (F^-) is known to inhibit the glycolytic enzyme enolase in vitro at concentrations of about 10^{-3} M. This report describes depressed glucose catabolism in intact rats when concentration of F^- in body fluids reached 10^{-4}–10^{-3} M. Rats (300 g) were brought to a metabolic steady state with respect to specifically labeled glucose-^{14}C, continuously infused iv at 150 mg/hr for 11 or 17 hr. F^- was infused from the fifth to eighth hr at rates of 0.025, 0.05, and 0.1 mmol/hr. The distribution and tissue concentration of F^- was measured in intact rats by using ^{18}F as a tracer in identical experiments. Depression of glucose catabolism by F^- is dose-dependent and is maximum at nonlethal fluoride concentrations in body fluids and soft tissues of about 10^{-3} M. At dose rates of 0.025 and 0.05 mmol F^-/hr, the catabolic rate of infused glucose

decreased sharply and returned to normal within 3 hr after the end of F^- administration. A higher dose of 0.1 mmol/kg caused a similar decrease in catabolism of glucose which persisted several hours longer. A direct relation between ^{18}NaF dose rates and blood fluoride concentration was apparent through 3 hr of infusion of up to 0.1 mmol F^-/hr/rat. Higher F^- intake exceeded capability for disposition in bone and by renal excretion, resulting in accumulation in soft tissues. It is suggested that the observed inhibition by F^- of glucose oxidation in intact rats is a manifestation of inhibition of enolase activity.

128. *Two Active Structures for Hemicholinium-like Action.* BARRIE BLASE, TED A. LOOMIS, and HAROLD Z. SOMMER, University of Washington School of Medicine, Seattle, Washington.

The hemiacetyl structure has been found necessary for compounds to exhibit hemicholinium action. This report describes the hemicholinium action of two bis-quaternary compounds (ie, 2-hydroxyethyldimethylamino derivatives) in which the positively charged nitrogens are connected by either the 4(tetramethylene-2-one)-phenacyl chain or by the same chain in which the ketonic groups have been reduced. The former compound exists in solution in the "hemiacetyl" or closed ring form, and the latter exists in solution as the "seco" or open ring form (verified by IR and UV absorption data). In vitro tests showed neither compound was an effective anticholinesterase agent. Studies in the intact rat anterior tibial nerve muscle preparation showed that the two compounds were equal in type of activity and potency, and the effect was reversed by choline. In the isolated nerve–diaphragm preparation of the rat, the "seco" form required a longer duration to produce maximal depression of tetanus; it also produced a significantly greater depression of tetanus amplitude than that produced by equal doses of the "hemiacetyl" form. These results indicate that both the "seco" and "hemiacetyl" forms of the 2-hydroxyethyldimethylamino analogues are active structures for hemicholinium action.

129. *Studies on the Nature of Cholinergic Receptors: Dissociation Constants of Fluoro-Chloro-, Bromo-, and Iodo-acetylcholines at Muscarinic and Nicotinic Receptors.* B. V. RAMA SASTRY, H. C. CHENG, and L. K. OWENS, Vanderbilt University School of Medicine, Nashville, Tennessee.

At the surface of both types of cholinergic receptors, muscarinic (M) and nicotinic (N), there is an anionic site and a site 2 (possibly cationic) where the quaternary ammonium group and one of the two oxygen atoms of the ester group (ether oxygen in case of M-receptor, carbonyl oxygen in case of N-receptor) of choline esters interact to produce the pharmacological response. The electron environment of the two oxygen atoms and the size of the acyl radical of acetylcholine (ACh) could be modified in a well defined manner by replacement of one of the hydrogen atoms of the acetyl group by F, Cl, Br, or I. Therefore, we synthesized monohalogeno-ACh (FACh, ClACh, BrACh, and IACh) and studied their dissociation constants (K_A) and relative intrinsic efficacies (E) at M-receptors of the guinea pig longitudinal ileal muscle and N-receptors of the frog rectus abdominis muscle. All monohalogeno-ACh had a higher K_A ($K_A \times 10^5$: 1.49–4.69) and lower E (0.40–0.46) than those of ACh (K_A: 1.08×10^{-6}, E: 1.00) at the M-receptor. The steric volume occupied by the acyl radical increases from ACh to IACh (49 to 321.6 cubic A). Therefore, the steric factors will significantly limit the interaction of acyl radicals of halogeno-ACh at the site 2 of the M-receptor. The K_A and E values at N-receptors have the following orders—K_A: IACh[<ACh] < BrACh ≤ ClACh ≤ FACh; E: IACh = BrACh = ClACh[>ACh] ≥ FACh. The electron density of the carbonyl oxygen decreases from IACh to FACh. The affinity ($1/K_A$) and E at the N-receptor decrease with decreasing electron densities of carbonyl oxygen atoms of halogeno-ACh. Therefore, halogeno-ACh bind at the site 2 of N-receptors through negative charges of their carbonyl oxygen atoms. (Supported by U.S. PHS Grant No. NS-04699.)

130. *Effect of Acetylcholine on the In Vitro Blood Clotting Time of Human Blood.* JASBIR M. SINGH, VICKY COSTANZA, and MELBRA DIANE SINGH, Xavier University College of Pharmacy, New Orleans, Louisiana.

In order to study the effect of acetylcholine (ACh) on human blood clotting time in vitro, two sets of experiments were performed. In the first, one stage prothrombin time (SPT),

different concentrations of ACh were mixed with thromboplastin (Simplastin), 0.2 ml of this mixture was added to 0.1 ml of plasma, and SPT was determined. In related experiments 0.2 ml of thromboplastin were added to plasma containing ACh, and SPT was determined with automatic clot timer. In the second set, thrombin solution (0.2, 1.0, and 2.0 units) was mixed with different concentrations of ACh, then 0.1 ml of this mixture was added to 0.2 ml of plasma and thrombin time was determined. Also, 0.2 ml of thrombin-ACh mixture was added to 0.2 ml of purified 1% fibrinogen solution, and the thrombin time was determined. It is suggested from our experimental results that ACh affects the clot-promoting activity of thromboplastin, enzyme thrombin and substrate fibrinogen, and these effects of ACh are dose dependent.

131. *The Action of Pesticides on Conduction in the Rat Superior Cervical Ganglion Preparation In Vitro.* ERNEST WHITCOMB, Environmental Protection Agency, P.O. Box 490, Perrine, Florida.

The findings of some investigators have suggested that DDT is capable of altering the stability of the nerve fiber. The present experiment was designated to determine if DDT, carbaryl, and chlordane, could alter the propagated action potential in the nerve fiber and at the synapsis using the superior cervical ganglion preparation of the rat. The in vitro recording from nontreated animals had the following characteristics: (1) preganglionic nerve fibers membrane requiring approximately 400 msec for 100% recovery; (2) no evidence of spontaneous preganglionic firing; (3) no synchronized firing of postganglionic fibers via stimulation of the preganglionic nerve. Carbaryl and chlordane alter the control pattern by shortening the recovery time. DDT lengthened the recovery time. The lengthened recovery time and absence of spontaneous firing in preparations from DDT-treated animals would not implicate peripheral nerve components as a source for tremors seen in the intact treated animal.

132. *Effect of Reserpine and Atropine Sulfate on the Development of Tolerance to Pentobarbital.* JASBIR M. SINGH and MELBRA DIANE SINGH, Xavier University College of Pharmacy, New Orleans, Louisiana.

During the development of tolerance to pentobarbital, the hypnotic effect (HE) or sleeping time (ST) is decreased on subsequent administrations. Percentage Tolerance Index (PTI) is determined as the ratio of HE or ST of first injection/second or subsequent injection times 100. If PTI is less than 100 it is indicative of cumulative effect; if it is greater than 100, it indicates tolerance. Significant PTI arises from paired comparison of ST or HE between groups. The tolerant effect of pentobarbital is reversed by acute administration ip of atropine sulfate (0.2, 0.4, and 0.6 mg/kg), since PTI of atropine sulfate-treated animals was nonsignificant, whereas PTI was significantly greater than 100 in animals not treated with atropine sulfate. In this experimental design, initial PTI was determined on days 1 and 2. No drug treatment was given from days 3–7. On days 8 and 9, atropine sulfate was administered with pentobarbital. Reserpine (2.5 mg/kg) treatment for 3 days in animals whose initial PTI had been determined produced cross tolerance to pentobarbital or significantly greater tolerance to HE. It is proposed from our experimental results that the development of tolerance to pentobarbital is affected by atropine and reserpine treatment.

133. *High Nicotine Tolerance of Syrian Golden Hamsters.* P. BERNFELD and F. HOMBURGER, Bio-Research Consultants, Inc., Cambridge, Massachusetts.

The availability of over twenty inbred strains of Syrian golden hamsters made it possible to document the remarkable lack of susceptibility of this species to nicotine poisoning and to demonstrate the strain-dependence of this phenomenon. Nicotine was administered in two ways: ip as the tartrate, followed by observation of the mortality within 30 min; and by exposure of the animals to 1:5.2 diluted, whole, fresh smoke from Kentucky Reference cigarettes and recording of the survival time. Smoke exposure was achieved in a Walton-Morrissey Reverse Smoking Machine which delivers successive 1-min cycles, each consisting of a 17-sec period of smoke followed by a 43-sec period of fresh air. The LD50 of ip nicotine tartrate, as determined

in groups of five animals, ranged from 125–320 mg/kg of body wt in five inbred hamster strains; the 15.16 line exhibited the highest value. It was significantly lower in rats (83 mg/kg) and considerably less in Swiss mice (13 mg/kg). The tolerance to whole cigarette smoke, expressed as mean survival times in groups of 12 animals, was again highest in the 15.16 line of hamsters 244 min); it ranged from 86 to 178 min in three other hamster strains, but these values did not parallel the corresponding LD50 values of ip nicotine tartrate. The tolerance of whole smoke was remarkably smaller in rats (20 min). It is apparent from these data that inbred hamster strains in general, and the 15.16 line in particular, lend themselves notably well to smoke inhalation studies.

134. *Early Effects of Thiamylal on Lidocaine Toxicity in Rhesus Monkeys.* MAJOR L. COHN, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. (M. Eisler.)

Lidocaine toxicity is manifested by convulsions, respiratory depression, and profound hypotension. Barbiturates are believed to protect against the toxic effects of local anesthetics in the central nervous system and enhance cardiovascular toxicity. The purpose of this research was to evaluate some early effects of thiamylal in lidocaine-induced toxicity in the Rhesus monkey. Rhesus female monkeys (*Macacca mulatta*) were premedicated with atropine 0.01 mg/kg iv. Anesthesia was induced with 40% oxygen, 60% nitrous oxide, and gallamine triethiodide, 2 mg/kg iv. Common bile duct, subarachnoidal space, and urinary bladder were catheterized, and the animals stabilized during a 1-hr period. Lidocaine was infused at the rate of 0.458 mg/kg/min until hypotension occurred. To the lidocaine infusion, thiamylal was added at the rate of 0.191 mg/min/kg. The administration of lidocaine–thiamylal to these animals resulted in a prompt rise in cardiac output and heart rate, and an increase of arterial blood pressure (ABP) of at least 30%. The lidocaine-induced seizures were not immediately reversed with the rise in ABP. There are several explanations of the mechanism of pharmacological antagonism of lidocaine by thiamylal. We have obtained evidence that thiamylal enhances the rate of excretion of lidocaine via the bile and the urine. We have also found significant alteration of lidocaine metabolites in the bile, blood, CSF, and urine of these monkeys. Lidocaine levels were significantly decreased in the blood and CSF secondary to thiamylal administration, whereas hydroxylated metabolite in bile, CSF, and urine rose acutely. Thiamylal is useful in reducing lidocaine toxicity as evidence by our findings of a two- to three-fold increase in survival time.

135. *Determination of the Ototoxicity of Ethacrynic Acid and Furosemide in the Dog.* W. J. GOLDMAN, T. C. BIELENSKI, and P. A. MATTIS, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

Studies have been carried out to determine the ototoxic effects of drugs in the dog. Silver–silver chloride ball electrodes were placed on the round window in the auditory bulla exposed by an inferior-ventral approach. Sound stimuli consisted of pure tones between 1.5 and 3.3 Hz. The input signal was carried to the external meatus by an earphone. Each dog was tested at its individual peak frequency determined prior to drug administration. After baseline levels had been determined for each animal, cochlear microphonic potentials (CMP) were followed for 4–6 hr following single or cumulative iv administration of the drugs. Studies assessing the ototoxocity of ethacrynic acid (EA) in the dog demonstrated that an iv dose of 40 mg/kg produces a 100% reduction of the CMP within 15 min. The minimum effective diuretic dose of EA in dogs is 0.5 mg/kg. This depression was followed by a slow recovery to 57% of predrug values within 3 hr, with no additional recovery observed at 4 hr. Lower doses of EA produced correspondingly lower reductions in the CMP at a slower rate with supracompensatory recovery to levels 21% greater than predrug levels at 10 mg/kg and no ototoxic effect at 5 mg/kg. On the basis of dose, the ototoxic effects of furosemide (F) were less than those observed with EA, but lasted longer. A 40 mg/kg iv dose yielded approximately a 50% reduction of the CMP, while at the same time an overall reduction of the CMP in terms of initial baseline values was observed after 4–6 hr. The minimum effective diuretic dose of F in dogs is 0.5 mg/kg. The

duration of the CMP reduction and subsequent recovery was quite rapid, occurring within 10–30 min. However, when recovery proceeded to values from 30 to 80% of the initial CMP, a secondary but variable reduction of the CMP occurred.

136. *Comparison of Ethanol and Acetaldehyde Induced Hyperglycemia in the Rabbit.* LAWRENCE W. MASTEN and HERBERT H. CORNISH, University of Michigan, Ann Arbor, Michigan.

In order to determine if acetaldehyde alone could account for the major hyperglycemic response in ethanol intoxication, 2.5–3.5 kg male white Flemish Giant rabbits were infused iv over a 4-hr period with acetaldehyde. Blood acetaldehyde levels roughly simulated those normally found in animals receiving 2.0 g/kg ethanol iv over a 1-hr period. Results of this study indicate that, based on comparable levels of blood acetaldehyde, hyperglycemia was significantly greater in the acetaldehyde infused animals than in those treated with ethanol. In addition the peak time for this hyperglycemic response in the ethanol treated animals lagged 8–10 hr behind that of the acetaldehyde infused animals, even though comparable acetaldehyde blood levels were maintained. Possible mechanisms to explain these two characteristic differences were explored. An attempt was made to differentiate between CNS mediation and hormonal control of the hyperglycemic response to ethanol or acetaldehyde. These studies include the use of stimulants and depressants in evaluating CNS involvement and the use of a variety of blocking agents in evaluating the hormonal control of the hyperglycemic response. (Supported in part by NIH Training Grant ES00106).

137. *In Vitro and In Vivo Structure–Activity Relationships in a Series of Methyl and Halogen Substituted Alcohols.* E. O. DILLINGHAM, R. W. MAST, G. E. BASS, and J. AUTIAN, University of Tennessee Medical Units, Memphis, Tennessee.

Series of methyl- and halogen-substituted alcohols were examined for toxicity in tissue culture (ID50, the concentration required to produce 50% inhibition of growth and T_i, the intrinsic toxicity or slope of the dose-response curve), for hemolytic potential (H50, the concentration required to produce 50% hemolysis) and for acute toxicity in mice (LD50). ID50 and H50 data were highly correlated ($r = 0.984$); however, hemolysis required approximately 20-fold higher concentrations. LD50 data were not highly correlated with ID50 or H50 data. Structure–activity analysis gave an explained variance of 92% with ID50 data, 94% with H50 data, and 25% with LD50 data. Structure–activity analyses were carried out by a linear free energy method employing the lipophilicity factor, P (the octanol–water partition coefficient), charge factors, and a steric factor. Explained variance was found to be more useful than the multiple correlation coefficient in assessing the contribution of the several factors to biological activity (BA). The steric parameter at C_2, a charge parameter at C_2 and P were found to be most highly correlated with BA. Charge factors and P^2 were significant factors in the analysis of LD50 data, although one subset of compounds showed highest correlation with P and the steric factor at C_2. In general, the significance of charge parameters was associated with higher lipophilicity. The ratio of T_i/P to LD50 for the methyl substituted compounds was approximately 10 (extreme variation of a factor of 3), whereas T_i, P, and LD50 values varied by factors of 60 to 100. The subset of halogen-substituted compounds gave an order of magnitude higher ratios, indicating a significantly different mechanism of action of those compounds. The halogen substituted compounds were less homogeneous with respect to structural homology and showed greater relative variation in the ratios. The results were consistent with a thermodynamic equilibrium model of structure-activity and indication of the usefulness of cellular toxicity in the prediction of in vivo toxicity.

138. *Actions of Dieldrin on the Uptake of Testosterone-1,2-H^3 by Prostate Glands of the Male Mouse.* J. A. THOMAS, M. T. SMITH, M. G. MAWHINNEY, C. G. SMITH, and J. J. MCPHILLIPS. West Virginia University Medical Center, Morgantown, West Virginia.

Male mice were treated with varying po doses of dieldrin for periods up to 10 days. An po regimen of 1.25, 2.5, or 5 mg/kg of this organochloride caused decreases in the prostatic uptake of radioactive testosterone. The highest dose (5 mg/kg daily × 10) caused a 42% reduction in

the assimilation of radiosteroid ($P \leq 0.05\%$). Lower dose regimens were not as effective in lowering androgen uptake by the prostate gland. While the chronic administration of dieldrin (10-day dose schedule) lead to reductions in prostatic uptake of testosterone-1,2-H^3, acute administration (2 hr) produced an increase in the assimilation of radiosteroid. This transient increase in the uptake of radiosteroid by dieldrin did not appear due to increased target organ protein metabolism since both pesticide-treated groups and control mice demonstrated similar incorporation rates of leucine-^{14}C. Preliminary studies with the herbicide 2,4,5-T revealed that it too may alter the assimilation of radiosteroid in male accessory glands of reproduction. This organochloride failed to alter testicular weights. (Supported by grants from the West Virginia School of Medicine and the Environmental Protection Agency, EP GM00871-01.)

139. *Gonadal Function in Mice Exposed Prenatally to p,p'-DDT.* J. A. McLachlan and R. L. Dixon, National Cancer Institute, Bethesda, Maryland.

It has been recently reported that DDT treatment during the neonatal period results in the persistent estrous syndrome and sterility in the adult female rat. Previous experiments have shown that exposure to DDT during the preimplantation stages of pregnancy resulted in retarded fetal growth. The current study was undertaken to evaluate the effects of prenatal treatment with this common pesticide on the reproductive capacity of the offspring. Pregnant Swiss-Webster mice were given *p,p'*-DDT (1 mg/kg) on days 10, 12, and 17 of gestation. At varying intervals postpartum representative male and female offspring were sacrificed, and the ovaries, testes, adrenals, and pituitaries processed for histolopathological examination. The onset of puberty was determined for the remaining newborn, and the estrous cycles of the females monitored. When the treated offspring attained puberty their reproductive capacity was evaluated. Fertility, forced breeding performance, birth intervals, fertility span, litter sizes, and lordosis quotients were determined for the treated female offspring. The fertility profiles of the males were obtained by serial mating techniques. The DDT treatment did not exert any gross teratogenic effect. The cellular components of the gonads were more significantly altered in the prenatally treated female offspring than in the corresponding males. Likewise, the decrease in fertility was more marked in the female. These results suggest that the functional status of the gonads may be altered in mice exposed prenatally to DDT.

140. *Localization of DDT-^3H in Male Reproductive Organs and its Actions Upon Androgenic Function.* M. T. Smith, J. A. Thomas, C. G. Smith, M. G. Mawhinney, and J. J. McPhillips, West Virginia University Medical Center, Morgantown, West Virginia.

Single po doses of radioactive DDT administered to male mice produced significant amounts of tritium in several organs of reproduction. The prostate glands and the testes contained very high amounts. Labeled DDT or its metabolites was also found in the seminal plasma. Residual amounts of radioactivity were detected in epididymal fat pads as long as 12 days after the single administration of DDT-^3H. Nonreproductive tissues such as the liver, kidney, and the adrenal gland also exhibited amounts of labeled pesticide or its metabolites. Ingestion of DDT (12.5, 25, or 50 mg/kg daily $\times$ 10) caused a decrease in the ability of the prostate gland to assimilate testosterone-1,2-^3H. This decrease in androgen uptake by prostatic tissue did not seem due to the reported inherent estrogenicity of DDT. The localization of DDT in sex accessory organs of the male mouse is correlated with altered androgenic activity. (Supported by grants from the West Virginia School of Medicine and the Environmental Protection Agency, EP GM00871-01.)

141. *Concentrations of Chlorinated Hydrocarbon Pesticides in Human Blood and Their Relation to the Concentration in Depot Fat.* John R. Brown, The University of Toronto, Toronto, Canada.

Blood samples have been taken from the populations of two agricultural areas and one pesticide industry in Ontario. In all, a total of 421 samples of blood have been taken, 50 of these from industry. Human fat samples have been taken from 52 persons and compared with blood samples taken from the same individuals with respect to their total DDT-derived material

content. It has been found that the ratio of total DDT-derived material fat/blood is 140:1. The majority of the DDT-derived material was present as DDE. Any DDT found usually was due to recent exposure to this material. The methods used for analysis give results which are approximately twice those obtained from current methods. The median value of DDT-derived materials for blood of nonexposed persons was about 0.02 ppm and for those exposed about 0.05 ppm. These results were lower for the Canadian population studied than for similar areas reported in Israel and in the United States. However, the relationship between blood and fat levels would appear to be constant, and, therefore, it would seem likely that blood determinations of total DDT derived material would give a useful indication of the body storage of this material. From examination of this work there does not seem to be any correlation between the amount of DDT-derived material in the biosphere and that found in man.

142. *Effects of o,p′-DDT on the Adrenal Cortex of the Beagle.* M. F. COPELAND and M. F. CRANMER, Environmental Protection Agency, Perrine, Florida.

The purpose of this study was to investigate the influence of the continued po administration of *o,p′*-DDT on the adrenal cortex of the beagle. Four of eight males received *o,p′*-DDT (50 mg/kg/day) dissolved in corn oil and administered in gelatin capsules; four received corn oil only. Plasma sodium, potassium and 17-hydroxycorticosteroids (17-OHCS), as well as hematocrit and circulating eosinophils, were determined before and after iv administration of 40 units of ACTH on each dog at intervals during the 32 days of treatment. After 32 days the animals were necropsied, and histological studies were performed on the adrenals of all dogs and adrenal 17-OHCS levels determined. GLC determinations were made of *o,p′*-DDT and its metabolites in adrenal, liver, testis, and adipose tissue. The treated dogs maintained body weights comparable to those of the controls, though treated dogs showed a decreased eosinopenic response to ACTH after day 16, when compared to controls. Two-way classification analysis of variance showed no significant difference at $p < 0.05$ in the 17-OHCS response to ACTH nor within group variation between treated and control dogs. There was no significant difference at $p < 0.05$ in plasma sodium and potassium levels, nor in the hematocrit in the treated as compared to controls before or 4 hr after ACTH on any of the test days, nor in the adrenal 17-OHCS levels at the conclusion of the experiment. The adrenals of the treated dogs were significantly larger ($p < 0.01$) than those of the controls. Histological studies showed cells in the fasciculata of the treated dogs to be larger, more vacuolated, and devoid of acidophilic cytoplasm with hyperchromatic nuclei, which frequently occupied a peripheral position in the cell. Of the tissues analyzed only fat contained a higher level of *o,p′*-DDT than the adrenals in treated dogs. Results from this study suggest that *o,p′*-DDT does not block the synthesis of corticosteroids by the adrenal cortex in the beagle dog as does *o,p′*-DDD.

143. *Morphological and Biochemical Effects of Sucrose Acetate Isobutyrate on the Liver.* B. G. PROCTER, P. DUSSAULT, G. RONA, and C. I. CHAPPEL, Bio-Research Laboratories Ltd., Pointe Claire, Quebec, Canada.

Sucrose acetate isobutyrate (SAIB) is employed as a flavor-suspending agent in the manufacture of soft drinks. Presently, Canadian regulations permit its use at a level of 50 ppm in conjunction with 15 ppm of brominated vegetable oils. To estimate its safety for use at higher levels, the effects of SAIB were investigated in rats and dogs. For 12 wk, SAIB was fed to beagles at dietary levels of 0.5, 1.0, 2.0, and 4.0% and to albino rats at levels of 2.5, 5.0, and 10.0%. In the dog, SAIB was responsible for increased activity of serum alkaline phosphatase and liver carboxylesterase and for impaired bromosulphthalein plasma clearance. There was also a dose-dependent increase in liver weight although the gross and light microscopic appearance of the liver was normal. Histochemical and electron microscopic studies revealed that SAIB caused slight cholestasis and induction of metabolic enzymes. All of these effects in the dog were fully reversed during 6 wk of drug withdrawal. In the rat, SAIB at dietary levels as high as 10.0% produced none of the changes described for the dog. Thus, SAIB produced mild and reversible cholestasis and other adaptive changes in the liver of the dog, not seen in the rat, which may represent a response to overloading. (Supported by the Canadian Soft Drink Association.)

144. *Inhibition Studies of Carboxyesterase Activity.* J. C. KILLEEN, JR. and T. B. GRIFFIN, Albany Medical College, Albany, New York.

The inhibitory effects on carboxyesterase activity exercised by po administered *O,O*-dimethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (I), its oxygen analog (II), and *O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (III) were examined in animals. Carboxyesterase and cholinesterase activities were determined using automated pH stat methods. Optimum conditions for determination of carboxyesterase activity in red cells, plasma, brain, and liver were established, using triacetic tributyrin, methyl butyrate, and other esters as substrates. Eserine was employed at a concentration suitable for distinguishing between carboxyesterase and cholinesterase activities. Studies in female Sprague-Dawley rats demonstrated that I or III given po for 15 days inhibited liver esterase activity in lower concentrations than were required to inhibit cholinesterase and carboxyesterase activities of red cells, plasma, and brain; III was the more potent esterase inhibitor. A single dose of II which elicited no inhibition of cholinesterase activity, reduced esterase activity in both liver and plasma. Further studies in rats which had received po 1.0 mg/kg of I for 15 days disclosed that reduction in liver esterase activity was primarily due to decrease in hydrolytic activity of an eserine-sensitive esterase; there was less marked inhibition of the eserine-insensitive component. These studies demonstrate that the nature of esterase activity in rat liver, with regard to eserine sensitivity, is different from that of the plasma, and substantiates the view that liver esterases are more sensitive than brain or blood cholinesterase to po-administered organophosphates. (Supported by Research Grant 5P01-ES00226-05 from the National Institute of Environmental Health Sciences, NIH, and by the National Institutes of Health Training Grant 5T01-ES00103-05.)

145. *Drug Induced Alkaline Phosphatase Activity in the Liver of the Beagle Dog.* M. H. LITCHFIELD and D. M. CONNING, Industrial Hygiene Research Laboratories, Alderley Park, Cheshire, England.

Our previous studies have shown that phenobarbital is capable of elevating alkaline phosphatase activity in liver microsomes and plasma of beagle dogs. This elevation was shown not to be associated with hepatobiliary or bone damage, suggesting that the rise in hepatic activity might be due to an enzyme induction effect, since microsomal drug metabolizing enzyme activity was also raised. To investigate this further, cycloheximide and phenobarbital have been given to beagle dogs. Two male dogs from a group of six were injected iv daily for 11 days with successively increasing doses of phenobarbital commencing at 5 mg/kg and rising to 40 mg/kg. Two other dogs were dosed similarly, but 1 mg/kg cycloheximide was injected 30 min before each dose of phenobarbital. The remaining two dogs were undosed controls. The microsomal activities of hepatic alkaline phosphatase and the drug metabolizing enzyme aminopyrine demethylase (APDM) were assayed at termination of the experiment. The alkaline phosphatase and APDM activities of the phenobarbital treated dogs showed four- and tenfold rises, respectively, but these activities rose only two- and threefold in those animals also given the protein synthesis inhibitor. Studies of plasma alkaline phosphatase by inhibition, heat inactivation, and electrophoretic assays showed an increased contribution from the hepatically derived enzyme in the phenobarbital dosed dogs where the activities were raised above control values. Histochemical and electron microscopical studies confirmed the absence of biliary obstructions or other hepatocellular damage. These results strongly indicate that raised plasma alkaline phosphatase in dogs following phenobarbital administration is derived from a drug-induced effect on the hepatic enzyme.

146. *Comparison of Plasma Enzyme Assays for the Detection of Liver Damage in the Beagle Dog.* M. H. LITCHFIELD and CAROLE J. GARTLAND. Industrial Hygiene Research Laboratories, Alderley Park, Cheshire, England.

The relative specificity and sensitivity of plasma enzyme assays to organ damage produced by the long-term administration of toxic agents is under investigation. An initial study has examined the changes in plasma enzyme activity following the effects of small doses of the

hepatotoxin carbon tetrachloride in dogs. Plasma GPT, GOT, OCT, and AP activities and BSP retention were assayed 24, 48, and 72 hr after single po doses of CCl_4. The smallest dose of CCl_4 (0.05 ml/kg) did not affect any of the enzyme activities, nor was BSP retention altered. GPT activity doubled 48 hr after 0.1 ml/kg CCl_4 but no other variation was detected. A dose of 0.2 ml/kg CCl_4 provoked a very marked rise in GPT activity, the value increasing fivefold in the first 24 hr and reaching a maximum increase of 20-fold within 3 days. There was little effect on OCT activity within 24 hr but this increased by a factor of 10 within 3 days. GOT activity doubled, but AP activity and BSP retention were unaffected. A dose of 0.3 ml/kg CCl_4 was required to alter AP activity and BSP, and then only one dog out of four was affected. Histological examination confirmed liver damage in all dogs, the degree ranging from low grade cirrhosis to acute necrosis in a dog given the largest dose of CCl_4. The results show GPT to be the most sensitive indicator among these assays for CCl_4 induced liver damage. Since AP activity was not affected by low doses of this hepatotoxin and yet is known to be sensitive to biliary obstruction, the combination of plasma GPT and AP assays should provide a suitable screen for a number of liver disorders arising from toxicological studies.

147. *Utilization of the Pig Zygote System for Mutagenic Testing.* D. J. KILIAN and C. B. JACOBSON, Dow Chemical Company, Freeport, Texas; George Washington University Medical Center, Washington, D.C.

Present mutagenic test systems have certain inherent defects. This work was undertaken to develop a better reproductive test system for chemical mutagenesis in an animal whose physiology and chromosome pattern are similar to man. In the Pig Zygote Test, fertilized ova can be exposed to the test chemical and observations made on cleavage progression and the effect on chromosome morphology. Treated and untreated zygotes from selected color matings may also be transplanted into a host gilt and embryonic development compared. The test chemical may be added to the tissue culture system to determine the direct effect, or it can be given to the gilt or boar, providing mutagenic information about its metabolic products. Implanting the developing zygotes into rabbit fallopian tubes and exposing this animal to the test chemical provides a host mediated assay system utilizing a mammalian reproductive indicator. Twenty-eight female pigs were given superovulatory hormone regime and up to 72 fertilized ova were recovered from a single animal following natural or artificial insemination and surgical recovery. Each zygote was used as an individual reproductive study model. The zygotes were grown and studied in tissue culture. Observations of cleavage and development of chemically treated and untreated blastomeres were compared. Successful preparation of chromosomes was achieved on both control and chemically treated zygotes. Results at this stage indicate that chromosomes from treated zygotes showed effects that may have been due to the chemical, while control zygotes were normal. So far, the data strongly indicates the promise of the pig zygote system as a practical mutagenic test system to screen industrial compounds and provide information that may be most relevant to the human species.

148. *Chronic Toxicity, Teratology, and Mutagenicity Studies with Cyclohexylamine in Rats.* D. E. BAILEY, K. MORGAREIDGE, G. E. COX, E. E. VOGIN, and B. L. OSER, Food and Drug Research Laboratories Inc., Maspeth, New York.

Two-year po toxicity studies are in progress with cyclohexylamine hydrochloride (CHA) in rats at dose levels of 0, 15, 50, 100, and 150 mg of base/kg body wt. As an adjunct to the toxicity evaluation, carcinogenicity, reproductive, teratogenic, and mutagenic studies are also being carried out. Ingestion of CHA at doses up to 150 mg of base/kg body wt. resulted in no adverse behavioral effects nor any evidence of increased sympathomimetic activity. There is a definite dose-related decrease in growth and food consumption at the two higher levels; however, no drug-induced changes were found in extensive series of hematological, biochemical and urine analytical examinations performed on the animals. Similar findings were seen in the F_1 and F_2 generation animals allowed to grow to maturity. Some tolerance to the growth-depressant effects appears evident in the F_3 and F_4 generations. Examination of urine samples of the F_0 animals revealed greater than 70% incidence of the bladder parasite, *Trichosomoides*

crassicauda. No dominant lethal, teratogenic, or cytogenetic effects have been observed. To date, all data indicate that the only apparent effect associated with the administration of this material at doses up to 150 mg/kg is a dose-related decrease in growth.

149. *Response of Neonatal Rats to Mycotoxins and Insecticides.* RODERICK M. FARB, JUDITH A. CAIN, BOBBY G. MOORE, and A. WALLACE HAYES, The University of Alabama, University, Alabama.

There is little information on the interaction of mycotoxins and pesticides in young animals or their effect on the offspring of exposed mothers. Studies, therefore, were initiated to compare day-old, 14- and 21-day animal responses to mycotoxins (aflatoxin, ochratoxin, rubratoxin) and to pesticides (aldrin, chlordan, dieldrin, DDVP). Various concentrations and combinations of the agents were administered by stomach tube to neonates, 14- or 21-day Sprague-Dawley rats. Daily weight gains, mortality, liver:body ratios and blood chemistry were monitored. The adult-neonate LD50 ratios were aldrin, 1.2; DDVP, 2.4; dieldrin, 3.4; ochratoxin, 6.5; and rubratoxin, 49.2. Rubratoxin (5.0 mg/kg) administered at day 1 followed by aflatoxin (4.0 mg/kg) at day 15 caused a reduction in average weight gains, whereas the reverse did not. Similar results were obtained with DDVP-aldrin and chlordan/dieldrin. The mortality rate of day-old rats given simultaneously po doses of rubratoxin (5.0 mg/kg) and ochratoxin (2.0 mg/kg) was greater than either compound alone. There was a 36% increase in WBC of rats treated with rubratoxin followed by aflatoxin. Similar increases in WBC were seen in animals treated with DDVP/aldrin and chlordan/dieldrin. It appears from our data that dual exposure of rats to these agents induces greater effects than equal doses of single agents. (Supported by Grant No. ES-00464-02.)

150. *Toxicity of Ochratoxin A in the Rat.* I. C. MUNRO, C. A. MOODIE, E. J. MIDDLETON, P. M. SCOTT, and H. C. GRICE, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

Studies were undertaken to examine the short-term and subacute toxicity of ochratoxin A in the rat. In a short-term study, groups of weanling male rats were fed a semipurified diet containing 0, 2.4, 4.8, 9.6, or 24.0 ppm of ochratoxin A for a period of 2 wk. Growth retardation and reduced food consumption were observed in rats fed 9.6 or 24.0 ppm but not in those fed 2.4 or 4.8 ppm of the compound. Rats fed 24.0 ppm of ochratoxin A had elevated serum BUN values and significantly increased kidney weights accompanied by marked degenerative changes involving the entire tubular system. Similar, less extensive changes were noted in the 4.8 and 9.6 ppm groups. In a subacute study groups of 8 weanling male and female rats were fed a semipurified diet for 90 days containing 0, 0.2, 1.0, or 5.0 ppm of ochratoxin A. Body weight was significantly reduced in the 5.0 ppm group. In contrast to the short-term study, relative kidney weights were reduced in the 1.0 and 5.0 ppm groups and the latter group had elevated BUN values. Dose-related pathological changes were noted in the kidneys of all treated rats. In the 0.2 ppm group a few scattered cells in the distal convoluted tubules underwent eosinophilic degeneration, and a very few cells developed bizarre giant nuclei. At the higher doses, similar, but more extensive changes were observed. Liver cells of the 0.2 ppm group were slightly smaller and the cytoplasm slightly denser than in controls. PAS staining, with and without diastase pretreatment, revealed that this change was related to reduced glycogen content. In addition, scattered single nuclei had undergone hyaline degeneration. Glycogen reduction was progressively greater in the higher dose groups, and there were more hyalinized cells. Liver cell nuclei were very variable in size with irregular chromatin arrangement and multiple nucleoli in the 1.0 and 5.0 ppm groups.

151. *Acute Toxicity of a Mycotoxin from Aspergillus glaucus.* T. GLINSUKON and R. C. SHANK, Massachusetts Institute of Technology, Cambridge, Massachusetts. (G. N. Wogan.)

A mycotoxin was isolated from *Aspergillus glaucus* originally cultured from leftover cooked rice presumably associated with the death of a 3-yr-old boy in Thailand. The pure compound has a mol wt of 485 and possible empirical formula $C_{26}H_{31}O_8$. The LD50 of this mycotoxin in

male weanling Fischer rats (55–65 g body weight) is 4.8 mg/kg body wt with 95% confidence limits of 4.4–5.2 mg/kg by ip injection and 17.4 mg/kg with 95% confidence limits of 14.7–20.5 mg/kg by po administration using dimethylsufoxide as solvent. After ip dosage the animals were in poor condition for 3–12 hr, during which time all deaths occurred. Animals receiving fatal doses po died 4–18 hr after administration; histopathologic changes observed were qualitatively the same for both groups. The most usual change seen in liver was necrosis with congestion in the centrolobular area: in parenchymal cells nuclei were large and hyperchromatic. There were clumps of bileduct epithelium and leukocytes in the portal tracts. The most striking change seen in kidney was in the cortical zone where there was a glomerularnephrosis, necrosis of epithelium in convoluted tubules and desquamation of epithelium in collecting ducts. Necrosis and desquamation also occurred in intestinal mucosa. The lung was characterized by thickened, hypercellular alveolar walls with congestion. (Supported by the National Institutes of Health Contract No. PH 43-62-468 and United States Public Health Service Contract No. PH 43-67-93.)

152. *The Oncogenic Effects of Aflatoxin Metabolites in the Rat.* C. ALASTAIR MOODIE, HAROLD C. GRICE, and DOROTHY C. SMITH, Food and Drug Directorate, Department of National Health and Welfare, Tunney's Pasture, Ottawa, Canada.

Aflatoxin was fed to pregnant and recently delivered female rats to determine the effect on the offspring of aflatoxin metabolites received only via the placenta, by milk, or both routes. The first group of rats received aflatoxin B_1 by mouth from day 15 of pregnancy until parturition, the second group from day 1 until day 10 postpartum, and the third group from day 15 of pregnancy until day 10 postpartum. Half of each group received 36.5 μg of aflatoxin daily, and the other half received 75 μg daily. Thereafter each group was fed normal diet. All offspring received normal diet on weaning. There was a heavy death toll from pneumonia among the offspring of the higher dose dams. The survivors had a multiplicity of benign and malignant tumors and tumor-like lesions involving most systems, but the organ most frequently involved was the pituitary gland which accounted for 39% of all tumors. Tumors and tumor-like lesions of the liver accounted for only 11% of all tumors, and 14.9% of all malignant tumors. This does not correspond with the experimental results of po aflatoxin administration where the liver is the most frequently involved organ.

153. *Preclinical Toxicologic Studies with Tobramycin.* J. S. WELLES, J. L. EMMERSON, W. R. GIBSON, R. NICKANDER, N. V. OWEN, and R. C. ANDERSON, The Lilly Toxicology Laboratories, Greenfield, Indiana.

Tobramycin, an aminoglycoside antibiotic, has been administered to mice, rats, cats, and dogs for toxicologic evaluation. The sc LD50 values in mice and rats were 441 $\pm$ 15 and 969 $\pm$ 50 mg/kg, respectively. Deaths were preceded by CNS depression and occurred within 1 hr of dose. A 100 mg/kg iv dose in chloralose-anesthetized cats produced a moderate, transient decrease in blood pressure and a significant decrease in inspiratory volume and soleus twitch force. Rats were given daily sc doses of 15–120 mg/kg for 3 mo. Renal tissue change, the only histologically evident drug effect, ranged in degree from a slight reparative nephrosis at the lowest dose to cortical tubular necrosis in some rats that received the highest dose. In a one-month study a daily im dose of 7.5 mg/kg had no apparent effect on dogs, but a 30 mg/kg dose produced severe renal damage. Vestibular injury occurred within less than 30 days in all cats that received daily sc doses of 50 mg/kg, but no vestibular changes were observed in cats that were given 65 doses of 25 mg/kg. The toxicologic effects observed in tobramycin-treated animals were qualitatively and quantitatively similar to those observed with gentamicin.

154. *Experimental Papilledema in the Dog Induced by a Salicylanilide.* W. R. BROWN, L. RUBIN, M. HITE, and R. E. ZWICKEY, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

The halogenated salicylanilide, 3,5-diiodo-3′-chloro-4′-(*p*-chlorophenoxy) salicylanilide (rafoxanide), has been shown extremely effective at low doses for the treatment of fascioliasis and hemonchosis in sheep and cattle. During the safety evaluation experiment of rafoxanide

in the dog, unusual morphologic changes were observed in the central nervous system and eye. A separate experiment was performed in which Beagle dogs were given high doses of rafoxanide in order to study the pathogenesis of these central nervous system changes. After 3–11 po doses of 100 mg/kg/day of rafoxanide, the dogs developed bilateral equatorial cataracts, papilledema, vacuolation of the optic nerve, optic chiasma, white matter of the brain and spinal cord, and focal vacuolation of sciatic nerve. Other toxicologic manifestations included increased SGOT and serum alkaline phosphatase, neutrophilia, lymphopenia, focal hepatic necrosis, and lymphoid necrosis in lymph nodes and the intestine. The pathogenesis of the ocular and neural lesions was related to increased cerebrospinal fluid pressure due probably to an increased amount of fluid in the cranial cavity, brain swelling and meningeal inflammation around optic nerve and optic chiasma.

155. *Aspirin: Three-Month Oral Toxicity Studies in Dogs and Rats.* D. L. BOKELMAN, W. J. BAGDON, R. D. JENSEN, and R. E. ZWICKEY, Merck Institute for Therapeutic Research, West Point, Pennsylvania.

These studies were performed to investigate, in detail, the toxic effects of aspirin in dogs and rats. Dogs and rats were given aspirin po at single daily doses of 100, 200, or 400 mg/kg for periods up to 3 mo. Most of the dogs given 400 mg/kg/day became moribund after 3–8 wk of treatment. Common clinical findings in these dogs included emesis, diarrhea, anorexia, sc abscesses, and ocular lesions. Emesis and anorexia were also observed in dogs given 100 or 200 mg/kg/day. Electrocardiographic changes were noted in dogs given 200 or 400 mg/kg/day. Anemia and other hematologic changes were seen in dogs at all dose levels. Serum biochemical changes noted in dogs given 400 mg/kg/day included increases in urea nitrogen, creatinine, glutamic oxaloacetic transaminase, alkaline phosphatase, ornithine carbamyl transferase, arginine succinic acid lyase, and creatine phosphokinase. Morphologic changes noted in dogs given 400 mg/kg/day included ulcerative gastritis, renal papillary necrosis, hepatic necrosis, arteritis, degenerative myopathy, and bone marrow changes. Less pronounced gastric and muscle lesions were found at the 100 or 200 mg/kg day dose level. Clinical and hematologic changes were not as pronounced in rats given 200 or 400 mg/kg/day as in dogs given similar doses. Whereas there were no clinical or hematologic changes in rats given 100 mg/kg/day, there was a low incidence of renal papillary lesions in rats at all dose levels, and gastric lesions were found at 200 or 400 mg/kg/day. This study demonstrated that dogs exhibit a wide range of hematologic, biochemical, and morphologic changes when given aspirin at doses up to 400 mg/kg/day. In comparison, the effects upon rats given the same doses were less pronounced.

156. *The Comparative Toxicity of the Pyrolysis Products of Three Copolymers of Vinylidene Fluoride and Hexafluoropropylene.* V. L. CARTER, JR., H. P. WARRINGTON, D. A. BAFUS, C. A. LEGG, and E. S. HARRIS, NASA Manned Spacecraft Center, Houston, Texas.

Three raw fluoropolymers were individually pyrolyzed at 550°C for 30 min in a 1 in. diameter stainless steel tube. They are identified as follows: (I), copolymer of vinylidene fluoride and hexafluoropropylene; (II) and (III), obtained from two different commercial sources, as copolymers of vinylidene fluoride and hexafluoropropylene with additives to improve properties. An air flow of 100 ml/min during pyrolysis moved the pyrolysis products into a 142-l chamber. Male rats weighing from 225–250 g were introduced into the chamber through an airlock where they were exposed to the pyrolysis products for 30 min under static conditions. They were then removed from the chamber and observed for 48 hr. Necropsies were performed on all subjects surviving the 48-hr observation period as well as those that died. Chamber atmosphere samples for carbon monoxide (CO), carbon dioxide (CO_2) and fluorocarbon analyses were obtained at 0, 15, and 30 min during the exposure. The 48-hr LD50 values for the fluoropolymers under the above test conditions were: I, 2.4 g; II, 1.1 g; and III, 1.4 g. CO_2 chamber concentrations, carboxyhemoglobin determinations, and the time course of death indicated that CO and/or CO_2 did not significantly contribute to fatalities. The lungs from all the necropsied rats exhibited varying, but significant pulmonary hemorrhage and edema. Preliminary results indicate these pulmonary changes as the primary cause of death.

157. *Toxicological Studies of Tryptamine and N,N-Dimethyltryptamine.* R. P. MAICKEL, E. CAMPAIGNE, and T. R. BOSIN, Indiana University, Bloomington, Indiana.

In a previous paper we have reported preliminary pharmacological studies on the benzo[b]-thiophene analogs of various indolealkylamines including tryptamine and *N,N*-dimethyl-tryptamine. Continuing along the line of studying isosteres, we have compared the indole, benzo[b]thiophene, *N*-alkylindole, and indene analogs of tryptamine and *N,N*-dimethyl-tryptamine. Test procedures included determination of LD50, measurement of motor activity effects (actophotometer), measurement of muscular coordination (rotorod) and interactions with benzoquinolizines and monoamine oxidase inhibitors. In the tryptamine series, the benzo-[b]thiophene and *N*-methylindole compounds were more toxic than the indole; in the *N,N*-dimethyltryptamine series, LD50 values were similar for all compounds. Pretreatment with reserpine increased the toxicity of tryptamine slightly, but decreased that of the sulfur analog; pargyline pretreatment increased the toxicity of both tryptamine compounds. Pretreatment with reserpine or pargyline increased the toxicity of the indolic dimethyltryptamines but reduced that of the non-indolic compounds. (Supported by USPHS grants K-02-MH-41083, MH-18852 and NS-09672.)

158. *A Review of the Toxicological Features of Some Drug-Induced Myocardial Papillary Muscle Necrosis in Dogs.* T. BALAZS, Food and Drug Administration, Washington, D.C.

The toxicological feature of isoproterenol induced cardiac necrosis in dogs has been reported. Similar events occurred in the preclinical testing of a novel antihypertensive agent SK&F 24260 [2,6-dimethyl-3,5-dicarboethoxy-4-(2-trifluoromethylphenyl)-1,4-dihydropyridine]. PO dosing with 10 mg/kg on two consecutive days induced necrosis in the left ventricular papillary muscle. The severe lesion was accompanied by extrasystoles lasing 2–3 days. In a 90-day study, the initial lesion healed and did not recur. However, the hypotension and tachycardia were maintained. PO doses of 10 mg/kg of hydralazine also induced necrosis of the papillary muscle in a 5-day test. This site is the most sensitive to ischemia, considered to be the cause of the lesion induced by the drugs described above. During long term administration, however, a resistance develops to this effect. Thus an acute test is necessary in preclinical studies to detect a cardio-toxic effect of this nature. The mechanism of resistance to the toxic effect of these agents may be related to a myocardial adaptation to hypoxia.

159. *Deficiency of Memory and Learning Functions Related to Lipofuscin Accumulation in Brains of Rats on Chronic Vitamin E Deficient Diet.* SURENDRA K. PURI, SRECKO POGACAR, and HARBANS LAL, University of Rhode Island, Kingston, Rhode Island, Rhode Island Medical Centre, Howard, Rhode Island.

In one respect chronic deficiency of vitamin E (E) resembles "aging": both promote accumulation of lipofuscin in the central nervous system (CNS). Since aging to senility induces disturbances in the functioning of CNS, we determined the effect of chronic E deficient diet on learning and memory related behaviors in the rat. Sprague-Dawley male rats (45 days old) were placed on commercially available E deficient diet for a total of 14 mo. An age-matched group on normal diet was used as control for normal aging. Normal aging promoted accumulation of lipofuscin in CNS. E deficient diet accelerated this process. No alteration due to E deficient diet was observed in weight gain, sensitivity to barbiturates, or locomotor activity. However, weight of brain, heart, kidney, or liver was reduced with respect to total body weight. Acquisition and retention of conditional avoidance response, retention of one-trial learning, and performance of delayed-alternate responding were impaired by chronic E deficient diet. (Supported by a grant from the University Research Committee of the University of Rhode Island.)

160. *Investigation of Extrapyramidal Toxicity and Depression of Shock Avoidance Behavior by Neuroleptic Drugs.* S. FIELDING, B. OUTWATER, T. MCGREEVY, and L. PACIFICO, CIBA-GEIGY Pharmaceutical Co., Summit, New Jersey. (H. Lal.)

The disruption of adaptive avoidance behavior in normal animals by drugs which usually produce an ameliorative action in human psychotic patients is used in detecting antipsychotic

drugs. The purpose of the present study was to determine whether the minimum dose effective in blocking this avoidance behavior also produces extrapyramidal side effects that may parallel those found in man. Four antipsychotic compounds (spiroperidol, haloperidol, chlorpromazine, and thioridazine) were administered po to eight squirrel monkeys working on a continuous shock avoidance procedure with RS and SS intervals both equal to 20 sec. The dose at which blockade of avoidance behavior occurred was determined during an uninterrupted session. During other sessions, a single-blind comparison of the four neuroleptic compounds was made involving 3–5 min neurological examinations before the start of the session and every hr thereafter for 4 consecutive hours. The presence of muscle tremors during these examinations was used as an indicator of extrapyramidal side effects. A determination of the minimal effective dose (MED) which caused inhibition of avoidance behavior and the MED which induced extrapyramidal symptoms (EPS) was made for each of the antipsychotic compounds. The MED for shock avoidance behavior for spiroperidol was 0.625 mg/kg, for haloperidol 1.25 mg/kg, for chlorpromazine 1.0 mg/kg, and for thioridazine 2.5 mg/kg. Although spiroperidol was more potent than haloperidol in blocking avoidance behavior, no significant dose separation was obtained, i.e., blockade of avoidance behavior and muscle tremors appeared at the same dose. The dose separation for chlorpromazine and thioridazine was $> 3 \leqslant 6$ and $> 4 \leqslant 8$, respectively. The ranking of these neuroleptic compounds in order of the relative extrapyramidal toxicity produced in the squirrel monkey parallels that found in man.

161. *The Toxicology of Metiapine A Dibenzothiazepine Tranquilizer.* JOHN P. GIBSON, JAMES W. NEWBERNE, and MICHAEL W. ROHOVSKY, Merrell–National Laboratories, Cincinnati, Ohio.

Metiapine, 2-methyl-11-(4-methyl-1-piperazinyl)-dibenzo [b,f] [1,4] thiazepine, is a central nervous system depressant that produces behavioral effects in a variety of species that are characteristic of the potent tranquilizers. The acute po LD50 after 24 hr in mice and rats was 680 and 934 mg/kg, respectively, but delayed deaths reduced the 7-day LD50 to 560 and 525 mg/kg, respectively. Continuous daily po administration in the diet to rats for 18 mo at doses of 3, 10, or 30 mg/kg/day produced a dose-related decrease in food consumption and body weight gain. When given single daily po doses of 5, 15, or 50 mg/kg/day for 1 yr, dogs exhibited varying degrees of depression and stimulation, and mammary enlargement with milk production was noted in some of the females. The 50 mg/kg group also exhibited slight increases in serum alkaline phosphatase. Single daily po doses of 30 mg/kg/day to pregnant rats and rabbits during embryogenesis increased fetal deaths, but 3 and 10 mg/kg/day did not; there was no indication of teratogenicity at any of these levels in either species. A reproduction study in rats in which 3, 10, or 30 mg/kg/day was administered in the diet to males for 106 and females for 14 days prior to mating resulted in a dose-related reduction in conception rates. Further study revealed that this was due to an effect on the female estrous cycle, and that once implantation had occurred there appeared to be no effect on pregnancy or fetal development except in the 30 mg/kg group, where there were more dead pups at birth and fewer survivors at weaning. Most of the changes noted in these studies were attributed to the psychotrophic effect of the drug, which greatly affected food consumption and endocrine activity.

162. *Interactions Between Diphenylhydantoin and Tolbutamide in the Mouse.* JOHN H. MENNEAR and THOMAS A. GOSSEL, Purdue University, Lafayette, Indiana.

Diphenylhydantoin (DPH) has been reported to induce alterations in carbohydrate metabolism in some humans and in most species of laboratory animals. These effects are often associated with high or even toxic doses of the drug. The present study was conducted to determine if the effects of DPH on carbohydrate metabolism could be detected in the mouse when the dose level of the drug was the anticonvulsant ED50 value. The ip ED50 value of DPH in the supramaximal electroshock test was found to be 5.5 mg/kg. In all subsequent experiments the ip dose of DPH was 5.0 mg/kg. The administration of DPH to normally feeding mice did not alter resting blood glucose levels, but it significantly reduced glucose tolerance. Similarly, DPH did not alter the hypoglycemic effect of exogenously administered insulin but it reversed the

hypoglycemic effect of tolbutamide. The reversal of tolbutamide-induced hypoglycemia appears to be mediated through the adrenal glands, since DPH reduced but did not reverse the hypoglycemic effect of tolbutamide in adrenalectomized mice. Also, DPH potentiates the hyperglycemic effect of epinephrine. The results of these studies suggest that there may be a clinically significant interaction between DPH and tolbutamide. (Supported by N.I.H. Grants GM 15005 and AM 14134.)

163. *Comparative Dermal Irritation/Sensitization Studies with Banomite (U-27415) in Guinea Pigs and Man.* T. J. KAKUK, R. L. JOHNSTON, T. E. WEDDON, and W. A. RYE, The Upjohn Co., Kalamazoo, Michigan. (G. C. Boxill.)

Benzoyl chloride(2,4,6-trichlorophenyl)hydrazone (Banomite) is effective against phytophageous arachnids on citrus, apples, and almonds. The aforementioned experiments in animals and man were conducted to elucidate and characterize the dermal irritation/sensitization potential of this compound. Rabbit, guinea pig, and human dermal irritation studies indicated that Banomite produced contact dermatitis in animals and man 5–16 days after exposure. The severity of the lesion was dose-related. The no-effect dermal dose was 0.1–0.25 mg applied as a single dose to an exposed area of 2 cm^2 on guinea pigs, approximately 40–100 times more than the field application rate. In man the single no-effect dermal dose was approximately 0.18 mg (2 cm^2 area) total dose for 24 hr exposure. This represented a 72× safety factor, over the recommended field application rate of 0.0025 mg per 2 cm^2. Sensitization studies in guinea pigs and man indicated that Banomite is a delayed dermal sensitizer if high enough doses are used and if irritation precedes the challenge. In this presentation we will describe the events necessary for eliciting sensitization and illustrate the pathogenesis of the dermal lesion. We have not seen irritation or sensitization under field conditions in 200–300 field workers totaling over 1000 exposures with Banomite. The aqueous insolubility and with the very dilute concentrations used under field conditions probably explains the lack of reported dermatitis in man.

164. *Effects of Vitamin C on Atrioventricular and Intraventricular Conduction in Dogs.* G. A. BOBB, S. H. LAU, and A. N. DAMATO, U.S. Public Health Service Hospital, Staten Island, New York. (E. J. Fairchild.)

Atrioventricular conduction (AVC) and intraventricular conduction (IVC) were studied in ten intact and open-chested dog hearts. Catheter and plunge wire recordings of the His bundle and left and right bundle branches were obtained during sinus rhythm and at atrial pacing rates (150–200/min). Stimulus to H (S–H) was used as a measurement of AVC. H to onset of Q wave (H–Q) and H to the terminal deflection of the QRS (H–S) were used as a measurement of IVC. Following 8 mg/kg iv vitamin C, sinus rate decreased; at rapid atrial pacing rates S–H interval shortened in 7 dogs, increased in three; two dogs developed prolongation of vagal escape time; two dogs manifested left bundle branch block and VPC. H–Q showed little or no change. Thus, vitamin C slows sinus rate, usually shortens AVC, prolongs vagal escape time, sometimes blocks conduction in the A–V node and causes aberrant ventricular conduction. These changes are reversible within 30 min.

165. *Studies on the Increased Toxicity of Ouabain in Newborn Rats.* CURTIS D. KLAASSEN, University of Kansas Medical Center, Kansas City, Kansas.

The 24-hr LD50 of ouabain in adult rats was approximately 125 mg/kg and 3.5 mg/kg in 3-day-old rats. There was a gradual increase in the LD50 from 3 to 33 days of age, after which LD50 values remained relatively constant. In an attempt to determine the mechanism of the increased sensitivity to the toxic action of ouabain, the distribution of ^3H at 5, 15, 30, and 45 min after administration of ^3H-ouabain (4 mg/kg, ip) was measured in 7- and 39-day-old rats. The concentrations of ouabain in the various tissues were relatively constant at the various times after ouabain administration. The plasma concentration of ouabain was approximately 0.7 μg/ml in the 39-day-old rats and much higher in the 7-day-old rats (5.0 μg/ml). The concentrations (μg/g) of ouabain in the various organs of 39- and 7-day-old rats, respectively, are as follows: liver 18 and 4, kidney 4 and 6, spleen 3.5 and 2.5, lung 0.5 and 2.5, heart 0.4 and 2.5,

and muscle 1.5 and 3.0. The organ to plasma concentration of ouabain in the various organs of the 39- and 7-day-old rats, respectively, are as follows: liver 30 and 1, kidney 7 and 1.5, spleen 7 and 0.9, lung 0.8 and 0.8, heart 0.6 and 0.6, and muscle 2.2 and 0.7. Therefore, it would appear that many organs in the newborn rat do not have the capacity of the adult rat to concentrate ouabain to a concentration greater than that in plasma. It appears that this inability of the various organs of the newborn rat to concentrate ouabain over the plasma, results in a higher plasma concentration of ouabain and a higher toxicity. Since the liver is the major organ of concentration and excretion of ouabain in the adult, it would appear that this defect is a major reason why ouabain is more toxic in newborn than adult rats. (Supported by USPHS Grant AM 14513.)

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